



Etude des acteurs et des interactions entre les voies de recombinaison chez *Arabidopsis thaliana*

Heïdi Serra

► To cite this version:

Heïdi Serra. Etude des acteurs et des interactions entre les voies de recombinaison chez *Arabidopsis thaliana*. Sciences agricoles. Université Blaise Pascal - Clermont-Ferrand II, 2014. Français. NNT : 2014CLF22483 . tel-01295033

HAL Id: tel-01295033

<https://theses.hal.science/tel-01295033>

Submitted on 14 Jun 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ÉCOLE DOCTORALE
DES SCIENCES DE LA VIE ET DE LA SANTÉ
Numéro d'ordre : 636

Thèse

Présentée à l'Université Blaise Pascal
pour l'obtention du grade de

DOCTEUR D'UNIVERSITÉ
Spécialité : Physiologie et Génétique Moléculaires

Soutenue le 5 septembre 2014

Heïdi SERRA

**ÉTUDE DES ACTEURS ET DES INTERACTIONS
ENTRE LES VOIES DE RECOMBINAISON
CHEZ *ARABIDOPSIS THALIANA***

Rapporteurs : Dr. Éric Coïc, CEA, Fontenay-aux-Roses
Dr. Emmanuel Guiderdoni, CIRAD, Montpellier

Membres : Dr. Mathilde Grelon, INRA, Versailles
Pr. Maria Eugenia Gallego, Université Blaise Pascal, Aubière
Dr. Charles I. White, CNRS, Aubière

Génétique, Reproduction & Développement
UMR CNRS 6293 - Clermont Université - INSERM U1103
24, avenue des Landais 63177 Aubière Cedex

Remerciements

Je souhaiterais tout d'abord remercier les membres du jury qui ont accepté d'évaluer mon travail de thèse, Emmanuel Guiderdoni, Eric Coïc et Mathilde Grelon. Merci pour cet échange et vos remarques constructives.

Je remercie également chaleureusement Holger Puchta et Pierre Sourdille pour l'intérêt qu'ils ont porté à l'avancement de mes travaux au cours des comités de thèse et leurs précieux conseils.

Je tiens à remercier tout particulièrement mes deux coachs, Maria E. Gallego et Charles White, co-directeurs de ma thèse. Merci de m'avoir fait confiance, pour l'autonomie que vous m'avez tout de suite accordée et pour votre disponibilité face à mes nombreuses questions. Merci pour le temps que vous m'avez consacré au cours de ces trois années. Charles, merci pour ton enthousiasme et ton optimisme à toute épreuve, pour nos discussions si enrichissantes, pour tes conseils avisés et ton aide précieuse dans la rédaction des articles. Maria, merci pour nos multiples échanges, pour ton soutien, et d'avoir pris au sérieux mes « réunions de crise » ! Merci à tous les deux pour votre passion pour la recherche que vous m'avez transmise.

Merci à tous les membres de l'équipe Recombinaison, Simon, Olivier, Fabienne, Chantal, Elisabeth, Margaux, Gunjita, Patrick, Imen et Annie. J'ai eu beaucoup de plaisir à travailler avec vous pendant ces trois ans, et je vous en remercie !

Simon et Olivier, un grand merci pour votre disponibilité, votre implication dans mon projet, vos précieux conseils et pour les discussions scientifiques et moins scientifiques que l'on a partagées ! Fabienne, je te remercie pour ton soutien quotidien, ta gentillesse, ton aide précieuse à la paillasse et la relecture minutieuse de ce manuscrit. Chantal, merci pour tes conseils-rando ! Quelle chance d'être ta voisine de paillasse Imen, ta bonne humeur quotidienne et ton optimisme sont contagieux ! Margaux, j'ai été ravie que tu rejoignes l'équipe. Merci pour ta spontanéité et ta gentillesse. Je te souhaite le meilleur pour la suite !

Je tiens également à remercier tous les membres de Biomove pour leur accueil chaleureux et les bons moments que nous avons partagés. Merci Leti et Angeles pour votre joie de vivre communicative ! Merci Axel pour ton humour ! Merci Zoli pour toutes nos discussions et tes conseils en clonage. Merci Sam pour ta gentillesse au quotidien et ton aide en phylogénie. Sylvie, merci de m'avoir intégré à l'équipe pédagogique et pour tes conseils dans la préparation des enseignements mais aussi de mes vacances ! Matthias, merci pour tes conseils-look et nos nombreux échanges ! Merci pour ta présence et ton soutien, de mon premier jour au laboratoire à celui de ma soutenance.

Un grand MERCI à mes « loulous », Coralie, Marine, Marion, Rafik et Kévin, pour tous ces moments partagés... nos pauses cappu, nos repas d'11h30, nos soirées en terrasse, nos virées shopping, nos escapades du week-end, ces veilles d'anniversaire de plus en plus folles ! Autant de joie, de rires et de fous rires, mais aussi parfois de doutes et d'appréhension. Merci pour votre présence, merci pour votre Amitié. Grâce à vous j'emporte avec moi d'excellents souvenirs de mes années clermontoises !

Je remercie aussi de tout cœur ma famille qui m'a toujours soutenue dans mes projets. Merci pour votre présence le jour J !

Et enfin, MERCI à toi Gaultier, pour tes encouragements, ton soutien quotidien, et d'être à mes côtés depuis tant d'années ! Merci pour TOUT, tout simplement...

Résumé

La réparation des cassures double brin (CDB) de l'ADN par recombinaison est essentielle au maintien de l'intégrité du génome de tous les êtres vivants. Ce processus doit cependant être finement régulé puisque la recombinaison peut générer des mutations ou des réarrangements chromosomiques, parfois extrêmement délétères pour la cellule. Les CDB peuvent être réparées par deux mécanismes : la recombinaison non homologue (ou jonction des extrémités d'ADN) ou la recombinaison homologue (impliquant une homologie de séquence entre les molécules recombinantes). Dans les cellules somatiques, les deux voies principales de recombinaison homologue (RH) sont la voie *Synthesis Dependent Strand Annealing* (SDSA) dépendante de la recombinase RAD51 et la voie *Single Strand Annealing* (SSA) indépendante de RAD51.

Nos résultats ont d'abord mis en évidence un rôle inattendu de XRCC2, RAD51B et RAD51D - trois paralogues de RAD51 - dans la voie SSA. Nous avons confirmé que la fonction de la protéine XRCC2 dans la voie SSA ne dépend pas de RAD51, ce qui démontre que certains paralogues de RAD51 ont acquis des fonctions indépendantes de la recombinase. La différence de sévérité des phénotypes des mutants individuels ainsi que les analyses d'épistasie menées sur le double et le triple mutant suggèrent des fonctions individuelles de ces protéines au cours du SSA. Nous proposons qu'elles facilitent l'étape d'hybridation des deux séquences complémentaires situées de part et d'autre de la cassure, bien que ceci reste à confirmer par des études *in vitro*.

L'étude des fonctions de l'hétérodimère XPF-ERCC1 - un complexe impliqué dans le clivage des extrémités d'ADN non homologues au cours des voies de RH - a révélé un rôle inhibiteur de ce complexe sur la voie SDSA. Cette action est dépendante de son activité endonucléasique et serait liée au clivage des longues extrémités 3' sortantes réalisant l'invasion d'un duplex d'ADN homologue, l'étape initiale de la voie SDSA. Notre étude a de plus confirmé que le rôle du complexe dépend de la longueur des extrémités non homologues chez *Arabidopsis*, comme chez les mammifères et la levure. Bien que le complexe XPF-ERCC1 soit essentiel au clivage des longues extrémités d'ADN non homologues, il n'est pas requis à l'élimination des courtes extrémités au cours de la RH.

Abstract

The repair of DNA double-strand breaks (DSB) by recombination is essential for the maintenance of genome integrity of all living organisms. However, recombination must be finely regulated as it can generate mutations or chromosomal rearrangements, sometimes extremely deleterious to the cell. DSB can be repaired by two classes of recombination mechanism: non-homologous recombination (or DNA End Joining) or homologous recombination (implicating DNA sequence homology between the recombining molecules). In somatic cells, the two main pathways of homologous recombination (HR) are RAD51-dependent Synthesis Dependent Strand Annealing (SDSA) and RAD51-independent Single Strand Annealing (SSA).

Our results have demonstrated an unexpected role of XRCC2, RAD51B and RAD51D - three RAD51 paralogs - in the SSA pathway. We confirmed that the function of XRCC2 in SSA does not depend upon RAD51, thus demonstrating that some RAD51 paralogs have acquired RAD51 recombinase-independent functions. The different severities of individual mutant phenotypes and epistasis analyses carried out on the double and triple mutants suggest individual functions of these proteins in SSA recombination. We propose that they facilitate hybridization of the two complementary sequences located on both sides of the break, although this remains to be confirmed by *in vitro* experiments.

Study of the roles of XPF-ERCC1 - a complex involved in the cleavage of non-homologous DNA ends during HR - revealed an inhibitory role of this complex on the SDSA pathway. This is dependent on its endonuclease activity and is probably due to the cleavage of long 3' ends performing the homologous DNA duplex invasion, the initial step of the SDSA pathway. Our analyses also confirmed that the role of the complex depends on the length of the non-homologous ends, as seen in mammals and yeasts. Although XPF-ERCC1 is essential for the cleavage of long non-homologous DNA ends, it is not required for the elimination of short ends during HR.

Mots-clés

Réparation de l'ADN, Cassure double brin, Recombinaison homologue, Single Strand Annealing, Synthesis Dependent Strand Annealing, XRCC2, RAD51B, RAD51C, XPF-ERCC1, *Arabidopsis thaliana*

Table des matières

LISTE DES ABREVIATIONS	3
TABLE DES ILLUSTRATIONS	5
SYNTHESE BIBLIOGRAPHIQUE	7
CHAPITRE I : LES CASSURES DOUBLE BRIN DE L'ADN : CAUSES ET CONSEQUENCES.....	11
I. ORIGINES DES CASSURES DOUBLE BRIN DE L'ADN	12
I.1. Les cassures double brin accidentelles	12
I.2. Les cassures double brin programmées	13
I.2.1. Au cours de la méiose : rôle de l'enzyme SPO11	13
I.2.2. Au cours de la différenciation des lymphocytes B et T : rôle de RAG1 / RAG2	14
I.2.3. Au cours de la commutation de type sexuel chez <i>S. cerevisiae</i> : rôle de HO	15
II. CONSEQUENCES DE L'ABSENCE OU D'UNE MAUVAISE REPARATION DES CASSURES DOUBLE BRIN D'ADN	16
CHAPITRE II : LES VOIES DE RECOMBINAISON NON HOMOLOGUE.....	18
I. VOIE DE JONCTION DES EXTREMITES DEPENDANTE DES PROTEINES KU	19
I.1. Reconnaissance de la cassure double brin	20
I.2. Maturation des extrémités d'ADN	20
I.3. Jonction des extrémités d'ADN	21
I.4. Fidélité de la réparation	22
II. VOIE(S) DE JONCTION DES EXTREMITES INDEPENDANTE(S) DES PROTEINES KU	23
II.1. Les acteurs des voies de jonction des extrémités indépendantes des protéines KU	23
II.2. Fidélité de la réparation	25
CHAPITRE III : LES VOIES DE RECOMBINAISON HOMOLOGUE.....	27
I. MECANISMES MOLECULAIRES DES VOIES DE RECOMBINAISON HOMOLOGUE	28
I.1. Double Strand Break Repair (DSBR)	28
I.2. Synthesis-Dependent Strand-Annealing (SDSA)	30
I.3. Break-Induced Replication (BIR)	31
I.4. Single-Strand Annealing (SSA)	32
II. LES ACTEURS DE LA RECOMBINAISON HOMOLOGUE	33
II.1. Les protéines de la reconnaissance et de la maturation des extrémités de la CDB	33
II.2. Les protéines impliquées dans la recherche d'homologie et l'invasion de brin	37

REVIEW: "RAD51 MEDIATORS IN EUKARYOTIC HOMOLOGOUS RECOMBINATION"	37
• RAD51 / DMC1 recombinases and homologous recombination pathways	39
• REPLICATION PROTEIN A	43
• RAD51 PARALOGUES	45
• SHU COMPLEX	51
• RAD52 AND RAD59	54
• BRCA1 AND BRCA2	58
• RAD54 AND RAD54B	63
II.3. Les protéines impliquées dans l'élimination des extrémités non complémentaires	79
II.4. Les protéines impliquées dans la dissociation des intermédiaires de recombinaison	83
II.4.1. Les protéines de la dissolution des jonctions de Holliday	84
II.4.2. Les protéines de la résolution des jonctions de Holliday	85
PROJET DE THESE	87
RESULTATS	90
CHAPITRE I : "ROLES OF XRCC2, RAD51B AND RAD51D IN RAD51-INDEPENDENT SSA RECOMBINATION" (ARTICLE 1)	91
RESULTATS COMPLEMENTAIRES	103
I. VALIDATION DU MATERIEL D'ETUDE	104
I.1. Vérification des mutants par complémentation	104
I.2. Vérification de l'induction des cassures double brin dans les plantes mutantes	105
II. XRCC2 N'EST PAS IMPLIQUE DANS LA JONCTION DES EXTREMITES D'ADN	107
III. XRCC2 JOUE UN ROLE INDEPENDANT DE RAD51 DANS LA VOIE SSA	109
CHAPITRE II : "XPF-ERCC1 INHIBITS SDSA RECOMBINATION" (ARTICLE 2)	111
CHAPITRE III : MISE AU POINT D'UN SYSTEME RAPPORTEUR D'EVENEMENTS DE JONCTION D'EXTREMITES D'ADN EN PROTOPLASTES D'ARABIDOPSIS THALIANA	147
I. PRESENTATION DU PLASMIDE RAPPORTEUR DE RECOMBINAISON	148
II. ANALYSES DE LA RECOMBINAISON EN MICROSCOPIE A FLUORESCENCE	149
III. ANALYSES DE LA RECOMBINAISON EN CYTOMETRIE EN FLUX	150
IV. UN SYSTEME RAPPORTEUR DE L'EFFICACITE DE JONCTION D'EXTREMITES D'ADN	152
DISCUSSION GENERALE & PERSPECTIVES	154
REFERENCES BIBLIOGRAPHIQUES	163

Liste des abréviations

ABL : Abelson leukemia virus tyrosine kinase	DSBR : Double strand break repair
ADN : Acide désoxyribonucléique	EME : Essential meiotic structure-specific endonuclease
ADNdb : ADN double brin	ERCC : Excision repair cross complementing
ADNg : ADN génomique	ES : Embryonic stem
ADNsb : ADN simple brin	EXO : Exonuclease
aNHEJ : alternative NHEJ	FANCD : Fanconi anemia group D
AP : Apurinique ou apyrimidique	GFP : Green fluorescent protein
ARN : Acide ribonucléique	H2B : Histone 2B
ARNm : Acide ribonucléique messenger	HML : Hidden MAT left
ATM : Ataxia telangiectasia mutated	HMR : Hidden MAT right
ATP : Adénosine triphosphate	HO : Homothallic switching endonuclease
BARD : BRCA1-associated ring domain	HU : Hydroxyurea
BCDX2 : RAD51B-RAD51C-RAD51D-XRCC2	ICL : Interstrand crosslink
BCR : Breakpoint cluster region	IG : Immunoglobuline
BER : Base excision repair	IR : Ionizing radiation
BIR : Break-induced replication	JH : Jonction de Holliday
BLM : Bloom	kb : kilobase
bNHEJ : backup NHEJ	kDa : kiloDalton
Boucle D : boucle de déplacement	KO : Knockout
BRCA : Breast cancer	LIF : Ligase interacting factor
CDB : Cassures double brin	MAT : Mating type
CENH : Centromeric histone	Mb : Mégabase
CFP : Cyan fluorescent protein	MEC : Mitosis entry checkpoint
CG : Conversion génique	MMC : Mitomycine C
CHO : Chinese hamster ovary	MMEJ : Microhomology-mediated end joining
C-NHEJ : Canonical NHEJ	MMR : Mismatch repair
CO : Crossing-over	MMS : Méthyl méthane sulfonate
CSB : Cassure simple brin	MRN : MRE11-RAD50-NBS1
CSM : Chromosome segregation in meiosis	MRX : MRE11-RAD50-XRS2
CtIP : CtBP-interacting protein	MSH : MutS homolog
CX3 : RAD51C-XRCC3	MUS : Mutagen sensitive
dJH : double jonction de Holliday	NCO : Non crossing-over
DMC : Disrupted meiotic cDNA	NER : Nucleotide excision repair
DNA-PKcs : DNA-dependent protein kinase catalytic subunit	NHEJ : Non homologous end joining
DNL : DNA ligase	NPF : Nucleoprotein filament
dRP : désoxyribose phosphate	P35S : Promoteur 35S

PALB : Partner and localizer of BRCA	SLX : Synthetic lethal of unknown function
PARP : Poly(ADP-ribose) polymerase	SPN : Spindle
pb : paire de bases	SPO : Sporulation
PCR : Polymerase chain reaction	SSB : Single-stranded DNA-binding
PEG : Polyéthylène glycol	SSA : Single strand annealing
PNKP : Polynucleotide kinase /phosphatase	SSC : Side scatter
PSY : Platinum sensitivity	SWS : SWIM domain-containing and Srs2-interacting protein
RAG : Recombination Activating Gene	SWSAP : SWS associated protein
RDH : RAD homologue	T35S : Termineur 35S
RDL : RAD51D-like protein	TdT : Terminal deoxynucleotidyl transferase
RFS : Rad55 suppressor	tdTomato : Tandem dimer Tomato
RH : Recombinaison homologue	TEL : Telomere maintenance
RHP : Rad homolog <i>S. pombe</i>	TID : Two-hybrid interaction with DMC1
RLP : RecA-like protein	T-NOS : Termineur de la nopaline synthétase
RMI : RecQ-mediated genome instability	TOP : Topoisomérase
ROS : Reactive oxygen species	UV : Ultraviolet
RPA : Replication protein A	V(D)J : Variable (Diversity) Joining
RSS : Recombination signal sequence	WT : Wild-type
RT : Reverse transcription	XL : XRCC4 like factor
SAE : Sporulation in the Absence of SPO Eleven	XPF : Xeroderma pigmentosum complementing group F
SAW : Single-strand annealing weakened	XRCC : X-ray repair cross-complementing
SDSA : Synthesis-dependent strand-annealing	YFP : Yellow fluorescent protein
SGS : Slow Growth Suppressor	
SHU : Suppressor of sgs1 hydroxyurea sensitivity	

Notations

Allèle sauvage des gènes : Gene
 Allèle mutant des gènes : *gene*
 Protéine de mammifères et de plantes : PROTEINE
 Protéine de levure : Protéine

Table des illustrations

Figure 1. Relations entre agents mutagènes, lésions de l'ADN et systèmes de réparation	9
Figure 2. Conversion d'une CDB en cassure double brin au cours de la réplication de l'ADN	12
Figure 3. Déclenchement de la recombinaison méiotique par la formation d'une CDB catalysée par SPO11	13
Figure 4. Induction d'une CDB par RAG1 et RAG2 et recombinaison V(D)J	14
Figure 5. Changement de type sexuel chez <i>Saccharomyces cerevisiae</i>	15
Figure 6. Modèle de réparation d'une cassure double brin par la voie de jonction des extrémités dépendante des protéines KU chez les Mammifères	19
Figure 7. Modèle de réparation d'une cassure double brin par la voie de jonction des extrémités indépendante des protéines KU chez les Mammifères	24
Figure 8. Voie DSBR (Double Strand Break Repair)	29
Figure 9. Voie SDSA (Synthesis-Dependent Strand-Annealing)	30
Figure 10. Voie BIR (Break Induced Replication)	31
Figure 11. Voie SSA (Single Strand Annealing)	32
Figure 12. Mécanisme de la résection des extrémités d'une CDB chez les Mammifères	33
Figure 13. La réparation d'une CDB par SSA implique l'élimination de deux ou d'une extrémité(s) non complémentaire(s) selon la localisation de la cassure	79
Figure 14. La réparation par SDSA d'une CDB située dans une séquence hétérologue implique l'élimination de deux ou d'une extrémité(s) non homologue(s)	80
Figure 15. Modèle d'élimination des longues extrémités 3' non homologues au cours du SSA chez <i>Sacharomyces cerevisiae</i>	81
Figure 16. Voies de dissolution et de résolution des doubles jonctions de Holliday chez les Mammifères	84
Figure 17. Les protéines de fusion XRCC2-GFP et RAD51B-GFP complètent la sensibilité à la Mitomycine C et les défauts de recombinaison des mutants <i>xrcc2</i> et <i>rad51b</i> , respectivement ...	104
Figure 18. La cassette d'expression inducible de d'I-SceI s'exprime dans les plantes mutantes <i>xrcc2</i> en présence d'inducteur et l'enzyme est fonctionnelle dans les mutants <i>xrcc2 rad51b rad51d</i>	106
Figure 19. XRCC2 n'est pas impliqué dans la jonction des extrémités d'ADN	108

Figure 20. La réduction de l'efficacité du SSA observée en absence de XRCC2 est indépendante de RAD51	110
Figure 21. Le plasmide rapporteur de recombinaison	148
Figure 22. Protocole de transfection des protoplastes d' <i>Arabidopsis thaliana</i>	149
Figure 23. Protocole d'isolation des noyaux de protoplastes d' <i>Arabidopsis thaliana</i>	150
Figure 24. Détection des protéines de fusion H2B-YFP, tdTomato-CENH3 et H2B-CFP par cytométrie en flux	151
Figure 25. Détection de la fluorescence YFP de protoplastes transfectés avec le plasmide rapporteur de recombinaison par cytométrie en flux	152

Synthèse bibliographique

Introduction

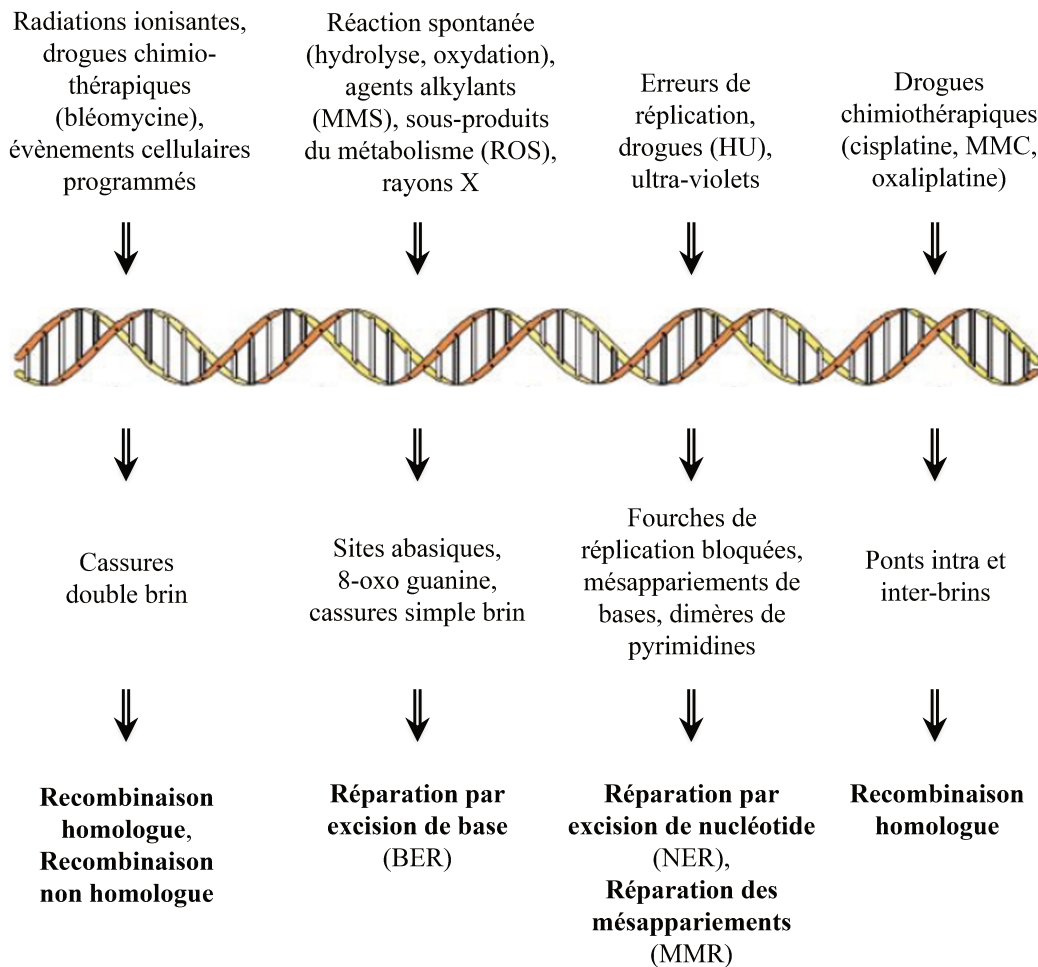


Figure 1 : Relations entre agents mutagènes, lésions de l'ADN et systèmes de réparation (d'après Ghosal *et al.*, 2013)

MMS (*Méthyl Méthane Sulfonate*), ROS (*Reactive Oxygen Species*), HU (*Hydroxyurea*), MMC (*Mitomycine C*)

L'ADN est le support de l'information génétique, nécessaire au développement et au fonctionnement de tous les êtres vivants. Le maintien de l'intégrité du génome est fondamental pour assurer la survie de l'organisme, mais également la transmission correcte de son patrimoine génétique à ses descendants. Cependant, les molécules d'ADN sont en permanence endommagées par de nombreux agents d'origine exogène (radiations ionisantes, agents chimiques...) et endogène (produits génotoxiques issus du métabolisme cellulaire notamment). Le nombre de lésions générées chez l'Homme est estimé à plusieurs milliers par cellule et par jour (Lindahl & Barnes, 2000). Celles-ci sont de natures très diverses : altérations des bases ou du squelette désoxyribophosphate (désamination, alkylation, oxydation), ponts intra ou inter-brins, cassures simple ou double brin de la molécule d'ADN. En réponse à ces dommages, un réseau coordonné de cascades de signalisation se met en place à l'échelle cellulaire. Après la détection des lésions de l'ADN, les points de contrôle du cycle cellulaire sont activés afin de retarder la progression du cycle cellulaire et permettre la réparation des dommages *via* le recrutement de la machinerie de réparation de l'ADN. Cet ensemble de processus constitue la réponse aux dommages de l'ADN.

Plusieurs systèmes de réparation de l'ADN prennent en charge les différents types de lésions : la réparation par excision de base (BER), la réparation par excision de nucléotide (NER), la réparation des mésappariements (MMR), la recombinaison homologue et la recombinaison non homologue - ces deux derniers processus étant impliqués dans la réparation des cassures double brin (CDB) (**Figure 1**) (Ciccia & Elledge, 2010 ; Symington & Gautier, 2011 ; Kamileri *et al.*, 2012 ; Dianov & Hübscher, 2013). La recombinaison homologue intervient également dans le redémarrage des fourches de réplication bloquées (pour revues, voir Branzei & Foiani, 2007 ; Petermann & Helleday, 2010). De nombreuses études ont porté sur l'identification et la caractérisation des protéines mises en jeu dans ces voies de réparation, et ont mis en évidence une grande conservation de ces activités au sein

des Procaryotes et des Eucaryotes. Ceci reflète le rôle essentiel des systèmes de réparation dans le maintien de l'intégrité du génome.

Mes travaux de thèse se sont focalisés sur les voies de réparation des cassures double brin de l'ADN, lésions les plus cytotoxiques puisqu'elles fragmentent les chromosomes. Après avoir décrit les origines des CDB de l'ADN, nous présenterons les conséquences possibles d'une absence ou d'une mauvaise réparation de ces cassures. Les principaux mécanismes de réparation des CDB seront ensuite présentés : les voies de recombinaison non homologue, ou voies de jonction des extrémités de la cassure, dans un premier temps, puis les voies de recombinaison homologue, c'est-à-dire impliquant une homologie de séquences pour la réparation.

Chapitre I

Les cassures double brin de l'ADN : causes et conséquences

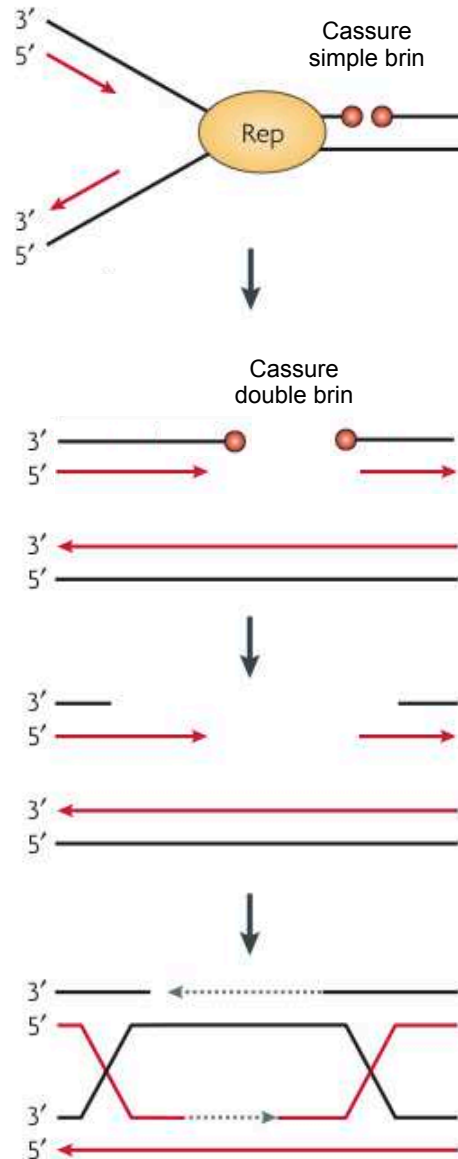


Figure 2 : Conversion d'une cassure simple brin en cassure double brin au cours de la réplication de l'ADN (d'après Caldecott *et al.*, 2008)

Cet exemple présente le cas d'une CSB du brin avancé ; le résultat serait similaire si celle-ci touchait le brin retardé (Rep : fourche de réplication ; lignes rouges : brins nouvellement synthétisés).

I. Origines des cassures double brin de l'ADN

I.1. Les cassures double brin accidentelles

Les cassures double brin (CDB) de l'ADN peuvent être la conséquence de l'action d'agents génotoxiques environnementaux (radiations ionisantes, produits radiomimétiques tels que la bléomycine) ou d'agents issus du métabolisme cellulaire (notamment les espèces réactives de l'oxygène : ion superoxyde, peroxyde d'hydrogène, radical hydroxyle). Des CDB sont également générées au cours des processus cellulaires : tensions dans la molécule d'ADN, accidents de réplication ou de réparation, action de nucléases. De plus, une exposition prolongée à des agents mutagènes ou une déficience des systèmes de réparation aboutit à la persistance d'autres types de lésions de l'ADN, qui peuvent être converties en CDB au cours de la réplication. C'est le cas des dimères de thymine, des ponts inter-brins et des cassures simple brin (CSB) (**Figure 2**). La rencontre d'une CSB par une fourche de réplication peut également générer une CDB à une seule extrémité d'ADN, comme discuté par Helleday *et al.* (2007).

A titre d'exemple, la létalité cellulaire induite par les rayonnements ionisants est liée aux nombreuses lésions que subissent les molécules d'ADN et notamment aux CDB. Le rendement de cassures produites directement par les rayonnements ionisants est estimé à 35 CDB par cellule diploïde humaine (en G1) et par Gray, contre environ 1000 CSB localisées sur les deux brins de la molécule d'ADN (Ward, 1988). Ces rayonnements sont également responsables de l'oxydation de nombreuses bases situées dans les mêmes régions que les CSB, formant ainsi des groupes de lésions (Sutherland *et al.*, 2000 ; Sutherland *et al.*, 2002). Ceux-ci sont plus difficiles à réparer que des lésions simples (Dianov *et al.*, 2001). Certaines lésions peuvent dans un second temps être converties en CDB, à travers l'action des enzymes impliquées dans la réparation par excision de base, ou au niveau des fourches de réplication de l'ADN, quand la machinerie de réplication rencontre ces lésions groupées. Cette capacité des rayonnements

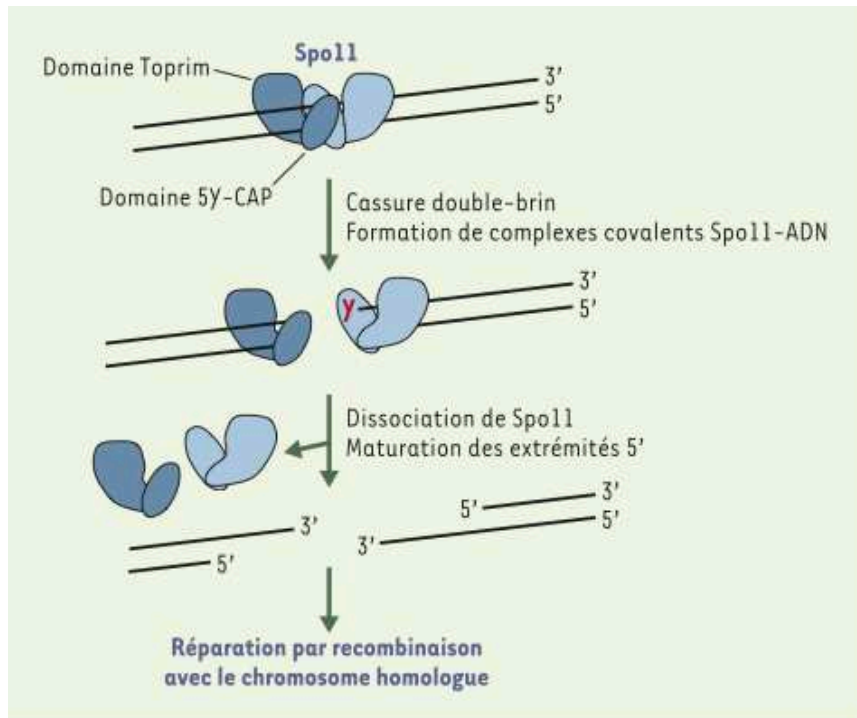


Figure 3 : Déclenchement de la recombinaison méiotique par la formation d'une cassure double brin catalysée par SPO11 (d'après Baudat & De Massy, 2004)

Le domaine carboxy-terminal Toprim (commun aux topoisomérases et aux primases) de chaque monomère de SPO11 interagit avec le domaine 5Y-CAP (*catabolite gene activator protein*) de l'autre monomère. Un dimère se fixe sur l'ADN, introduit une CSB par l'intermédiaire des tyrosines catalytiques (Y) situées dans les domaines 5Y-CAP et forme deux intermédiaires covalents avec les deux extrémités 5' d'ADN. A ce stade, les monomères peuvent rester associés ou se séparer. SPO11 se dissocie ensuite de l'ADN. Les cassures double brin ainsi formées sont ensuite réparées par recombinaison homologue.

ionisants (ainsi que des agents chimiques tels que la bléomycine) à induire des CDB extrêmement délétères est exploitée dans de nombreux traitements anticancéreux pour éliminer les cellules tumorales.

I.2. Les cassures double brin programmées

A ces CDB d'origine accidentelle s'ajoutent des cassures dites programmées. Celles-ci sont générées par des enzymes cellulaires de manière extrêmement contrôlée et sont essentielles dans plusieurs processus du développement ou de la différenciation cellulaire.

I.2.1. Au cours de la méiose : rôle de l'enzyme SPO11

La ségrégation correcte des chromosomes homologues au cours de la première division de méiose ainsi que l'échange de matériel génétique (conversion génique et crossing-over) sont dépendants de la formation de CDB (Cao *et al.*, 1990 ; Page & Hawley, 2003). Ces CDB méiotiques sont générées au stade leptotène de la prophase I par l'enzyme SPO11 (**Figure 3**) (Bergerat *et al.*, 1997 ; Keeney *et al.*, 1997). Cette protéine est extrêmement bien conservée chez les champignons, les animaux et les plantes (pour revue, voir Keeney, 2008). Le rôle fondamental de SPO11 en méiose est révélé par le phénotype méiotique sévère des mutants : d'une fertilité fortement réduite chez *S. pombe* et *A. thaliana* à une stérilité totale chez la souris (pour revue, voir Baudat & de Massy, 2004). L'induction de ces CDB au cours de la méiose est donc essentielle à la production de gamètes viables et à la reproduction.

Des analyses plus récentes ont montré que les CDB méiotiques ne se produisent pas aléatoirement, mais sont localisées préférentiellement dans certaines régions du génome, appelées "points chauds" (Pan *et al.*, 2011 ; Smagulova *et al.*, 2011). Le domaine de liaison à l'ADN de SPO11 ne présente pas ou peu de spécificité de séquence mais l'activité de cette enzyme est dépendante de nombreux facteurs (pour revue, voir Borde & de Massy, 2013). La structure de la chromatine joue également un rôle clé dans la régulation de la formation des

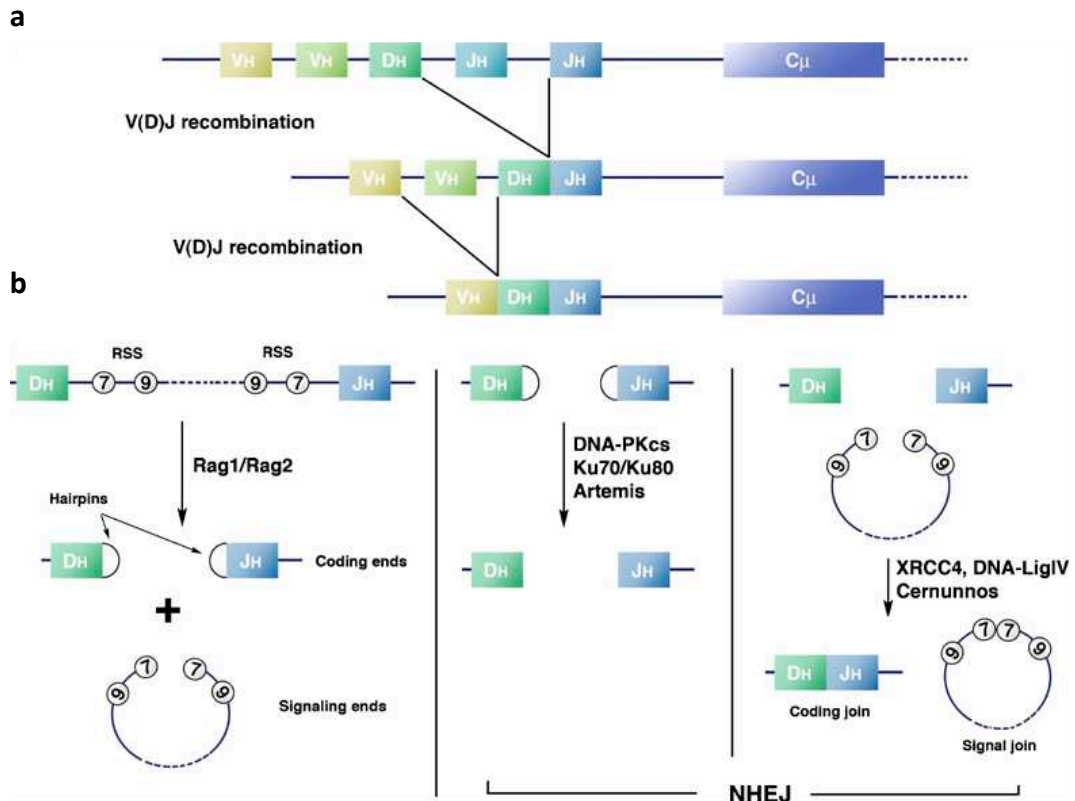


Figure 4: Induction d'une CDB par RAG1 et RAG2 et recombinaison V(D)J (d'après Soulas-Sprauel *et al.*, 2007)

- (a) Représentation schématique du locus de la chaîne IgH (Immunoglobuline H) et processus de la recombinaison VDJ.
- (b) La recombinaison V(D)J peut être résumée en trois étapes. Le complexe RAG1/RAG2 réalise une coupure à la bordure entre les segments V_H et D_H et leurs RSS respectifs, générant ainsi une CDB avec des extrémités en épingle à cheveux (hairpins). Le complexe KU70-KU80 reconnaît la cassure, la DNA-PKcs et Artemis permettent la maturation des extrémités d'ADN, puis le complexe XRCC4-LigaseIV-XLF assure leur jonction. RSS (*recombination signal sequence*) ; NHEJ (*non homologous end joining*) : voie de recombinaison non homologue dépendante de l'hétérodimère KU.

CDB méiotiques en influençant l'activité de SPO11 (aussi bien à l'échelle locale des points chauds qu'à l'échelle chromosomique) (pour revue, voir Yamada & Ohta, 2013). Puisque ces CDB programmées initient la recombinaison méiotique (mécanismes détaillés dans le chapitre III), elles sont à l'origine des crossing-over et des événements de conversion génique participant à la variabilité génétique des gamètes.

I.2.2. Au cours de la différenciation des lymphocytes B et T : rôle de RAG1 / RAG2

L'induction de CDB dans la région des gènes codant pour les régions variables des immunoglobulines et des récepteurs des lymphocytes constitue un autre exemple de CDB programmées chez les Vertébrés. Elle se produit dans les cellules B et T immatures (futurs lymphocytes) et correspond à l'étape initiale de la recombinaison V(D)J. La génération de ces CDB est attribuée à RAG1 et RAG2, deux protéines exprimées uniquement durant la phase de différenciation des lymphocytes B et T (Schatz *et al.*, 1989 ; Oettinger *et al.*, 1990 ; Van Gent *et al.*, 1996). Elles sont capables de se lier spécifiquement aux régions RSS (*recombination signal sequence*) et d'induire une CDB de la double hélice d'ADN entre une région RSS et un segment codant V (*variable*), D (*diversity*) ou J (*joining*). Les CDB générées sont prises en charge par la voie de recombinaison non homologue dépendante des protéines KU (mécanismes détaillés dans le chapitre II) et permet la liaison de segments codants non contigus (**Figure 4**).

Ces réarrangements génomiques sont à l'origine du vaste répertoire des immunoglobulines et des récepteurs des lymphocytes B et T, impliqués dans la reconnaissance spécifique d'une grande diversité d'antigènes que peut rencontrer l'individu au cours de sa vie. L'induction de ces CDB (puis leur réparation par recombinaison) est donc essentielle à la réponse immunitaire acquise des Vertébrés (pour revue, voir Nishana & Raghavan, 2012).

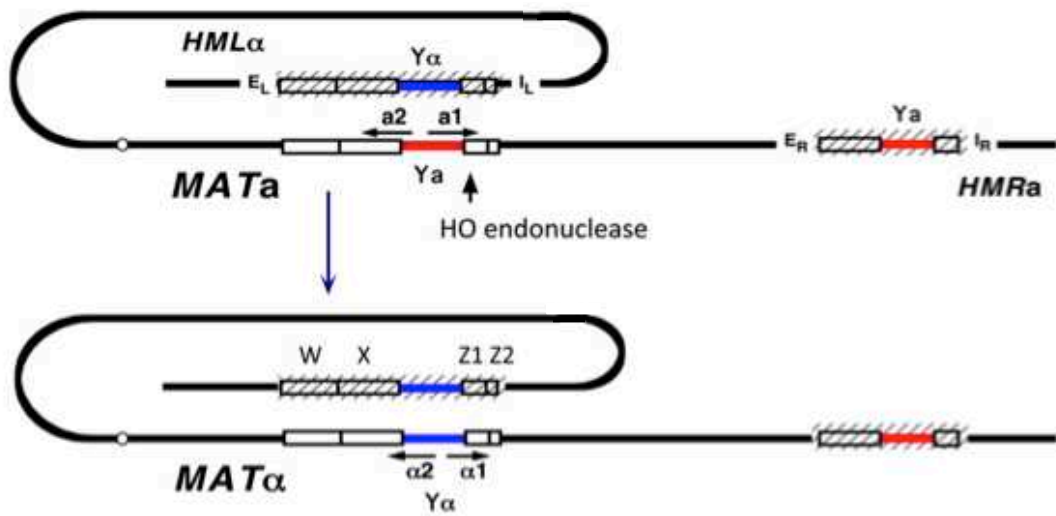


Figure 5 : Changement de type sexuel chez *Saccharomyces cerevisiae* (d'après Haber, 2012)

L'endonuclease HO réalise un cassure double brin entre les régions Y et Z1 au locus MAT. Après maturation des extrémités de la cassure, la région simple brin envahit le locus donneur HML (dans l'exemple présenté ici) et amorce une synthèse d'ADN qui permet de remplacer entièrement la région Y du locus MAT. Zone hachurée : région hétérochromatique.

I.2.3. Au cours de la commutation de type sexuel chez *S. cerevisiae* : rôle de HO

La levure *Saccharomyces cerevisiae* a la particularité de changer de type sexuel au cours de sa croissance végétative à l'état haploïde. La commutation de type sexuel est un mécanisme initié par l'induction d'une CDB par l'endonucléase HO (*homothallic switching endonuclease*) au niveau d'une séquence spécifique (Strathern *et al.*, 1982 ; Kostriken *et al.*, 1983). Cette séquence se situe sur le chromosome III au niveau du locus MAT (*mating-type*), entre les régions Y et Z1 (**Figure 5**). Bien que cette séquence spécifique soit également présente au niveau des loci hétérochromatiques HML et HMR, aucun clivage n'a lieu du fait de l'inaccessibilité de ces régions à l'enzyme HO (**Figure 5**, régions hachurées). L'un de ces loci sert de matrice à l'évènement de recombinaison homologue de type conversion génique que subi le locus MAT (mécanismes moléculaires détaillés dans le chapitre III) (pour revue, voir Haber, 2012).

La commutation de type sexuel chez la levure est dépendante de l'induction de la CDB programmée par l'endonucléase HO, donc directement liée au profil de transcription du gène codant cette enzyme. L'expression de ce gène est hautement contrôlée et n'est active que dans la cellule mère (à la fin de la phase G1) (Nasmyth, 1993). Ainsi, seule la cellule mère peut subir un changement de type sexuel (MAT_a vers MAT_α ou inversement). Les deux cellules de type sexuel différent peuvent alors fusionner, générant ainsi un zygote diploïde a/α pouvant subir la méiose (pour revue, voir Haber, 2012). Ce processus permet à une spore haploïde isolée de devenir rapidement diploïde et de se multiplier par bourgeonnement jusqu'à ce que les conditions environnementales (appauvrissement du milieu) l'amène à sporuler.

Depuis sa découverte, l'endonucléase site-spécifique HO s'est avérée être un outil de choix pour l'étude des mécanismes de recombinaison *in vitro* et *in vivo*.

II. Conséquences de l'absence ou d'une mauvaise réparation des cassures double brin d'ADN

L'absence de réparation d'une CDB de la double hélice d'ADN induit une discontinuité dans la molécule d'ADN pouvant mener à la perte de fragments chromosomiques au cours des divisions cellulaires. Cette perte de matériel génétique, qui concerne parfois un bras entier de chromosome, a des conséquences désastreuses évidentes pour la cellule. Bien que de moindre importance, la présence d'une discontinuité au niveau de la molécule d'ADN affecte également les processus biologiques tels que la réplication et la transcription. Il a été montré par exemple que l'induction d'une seule CDB dans une cellule humaine entraîne l'inhibition de la transcription de la région *via* la dissociation de l'ARN polymérase II de la molécule d'ADN (Pankotai *et al.*, 2012).

La réparation incorrecte des CDB peut engendrer des réarrangements chromosomiques de différentes échelles : de la mutation d'un ou de quelques nucléotides à la duplication ou la translocation de bras chromosomiques. Ces mutations peuvent induire des modifications de l'expression des gènes ayant des conséquences à l'échelle cellulaire, voire de l'organisme. La perte d'un gène suppresseur de tumeur et/ou la surexpression d'un oncogène peuvent être à l'origine d'un processus de tumorigenèse. Les cellules cancéreuses présentent en effet de nombreux réarrangements génomiques (délétions, inversions, duplications et translocations). La progression de la malignité est souvent corrélée à l'augmentation de l'instabilité génomique, résultat de l'escalade des défauts dans les systèmes de réparation de l'ADN et au niveau des points de contrôle du cycle cellulaire (pour revues, voir Hoeijmakers, 2001 ; Jackson & Bartek, 2009 ; Bohgaki *et al.*, 2010).

A titre d'exemple, la translocation réciproque d'un fragment des bras longs des chromosomes 9 et 22 juxtapose l'oncogène Abl (*Abelson leukemia virus tyrosine kinase*) et le gène Bcr (*Breakpoint cluster region*), générant un oncogène de fusion Bcr-Abl. L'activité

kinase de la protéine de fusion (nettement augmentée par rapport à celle de la protéine ABL) stimule la prolifération incontrôlée des leucocytes, ce qui initie la leucémie myéloïde chronique chez l'Homme. L'accumulation de modifications génétiques et/ou épigénétiques supplémentaires (de manière BCR-ABL dépendante ou indépendante) contribuent à la progression de la maladie dans une phase plus agressive (pour revue, voir Perrotti *et al.*, 2010).

Il semble donc clair que l'absence de réparation d'une CDB ou une réparation incorrecte peut avoir des conséquences dramatiques à l'échelle de la cellule et de l'organisme. Pour lutter contre ces effets néfastes, la cellule dispose d'une machinerie complexe spécialisée dans la réparation des CDB de l'ADN et impliquant l'action coordonnée de nombreuses protéines. Deux grands mécanismes peuvent être distingués selon l'utilisation ou non de séquences homologues pour la réparation : la recombinaison homologue et la recombinaison non homologue.

Chapitre II

Les voies de recombinaison non homologue

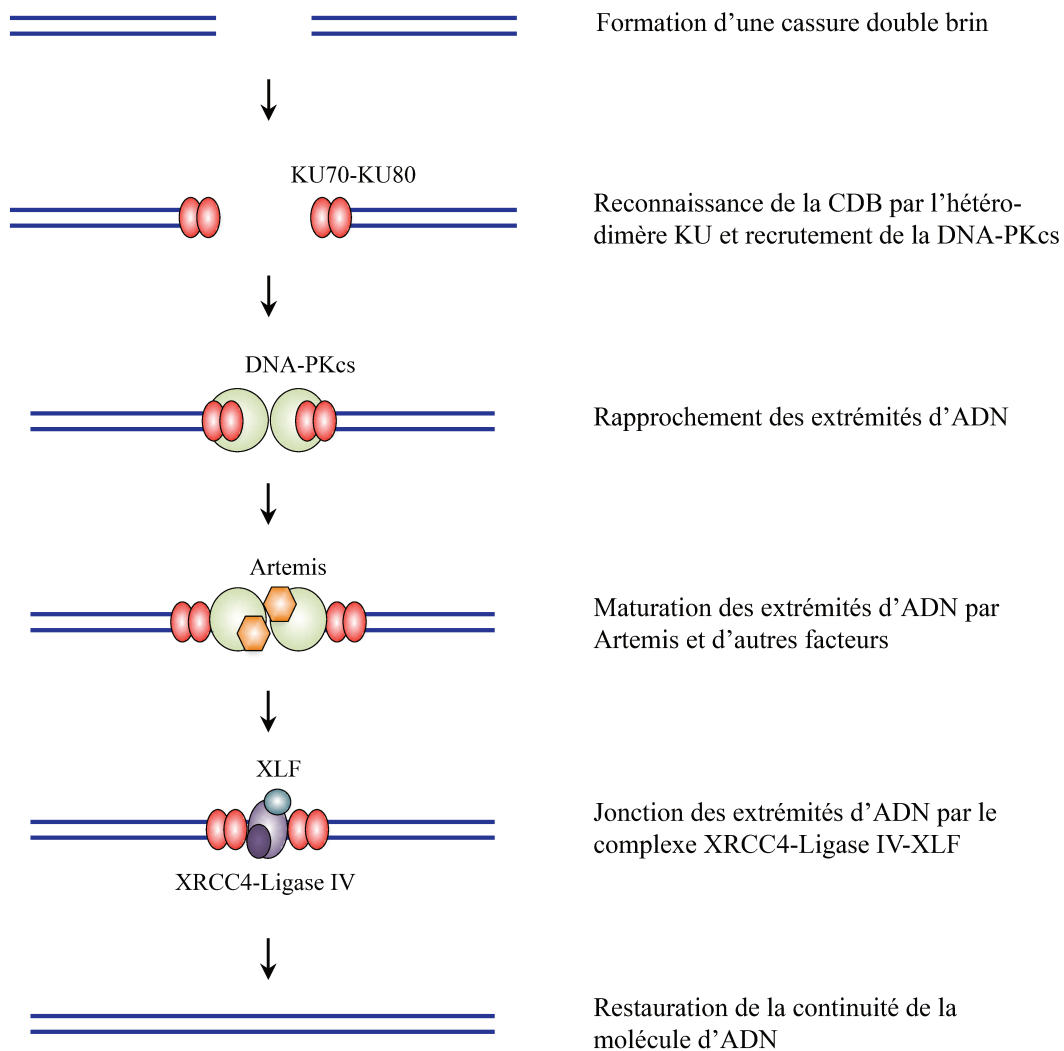
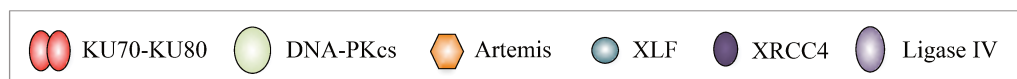


Figure 6 : Modèle de réparation d'une cassure double brin par la voie de jonction des extrémités dépendante des protéines KU chez les Mammifères (d'après Mladenov *et al.*, 2011)



La réparation des CDB par recombinaison non homologue consiste en la jonction des deux extrémités de la cassure. Ce système de réparation est largement majoritaire chez les Eucaryotes pluricellulaires et a été, de ce fait, largement étudié au cours des dernières décennies. La première voie de recombinaison non homologue identifiée est la voie canonique de jonction d'extrémités non homologues (ou C-NHEJ), voie dépendante des protéines KU. Celle-ci existe chez tous les Eucaryotes étudiés et est très bien décrite. Il existe également une ou plusieurs voies indépendantes des protéines KU, qui font actuellement l'objet de nombreuses analyses.

I. Voie de jonction des extrémités dépendante des protéines KU

Cette voie de recombinaison est fonctionnelle à tous les stades du cycle cellulaire mais semble particulièrement active au cours de la phase G1 (Delacôte & Lopez, 2008). Elle est impliquée dans la réparation des CDB mais aussi dans la génération de la diversité des immunoglobulines et des récepteurs des lymphocytes B et T *via* la recombinaison V(D)J (Soulas-Sprauel *et al.*, 2007) (**Figure 4**). L'inactivation d'un des acteurs de cette voie entraîne un phénotype sévère des mutants (d'une hypersensibilité aux radiations ionisantes à la létalité embryonnaire), ce qui met en évidence le rôle essentiel de ce type de réparation (pour revue, voir Brugmans *et al.*, 2007).

Les protéines majeures de cette voie et leurs fonctions sont extrêmement bien conservées chez les Eucaryotes, bien que les homologues de certaines des protéines décrites chez les Mammifères n'ont pas été identifiés chez tous les organismes. Le C-NHEJ, ou voie de jonction des extrémités dépendante des protéines KU, peut être subdivisé en trois étapes principales : la reconnaissance de la CDB, la maturation des extrémités d'ADN puis la jonction de ces extrémités, restaurant l'intégrité de la molécule d'ADN. Chacune de ces étapes implique l'action coordonnée de plusieurs protéines (**Figure 6**).

I.1. Reconnaissance de la cassure double brin

L'hétérodimère KU, composé des sous-unités KU70 et KU80, est capable de détecter et de se lier aux extrémités d'ADN avec une haute affinité et de manière indépendante de la séquence nucléotidique (Mimori & Hardin, 1986 ; Paillard & Strauss, 1991). Les deux sous-unités forment un anneau qui encercle les extrémités d'ADN (Walker *et al.*, 2001 ; Tamura *et al.*, 2002), les protégeant ainsi de la dégradation par des exonucléases. Il a en effet été montré que l'inactivation de KU chez la souris ou la levure provoque une dégradation accélérée des extrémités des CDB (Liang & Jasin, 1996 ; Lee *et al.*, 1998).

L'hétérodimère est également responsable du recrutement de la DNA-PKcs (*DNA-dependent protein kinase catalytic subunit*) au niveau de la CDB (Uematsu *et al.*, 2007) et stimule son activité kinase (Gottlieb & Jackson, 1993). Le complexe multiprotéique ainsi formé stabilise les extrémités d'ADN et assure leur rapprochement (Getts & Stamato, 1994 ; Rathmell & Chu, 1994). Malgré la présence de KU, aucun orthologue de la DNA-PK n'a cependant été identifié chez les plantes et les levures.

I.2. Maturation des extrémités d'ADN

Selon la nature des extrémités (franches ou cohésives, 5' ou 3' sortantes, présence ou absence de modifications...), différents facteurs sont ensuite recrutés. La protéine Artemis est une endonucléase agissant au niveau des extrémités en épingle à cheveux, 3' et 5' sortantes, et 3'-phosphoglycolate (Ma *et al.*, 2002 ; Povirk *et al.*, 2007). Artemis interagit avec la DNA-PKcs qui stimule son activité endonucléasique (Ma *et al.*, 2002). Bien qu'une activité exonucléasique d'Artemis ait été suggérée, elle a récemment été remise en question (Pawelczak & Turchi, 2010). Aucun homologue d'Artemis n'a été identifié chez la plante modèle *Arabidopsis thaliana* à ce jour. La protéine PNKP (*polynucleotide kinase /phosphatase*) catalyse la phosphorylation des extrémités 5'-OH et élimine les groupements

phosphate des extrémités 3' (Chappell *et al.*, 2002). Des études récentes ont révélé que, en plus de son rôle essentiel dans la détection des CDB, KU joue un rôle direct dans la maturation de certaines extrémités modifiées *via* son activité lyase 5'-dRP/AP (5'-*desoxyribose-5-phosphate / apurinic/apyrimidic sites*) (Roberts *et al.*, 2010 ; Strande *et al.*, 2012). Les lacunes nucléotidiques éventuellement générées au cours de la maturation des extrémités sont comblées par les ADN polymérases μ , λ et TdT (*terminal deoxynucleotidyl transferase*) chez les Vertébrés (Capp *et al.*, 2006 ; Andrade *et al.*, 2009). TdT agit spécifiquement au cours de la recombinaison V(D)J, son expression étant restreinte au développement lymphocytaire (Gilfillan *et al.*, 1995). Chez les plantes et les levures, ces lacunes sont prises en charge par les ADN polymérases Pol λ et Pol IV, respectivement (Wilson & Lieber, 1999 ; Roy *et al.*, 2013).

I.3. Jonction des extrémités d'ADN

Une fois que des extrémités compatibles sont générées, le complexe XRCC4-Ligase IV permet leur jonction grâce à la formation d'une liaison covalente entre les deux molécules (Grawunder *et al.*, 1997 ; Grawunder *et al.*, 1998). En effet, XRCC4 interagit physiquement avec la Ligase IV avec une haute affinité (Critchlow *et al.*, 1997 ; West *et al.*, 2000 ; Sibanda *et al.*, 2001 ; Wu *et al.*, 2009), permet la stabilisation de la protéine et stimule son activité (Grawunder *et al.*, 1997). Ce complexe et son activité sont conservés chez les plantes et les levures (complexe Dnl4-Lif1 (*DNA ligase 4 - ligase interfacing factor 1*)).

La protéine XLF (*XRCC4 like factor*, aussi nommé Cernunnos) est également capable d'interagir avec le complexe XRCC4-Ligase IV, mais avec une plus faible affinité (Riballo *et al.*, 2009). Cette interaction stimulerait l'activité du complexe (Ahnesorg *et al.*, 2006 ; Lu *et al.*, 2007). Des études *in vitro* ont suggéré que XLF serait seulement nécessaire à la réparation d'un sous-ensemble de CDB impliquant des mésappariements ou des extrémités non-

cohésives (Tsai *et al.*, 2007). XLF est conservé chez *S. cerevisiae* (Nej1) mais le génome d'*Arabidopsis* ne semble pas posséder d'orthologue de XLF (Kegel *et al.*, 2001).

Le recrutement de XRCC4-Ligase IV et de XLF au niveau du site de la cassure est dépendant de l'hétérodimère KU mais indépendant de la DNA-PK (Mari *et al.*, 2006 ; Yano *et al.*, 2008). Ceci indique que KU est non seulement impliqué dans la détection de la cassure et la maturation des extrémités d'ADN, mais également indirectement dans la dernière étape du C-NHEJ (pour revue, voir Radhakrishnan *et al.*, 2014).

I.4. Fidélité de la réparation

Il a été établi que la voie de jonction des extrémités dépendante de KU favorise la stabilité génomique en limitant les translocations chromosomiques (Simsek & Jasin, 2010). En effet, au cours de la réparation d'une CDB par C-NHEJ, les deux extrémités d'ADN sont très rapidement prises en charge et maintenues ensemble par le complexe protéique KU-DNA-PK. Ainsi, dans la majorité des cas, les deux extrémités d'ADN initialement contiguës sont liées entre elles (pour revues, voir Iliakis *et al.*, 2004 ; Lieber, 2010 ; Gostissa *et al.*, 2011). En revanche, le C-NHEJ est traditionnellement considéré comme un mécanisme générant des erreurs puisque les étapes de maturation des extrémités modifient la séquence nucléotidique au niveau de la CDB. Cependant, de nombreuses études suggèrent que les protéines KU protègent les extrémités d'ADN contre une dégradation étendue, limitant ainsi les effets néfastes potentiels des mutations de type délétion (Guirouilh-Barbat *et al.*, 2004 ; Schulte-Uentrop *et al.*, 2008). La voie du C-NHEJ est donc un mécanisme de réparation générant relativement peu d'erreurs (à l'échelle nucléotidique) et évitant les mutations de plus grande échelle.

II. Voie(s) de jonction des extrémités indépendante(s) des protéines KU

La détection de produits de réparation de type jonction d'extrémités dans des cellules déficientes pour *ku80* a permis de mettre en évidence l'existence d'une ou de plusieurs voies de réparation indépendante(s) de KU (Liang *et al.*, 1996 ; Kabotyanski *et al.*, 1998). L'analyse de ces produits, présentant majoritairement des délétions ou insertions de quelques nucléotides, implique des remaniements au niveau des extrémités de la CDB et suggère une réparation par microhomologies (c'est-à-dire *via* l'appariement de quelques bases situées de part et d'autre de la CDB) (Kramer *et al.*, 1994 ; Moore & Haber, 1996 ; Feldmann *et al.*, 2000). Cette (ces) voie(s) semble(nt) particulièrement active(s) en absence de la voie dépendante de KU (Soulas-Sprauel *et al.*, 2007 ; Yan *et al.*, 2007). Cependant, le nombre de voies indépendantes des protéines KU, ainsi que les protéines impliquées sont encore mal définies. De multiples noms ont été utilisés dans la littérature : alternative NHEJ (aNHEJ ou alt-NHEJ), back-up NHEJ (bNHEJ) et microhomology-mediated end-joining (MMEJ) (Ma *et al.*, 2003 ; Corneo *et al.*, 2007 ; Iliakis, 2009). Nous regrouperons ici ces voies sous le terme "Voies de jonction des extrémités indépendantes des protéines KU".

II.1. Les acteurs des voies de jonction des extrémités indépendantes des protéines KU

Plusieurs études ont d'abord suggéré les rôles de la protéine PARP-1 (*Poly(ADP-Ribose) Polymerase 1*), de la Ligase III et de XRCC1 (*X-ray Repair Cross-Complementing protein 1*) dans les voies indépendantes des protéines KU (pour revue, voir Mladenov & Iliakis, 2011). Ces trois protéines sont par ailleurs impliquées dans la réparation des CSB et dans le système de réparation par excision de base (pour revue, voir Caldecott, 2003). PARP-1 est capable de se fixer au CSB et aux CDB, mais se lie aux CDB avec une plus haute affinité (Benjamin & Gill, 1980). L'inhibition de PARP-1 diminue l'efficacité de réparation des CDB mais dans une moindre mesure par rapport à l'inhibition de PARP-1 et DNA-PKcs

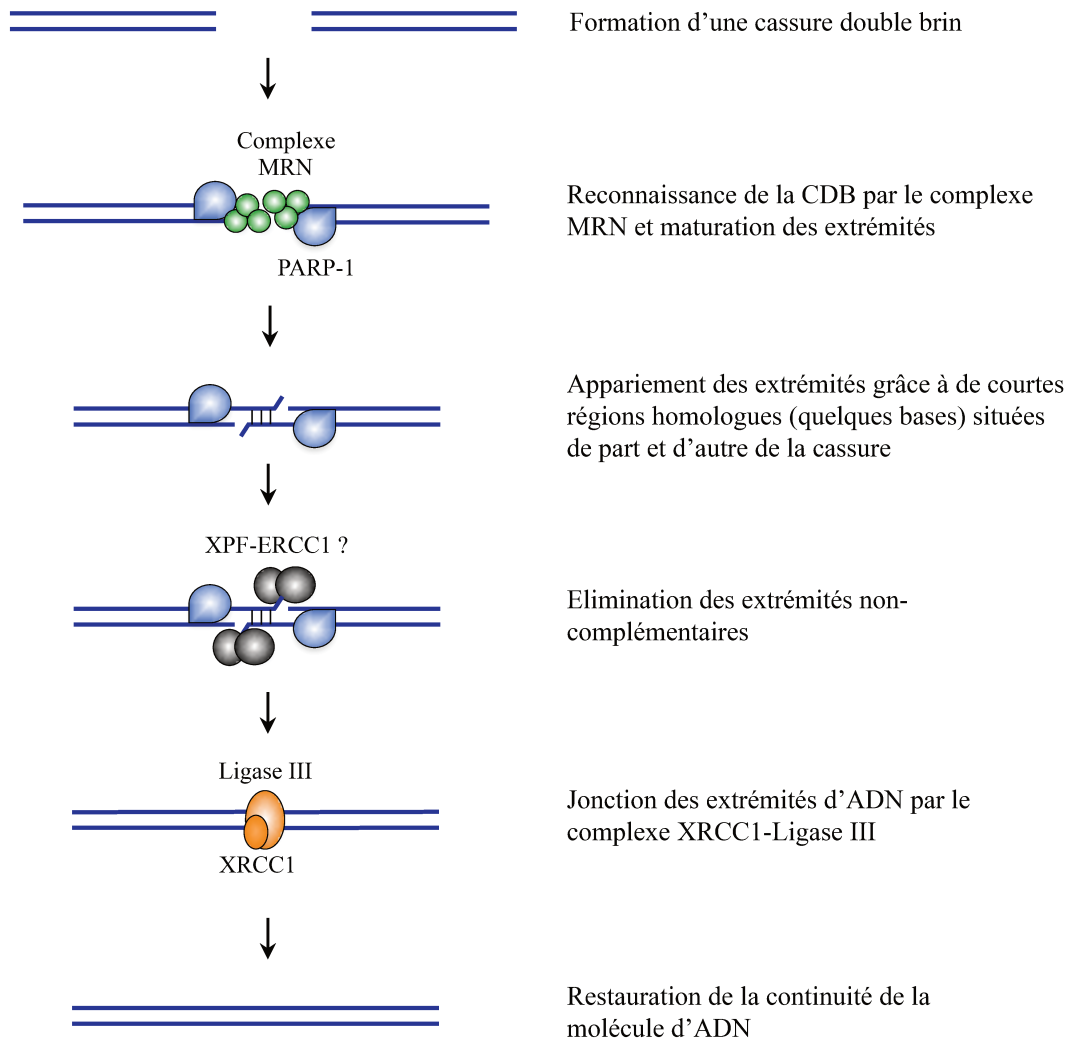


Figure 7 : Modèle de réparation d'une cassure double brin par la voie de jonction des extrémités indépendante des protéines KU chez les Mammifères (d'après Mladenov *et al.*, 2011)

Les principaux acteurs impliqués dans les voies de jonction des extrémités indépendantes de KU sont présentés ici. Il est cependant possible qu'ils interviennent dans des voies parallèles.



(Boulton *et al.*, 1999 ; Veuger *et al.*, 2003 ; Veuger *et al.*, 2004). Ces résultats indiquent que PARP-1 agit dans une voie alternative à la voie C-NHEJ. Plus précisément, PARP-1 serait impliqué dans les premières étapes de la voie (reconnaissance de la CDB et/ou maintien des extrémités) mais son rôle exact reste à identifier (**Figure 7**) (pour revue, voir Ko & Ren, 2012). Le rôle de l'ADN Ligase III dans les voies indépendantes de KU a été mis en évidence *in vitro* et *in vivo* (Wang *et al.*, 2005). La Ligase III interagit physiquement avec XRCC1 et cette interaction stimule l'activité de ligature des brins d'ADN de la ligase (Caldecott *et al.*, 1994). Aubebert *et al.* ont montré que les protéines humaines PARP-1, XRCC1 et la Ligase III sont suffisantes à la réparation d'une CDB par jonction des extrémités *in vitro* (Audebert *et al.*, 2004). Cependant, le rôle de la Ligase III et de XRCC1 dans ces voies indépendantes de KU chez les Mammifères est actuellement controversé (Gao *et al.*, 2011 ; Simsek *et al.*, 2011 ; Boboila *et al.*, 2012 ; Han *et al.*, 2012). D'autres analyses sont donc nécessaires pour comprendre ces apparentes contradictions.

Plusieurs études ont révélé l'implication du complexe MRX (composé des protéines Mre11, Rad50 et Xrs2) dans des voies indépendantes de KU chez la levure (Moore & Haber, 1996 ; Paull & Gellert, 1998 ; Ma *et al.*, 2003). Ce complexe, et plus particulièrement Mre11, favoriserait l'initiation de la réparation en stimulant la résection 5'-3' des extrémités de la CDB - étape nécessaire à la recherche de microhomologies dans les régions bordant la cassure. Un rôle similaire du complexe MRN (MRE11, RAD50, NBS1) a été observé chez les Mammifères (Deriano *et al.*, 2009 ; Rass *et al.*, 2009 ; Rahal *et al.*, 2010).

Un rôle du complexe Rad1-Rad10 de levure dans le clivage des séquences non homologues de part et d'autre des bases appariées a été proposé, mais ce complexe ne serait pas essentiel (Ma *et al.*, 2003). Un test de recircularisation de plasmides chez les Mammifères a également suggéré que XPF-ERCC1, l'homologue de Rad1-Rad10, favoriserait les mécanismes de jonction des extrémités indépendants des protéines KU (Ahmad *et al.*, 2008).

D'autres analyses sont cependant requises, chez les Mammifères comme chez la levure, pour confirmer et préciser le rôle de ces différentes protéines et définir les différentes voies de réparation.

Chez les plantes, des orthologues de MRE11, RAD50, NBS1, XRCC1, XPF et PARP-1 ont été identifiés (Babiychuk *et al.*, 1998 ; Fidantsef *et al.*, 2000 ; Gallego *et al.*, 2001 ; Bundock & Hooykaas, 2002 ; Waterworth *et al.*, 2007 ; Uchiyama *et al.*, 2008). AtMRE11 a été impliqué dans la jonction d'extrémités impliquant des courtes homologies de séquences puisque le nombre de produits de fusion chromosomiques présentant des microhomologies est nettement réduit dans le mutant *mre11* (Heacock *et al.*, 2004). L'utilisation de simple et double mutants chez *Arabidopsis* a révélé que la réparation des CDB radio-induites dans un mutant *ku80* est dépendant de XRCC1 (Charbonnel *et al.*, 2010) et XPF (Charbonnel *et al.*, 2011). Ces protéines sont donc impliquées dans des voies de réparation indépendantes de KU. L'analyse des relations d'épistasie entre ces trois protéines a permis de démontrer que chacune d'elles agit dans une voie indépendante, mettant en évidence pour la première fois l'existence d'au moins deux voies de jonction des extrémités indépendantes des protéines KU chez les plantes (Charbonnel *et al.*, 2011).

II.2. Fidélité de la réparation

Il a été établi que la probabilité de lier deux extrémités d'ADN initialement non contigües au cours de la réparation d'une CDB est nettement augmentée en absence des protéines KU. Les voies de recombinaison non homologue indépendantes de KU sont en effet impliquées dans l'augmentation de l'instabilité génomique (en favorisant notamment les translocations) et donc dans les processus de tumorigenèse (Weinstock *et al.*, 2007). Ceci serait lié à la cinétique de réparation : les voies indépendantes de KU sont plus lentes et moins

efficaces que la voie dépendante de KU, et génèrent donc plus d'erreurs (pour revue, voir Schipler & Iliakis, 2013).

A l'échelle nucléotidique, les voies indépendantes de KU sont à l'origine de mutations de quelques nucléotides à quelques dizaines de nucléotides, principalement des petites délétions ou insertions. Les voies de jonction d'extrémités indépendantes de KU sont donc considérées comme des voies de réparation très mutagènes (pour revues, voir Iliakis *et al.*, 2007 ; Lieber, 2010).

Chapitre III

Les voies de recombinaison homologue

Le terme "recombinaison homologue" au sens large regroupe toutes les voies de réparation des CDB impliquant une homologie de séquence pour la réparation de la molécule endommagée. D'abord, la voie d'hybridation d'ADN simple brin (SSA, *single strand annealing*) permet la réparation des CDB situées entre deux régions homologues. C'est une voie mutagène puisqu'elle aboutit à la délétion de la région située entre les répétitions et d'une des répétitions. En revanche, les voies de recombinaison homologue *sensu stricto* réparent la CDB grâce à la copie d'une séquence homologue non endommagée, située généralement sur la chromatide sœur ou le chromosome homologue. Ces voies de réparations sont dites fidèles puisqu'elles préservent l'intégrité de l'information génétique. Elles sont actives dans les cellules somatiques (principalement au cours des phases S et G2 où la chromatide sœur est présente) mais aussi dans les cellules méiotiques.

Depuis la proposition du modèle moléculaire de la recombinaison homologue établi par Robin Holliday en 1964, de nouvelles données expérimentales ont permis d'améliorer ce modèle en y apportant des modifications. Ces études ont révélé l'existence de plusieurs voies de recombinaison homologue, plus ou moins indépendantes, impliquant les activités de nombreuses protéines.

I. Mécanismes moléculaires des voies de recombinaison homologue

I.1. Double Strand Break Repair (DSBR)

Le modèle DSBR a été proposé par Jack Szostak *et al.* (1983). Celui-ci est basé sur des études de transformation et de ciblage de gènes chez la levure (Orr-Weaver & Szostak, 1983) et fournit des explications à de nouvelles observations non prises en compte par le modèle de Holliday (Haber *et al.*, 2004).

Les principales étapes de ce modèle sont présentées **figure 8**. Suite à une CDB de l'ADN, les deux extrémités subissent une résection 5'-3' générant des extrémités d'ADN

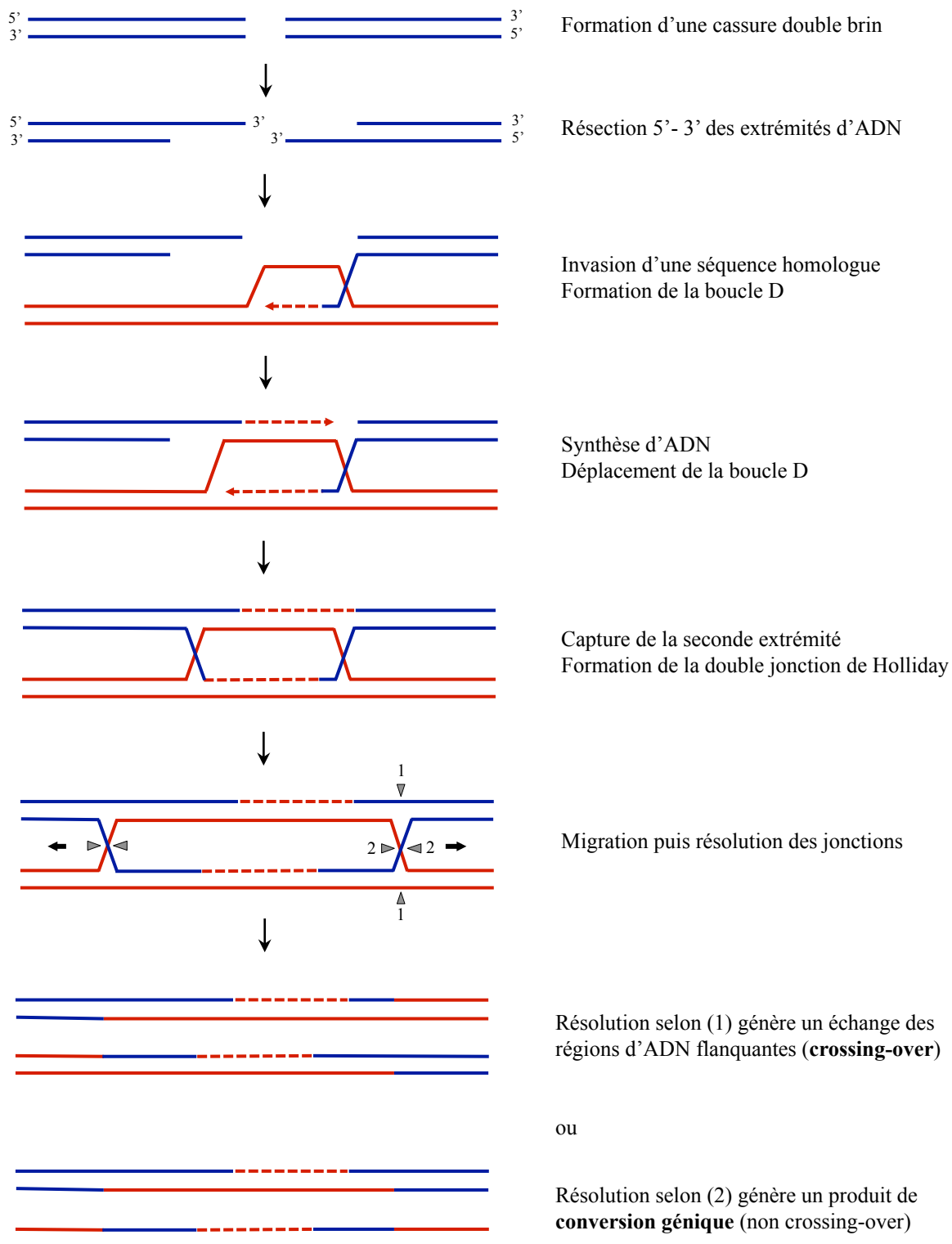


Figure 8 : Voie DSBR (Double Strand Break Repair)
(d'après Symington, 2002)

simple brin (ADNsb) 3' sortantes. Ces extrémités recherchent une région homologue dans une double hélice d'ADN, généralement la chromatide sœur ou le chromosome homologue. Quand une région homologue est identifiée, l'extrémité d'ADNsb l'envahit par appariement avec le brin complémentaire, formant ainsi une structure appelée boucle de déplacement, ou boucle D. La région d'ADN hétérologue est étendue par une synthèse d'ADN utilisant comme amorce, l'extrémité 3' du brin envahisseur et comme matrice, la copie homologue non endommagée. La poursuite de la polymérisation d'ADN conduit au déplacement de la boucle D qui pourra s'apparier avec la seconde extrémité de la cassure et servir de matrice à son extension. L'intermédiaire ainsi formé est nommé double jonction de Holliday. Les deux simples jonctions peuvent migrer, généralement en sens opposé, conduisant à l'extension du duplexe d'ADN hétérologue (étape de migration des branches). La résolution de cet intermédiaire de recombinaison peut aboutir à différents types de produits selon la localisation des clivages au niveau des deux jonctions (triangles gris, **Figure 8**). Les crossing-over (CO) génèrent un échange réciproque de matériel génétique entre les deux molécules d'ADN homologues. En revanche, les produits de non crossing-over (NCO) résultent d'un transfert non réciproque d'information génétique entre les deux molécules, c'est la conversion génique.

Selon ce modèle, la résolution aléatoire des deux jonctions de Holliday devrait produire un nombre équivalent de produits de CO et de produits de NCO. Or, des études portant sur la commutation de type sexuel chez *S. cerevisiae* (Nasmyth, 1982 ; McGill *et al.*, 1989) ou la recombinaison des éléments transposables chez la drosophile (Gloor *et al.*, 1991) ont révélé que les événements de conversion génique sont plus nombreux que les CO. Ces travaux ont mené à la proposition d'un nouveau modèle dans lequel la réparation des CDB par recombinaison homologue ne génère que des événements de conversion génique : il s'agit de la voie SDSA (Synthesis-Dependent Strand-Annealing).

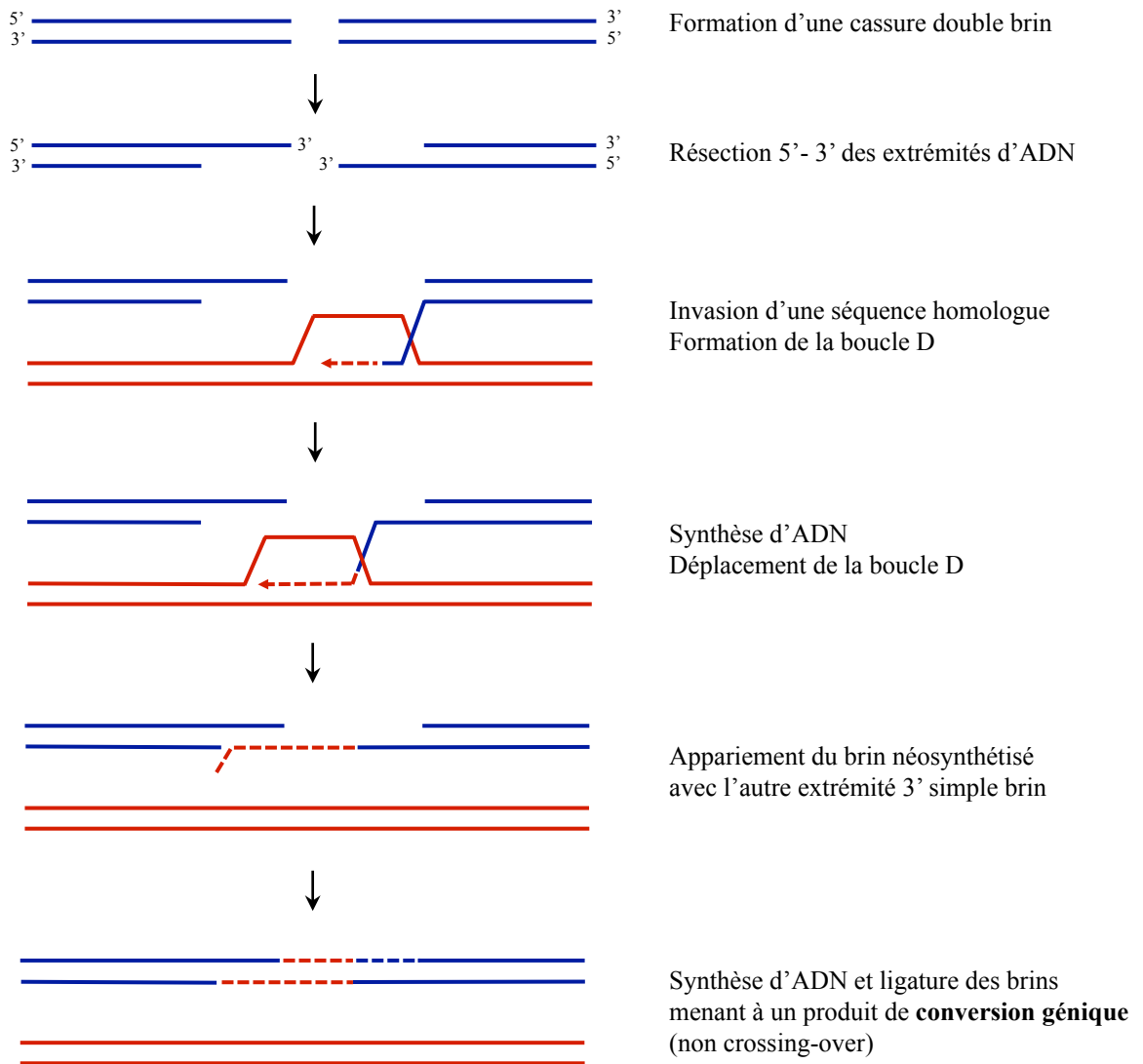


Figure 9 : Voie SDSA (Synthesis-Dependent Strand-Annealing)
(d'après Symington, 2002)

I.2. Synthesis-Dependent Strand-Annealing (SDSA)

Le modèle SDSA, proposé dans les années 90 (Nassif *et al.*, 1994 ; Ferguson & Holloman, 1996), est présenté **figure 9**. Les premières étapes de la voie SDSA sont communes à celles de la voie DSBR : résection 5'-3' des extrémités de la CDB, recherche d'homologie et invasion d'une séquence homologue non endommagée par l'une des extrémités d'ADNsb 3' sortantes. Ceci aboutit à la formation de la boucle D. La synthèse d'ADN initiée à partir de l'extrémité 3' du brin envahisseur provoque le déplacement de la boucle D le long de la matrice d'ADN. Le brin nouvellement synthétisé est ensuite déplacé et peut s'hybrider par complémentarité de bases avec l'autre extrémité d'ADNsb 3' sortante de la chromatide endommagée. La synthèse d'ADN est alors amorcée à partir de l'extrémité 3' du brin non envahisseur et permet de combler les lacunes. La ligature des brins restaure la continuité de la molécule d'ADN.

Une différence essentielle entre ce mécanisme et le modèle de Szostak est qu'il génère exclusivement des produits de NCO (conversion génique), puisqu'il n'implique ni la formation ni la résolution d'une double jonction de Holliday. De plus, l'ensemble de l'ADN nouvellement synthétisé se retrouve finalement dans la molécule "destinataire", c'est-à-dire dans la chromatide initialement endommagée (lignes pointillées, **Figure 9**). En accord avec ces prédictions, les séquences "donneuses" sont généralement non modifiées au cours d'évènements de conversion génique induits par une CDB (Pâques *et al.*, 1998).

Dans les cellules somatiques, la réparation des CDB est assurée principalement par la voie SDSA, prévenant ainsi les potentiels effets négatifs des CO tels que les réarrangements chromosomiques issus d'évènements de recombinaison entre séquences homologues non-alléliques. Les travaux de Allers et Lichten (2001) ont montré que les produits de NCO méiotiques sont principalement générés par SDSA, révélant le rôle clé de cette voie (pour revue, voir Andersen & Sekelsky, 2010).

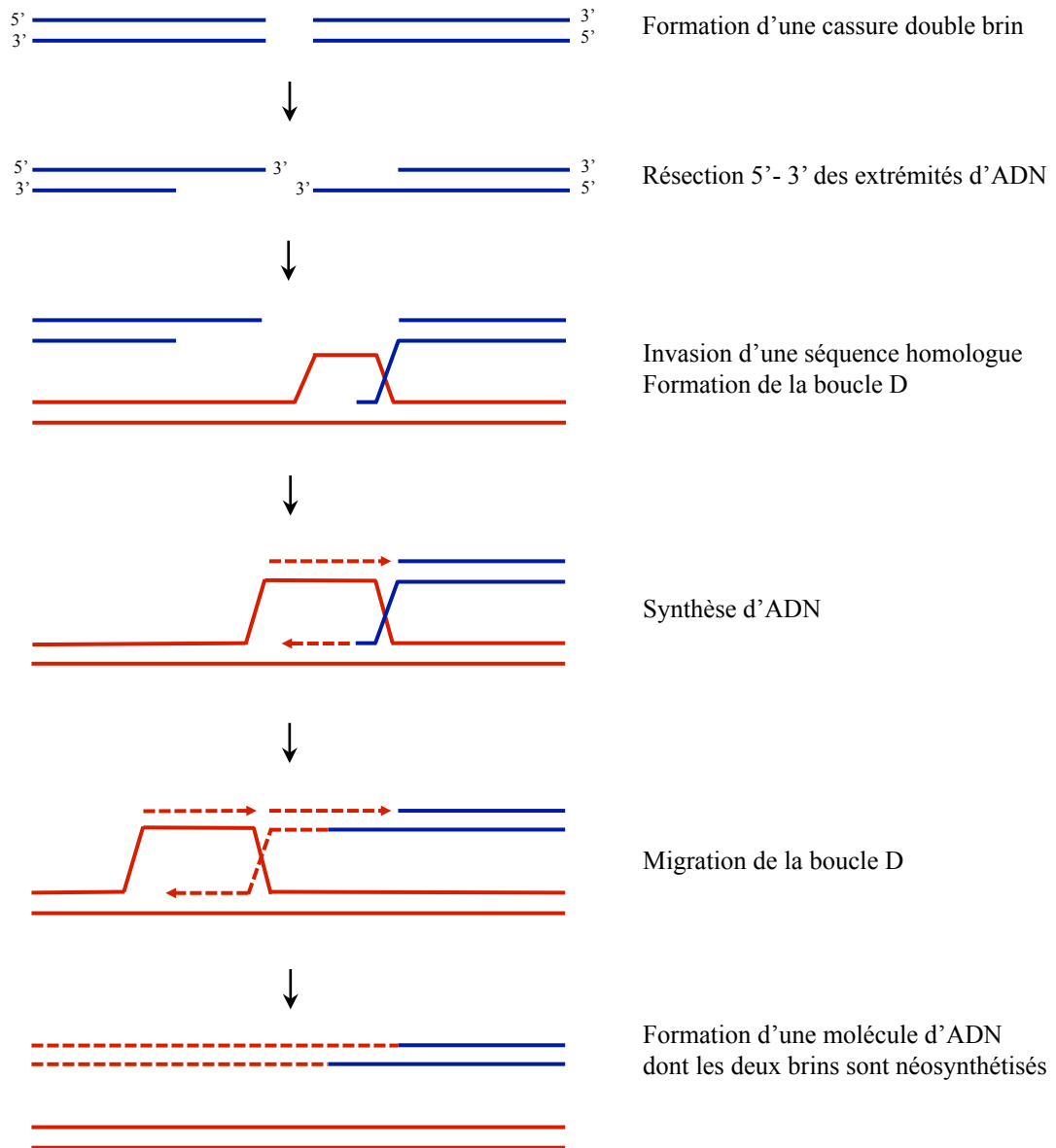


Figure 10 : Voie BIR (Break-Induced Replication)
(d'après Malkova & Ira, 2013)

Alors que les événements de conversion génique générés par les voies SDSA et DSBR ne concernent que des régions de quelques kilobases, des événements de plus grande échelle (d'une centaine de kilobases à un bras de chromosome entier) ont été décrits (Esposito, 1978 ; Voelkel-Meiman & Roeder, 1990 ; Malkova *et al.*, 1996). Ces observations ont servi de base à l'élaboration de nouveaux modèles nommés BIR (*Break-Induced Replication*).

I.3. Break-Induced Replication (BIR)

Inspirés des mécanismes de réplication dépendante de la recombinaison d'*E.coli* et du phage T4, plusieurs modèles de réplication d'ADN induite par une CDB ont été envisagés (Malkova *et al.*, 1996 ; Morrow *et al.*, 1997). Des travaux récents menés chez la levure ont démontré que la voie BIR aboutit à l'héritage conservatif du matériel génétique néosynthétisé, en accord avec le modèle de synthèse d'ADN conservative (Donnianni & Symington, 2013 ; Saini *et al.*, 2013). Comme dans les voies DSBR et SDSA, la voie BIR implique la résection 5'-3' des extrémités de la cassure suivie de la recherche puis de l'invasion d'une séquence homologue (**Figure 10**). La synthèse d'ADN initiée à partir de l'extrémité 3' du brin envahisseur induit la migration de la boucle D. Le mouvement de la jonction non résolue provoque le déplacement du brin nouvellement synthétisé. Ce processus aboutit à la formation d'une molécule d'ADN dont les deux brins sont néosynthétisés, tandis que l'ADN matrice conserve ses deux brins d'origine (Smith *et al.*, 2007). Le BIR peut ainsi être à l'origine de la réplication de grandes régions chromosomiques. La recombinaison de type BIR prendrait en charge les CDB dont une seule extrémité est disponible et serait notamment impliqué dans la réparation des fourches de réplication bloquées et l'allongement des télomères en absence de télomérase (pour revue, voir Malkova & Ira, 2013).

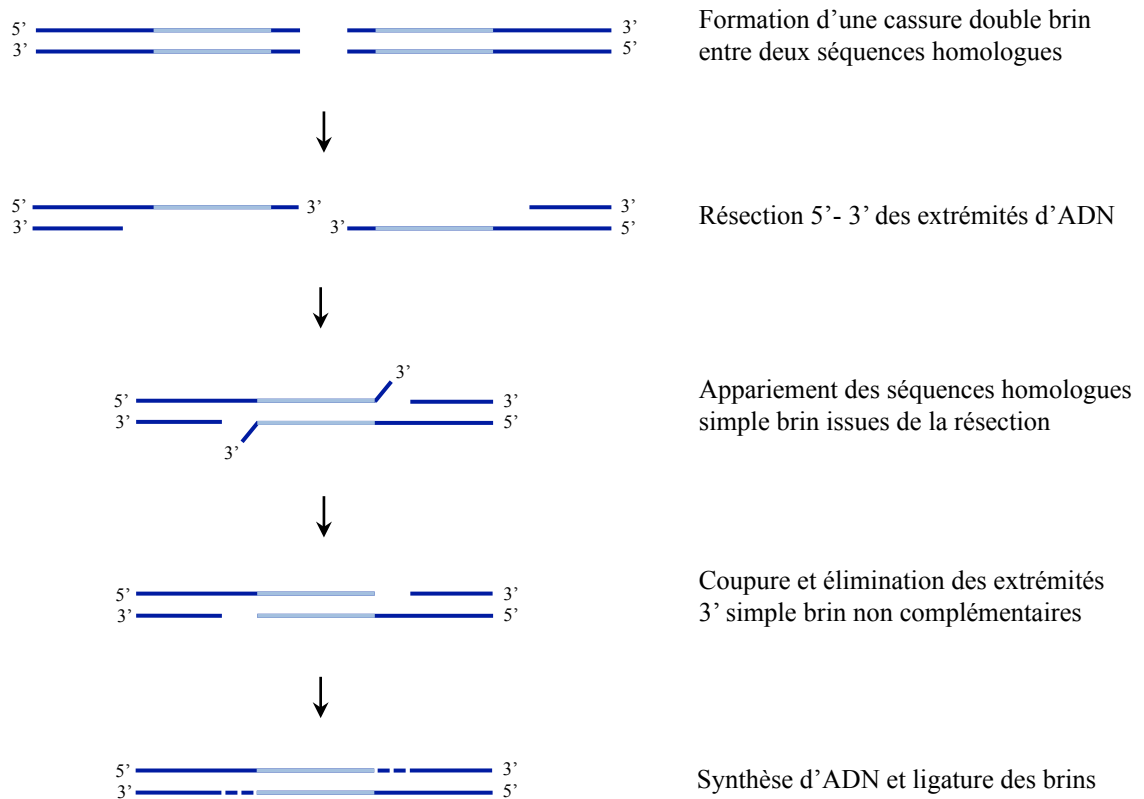


Figure 11 : Voie SSA (Single Strand Annealing)
(d'après Symington, 2002)

I.4. Single-Strand Annealing (SSA)

Une autre voie impliquant une homologie de séquence pour la réparation d'une CDB est la voie d'hybridation simple brin (SSA). Le modèle SSA, proposé initialement par Lin *et al.* (1984), décrit la recombinaison entre deux séquences répétées dans la même orientation situées sur la même molécule d'ADN (SSA intramoléculaire) ou sur deux molécules différentes (SSA intermoléculaire) (Lin *et al.*, 1984). Les principales étapes de la voie SSA intramoléculaire sont présentées dans la **figure 11**. Le processus est initié par la résection 5'-3' des extrémités d'ADN générant des queues simple brin 3' sortantes. Quand la résection est suffisante, les séquences complémentaires situées de part et d'autre de la cassure s'apparient. Les extrémités d'ADNs non complémentaires sont ensuite clivées et éliminées. La synthèse d'ADN permet de combler les lacunes potentielles avant la ligature des brins. Ce processus aboutit à la délétion du matériel génétique situé entre les séquences répétées ainsi que d'une des répétitions. Contrairement aux voies conservatives précédemment décrites, le SSA est donc un système de réparation mutagène. Il peut également être à l'origine d'importants réarrangements chromosomiques dans le cas où les séquences répétées se situent sur deux chromosomes différents (Richardson & Jasin, 2000).

Des évènements de SSA ont été détectés chez les levures, les animaux et les plantes ; ce mode de réparation des CDB serait particulièrement important dans les génomes présentant de nombreuses séquences répétées (Lin *et al.*, 1984 ; Maryon & Carroll, 1991 ; Fishman-Lobell *et al.*, 1992). L'efficacité du SSA est largement influencée par la longueur des régions répétées, leur degrés d'identité de séquence (Sugawara & Haber, 1992 ; Sugawara *et al.*, 1997) et par la distance séparant les deux répétitions (Fishman-Lobell *et al.*, 1992). A titre d'exemple, il a été montré chez la levure que l'efficacité du SSA est de 100% quand les régions homologues ont une taille supérieure à 400 paires de bases mais seulement de 5% quand celle-ci est de 60 pb (Sugawara & Haber, 1992).

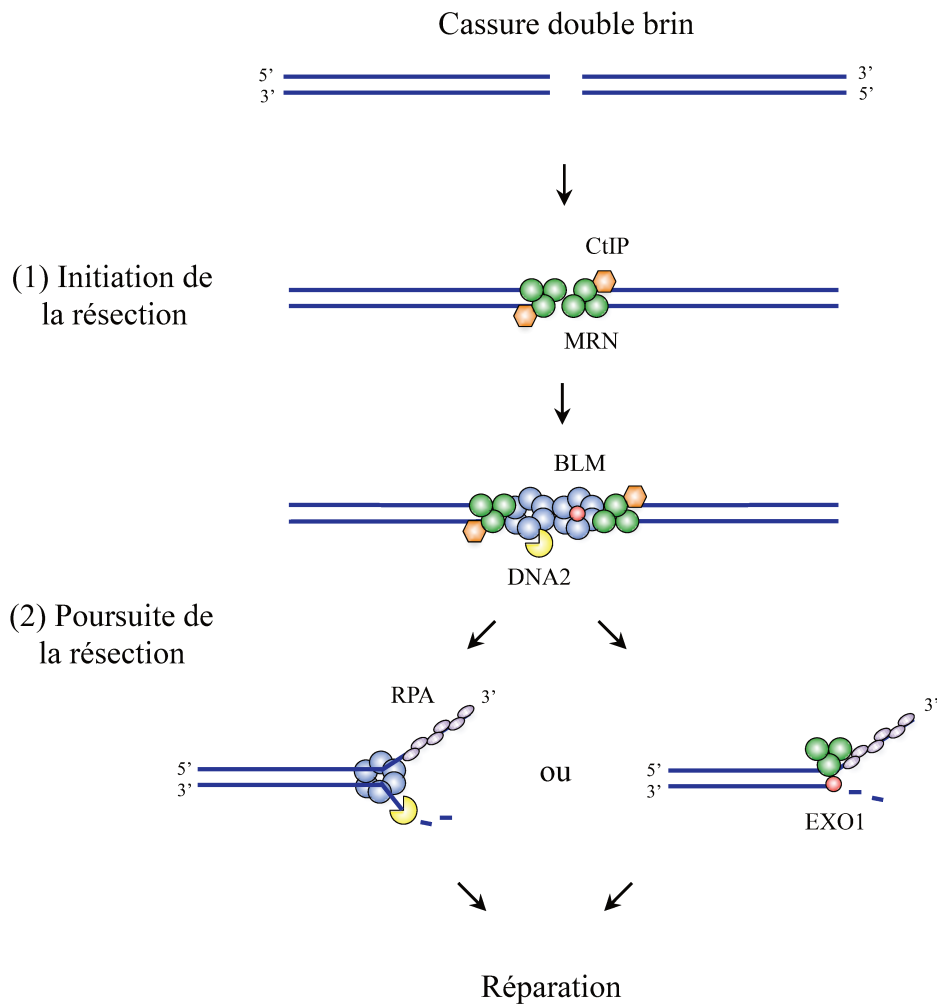


Figure 12 : Mécanisme de la résection des extrémités d'une cassure double brin chez les Mammifères (d'après Nimonkar *et al.*, 2011)

- (1) **Initiation de la résection.** La cassure double brin est reconnue par le complexe MRN qui recrute ensuite CtIP. Le complexe ainsi formé initie la résection sur quelques centaines de nucléotides.
- (2) **Poursuite de la résection.** MRN recrute ensuite BLM-DNA2 ou EXO1-BLM. La résection se poursuit sur plusieurs kilobases grâce à l'activité exonucléasique de DNA2 ou EXO1.



II. Les acteurs de la recombinaison homologue

Les voies de recombinaison homologue (RH) font intervenir un grand nombre de protéines dont les fonctions ont été largement étudiées ces dernières décennies. Pour les Eucaryotes, la majorité de ces protéines ont été identifiées grâce à des expériences de sensibilité aux radiations ionisantes chez *Saccharomyces cerevisiae* ; il s'agit de Rad50, Rad51, Rad52, Rad54, Rdh54 (Tid1), Rad55, Rad57, Rad59, Mre11 (Rad58) et Xrs2. Ces protéines appartiennent au groupe épistatique de Rad52 et sont hautement conservées chez les Eucaryotes (pour revue, voir Krogh & Symington, 2004).

Les principaux acteurs de la recombinaison homologue seront traités chronologiquement, en fonction de l'étape dans laquelle ils interviennent : (1) la reconnaissance et la maturation des extrémités de la CDB, (2) la recherche d'homologie et l'invasion de brin, (3) l'élimination des extrémités non complémentaires, puis (4) la résolution des intermédiaires de recombinaison.

II.1. Les protéines de la reconnaissance et de la maturation des extrémités de la CDB

La détection de la CDB est assurée par le complexe MRN (MRE11-RAD50-NBS1) chez les animaux, ou le complexe MRX (Mre11-Rad50-Xrs2) chez les levures (Lisby *et al.*, 2004 ; Lisby & Rothstein, 2009). Celui-ci se lie rapidement aux extrémités de la CDB et permet dans un premier temps de les maintenir ensemble (De Jager *et al.*, 2001) (**Figure 12**). Il joue ensuite un rôle fondamental dans la signalisation de la cassure en recrutant notamment la protéine ATM (*Ataxia telangiectasia mutated*) (pour revue, voir Lamarche *et al.*, 2010). De plus, le complexe MRN (MRX) participe à l'étape d'initiation de la résection des extrémités de la cassure. Bien que MRE11 possède des activités endonucléase et exonucléase 3'-5' (Paull & Gellert, 1998 ; Trujillo *et al.*, 1998 ; Trujillo & Sung, 2001), aucune activité exonucléase 5'-3' - sens de la résection des extrémités de la cassure - n'a été démontrée. Le complexe MRN

contribuerait à la résection en recrutant ou en facilitant l'activité d'autres facteurs de la RH (pour revue, voir Lamarche *et al.*, 2010). Parmi eux, l'endonucléase CtIP (*CtBP-interacting protein*) (Sae2 chez la levure) initie la résection 5'-3' des extrémités grâce à une interaction physique et fonctionnelle avec le complexe MRN (Sartori *et al.*, 2007). CtIP (Sae2) serait également impliqué dans la maturation de certaines extrémités d'ADN modifiées (Clerici *et al.*, 2005 ; Lengsfeld *et al.*, 2007). De plus, CtIP sous sa forme phosphorylée est capable d'interagir avec BRCA1 (*Breast Cancer 1*) (Yu & Chen, 2004), suggérant que cette protéine pourrait également participer à l'initiation de la résection des extrémités d'ADN (pour revue, voir Huen *et al.*, 2010). Cette étape d'initiation de la résection est essentielle dans le choix de la voie de réparation de la CDB puisqu'elle prévient les voies de recombinaison non homologue et favorise la recombinaison homologue (pour revue, voir Symington & Gautier, 2011).

La poursuite de la résection nécessite le recrutement d'autres protéines au niveau du site de la cassure : les exonucléases EXO1 et DNA2 ainsi que le complexe BLM-TOP3-RMI1 (Sgs1-Top3-Rmi1 chez la levure) (Zhu *et al.*, 2008 ; Shim *et al.*, 2010). De nombreuses études *in vitro* et *in vivo* ont permis de préciser les fonctions de ces protéines, notamment chez les Mammifères et les levures. L'exonucléase Exo1 et l'hélicase Sgs1 participent à la résection de manière indépendante puisque le double mutant *exo1 sgs1* accumule davantage de produits dont la résection est inachevée (seulement une centaine de nucléotides éliminés) par rapport aux simples mutants (Mimitou & Symington, 2008 ; Zhu *et al.*, 2008). Chez la souris, EXO1 serait également impliqué dans le recrutement de la protéine de liaison à l'ADNsb RPA (*Replication protein A*) (Schaezlein *et al.*, 2007), une protéine jouant un rôle clé dans la résection (Cejka *et al.*, 2010a ; Niu *et al.*, 2010 ; Nimonkar *et al.*, 2011). L'hélicase BLM interagit physiquement avec EXO1 et DNA2 et stimule leurs activités nucléases (Nimonkar *et al.*, 2008 ; Nimonkar *et al.*, 2011).

L'ensemble de ces études a permis de montrer que la poursuite de la résection 5'-3' des extrémités de la cassure peut être prise en charge par deux voies parallèles (**Figure 12**). Dans la première voie, l'hélicase BLM (Sgs1) et la nucléase DNA2 interagissent physiquement et spécifiquement pour stimuler la résection. RPA favorise le déroulement de l'ADN assuré par BLM (Sgs1) ainsi que la résection 5'-3' réalisée par la DNA2. Le complexe MRN (MRX) accélère le processus en recrutant BLM (Sgs1) au niveau des extrémités. Dans la seconde voie, EXO1 dégrade l'un des brins de la double hélice grâce à son activité 5'-3' exonucléasique. Le complexe MRN (MRX) recrute EXO1 au niveau de la cassure et stimule son activité nucléase. BLM (Sgs1) favoriserait également cette voie en augmentant l'affinité d'EXO1 pour les extrémités d'ADN (pour revue, voir Symington & Gautier, 2011). Ces deux voies génèrent des extrémités d'ADNsb 3' sortantes de plusieurs dizaines de kilobases, nécessaires à la recombinaison homologue. Il a été rapporté qu'en absence de recombinaison ou de séquence homologue, la résection pouvait se poursuivre sur plusieurs milliers de nucléotides à une vitesse d'environ 4 kilobases par heure chez la levure (Zhu *et al.*, 2008).

Chez *Arabidopsis thaliana*, des orthologues de chacune des protéines présentées précédemment ont été identifiés. AtRAD50 (Gallego *et al.*, 2001), AtMRE11 (Bundock & Hooykaas, 2002) et AtNBS1 (Waterworth *et al.*, 2007) sont les homologues des constituants du complexe MRN / MRX des Mammifères et des levures. AtCOM1 est l'homologue de CtIP/Sae2 (Uanschou *et al.*, 2007) et AtRecQ14A, l'homologue de BLM/Sgs1 (Bagherieh-Najjar *et al.*, 2003 ; Bagherieh-Najjar *et al.*, 2005). Ces différentes études ont montré que l'absence d'une de ces protéines conduit à une hypersensibilité à différents agents génotoxiques (notamment la mitomycine C) et une instabilité chromosomique en mitose ainsi qu'en méiose. La formation de foci RAD51 (la recombinaison essentielle de l'invasion de brin) est très nettement réduite dans les cellules *atcom1* par rapport aux cellules sauvages, malgré la présence de CDB non réparées (Uanschou *et al.*, 2007). Ces analyses démontrent que

l'ensemble de ces protéines est impliqué dans le maintien de la stabilité du génome des plantes, et probablement dans les voies de recombinaison homologue. Deux orthologues du gène Exo1 ont également été identifiés dans le génome d'*Arabidopsis*, AtExo1A et AtExo1B (Furukawa *et al.*, 2008), ainsi qu'un orthologue du gène Dna2 (Cupp & Nielsen, 2014), mais les protéines correspondantes n'ont pas encore été caractérisées. Bien que l'étude des acteurs de la résection chez les plantes soit bien moins avancée que chez les animaux et les levures, les informations disponibles suggèrent une haute conservation de ces mécanismes au sein des Eucaryotes.

II.2. Les protéines impliquées dans la recherche d'homologie et l'invasion de brin

Review: "RAD51 mediators in eukaryotic homologous recombination"

Heidi Serra & Charles I. White

(en préparation)

DNA double-strand breaks (DSB) are cytotoxic lesions, which arise from both exogenous (ionizing radiation, genotoxic chemicals) and endogenous sources (free radicals derived from metabolism, replicative damages) (Whitaker, 1992 ; Cox *et al.*, 2000). DSB can result in mutations, chromosomal rearrangements and/or loss of chromosomes, causing tumorigenesis or cell death. The repair of DSB by recombination is thus crucial to the maintenance of genome integrity in living organisms. Two principal mechanisms are involved in DSB repair: non-homologous end-joining and homologous recombination. The first leads to a direct joining of the break's ends, while the second implicates DNA sequence homology of the recombining molecules (for reviews, see San Filippo *et al.*, 2008 ; Waterworth *et al.*, 2011). The primary function of homologous recombination in mitotic cells is to repair single and double strand breaks but this process is also required for telomere maintenance, and consequently proliferation, in absence of telomerase. During meiosis, homologous recombination is essential to establish a physical connection between homologous chromosomes allowing their correct disjunction during the first meiotic division. Moreover, meiotic recombination creates mutations and/or new allelic combinations between genes, contributing to the generation of genetic diversity.

Among the pathways using homology for repair of DSB, Single Strand Annealing (SSA) is believed to play a particularly important role in repair of DSB flanked by direct repeat DNA sequences. SSA has been studied in yeasts, plants and animals using artificial direct repeats and could be particularly important for DSB repair in genomes that contain many repeated sequences (Lin *et al.*, 1984 ; Maryon & Carroll, 1991 ; Fishman-Lobell *et al.*, 1992). The efficiency of SSA is strongly influenced by the length of the flanking repeats, by their degree of sequence identity (Sugawara & Haber, 1992 ; Sugawara *et al.*, 1997) and by the distance separating the two repeats (Fishman-Lobell *et al.*, 1992). In the SSA model, after bidirectional 5'-3' resection of the DSB ends, the exposed complementary sequences anneal.

This step requires homologous recombination mediators as RAD52 (and Rad59 in yeast) (Ivanov *et al.*, 1996 ; Bai *et al.*, 1999 ; Liu *et al.*, 2012). Non-homologous 3' single strand DNA tails are subsequently removed by nucleases and single strand gaps filled. Strand ligation restores integrity of the DNA molecule. As DNA strand invasion and exchange are not involved, SSA is independent of RAD51 (Ivanov *et al.*, 1996 ; Stark *et al.*, 2004 ; Mansour *et al.*, 2008 ; Roth *et al.*, 2012). SSA recombination is a mutation prone pathway due to deletion of the interstitial DNA sequence lying between the repeats and one of the repeated sequences (for reviews, see Krogh & Symington, 2004 ; Heyer *et al.*, 2010).

The gene conversion homologous recombination pathway (HR) notably differs from SSA by its dependence on strand invasion catalysed by RAD51 recombinase (and/or DMC1 in meiosis). RAD51 activity has a central role in the HR process and is tightly regulated by a number of mediator proteins. In this review, we focus on the key mediators of RAD51 and propose an up-to-date synthesis of their functions in wide range of eukaryotes, including yeasts, plants, vertebrates and invertebrates. The recently described SHU complex is also reviewed and the phylogenetic relationships between components of the SHU complex and the RAD51 paralogues are discussed.

RAD51 / DMC1 recombinases and homologous recombination pathways

RAD51 is the universal recombinase of the HR pathway, highly conserved in all eukaryotes (yeast, animals and plants). RAD51 is an orthologue of the prototypic bacterial recombinase RecA and archaeal RadA (Aboussekhra *et al.*, 1992 ; Basile *et al.*, 1992 ; Shinohara *et al.*, 1992). Budding yeast *rad51* null mutants are viable but show high sensitivity to ionizing radiation and severe meiotic recombination defects (Symington, 2002a). Inactivation of RAD51 in *A. thaliana*, *D. melanogaster* and *C. elegans* has no effect on viability but mutants are completely sterile (Alpi *et al.*, 2003 ; Staeva-Vieira *et al.*, 2003 ; Li

et al., 2004). In vertebrates however, knockout of RAD51 results in cell inviability and early embryonic lethality (Tsuzuki *et al.*, 1996 ; Sonoda *et al.*, 1998). The severity of the *rad51* phenotype, together with the very high conservation of Rad51 protein throughout evolution, highlights the importance of RAD51/RecA activity in recombination.

RAD51 is a relatively small protein (37 kDa in human and Arabidopsis and 43 kDa in yeast) with a catalytic ATPase core region that includes Walker A/B motifs and a single-strand DNA binding domain (Shinohara *et al.*, 1992 ; Benson *et al.*, 1994 ; Sato *et al.*, 1995). RAD51 is able to nucleate on both single (ssDNA) and double strand DNA (dsDNA) *in vitro*, forming a nucleoprotein filament (NPF) (Ogawa *et al.*, 1993 ; Sung & Robberson, 1995 ; Yu *et al.*, 2001). Electron microscopic images revealed that this NPF is right-handed and composed of 18bp and approximately six RAD51 monomers per helical turn, each monomer covering three nucleotides (Ogawa *et al.*, 1993 ; Benson *et al.*, 1994 ; Yu *et al.*, 2001). The RAD51 NPF helical pitch ranges between 90 and 130 Å (Yu *et al.*, 2001 ; Conway *et al.*, 2004). ssDNA and dsDNA are thus extensively stretched within the filament, (extended in length by a factor of 1.5 compared to the B-form DNA) and dsDNA unwound (Ogawa *et al.*, 1993 ; Sung & Robberson, 1995). Subsequent real-time analyses have revealed the high flexibility and the dynamic nature of the NPF, with assembly and disassembly of RAD51 monomers regulated by many recombination mediators (Miné *et al.*, 2007 ; van der Heijden *et al.*, 2007).

The meiotic specific recombinase, DMC1 (disrupted meiotic cDNA), is present in most studied eukaryotes including yeast, vertebrates and plants (but not in *S. macrospora*, *D. melanogaster* and *C. elegans*) (Villeneuve & Hillers, 2001 ; Ramesh *et al.*, 2005 ; Malik *et al.*, 2008). DMC1 is a ~37 kDa protein, functionally, structurally and evolutionarily related to RAD51 (Bishop *et al.*, 1992 ; Story *et al.*, 1993). Ablation of DMC1 in *S. cerevisiae*, *A. thaliana* and mice produces a wide range of meiotic abnormalities that reflect the

indispensable role of DMC1 in meiotic recombination and chromosome segregation (Bishop *et al.*, 1992 ; Sehorn & Sung, 2004 ; Siaud *et al.*, 2004 ; Neale & Keeney, 2006). Human DMC1 has a weak ATPase activity, binds to ssDNA and dsDNA, and catalyses strand exchange between oligonucleotides *in vitro* (Li *et al.*, 1997 ; Masson *et al.*, 1999). The yeast DMC1 also promotes annealing of complementary ssDNA (Hong *et al.*, 2001). DMC1 exists as an octamer in solution (Passy *et al.*, 1999), and, like RAD51, is able to form right-handed helical filaments on ssDNA in an ATP-dependent manner (Masson *et al.*, 1999 ; Sehorn *et al.*, 2004 ; Sheridan *et al.*, 2008). In addition, DMC1 functions similar to RAD51 in assays for recombination and ATPase activity *in vitro* (Sehorn *et al.*, 2004 ; Bugreev *et al.*, 2005 ; Sauvageau *et al.*, 2005). DMC1 and RAD51 are both required for high rates of meiotic recombination and have overlapping, but non-identical, functions in meiosis (Shinohara *et al.*, 1997a ; Tsubouchi & Roeder, 2003 ; Bishop, 2012 ; Cloud *et al.*, 2012 ; Da Ines *et al.*, 2012). One of the key differences between both recombinases is their opposite preferential polarities of strand exchange which may explained their divergent roles in homologous recombination (Murayama *et al.*, 2011).

In the HR pathway, resection of 5'-ended DNA strands flanking the DSB generates 3' ssDNA overhangs that are rapidly bound by the single-strand binding protein, Replication Protein A (RPA). Assisted by mediator proteins, RAD51 (or DMC1) is loaded onto exposed ssDNA by displacing RPA, and forms the helical NPF (Sugiyama & Kowalczykowski, 2002) (Yu *et al.*, 2001). Presynaptic filament assembly requires ATP binding but not hydrolysis (Chi *et al.*, 2006b). Formation and maintenance of the RAD51-ssDNA NPF is required for the DNA homology search and strand exchange (Baumann *et al.*, 1996). Homology search is by random collision that involved transient nonspecific interactions with dsDNA (Bianco *et al.*, 1998). Human RAD51 facilitates homology search by a rapid A:T base-flipping mechanism (Gupta *et al.*, 1999). Once a homologous sequence is found, RAD51 promotes the formation

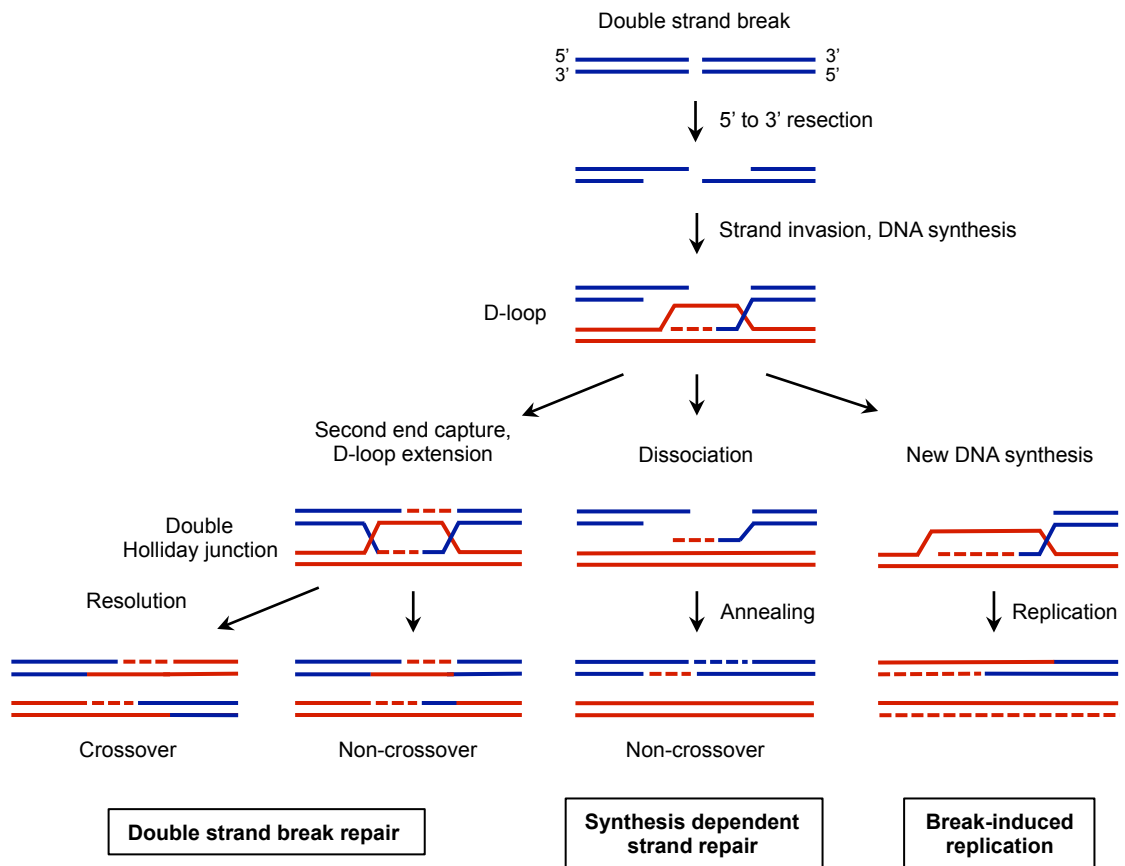


Figure 1: Models for the repair of double strand break by homologous recombination.

of a physical connection between the invading ssDNA and homologous duplex DNA template (Baumann *et al.*, 1996). This results in the generation of heteroduplex DNA with D-loop (displacement-loop) structure (**Figure 1**). Subsequent dissociation of RAD51 from DNA, correlated to ATP hydrolysis, permits to expose the invading 3'-OH end (Kowalczykowski, 1991). This extremity is used as a primer for DNA synthesis with the homologous sequence as a template.

As illustrated in Figure 1, the recombination intermediate (D-loop) can be resolved by three HR sub-pathways: DSBR, SDSA and BIR. (1) The double-strand break repair model (DSBR) involved the formation of stable double Holliday junctions (dHJ) by capture of the second-end of the DSB. DNA heteroduplex can be subsequently extended by branch migration, an ATP-dependent process driven by RAD51 recombinase (via multiple rounds of RAD51 polymerisation and dissociation) and other recombination mediators (Murayama *et al.*, 2008 ; Rossi *et al.*, 2011). The dHJs can be dissolved by the combined action of helicase/topoisomerase (only NCO products), or resolved by structure-specific endonucleases, producing crossover or non-crossover products (Szostak *et al.*, 1983 ; Schwacha & Kleckner, 1995 ; Hunter & Kleckner, 2001 ; Börner *et al.*, 2004 ; Chang *et al.*, 2005 ; Mullen *et al.*, 2005 ; Hartung *et al.*, 2008). (2) Alternatively, the invading strand can dissociate from the D-loop and anneal with the complementary 3' single strand tail on the other side of the break as described by the synthesis-dependent strand-annealing model (SDSA) (Nassif *et al.*, 1994 ; Pâques & Haber, 1999 ; Allers & Lichten, 2001 ; Puchta, 2005). The remaining gaps are filled by DNA synthesis and the nicks ligated. SDSA leads to gene conversion events not accompanied by crossovers. In somatic cells, the prevalence of gene conversion events avoids unpredictable deleterious genomic change by crossovers between ectopic sequences (Richardson *et al.*, 1998). This might be particularly important for preserving the genome stability of species containing ample amounts of repetitive DNA. During meiosis, non-

crossovers are primarily produced via SDSA processing while crossovers, prerequisite for alterations in genetic coupling arrangements, are formed by resolution of dHJ via the DSBR mechanism (Allers & Lichten, 2001 ; Hunter & Kleckner, 2001). (3) The third possibility for the resolution of the D-loop structure is the copy of the entire chromosome arm via establishment of a processive replication fork (Llorente *et al.*, 2008). This process, called break-induced replication (BIR), required leading and lagging strand DNA synthesis, in contrast to SDSA that uses only leading strand DNA synthesis (Wang *et al.*, 2004). This extensive replication primed from the invading 3' end occurs when only one DNA is available, because of the loss of the other end or in the case of the process of lengthening telomeres in telomerase-deficient cells.

REPLICATION PROTEIN A

Replication Protein A (RPA) has been identified in numerous organisms, including mammals, zebrafish, flies, nematodes, plants and budding and fission yeasts (**Table 1**). RPA is a heterotrimeric protein that binds to ssDNA with high affinity but binds poorly dsDNA and RNA (Fairman & Stillman, 1988 ; Wold & Kelly, 1988 ; Erdile *et al.*, 1990 ; Marton *et al.*, 1994 ; Sugiyama *et al.*, 1997 ; Sung, 1997a ; Hyun *et al.*, 2012). Extensive analyses have shown that RPA binds ssDNA in a sequential binding manner with a 5' to 3' polarity (De Laat *et al.*, 1998b ; Kolpashchikov *et al.*, 2000). RPA functions in a wide range of DNA processing pathways, including DNA replication, repair and recombination (see **Table 1**). In addition, a potential role of RPA in the regulation of gene transcription has been suggested in yeast and human (Singh & Samson, 1995 ; Tang *et al.*, 1996). Only its role in RAD51-dependent homologous recombination will be treated here.

RPA binds rapidly to the ssDNA resulting from the resection of the DSB extremities. RPA has higher affinity for ssDNA than Rad51 (Sugiyama *et al.*, 1997 ; Sung, 1997a) and

arrives first at DSB sites during the presynaptic phase (Wolner *et al.*, 2003). This binding to exposed ssDNA acts to protect it against degradation from endo/exo-nuclease activities and to prevent formation of DNA secondary structures that could potentially lead to inhibitory effects on Rad51 nucleoprotein filament formation (Sugiyama *et al.*, 1997). As RPA has higher affinity for ssDNA than Rad51, it inhibits Rad51 nucleoprotein complex formation by prior binding to ssDNA (Sugiyama & Kowalczykowski, 2002). This inhibition is overcome *in vivo* by RAD51 mediators such as Rad52 and Rad55-Rad57 in yeast, or BRCA2 and RAD51 paralogues in mammals (see corresponding sections below). Sugiyama and colleagues have shown that yeast Rad51 NPF formation is simultaneous with displacement of RPA from ssDNA. This displacement is initiated by a rate-limiting nucleation of Rad51 proteins onto ssDNA, followed by rapid elongation of the filament (Sugiyama & Kowalczykowski, 2002).

Once the RAD51 NPF is formed, human RPA stimulates the strand exchange activity of RAD51 by physical interaction (Baumann & West, 1997 ; Stauffer & Chazin, 2004). A similar stimulatory role was also observed in budding yeast where RPA ensures unidirectional heteroduplex extension by binding to the displaced ssDNA during the strand exchange phase (Sung, 1994 ; Eggler *et al.*, 2002 ; Van Komen *et al.*, 2002). However, during *in vitro* recombination assays, the addition of RPA to ssDNA prior to RAD51 inhibits the RAD51 reactions (Haruta *et al.*, 2006) due to inefficient displacement of RPA from ssDNA in the absence of RAD51 mediators. RPA can thus be considered as a positive regulator of HR (notably by preventing DNA secondary structure formation at all stages) and RAD51 functions, despite its apparently paradoxical role in inhibition of RAD51 NFP formation.

RAD51 PARALOGUES

The existence of RAD51/DMC1-related proteins has been described in a variety of organisms. Although clearly of common origin, these proteins show a high degree of evolutionary divergence from RAD51 as well as from each other (Thompson & Schild, 2001). Genes encoding these factors appear to be the result of gene duplication events of an ancestral RecA/RAD51 gene and the corresponding proteins have subsequently acquired new functions (Lin *et al.*, 2006).

The budding yeast *Saccharomyces cerevisiae* encodes two well-known Rad51 paralogues, Rad55 and Rad57 (Kans & Mortimer, 1991 ; Lovett, 1994). They form a stable heterodimeric complex (Sung, 1997b), which is able to integrate into the Rad51-DNA nucleofilament (Liu *et al.*, 2011a). They are positive regulators of mitotic and meiotic recombination, promoting nucleation and elongation of Rad51 NPF and stabilising it against disruption by the Srs2 antirecombinase (Liu *et al.*, 2011a). They act also in promoting of DNA strand exchange mediated by Rad51 (Sung, 1997b) (**Table 2**). Two structural and functional orthologues of Rad55 and Rad57 have been identified in the fission yeast *Schizosaccharomyces pombe*, Rhp55 and Rhp57, respectively (Khasanov *et al.*, 1999 ; Tsutsui *et al.*, 2000).

Five RAD51 paralogues have been identified in vertebrates and plants: RAD51B (RAD51L1/REC2), RAD51C (RAD51L2), RAD51D (RAD51L3), XRCC2 and XRCC3. They share 20% to 30% identity at amino acid level with RAD51 and with each other. XRCC2 and XRCC3 (X-ray Repair Cross Complementing) were first identified by their ability to complement the high sensitivity of *irs1* and *irs1SF* hamster cell lines to diverse DNA damaging agents (Tebbs *et al.*, 1995 ; Tambini *et al.*, 1997). Homology searches further identified RAD51B (Albala *et al.*, 1997 ; Rice *et al.*, 1997 ; Cartwright *et al.*, 1998a), RAD51C (Dosanjh *et al.*, 1998) and RAD51D (Cartwright *et al.*, 1998a ; Pittman *et al.*,

A. RAD51 paralogue complexes

BCDX2 complex
H. sapiens



CX3 complex
H. sapiens



Rad55-Rad57
complex
S. cerevisiae



Rhp55-Rhp57
complex
S. pombe



B. SHU complexes

H. sapiens



S. cerevisiae



S. pombe



Figure 2: Composition of RAD51 paralogue complexes and SHU complexes in human and yeast

1998). The relatively low conservation of these proteins makes detailed evolutionary relationships difficult to determine with certitude, however Thacker (1999) and Tsutsui et al. (2000) have suggested that Rad55/Rhp55 are closest to XRCC2 and Rad57/Rhp57 are closest to RAD51D and XRCC3. More recent analyzes confirm this and suggest that Rad55 and Rad57 are orthologous to XRCC2 and XRCC3, respectively (Lin *et al.*, 2006).

Two-hybrid and co-immunoprecipitation studies have shown that the five RAD51 paralogues form two major complexes: RAD51B-RAD51C-RAD51D-XRCC2 (BCDX2) and RAD51C-XRCC3 (CX3), as well as RAD51B-RAD51C (BC) and RAD51D-XRCC2 (DX2) sub-complexes (Schild *et al.*, 2000 ; Masson *et al.*, 2001 ; Liu *et al.*, 2002 ; Miller *et al.*, 2002 ; Osakabe *et al.*, 2002 ; Wiese *et al.*, 2002 ; Miller *et al.*, 2004 ; Osakabe *et al.*, 2005) (**Figure 2**). No self-assembly of individual paralogues has been detected (in contrast to RAD51).

Phenotypic analyses of RAD51 paralogue mutants clearly indicate that these proteins are key mediators of the recombinational repair process in mitotic cells, but their exact roles remain to be identified (Brenneman *et al.*, 2002 ; Bleuyard & White, 2004 ; Liu *et al.*, 2004 ; Liu *et al.*, 2007 ; Badie *et al.*, 2009 ; Nagaraju *et al.*, 2009 ; Rodrigue *et al.*, 2012 ; Chun *et al.*, 2013 ; Da Ines *et al.*, 2013a). A synthesis of the properties and functions of the RAD51 paralogues are presented in Table 2. In the presynaptic phase of HR, the BCDX2 (but not the CX3 complex) is responsible for RAD51 recruitment at damage sites (Chun *et al.*, 2013). Formation and stabilization of RAD51 NPF are mediated by RAD51 paralogues, probably through counteracting disruption of the filaments by helicases (Hu *et al.*, 2007 ; Moldovan *et al.*, 2012 ; Chun *et al.*, 2013). Both CX3 (Kurumizaka *et al.*, 2001) and DX2 (Kurumizaka *et al.*, 2002) complexes catalyse homologous strand pairing *in vitro*. The human BC complex also enhances the homologous DNA pairing activity of RAD51 by suppressing the inhibitory effect of RPA (Sigurdsson *et al.*, 2001). In addition, the RAD51 paralogues are involved in regulation of gene conversion tract length (Brenneman *et al.*, 2002 ; Nagaraju *et al.*, 2009).

The association of CX3 complex with HJ in human cell extracts and the defects of HJ resolution activity in *rad51c*-deficient cells strongly suggest that CX3 is also involved in the later steps of HR in resolution of HR intermediary structures (Liu *et al.*, 2004 ; Yokoyama *et al.*, 2004 ; Kuznetsov *et al.*, 2007 ; Liu *et al.*, 2007 ; Compton *et al.*, 2010). A role of Rad51 paralogues distinct from stabilization of Rad51 nucleofilament was also reported in yeast (Mozlin *et al.*, 2008 ; Fung *et al.*, 2009).

Are these RAD51 paralogues involved in meiosis in addition to their key roles in somatic recombination? Consistent with the sterility phenotype of Arabidopsis *rad51c* and *xrcc3* mutants, a number of studies have demonstrated that RAD51C and XRCC3 are essential for meiotic recombination in plants, but also in vertebrates (Bleuyard & White, 2004 ; Abe *et al.*, 2005 ; Bleuyard *et al.*, 2005 ; Li *et al.*, 2005 ; Kuznetsov *et al.*, 2007 ; Liu *et al.*, 2007 ; Da Ines *et al.*, 2012). While *rad51c* and *xrcc3* mutants exhibit severe chromosomal fragmentation during meiotic prophase (Bleuyard & White, 2004 ; Li *et al.*, 2005), *xrcc2*, *rad51b* and *rad51d* mutants are however fully fertile and present no detectable meiotic defect (Bleuyard *et al.*, 2005 ; Osakabe *et al.*, 2005). These observations have led to the assumption that XRCC2, RAD51B and RAD51D play roles only in mitotic recombination (Osakabe *et al.*, 2002 ; Bleuyard *et al.*, 2005 ; Osakabe *et al.*, 2005 ; Durrant *et al.*, 2007). These three genes are however highly expressed in meiotic tissues in animals (Cartwright *et al.*, 1998a ; Cartwright *et al.*, 1998b ; Tarsounas *et al.*, 2004a) and plants (Yang *et al.*, 2011), and absence of AtXRCC2, and to a lesser extent AtRAD51B, increases rates of meiotic crossing over in Arabidopsis (Da Ines *et al.*, 2013a). The authors propose that this hyper-recombination phenotype could be due to an increase in DMC1-dependent recombination, promoted by a decrease in RAD51-dependent recombination, via the modulation of RAD51 activities by RAD51 paralogues (Da Ines *et al.*, 2013a). Although the precise roles of these proteins in

meiosis remain to be clarified, these results represent the first evidence that XRCC2 and RAD51B are involved in both mitotic and meiotic recombination.

In addition to the functions of the RAD51 paralogue complexes, individual roles for the proteins have also been reported. RAD51D is involved in telomere integrity in human cells, and appears to be the only RAD51 paralogue so involved (Tarsounas *et al.*, 2004b). The greater sensitivity of the *xrcc2 rad51b rad51d* triple mutant to bleomycin-induced DNA damage compared to corresponding single mutants (Wang *et al.*, 2014) indicates that these RAD51 paralogues have non-epistatic functions, also suggesting individual roles. In accordance to this, non-epistatic functions of Arabidopsis XRCC2, RAD51B and RAD51D have also been reported in Single Strand Annealing recombination (Serra *et al.*, 2013). Finally, a recent report shows opposing effects on cell cycle regulation of the inhibition of XRCC3 and RAD51C in HeLa cells, with inhibition of XRCC3 eliciting checkpoint defects and inhibition of RAD51C inducing G2/M cell cycle arrest (Rodrigue *et al.*, 2012).

Drosophila melanogaster encodes four RAD51-related proteins: SPN-B, SPN-D, CG2412 and CG6318 (Radford & Sekelsky, 2004). Phylogenetic analysis of RAD51 family members revealed that SPN-B is most similar to hXRCC3 (Thacker, 1999 ; Abdu *et al.*, 2003 ; Lin *et al.*, 2006) and SPN-D to hRAD51C (Abdu *et al.*, 2003 ; Lin *et al.*, 2006). Moreover, CG2412 and CG6318 appear orthologous to RAD51D and XRCC2, respectively (Sekelsky *et al.*, 2000 ; Lin *et al.*, 2006), although they are extremely divergent. Spn-B and CG2412 appear to be expressed ubiquitously, whereas Spn-D and CG6318 are expressed exclusively in the female germline (Staeve-Vieira *et al.*, 2003), suggesting meiosis-specific expression. No mutation in CG2412 and CG6318 genes have been reported and in general, very little information is available in the literature regarding the functions of the *Drosophila* RAD51 paralogues (**Table 2**). Staeve-Vieira *et al.* (2003) suggested that SPN-B, but not SPN-D, plays a role in DNA repair in somatic cells. As *spn-B* and *spn-D* mutations have identical meiotic

phenotypes (Ghabrial *et al.*, 1998 ; Abdu *et al.*, 2003), the proteins may function together in meiotic recombination. A hypothesis supported by the recent report of physical interaction between SPN-B and SPN-D (Joyce *et al.*, 2012). Further analyses will however be required to determine the functional relationships of the *Drosophila* RAD51 paralogues.

Contrary to all other analysed organisms, *Caenorhabditis elegans* appears to possess a unique RAD51 paralogue, RFS-1, most similar to RAD51D (Ward *et al.*, 2007). Surprisingly, RFS-1 is dispensable for mitotic and meiotic HR-mediated DSB repair (Ward *et al.*, 2007). RFS-1 is thus not a general mediator of HR-mediated repair, but performs specific roles in promoting HR repair of lesions encountered by the replication fork during S-phase (Ward *et al.*, 2007). In addition, RFS-1 is involved in telomere integrity maintenance (Yanowitz, 2008), in accordance with the implication of human RAD51D in telomere-length regulation (Tarsounas *et al.*, 2004b).

Analyses of RAD51 paralogues in various species highlight some interesting points, including differences in the severity of mutant phenotypes. Indeed, animal cells defective in any of the RAD51 paralogues are hypersensitive to DNA cross-linking agents, such as cisplatin and Mitomycin C, and show spontaneous chromosomal aberrations (Tebbs *et al.*, 1995 ; Liu *et al.*, 1998 ; Johnson *et al.*, 1999 ; Pierce *et al.*, 1999 ; Takata *et al.*, 2000 ; Takata *et al.*, 2001 ; Godthelp *et al.*, 2002). Mouse *xrcc2*, *rad51b*, *rad51c* and *rad51d* mutants are embryonic lethal (Shu *et al.*, 1999 ; Deans *et al.*, 2000 ; Pittman & Schimenti, 2000 ; Kuznetsov *et al.*, 2009). In contrast, RAD51 paralogue mutants are viable but infertile in *Drosophila* and the nematode *C. elegans* (Ghabrial *et al.*, 1998 ; Yanowitz, 2008). In *Arabidopsis*, all five RAD51 paralogue mutants grow and develop normally and only *rad51c* and *xrcc3* mutant plants are sterile due to meiotic recombination defects (Bleuyard & White, 2004 ; Bleuyard *et al.*, 2005 ; Li *et al.*, 2005). The reasons for such differences in phenotypes between organisms (such as mutant lethality/viability between vertebrates and plants) have

not to date been elucidated. A first explanation is that vertebrate RAD51 paralogues may provide additional function(s) not associated with the invertebrate, yeast and plant orthologues, but essential for organism development. It is also possible that functions of RAD51 paralogues are compensated by other protein(s) in plants and that these redundant activities do not exist in vertebrates. On the other hand, the impact on development of cell death due to DNA damage differs in vertebrates and plants. Indeed, death of individual cell in plants does not translate simply to death of the plant, but is compensated by division of other cell and at most lead to a temporary slowing of the plant's growth. Cell death potentially induced by the absence of RAD51 paralogues in the vertebrate embryo can cause developmental arrest and/or lethal abnormalities. Due to this inviability of vertebrates lacking one of the RAD51 paralogues (Shu *et al.*, 1999 ; Deans *et al.*, 2000 ; Pittman & Schimenti, 2000 ; Kuznetsov *et al.*, 2009), plants thus provide an interesting model to study functions of the RAD51 paralogues.

Comparison of RAD51 paralogue functions between eukaryotes both confirms their high conservation and, highlights interesting functional divergences. For example, *Arabidopsis* XRCC2 is clearly involved in RAD51-independent SSA recombination (Serra *et al.*, 2013) whereas its yeast counterpart, Rad55, has an inhibitory effect on SSA via promotion of HR (Godin *et al.*, 2013). Concerning the requirement of RAD51 paralogues in formation and stabilization of RAD51 NPF, some differences have been reported between organisms. Yeast Rad55 and Rad57 are required for the formation of RAD51 foci in meiosis, but only partially in mitotic cells (Gasior *et al.*, 1998 ; Lisby *et al.*, 2004). Chinese hamster ovary (CHO) and mouse embryonic fibroblast (MEF) cells defective in any of the five RAD51 paralogues exhibit defects in the formation of DNA damage-induced RAD51 foci (Bishop *et al.*, 1998 ; Tarsounas *et al.*, 2004a ; Smiraldi *et al.*, 2005 ; Van Veelen *et al.*, 2005). However, the requirement of RAD51 paralogues in RAD51 focus formation is by-passed in

the context of spontaneous S-phase replication-associated RAD51 foci in CHO (Tarsounas *et al.*, 2004a). Experiments in chicken DT40 cells showed that mutation of any of the RAD51 paralogues significantly reduces, but doesn't eliminate the formation of RAD51 foci (Takata *et al.*, 2000 ; Takata *et al.*, 2001 ; Yonetani *et al.*, 2005 ; Qing *et al.*, 2011). Recent work in plants has revealed that Arabidopsis RAD51B, RAD51D and XRCC2 are not required for the formation of radio-induced RAD51 foci in somatic cells (Da Ines *et al.*, 2013a). Taken together, these results highlight an apparent variability in the need for the RAD51 paralogues in recruitment/stabilization of RAD51 at DNA damage sites. Given the transient presence of the foci and that the kinetics of focus appearance/disappearance can be modified in mutants, this may however be an artefact due to the experimental conditions. In agreement with this, it has recently been demonstrated that mutation of XRCC2 leads to a delay (not absence) in RAD51 focus formation in hamster cells (Tambini *et al.*, 2010). It is however also possible that these data reflect real differences in the requirement for RAD51 paralogues in RAD51 NPF formation/stabilization between yeasts, animals and plants.

SHU COMPLEX

Considerable interest is being given to four additional RAD51 mediators characterised in *Saccharomyces cerevisiae*: Shu1, Shu2, Psy3 and Csm2, components of the SHU (or PCSS) complex (Shor *et al.*, 2005). Shu1 and Shu2 (suppressor of *sgs1* hydroxyurea sensitivity) were first identified by a screen for suppressors of the slow-growth phenotype of yeast topoisomerase III (*top3*) mutants (Shor *et al.*, 2005). Psy3 and Csm2 were initially discovered by genome-wide screens for mutants sensitive to platinum (Wu *et al.*, 2004) or affecting chromosome segregation in meiosis (Rabitsch *et al.*, 2001), respectively. Yeast two-hybrid experiments have identified the physical interactions between these proteins (Ito *et al.*, 2001 ; Shor *et al.*, 2005 ; Ball *et al.*, 2009), and thus permit specification of the organisation

of the complex (see **Figure 2**): Psy3 would be the core protein, the sole that simultaneously interacts with the three other components. This stable complex is able to form a nucleofilament by binding non-specifically to DNA, like Rad51 (Tao *et al.*, 2012). The key function of the SHU complex is to promote RAD51 NPF formation by inhibiting Srs2 anti-recombinase recruitment at inducible DSB sites (Bernstein *et al.*, 2011). The SHU complex could directly inhibit the action of Srs2 through its interaction with Shu2 (Ito *et al.*, 2001), like Rad55-Rad57, and/or be a stabilizer of RAD51 filament. This complex clearly promotes HR while suppressing error-prone DNA repair (Shor *et al.*, 2005 ; Mankouri *et al.*, 2007 ; Ball *et al.*, 2009) and given that its absence leads to sensitivity to replication blocking agents, it may have a specialized role in HR during replicative stress (Ball *et al.*, 2009 ; Choi *et al.*, 2010 ; Xu *et al.*, 2013). Recent reports have also revealed a crucial role in meiotic recombination in mediating assembly of Rad51 NPF (Sasanuma *et al.*, 2013) and in promoting the establishment of homolog bias during meiotic HR (Hong & Kim, 2013). Available data about SHU complex functions are summarized in Table 3. Further work will however be needed to fully establish the roles of the SHU proteins.

Some components of the SHU complex are evolutionally conserved in fission yeast and in human: Rlp1, Sws1 and Rdl1 are the three identified components of *Schizosaccharomyces pombe* SHU complex and SWS1 and SWSAP1 are members of human SHU complex (Martín *et al.*, 2006 ; Liu *et al.*, 2011b ; Sasanuma *et al.*, 2013). These proteins form stable complexes *in vivo*: Rlp1-Sws1-Rdl1 in fission yeast and SWS1-SWSAP1 in human (**Figure 2**) and have an evolutionarily conserved function required for efficient HR repair (Martín *et al.*, 2006 ; Liu *et al.*, 2011b) (**Table 3**). Liu *et al.* (2001) suggest that human SHU complex is a non-essential HR accessory factor, which affects the efficiency and/or timing of HR.

What are the phylogenetic relationships between human SHU complex components, their yeast counterparts and well-known RAD51 paralogues? Data from literature on this subject show some discrepancy. Shu1 and Psy3 would be RAD51 paralogues (Martín *et al.*, 2006). Sequence homology analyses indicate that budding yeast Shu1, Shu2, Psy3 and fission yeast Rlp1, Sws1, Rdl1 correspond to human XRCC2, SWS1 and RAD51D, respectively (Khasanov *et al.*, 2004 ; Martín *et al.*, 2006). Rdl1 and Psy3 are significantly similar whereas Rlp1 shares no obvious sequence similarities to Shu1 (or Csm2) (Martín *et al.*, 2006). No human or fission yeast protein related to Csm2 has yet been found. Although the yeast proteins are associated as a complex, XRCC2, RAD51D (or other RAD51 paralogues) were not detected in the mass spectrometry analysis of hSWS1-containing complexes (from human 293T cells) (Liu *et al.*, 2011b). This screen did however identify SWSAP1 (SWS associated protein 1), a new interacting partner which has similarity to RadA, a RecA family protein in *archaea* (Liu *et al.*, 2011b). Divergences of protein domains and sequences have thus frustrated efforts to identify reliable phylogenetic correlations between the components of SHU complex and RAD51 paralogues. The recent resolution of the crystal structures of Psy3 and Csm2 have revealed that these proteins share a similar structure with Rad51 (She *et al.*, 2012 ; Tao *et al.*, 2012) and that Psy3-Csm2 sub-complex is a structural mimic of the Rad51-dimer (Sasanuma *et al.*, 2013). These results suggest that the structure (and functions), but not the sequence, of these proteins has been conserved.

The existence of several RAD51-like protein complexes, like SWS1-SWSAP1, BCDX2 and CX3 in human, raises the question of their functional relationships. Many lines of evidence argue for an interconnected and coordinated action of the SHU complex with the other RAD51 mediators. Rad55 physically interacts with SHU complex via Csm2 protein (Godin *et al.*, 2013). *csm2* and *psy3* are epistatic to *rad55* (Godin *et al.*, 2013 ; Xu *et al.*, 2013) and these two proteins are needed for efficient recruitment of Rad55 to DNA repair foci

after DNA damage (Godin *et al.*, 2013). Shu2 favours Rad51 focus formation (Bernstein *et al.*, 2011), but also Rad52 focus formation (Shor *et al.*, 2005). In addition, Shor *et al.* (2005) highlighted an epistatic relationship between *rad52* and *shu* mutations, suggesting that Shu proteins function together with Rad52. Human SWS1 is able to associate with RAD51D and XRCC3, and SWSAP1 interacts with RAD51B, RAD51C, RAD51D and XRCC3 (Martín *et al.*, 2006 ; Liu *et al.*, 2011b). Moreover, chicken SWS1 requires BRCA2 to contribute to HR (Qing *et al.*, 2011). Taken together, these data underscore the interconnected roles of mediators and the importance of controlling HR repair.

In view of the high conservation of RAD51 mediators, especially RAD51 paralogues, between animals and plants, it is very likely that the components of the SHU complex also exists in other mammals and plants.

RAD52 AND RAD59

The RAD52 protein has been identified in most eukaryotic organisms, including yeasts, vertebrates and recently in plants (Adzuma *et al.*, 1984 ; Bezzubova *et al.*, 1993 ; Muris *et al.*, 1994 ; Samach *et al.*, 2011), but appears to be absent in invertebrates (Lok & Powell, 2012). Structure and biochemical activities of RAD52 proteins from different species are very similar. Electron microscopic evidence indicates that both yeast and human RAD52 form oligomeric ring structures (Shinohara *et al.*, 1998 ; Stasiak *et al.*, 2000 ; Ranatunga *et al.*, 2001). RAD52 proteins bind ssDNA and dsDNA in a sequence-independent manner and stimulate annealing of complementary ssDNA *in vitro* (Mortensen *et al.*, 1996 ; Reddy *et al.*, 1997 ; Shinohara *et al.*, 1998 ; Sugiyama *et al.*, 1998). RAD52 is also able to interact directly with the Replicative Protein A (RPA) (Hays *et al.*, 1998 ; Sugiyama *et al.*, 1998 ; Jackson *et al.*, 2002 ; van den Bosch *et al.*, 2002). It accumulates at DNA damage sites, forms discrete nuclear foci and co-localizes with RAD51 *in vivo* (Liu *et al.*, 1999 ; Liu & Maizels, 2000 ; Lisby *et al.*, 2001 ; Essers *et al.*, 2002).

Budding yeast Rad52 plays an essential role in DSB repair and HR with two main functions which both use RPA-coated ssDNA as a substrate: it facilitates the Rad51 NPF formation in the presence of RPA and promotes DNA annealing (Sung, 1997a ; New *et al.*, 1998 ; Shinohara *et al.*, 1998 ; Sugiyama *et al.*, 1998 ; Sugiyama & Kowalczykowski, 2002) (**Table 1**). Rad52 mediator activity is crucial to overcome the inhibitory action of RPA (when RPA is added prior to Rad51). Rad51 recruitment is dependent on Rad52 during both mitotic and meiotic DSB repair (Gasior *et al.*, 1998 ; Sugawara *et al.*, 2003 ; Wolner *et al.*, 2003 ; Fung *et al.*, 2009). Yeast Rad52 is able to interact directly with Rad51 (Shinohara *et al.*, 1992 ; Milne & Weaver, 1993 ; van den Bosch *et al.*, 2002) and both recruitment and nucleation of Rad51 onto RPA-coated DNA requires Rad51-Rad52 interaction (Shinohara *et al.*, 1998 ; Krejci *et al.*, 2002). Moreover, the strand-annealing activity of Rad52 facilitates the second-end capture leading the formation of a HJ during SDSA pathway (Wu *et al.*, 2008 ; Nimonkar *et al.*, 2009). In addition to its essential role in HR, yeast Rad52 has also been implicated in RAD51-independent events, such as BIR (McEachern & Haber, 2006) and SSA (Sugawara & Haber, 1992), where its DNA annealing activity is required for annealing of the two tandemly repeated ssDNA sequences covered by RPA. *Schizosaccharomyces pombe* possess two homologues of *S. cerevisiae* Rad52, Rad22A⁺ and Rad22B⁺, which share with Rad52 similar functions in HR and SSA (Ostermann *et al.*, 1993 ; Kim *et al.*, 2000 ; van den Bosch *et al.*, 2001) (**Table 1**). While Rad22A⁺ plays a more important role during vegetative growth (van den Bosch *et al.*, 2001), Rad22B⁺ would essentially act during meiosis (Octobre *et al.*, 2008).

Rad59 was identified in *S. cerevisiae* as a Rad52 protein homologue (Bai & Symington, 1996), but surprisingly, no Rad59 homologue has been found in fission yeast. It shares sequence similarity with the conserved N-terminal domain of Rad52 protein but lacks the C-terminal Rad51-interacting domain (Feng *et al.*, 2007). Rad52 and Rad59 bind ssDNA and can mediate annealing of complementary ssDNA, but only Rad52 interacts with Rad51

and RPA (Mortensen *et al.*, 1996 ; Petukhova *et al.*, 1999a ; Davis & Symington, 2001 ; Wu *et al.*, 2006). *rad59* displays subtle epistasis interactions with *rad52* in a variety of DSB repair events, suggesting that the two proteins cooperate in multiple contexts (Feng *et al.*, 2007 ; Manthey & Bailis, 2010). Mutation of Rad59 can in addition be partially complemented by overexpression of Rad52, suggesting that the functions of both proteins overlap (Bai & Symington, 1996). Thus, Rad59 possesses distinct Rad52-dependent and -independent functions (Pannunzio *et al.*, 2012). Rad59 and Rad52 interact *in vivo* (Davis & Symington, 2001) and Rad59 plays a critical role in localization of Rad52 to DSB (Pannunzio *et al.*, 2012). Unlike yeast Rad52, Rad59 is dispensable in Rad51-dependent HR pathways but is a crucial factor of the RAD51-independent SSA pathway (Bai & Symington, 1996 ; Jablonovich *et al.*, 1999 ; Petukhova *et al.*, 1999a ; Sugawara *et al.*, 2000 ; Davis & Symington, 2001).

Although Rad52 plays an essential role in HR in budding yeast, it plays a more modest role in vertebrates, where additional proteins like BRCA2 have evolved to perform similar functions. Indeed, RAD52 knockout in mouse embryonic cells and in chicken DT40 cells does not cause an apparent sensitivity to IR or DSB-causing chemical agents (Rijkers *et al.*, 1998 ; Yamaguchi-Iwai *et al.*, 1998). Despite structural and some biochemical similarity between human and yeast RAD52, the human protein appears functionally similar to the yeast Rad59 protein. Human RAD52 does not appear to have a recombination mediator activity in reconstituted biochemical assays (Jensen *et al.*, 2010), while it conserves a strand annealing activity (Van Dyck *et al.*, 2001 ; Singleton *et al.*, 2002). A recent report has shown that human RAD52 acts in parallel to BRCA2 and that its inactivation is lethal in BRCA2 deficient cells (Feng *et al.*, 2011). These observations argue for a model in which RAD52 functions in an alternative pathway to BRCA2 (Liu & Heyer, 2011) which is suggested to support the proliferation of human tumour cells deficient in BRCA2 *via* mediation of RAD51

functions (Feng *et al.*, 2011). RAD52 however also has a function in the late stages of DSB repair at stalled or collapsed replication forks that does not appear to be shared by BRCA2 (Fujimori *et al.*, 2001). The inviability and extensive chromosomal breaks of *rad52 xrcc3* double knockout DT40 cells implies that RAD52 might function as a recombination mediator in conjunction with one or a combination of the RAD51 paralogues (Fujimori *et al.*, 2001 ; Liu & Heyer, 2011).

The question of whether plants possess a RAD52 homologue has long left open (Iyer *et al.*, 2002 ; Mortensen *et al.*, 2009). In 2011, Samach *et al.* reported that RAD52 homologues are present in all plants whose genomes have undergone extensive sequencing. The RAD52 gene apparently underwent very early duplication, followed by later duplication in specific lineages, during the evolution of higher plants (Samach *et al.*, 2011). Plant RAD52 proteins share high sequence similarity to the known RAD52 proteins (Samach *et al.*, 2011), suggesting similar function for these two families. Two RAD52 homologues have been identified in *Arabidopsis thaliana*: RAD52-1 and RAD52-2. Mutants or RNA interference lines defective in the expression of one or both of these showed mildly reduced fertility, sensitivity to MMC and decreased levels of intrachromosomal recombination compared to the wild type (Samach *et al.*, 2011). In addition, Arabidopsis RAD52-1A partially complemented the yeast *rad52* mutant. These functional assays provide evidence for the role of Arabidopsis RAD52 in DNA recombination and repair and suggest conserved functions of RAD52 in eukaryotes. Given the presence of BRCA2 (see next section) and the moderate effect of plant *rad52* mutants on HR, the plant RAD52 genes appear to more closely resemble human RAD52 (Samach *et al.*, 2011). Further work will however be required to identify the mechanisms through which the diverse RAD52 proteins affect DNA recombination in plants and animals.

BRCA1 AND BRCA2

Despite their absence in budding and fission yeasts, the BRCA1 and BRCA2 proteins appear relatively well conserved in eukaryotes (Lo *et al.*, 2003 ; Trapp *et al.*, 2011 ; Lok & Powell, 2012) (**Table 4**). However, no orthologue of BRCA1 has yet been identified in flies and zebrafish (Meetei *et al.*, 2004 ; Howe *et al.*, 2013). These two proteins are encoded by tumour suppressor genes in mammals, with hereditary mutations in the *brca1* or *brca2* genes being associated with high breast cancer susceptibility and increased risk of ovarian, pancreatic, stomach, laryngeal, fallopian tube and prostate cancer (Roy *et al.*, 2012). Extensive evidence shows that BRCA1 and BRCA2 are key actors in maintaining genome stability, especially by promoting efficient and precise repair of DSB. Many outstanding reviews on human BRCA1 and BRCA2 proteins have been written (Gudmundsdottir & Ashworth, 2006 ; Roy *et al.*, 2012 ; Caestecker & Van de Walle, 2013) and only a brief overview, including more recently described aspects, will be given here. We will take care to include less well-known data from invertebrates and plants.

The BRCA1 protein is an E3 ubiquitin ligase which catalyses protein ubiquitylation *in vitro* (Hashizume *et al.*, 2001 ; Ruffner *et al.*, 2001). BRCA1 has been implicated in interactions with more than 20 proteins (Welsh & King, 2001), including RAD51 (Scully *et al.*, 1997b), RAD50 (Zhong *et al.*, 1999) and BRCA2 (Chen *et al.*, 1998a). *In vivo*, BRCA1 associates and colocalizes with RAD51 in nuclear foci in mitotic cells (Scully *et al.*, 1997b) and these foci also contain BRCA2 and BARD1 (BRCA1-binding protein) (Jin *et al.*, 1997 ; Scully *et al.*, 1997a ; Chen *et al.*, 1998a). Subsequent studies have revealed that BRCA1 is required for RAD51 foci formation in mouse cells (Bhattacharyya *et al.*, 2000). Most homozygous *brca1* mutations lead to embryonic lethality in mice (Hakem *et al.*, 1996 ; Deng & Scott, 2000 ; Evers & Jonkers, 2006), clearly showing the biological importance of this protein. In *C. elegans*, inactivation of BRC-1 (the nematode BRCA1 orthologue) results in

high incidence of males due to nondisjunction of X chromosomes and elevated levels of germ cell death (Boulton *et al.*, 2004). In addition to high sensitivity to IR and chromosomal instability (Shen *et al.*, 1998 ; Xu *et al.*, 1999), *brca1* mouse embryonic stem cells exhibit impaired DSB repair by HR (Moynahan *et al.*, 1999 ; Moynahan *et al.*, 2001). As DSB repair by SSA is also reduced in *brca1* deficient cells, BRCA1 may act before the branch point of HR and SSA (Stark *et al.*, 2004). In accordance with this, BRCA1 promotes CtlP-mediated 5'-end resection of DSB in human cells (Yun & Hiom, 2009). BRCA1 does not seem to be involved in End Joining recombination pathways, although data on this subject are conflicting (Moynahan *et al.*, 1999 ; Baldeyron *et al.*, 2002 ; Zhong *et al.*, 2002 ; Stark *et al.*, 2004 ; Wang *et al.*, 2006 ; Zhuang *et al.*, 2006 ; Yun & Hiom, 2009). In addition to its role in DSB repair, BRCA1 has also been connected to various cellular processes in response to DNA damage in animal cells, such as DNA damage signalling, chromatin remodelling and checkpoint control. Roles for human BRCA1 in the regulation of telomere length (Ballal *et al.*, 2009) and in meiotic sex chromosome inactivation during spermatogenesis (Turner, 2007) have also been described. It is however not excluded that the action of BRCA1 in these processes is indirect. For more details concerning these other functions, see Table 4 and specific reviews (Gudmundsdottir & Ashworth, 2006 ; Roy *et al.*, 2012 ; Caestecker & Van de Walle, 2013).

Putative homologues of BRCA1 have been identified in almost all sequenced plant genomes (Trapp *et al.*, 2011). Plant BRCA1 differs from animal BRCA1 by the presence of the PHD domain (plant homeodomain). PHD domains were previously shown to bind lysine 4 trimethylated histone H3 (H3K4me3), which can act as a dynamic signal for transcriptionally active genes and mark initiation sites for meiotic recombination (Santos-Rosa *et al.*, 2002 ; Borde *et al.*, 2009). Although an exciting possibility, such a role of the plant BRCA1 PHD domain has not however been tested to date. Arabidopsis BRCA1 shows an overall identity of

approximately 20% at the amino acid level with its human counterpart and the BRCT and RING domains are conserved (Lafarge & Montané, 2003). It is thus likely that this RING domain also exhibits E3-ligase activity in Arabidopsis. Interestingly, homozygous *brca1* Arabidopsis plants show no developmental abnormalities (Lafarge & Montané, 2003 ; Reidt *et al.*, 2006). Similarly, BRCA1 is not essential for meiosis as the amount of viable seeds and pollen are similar in mutants and wild-type plants (Reidt *et al.*, 2006), although possible minor functions may exist. Arabidopsis *brca1* mutants do however have defects in HR, especially prominent after induction of DSB by bleomycin (Block-Schmidt *et al.*, 2011). Also, Reidt *et al.* (2006) have shown that BRCA1 is involved in DNA crosslink repair, in accordance with data in human (Westermarck *et al.*, 2003). Taken together, these results indicate that animal and plant BRCA1 are required for efficient DSB repair by HR in somatic cells and the existence of plant-specific domain could suggest more extensive roles in plants.

BRCA2 was first identified in a screen for high-penetrance germline mutations associated with breast cancer susceptibility (Wooster *et al.*, 1995). Orthologues of BRCA2 have subsequently been identified in various organisms, including mouse, chicken, zebrafish, *Drosophila*, *C. elegans* and some plant species (**Table 4**) (Sharan & Bradley, 1997 ; Warren *et al.*, 2003 ; Siaud *et al.*, 2004 ; Martin *et al.*, 2005 ; Titus *et al.*, 2006 ; Klovstad *et al.*, 2008). Comparison of BRCA1 and BRCA2 proteins indicates that they are unrelated proteins (for a review, see Powell & Kachnic, 2003). Indeed, while BRCA1 is characterized by the BRCT motif and the RING domain (with E3 ubiquitin ligase structure), BRCA2 possess a ssDNA binding domain and several BRC peptide motifs (crucial for RAD51 binding). *In vivo* BRCA2-RAD51 interaction have been reported in all analysed animals (Wong *et al.*, 1997 ; Chen *et al.*, 1998b ; Martin *et al.*, 2005 ; Brough *et al.*, 2008). Further analyses precise that one human BRCA2 molecule can bind 6 molecules of RAD51 (Liu *et al.*, 2010). BRCA2 seems to be required for the correct localization of RAD51 and DMC1 recombinases to DSB

in mice (Sharan *et al.*, 2004). According to this, numerous studies have demonstrated an early role of BRCA2 in HR by promoting the assembly of RAD51 (and probably DMC1) onto RPA-coated ssDNA and stabilization of RAD51 NPF (Xia *et al.*, 2006 ; Petalcorin *et al.*, 2007 ; Jensen *et al.*, 2010 ; Liu *et al.*, 2010 ; Thorslund *et al.*, 2010). A recent study revealed that human BRCA2 is also a key regulator of the extension step after strand invasion at replication-dependent DSB (Buisson *et al.*, 2014), therefore BRCA2 would be involved not only in stimulation of RAD51 activities but also in subsequent DNA synthesis. Consistent with its key mediator role in the core mechanism of HR, inactivation of *brca2* in mice results in an early embryonic lethality associated with chromosomal instability (Hakem *et al.*, 1998 ; Evers & Jonkers, 2006).

In addition to its interaction with DMC1, numerous data support a role of BRCA2 in meiotic recombination: it forms foci associated with meiotic chromosomes during synaptonemal complex formation (Chen *et al.*, 1998a), it is involved in ovarian development and tumorigenesis in zebrafish reproductive tissues (Shive *et al.*, 2010) and the expression of a truncated BRCA2 in mouse leads to a failure in spermatogenesis (Connor *et al.*, 1997). In addition, Ko *et al.* have shown that BRC-2, the BRCA2 homologue in *C. elegans*, plays an essential role in chromosome integrity of germ cells and is crucial in meiotic progression (Ko *et al.*, 2008). *Drosophila* BRCA2 has also been connected to efficient activation of the meiotic recombination checkpoint (Klovstad *et al.*, 2008). The potential function of BRCA2 in SSA is not clear: it seems to suppress this pathway in mouse (Stark *et al.*, 2004) while CeBRC-2 promotes the SSA pathway in nematode cells (Petalcorin *et al.*, 2006). One possibility is that CeBRC-2 may have replaced the role of vertebrate RAD52 (missing from *C. elegans*) in SSA.

Two BRCA2 homologues have been identified in the plant *Arabidopsis thaliana*: BRCA2A (or BRCA2(IV), localised in the subtelomeric region of chromosome IV) and BRCA2B (BRCA2(V), on chromosome V) (Siaud *et al.*, 2004). *Arabidopsis* is the only

species reported to have two homologues of the BRCA2 gene. Although much research has been conducted on Arabidopsis BRCA2 proteins, no data are available on the BRCA2 homologues in other plants. Like mammalian BRCA2, both Arabidopsis BRCA2 proteins are able to interact with AtRAD51 and AtDMC1 in yeast two-hybrid assays, suggesting a conserved role of plant BRCA2 in mitosis and meiosis (Siaud *et al.*, 2004). Silencing of the BRCA2 genes specifically in meiosis (to prevent potential somatic phenotypes) results in a partial sterility phenotype, associated with dramatic meiotic alterations including the absence of pairing, synapsis and bivalent formation, defects in anaphase I and II and chromosome fragmentation (Siaud *et al.*, 2004). This *brca2* mutant phenotype is suppressed in a *spo11-1* background (Siaud *et al.*, 2004), demonstrating that BRCA2 function is dependent upon meiotic DSB. More recently, Seeliger *et al.* (2012) have generated simple and double *brca2* mutant plants and showed that they are viable throughout somatic growth and that only the double mutant exhibits sterility phenotype. Therefore, it appears that the role of the two Arabidopsis BRCA2 proteins in meiosis is redundant. The double mutants also showed hypersensitivity to MMC and a dramatic reduction in somatic HR frequency, especially after DSB induction (Seeliger *et al.*, 2012). Taken together, these results demonstrate an important role of BRCA2 in both somatic and meiotic RAD51/DMC1-dependent HR in plants. In addition to evolutionarily conserved functions in DNA repair and recombination, recent research has surprisingly revealed a function of Arabidopsis BRCA2 in plant defense mechanisms. BRCA2A, but not BRCA2B, specifically regulates pathogenesis-related gene transcription in response to salicylic acid signalling during plant immune responses (Wang *et al.*, 2010). This discovery challenges the idea that BRCA2 only a specific role in mediating HR - like BRCA1, Arabidopsis BRCA2 appears to be involved in various cellular processes.

The normal vegetative development of plants carrying homozygous *brca1* or *brca2* mutations (in comparison to the death at a very early developmental stage observed in

mammals) highlights the spectacular resistance of plant development to DNA damage in, the absence of key DNA metabolism proteins. Thanks to this capacity, plants offer a chance to study the function of these proteins during the complete life cycle of a multicellular eukaryote.

RAD54 AND RAD54B

RAD54 is a highly conserved gene: RAD54 homologues have been identified in most eukaryotes including human, mice, chicken, zebrafish, *Drosophila*, *Arabidopsis* and yeast (**Table 1**) (Kanaar *et al.*, 1996 ; Muris *et al.*, 1996 ; Bezzubova *et al.*, 1997 ; Kooistra *et al.*, 1997 ; Thoma *et al.*, 2005 ; Shaked *et al.*, 2006). The *Drosophila* homologue of RAD54 is termed *okra* (Ghabrial *et al.*, 1998). RAD54 is a member of the SWI2/SNF2 protein family and exhibits, as do the other members of this family, a dsDNA-dependent ATPase, DNA translocase, DNA supercoiling and chromatin remodeling activities (for reviews, see Heyer *et al.*, 2006 ; Mazin *et al.*, 2010 ; Ceballos & Heyer, 2011). RAD54 is an ATP-dependent motor protein that tracks along dsDNA but is unable to catalyse the strand displacement reactions typical of DNA helicases (Flaus *et al.*, 2006). Its ATPase activity requires dsDNA and is not supported by ssDNA (Swagemakers *et al.*, 1998), whereas ATPase activity of classical DNA helicases is stimulated by ssDNA (for a review, see Singleton *et al.*, 2007). Single molecule experiments directly visualized *S. cerevisiae* Rad54 translocation on dsDNA, demonstrating highly processive movement at 300 bp/second (Amitani *et al.*, 2006). dsDNA tracking by RAD54 is associated with topological changes in the DNA, forming positive and negative supercoils in topologically constrained DNA molecules (Tan *et al.*, 1999 ; Van Komen *et al.*, 2000 ; Ristic *et al.*, 2001 ; Jaskelioff *et al.*, 2003). Extensive biochemical studies have demonstrated that the presence of RAD51-DNA filament significantly enhances ATP hydrolysis activity and Rad54-induced topological changes of yeast Rad54 (Mazin *et al.*,

2000 ; Van Komen *et al.*, 2000 ; Kiianitsa *et al.*, 2002), suggesting that Rad54 functions in concert with RAD51 *in vivo*.

Similar to *rad51* mutants, *S. cerevisiae rad54* mutants are extremely sensitive to ionizing radiation but also to cross-linking (MMC, cisplatinium) or alkylating agents (MMS), and other agents that are able of causing DSB (Krogh & Symington, 2004). Yeast *rad54*-deficient cells are defective in intra- and inter-chromosomal gene conversion (Shinohara *et al.*, 1997b). In mice, inactivation of RAD54 is viable (in contrast to the *rad51* mutant) but animals exhibit an hypersensitivity to DNA cross-linking agents at all development stages and to IR only at embryonic stages, due to rescue by End-Joining repair in adult stage (Essers *et al.*, 1997 ; De Silva *et al.*, 2000 ; Essers *et al.*, 2000 ; Essers *et al.*, 2002). The impact of absence of RAD54 on fertility differs depending on the organism studied: *rad54* mutant mice and Arabidopsis are fertile (Essers *et al.*, 1997 ; Osakabe *et al.*, 2006) and *rad54* yeasts are able to produce some viable spores (Muris *et al.*, 1997), while *rad54* Drosophila females are sterile (Kooistra *et al.*, 1997) (**Table 1**). Taken together, these data suggest that RAD54 is involved in DSB repair, but does not play an essential role in meiosis, at least in mammals, plants and yeasts. In mammalian cells, RAD54 proteins accumulate at DNA damage sites and colocalize with RAD51 foci following IR-treatment (Tan *et al.*, 1999 ; Essers *et al.*, 2002). Although RAD54 focus formation is dependent on Rad51, cytological studies in yeast, chicken DT40 cells and mouse ES cells suggest that RAD54 is not required for the formation of Rad51 foci, indicating that Rad54 acts downstream of Rad51 (Shinohara *et al.*, 2000 ; Takata *et al.*, 2000 ; Lisby *et al.*, 2004 ; Miyazaki *et al.*, 2004 ; Van Veelen *et al.*, 2005).

Many studies, conducted primarily in mammals and yeast, have demonstrated that RAD54 acts at all stages of homologous recombination, although some of its roles are not yet well understood. *In vitro* assays have showed that RAD54 facilitates RAD51 binding to ssDNA in the presence of RPA and stabilizes the RAD51 NPF without requiring RAD54

ATPase activity (Mazin *et al.*, 2003 ; Wolner *et al.*, 2003). However, the significance of these biochemical studies is unclear because Rad51 focus formation in response to DSB does not seem to be dependent on Rad54 (Shinohara *et al.*, 2000 ; Takata *et al.*, 2000 ; Lisby *et al.*, 2004 ; Miyazaki *et al.*, 2004 ; Van Veelen *et al.*, 2005). RAD54 is involved in stimulation of RAD51-mediated three-strand exchange activity and D-loop formation. This RAD54 function was first demonstrated with recombinant yeast proteins (Petukhova *et al.*, 1998) and then extended to all the examined systems (**Table 1**) (Alexiadis & Kadonaga, 2002 ; Sigurdsson *et al.*, 2002 ; Mazina & Mazin, 2004). The stimulation depends on the ATPase activity of RAD54 and required species-specific protein interactions between the RAD51 and RAD54 proteins (Jiang *et al.*, 1996 ; Clever *et al.*, 1997 ; Golub *et al.*, 1997 ; Petukhova *et al.*, 1998 ; Petukhova *et al.*, 1999b). More precisely, thanks to its chromatin remodeling activity, RAD54 induces changes in DNA topology, which facilitates accessibility for strand exchange and RAD51-dependent homologous DNA pairing (Petukhova *et al.*, 1999b ; Zhang *et al.*, 2007). Conversely, RAD51 improves ATP hydrolysis and translocation ability of RAD54 on dsDNA (Van Komen *et al.*, 2000 ; Mazina & Mazin, 2004). Biochemical studies have revealed that RAD54 is able to dissociate RAD51 from dsDNA in an ATP-dependent manner (Solinger *et al.*, 2002 ; Li *et al.*, 2007). This action is believed to prevent the nonspecific association of RAD51 with bulk chromatin and to provide DNA polymerase access to the 3'-OH primer terminus to initiate the DNA synthesis reaction in the nascent D-loop (Heyer *et al.*, 2006). In accordance to this, Rad51 foci fail to disappear in yeast *rad54* mutants (Miyazaki *et al.*, 2004). In addition, RAD54 mediates the ATP hydrolysis-driven branch migration of Holliday junctions (Bugreev *et al.*, 2006), which is consistent with its high affinity for branched DNA molecules (Mazina *et al.*, 2007). Finally, RAD54 stimulates resolution of Holliday junctions *via* physical interactions with structure specific resolvase MUS81-EME1 (Mus81-Mms4 in yeast) (Mazina & Mazin, 2008 ; Matulova *et al.*, 2009). RAD54-mediated enhancement of

HJ-specific resolvase function is evolutionarily conserved, although some differences exist. For example, this nuclease enhancement does not require ATP binding nor its hydrolysis by RAD54 in budding yeast (Matulova *et al.*, 2009) while this activity is ATP-dependent in human (Mazina & Mazin, 2008).

Relatively little information is available regarding RAD54 in the plant kingdom. Osakabe *et al.* (2006) have identified and characterized the RAD54 orthologue in *Arabidopsis thaliana*. AtRAD54 is expressed in all tissues examined and its expression can be induced by γ -irradiation (Osakabe *et al.*, 2006). *Arabidopsis rad54* mutants are viable and fertile, but exhibit increased sensitivity to γ -irradiation and the cross-linking reagent cisplatin (Osakabe *et al.*, 2006). Two-hybrid experiments revealed that AtRAD54 interacts physically with AtRAD51, as well as interactions between alien proteins (i.e. yeast Rad54 with AtRAD51 and yeastRad51 with AtRAD54) (Osakabe *et al.*, 2006 ; Klutstein *et al.*, 2008). In addition, AtRAD54 is able to complement some DNA repair deficiencies of yeast *rad54* mutant cells (Klutstein *et al.*, 2008), suggesting that RAD54 functions are conserved across species. Subsequent analyses in *Arabidopsis* have shown that AtRAD54 is extremely important in SDSA, but not required in SSA recombination (Roth *et al.*, 2012) and finally, a recent study has demonstrated that AtRAD54 is also involved in geminiviral DNA replication (Kaliappan *et al.*, 2012). Further investigations are needed to specify the AtRAD54 functions at molecular level and to expand these observations to other plants.

A RAD54 paralogue has been identified in budding and fission yeasts and in mammals (**Table 1**), but not in plants and invertebrates to date. RAD54B, the mammal RAD54 paralogue, shares similar biochemical activities with RAD54 (Tanaka *et al.*, 2002 ; Wesoly *et al.*, 2006 ; Zhang *et al.*, 2007). It is also a dsDNA-dependent ATPase that translocates on duplex DNA leading to DNA topological changes and transient opening of the DNA strands. Similar to RAD54, RAD54B interacts with RAD51 (although this interaction may be indirect)

(Tanaka *et al.*, 2000) and promotes RAD51-mediated D-loop formation (Wesoly *et al.*, 2006). Conversely, RAD51 also enhances the activities of RAD54B in human (Wesoly *et al.*, 2006). In addition, RAD54B also stimulates the DNA strand-exchange activity of DMC1, the meiosis-specific recombinase (Sehorn *et al.*, 2004 ; Sarai *et al.*, 2006). In mouse embryonic stem cells, ablation of RAD54 leads to a mild reduction of HR efficiency while the absence of RAD54B has very little effect. However, the absence of both proteins dramatically reduces HR efficiency (Wesoly *et al.*, 2006). The involvement of RAD54B in HR is thus revealed in absence of RAD54. Experiments of MMC sensitivity of single and double mutants indicate that RAD54 and RAD54B function synergistically to protect mice from DNA damages (Wesoly *et al.*, 2006).

The RAD54 homologue identified in *S. cerevisiae* and *S. pombe* is called Rdh54 (Rad homologue 54) or Tid1 (two-hybrid interaction with Dmc1) (Dresser *et al.*, 1997 ; Klein, 1997 ; Catlett & Forsburg, 2003). Despite their biochemical similarities, biological functions of yeast Rad54 and Rdh54 exhibit important differences (**Table 1**). Whereas Rad54 is involved in mitotic DSB repair, Rdh54 appears to act in both mitotic and meiotic recombination. In accordance to this, Rad54 and Rdh54 physically and functionally interact with Rad51 (Jiang *et al.*, 1996 ; Clever *et al.*, 1997 ; Petukhova *et al.*, 2000 ; Mazin *et al.*, 2003 ; Raschle *et al.*, 2004 ; Santa Maria *et al.*, 2013) while only Rdh54 has been found to interact with Dmc1 (Dresser *et al.*, 1997 ; Catlett & Forsburg, 2003). Although its role in meiosis is not fully understood, Rdh54 appears to promote colocalization of Rad51 and Dmc1 (Shinohara *et al.*, 2000) and to control sister chromatid cohesion to facilitate interhomologue recombination and chromosome segregation (Shinohara *et al.*, 2000 ; Shinohara *et al.*, 2003 ; Kateneva *et al.*, 2005). Rdh54 is also implicated in Rad51 and Dmc1 dissociation from dsDNA to avoid recombinase association at nonrecombinogenic sites and to facilitate DNA synthesis initiation in the nascent D-loop structure (Chi *et al.*, 2006a ; Holzen *et al.*, 2006 ;

Shah *et al.*, 2010). Thanks to meiotic functions of Rdh54, the yeast *rad54* mutant only exhibits minor defects in meiotic recombination (Klein, 1997). Moreover, Rdh54 seems to be involved in broader processes: it participates in the coordination of some repair pathways of DNA lesions (Latypov *et al.*, 2010) and may facilitate communication between DNA repair and checkpoint control (Lee *et al.*, 2001). These findings indicate that (1) aside from some functional overlap, yeast Rad54 and Rdh54 have independent functions in HR, DSB repair and other processes and that (2) yeast Rdh54 does not appear to be the functional equivalent to mammal RAD54B, based on mutant phenotypes, particularly the meiotic mutant phenotype.

	RPA	RAD52	RAD59	RAD54	RAD54B
<i>H. sapiens</i>	<i>RPA</i> = replication protein A, which is comprised of three subunits of 70, 32 and 14kDa^[1], was first identified as an indispensable component of simian virus 40 DNA replication^[2] • forms nuclear foci which colocalize with RAD51 foci after γ-irradiation^[3,4], is present during meiosis throughout prophase I until mid-pachytene^[5] • interacts with RAD51^[6],^[3], with BRCA1 and BRCA2^[7,8], with XPF-ERCC1^[9] and with WRN and BLM helicases^[10-12] • binds to ssDNA with high affinity^[2,13,14], and in a sequential binding manner with a 5' to 3' polarity^[15] • is activated by phosphorylation at the G1- to S-phase transition, and dephosphorylation occurs at mitosis^[16] • functions in DNA replication^[2,13,17,18], nucleotide excision repair^[19] and homologous recombination^[20] Replication: its phosphorylation is a critical early step for full ATR activation in response to replication stress and subsequent replication checkpoint arrest^[21], UV-induced hyperphosphorylation of RPA depends on DNA replication and expression of ATM protein^[22] NER: plays a role in DNA damage recognition^[23,24] and in recruiting and positioning of XPG and ERCC1-XPF endonucleases to the lesion site for incision reactions^[15,25], promotes junction cutting by XPF-ERCC1 and XPG nucleases of "loop" substrates^[25], participates in the gap-filling reaction^[26] BER: stimulates the long-patch BER^[27] HR: is required for the recruitment of ATR to sites of DNA damage and for ATR-mediated Chk1 activation^[28], modulates RAD51 activity by physical interaction^[3,6], stimulates the strand transfer activity of RAD51^[29], stimulates WRN and BLM helicase-catalyzed DNA unwinding^[10-12]	• expression: low in G0/G1, increased in S and maximum in G2/M^[30], expression not induced by γ-irradiation^[30] • directly interacts with RPA^[31] and RAD51^[32] • preferentially binds ssDNA^[33,34] • has strand-annealing activity <i>in vitro</i>^[35-37] and strand exchange activity^[34] • does not show recombination mediator activity in reconstituted biochemical assays^[38] but mediates RAD51 function in human cancer cells deficient in BRCA1, PALB2^[39], or BRCA2^[40], mediates second end capture via its strand annealing activity^[41], overexpression of RAD52 enhances spontaneous HR but reduces I-SceI-induced HR^[42]		• accumulates as foci at sites of DNA damage in the nucleus after IR-treatment^[43], focus formation is impaired in <i>xrcc2</i>, <i>xrcc3</i>, <i>rad51b</i>, <i>rad51c</i>, <i>rad51d</i> and <i>brca2</i> mutant cells but is RAD52-independent^[44] • interacts <i>in vivo</i> and <i>in vitro</i> with RAD51 and this association is induced by IR^[45], interacts with and stimulates DNA cleavage activity of MUS81-EME1^[46] • dsDNA-dependent ATPase^[47] which translocates along the helix DNA (ATP-dependent manner)^[48] • binds ssDNA and dsDNA with similar affinities, but binding to ssDNA does not stimulate its ATP hydrolysis^[49], has a higher binding affinity for branched DNA structures^[50] • induces a change in the topology of the DNA by binding to dsDNA^[51] which facilitates homologous DNA pairing by the RAD51 protein directly^[48], is recruited by RAD51-ssDNA filament to the chromatin of the intact chromosome and that it remodels that chromatin to facilitate accessibility for strand exchange^[52], binds Holliday junctions and drives their branch migration^[53]	<i>RAD54</i> homologue, more closely related to <i>ScRdh54/Tid1</i> than to <i>ScRad54</i>^[54-56] • forms nuclear foci that colocalize with RAD51, RAD54, and BRCA1^[54,55] • constitutively interacts with RAD51 but this interaction may be indirect^[54], interacts with DMC1^[57,58] • shares similar biochemical activities with RAD54^[59], is a DNA-binding protein and hydrolyzes ATP in the presence of dsDNA^[55] • seems to play a unique role in homologous recombination^[56], stimulates the DNA strand-exchange activity of DMC1 by stabilizing the DMC1-ssDNA complex^[57,58]
<i>M. musculus</i>	• forms foci during prophase I^[60-62] and colocalizes with RAD51 on newly synapsed axes formed during meiotic prophase^[61], accumulates during late leptotene/early zygotene and persists through to pachytene, before dissociating from the chromatin at around the time CO-designated recombination intermediates^[63] • <i>rpa1</i> heterozygote: accumulation of lymphoid tumours and early embryonic lethality of the litters^[64]	• <i>rad52</i> mutant is viable^[65] • role in HR is uncertain but mutant has decreased gene targeting^[65], expression of two splice variants from the RAD52 gene (normally expressed in adult mouse tissues) in tissue culture cells elevates the frequency of recombination that uses a sister chromatid template^[66]		• expression elevated in organs of germ cell and lymphoid development, expression correlated with the meiotic phase of spermatogenesis^[67], colocalizes with RAD51 foci following IR^[51] • adult <i>rad54</i> mice: hypersensitive to MMC^[68] but resistant to IR^[69], <i>rad54</i> embryonic stem cells: sensitive to IR, MMC, and MMS, but not to UV light, reduction of gene targeting^[70], <i>rad54 rad54b</i> double KO: stronger defects on HR^[71] • RAD54 affects DNA DSB repair and sister chromatid exchange^[72], RAD54 (but not RAD54B) is needed for a normal distribution of RAD51 on meiotic chromosomes^[71], plays a minor role in meiotic recombination as well, mutants are fertile^[70], role in telomere length maintenance in mammals^[73]	• <i>rad54b</i> mice viable with no macroscopic abnormalities up to at least 6 months of age^[71], <i>rad54b</i> cells: modest to no HR defects, but <i>rad54 rad54b</i> cells have stronger defects^[71] • contributes to repair of IR and MMC-induced DNA damage, involvement of RAD54B in homologous recombination was revealed in absence of RAD54 so functions synergistically with RAD54 to protect mice from the deleterious effects of MMC^[71], Rad54B-mediated HR is not essential for somatic hypermutation^[74]

Table 1: RPA, RAD52, RAD59, RAD54 and RAD54B proteins in eukaryotes (1/4)

	RPA	RAD52	RAD59	RAD54	RAD54B
<i>C. griseus</i>	interacts with XPF and this interaction contributes to <u>efficient NER</u> ^[74]	expression: low in G0/G1, increased in S and reached a maximum in G2/M and this increase does not require DNA-PK ^[30]			
<i>G. gallus</i>		- high abundance of the mRNA in testis ^[75] <i>rad52</i> mutant cells: no hypersensitive to γ-irradiation, MMS, or cisplatin ^[76, 77], reduction of targeted integration frequency ^[76], <i>rad52-xrcc3</i> double knockdown cells: non viable and exhibit severe HR defects ^[78] - RAD52 may play a role in meiotic recombination ^[75]		<i>rad54</i> mutant: X-Ray sensitive, reduced IG gene conversion rate and targeted integration ^[79], spontaneous chromosome aberrations ^[80, 81] and cell death ^[80], RAD54 deficiency is associated with a loss of the S phase dependent radioresistance to killing ^[77] - RAD54 is not required for IR-induced DNA DSB repair ^[77]	
<i>D. rerio</i>	<i>High. similarity to human RPA (identical, 55%; conservative, 70%)</i> ^[82] - is ubiquitously expressed in embryo, liver, ovary, intestine and in cultured fibroblast cell ^[82] - inhibits annealing of oligonucleotides <i>in vitro</i> ^[83]	- Loss of function of RAD52 impairs the SSA pathway and results in abnormal embryo development ^[84] - facilitates annealing of oligonucleotides in the presence of RPA <i>in vitro</i> ^[83]		belongs to SWI2/SNF2P family ^[58], uses a mechanism analogous to helicases to translocate on dsDNA ^[85]	
<i>D. melanogaster</i>	- binds ssDNA very tightly ^[86] - stimulates the extent and processivity of DNA synthesis of the DNA polymerase alpha ^[86, 87]			Okra <i>drosophila</i> homolog of RAD54 ^[88] <i>rad54</i> deficient flies: normal development, but the females are sterile ^[89] - is involved in the repair of radiation damages and recombination ^[89], chromatin remodeling and homologous strand pairing ^[90, 91], required for both mitotic DNA repair and meiotic recombination ^[88]	
<i>C. elegans</i>	- forms nuclear foci in response to IR, dependent on WRN and MRE11 ^[92] but independently of ATM ^[93] - physically interacts with WRN, a member of the RecQ helicase family ^[94] - binds ssDNA ^[94] - stimulates WRN helicase activity ^[94], stimulates both unwinding and endonuclease activities of CeDNA2, an helicase/endonuclease which remove RNA-DNA primers of Okazaki fragments during DNA replication ^[95]				
<i>A. thaliana</i>	<i>Arabidopsis</i> has multiple genes for most RPA subunits ^[96, 97], two RPA complexes depending of RPA-70kDa subunit: RPA70a and RPA70b - is present during prophase I of meiosis ^[98] <i>rpa70a</i> mutation: lethal, <i>rpa70b</i> mutation: morphologically normal, hypersensitive to UV-B and MMS ^[99] - complex containing RPA70a is essential for <u>DNA replication</u>, complex containing RPA70b may function mostly in <u>DNA repair</u> ^[99], is not required for the meiotic DSB repair, but plays an essential role at <u>later stages in the meiotic recombination pathway</u> that is required for the formation of class I COs ^[98]	RAD52-1 & RAD52-2 <i>the 2 genes encode four ORFs through differential splicing</i> - are localized in all DNA-containing compartments (nucleus, mitochondria, or chloroplast) ^[104] <i>rad52-1</i> or <i>rad52-2</i> mutant: reduced fertility and mild MMC hypersensitivity ^[104]		- is expressed in all tissues examined, with the highest levels of expression in flower buds, expression induced by γ-irradiation ^[100] - physically interacts with RAD51 ^[100] <i>rad54</i> mutant: viable and fertile ^[100] - predicted to be a DNA-stimulated ATPases (SWI2/SNF2 family) ^[101] - not required for SSA, but <u>important for SDSA</u> ^[102], involved in geminiviral DNA replication ^[103]	

Table 1: RPA, RAD52, RAD59, RAD54 and RAD54B proteins in eukaryotes (2/4)

	RPA	RAD52	RAD59	RAD54	Rdh54 / Tid1
<i>S. cerevisiae</i>	<i>S. cerevisiae</i> has a single gene for each subunit of RPA • forms foci in nuclei at meiotic S-phase and in early prophase I which colocalize with Rad51 and Rad52^[105] • is activated by phosphorylation at the G1- to S- phase transition, and dephosphorylation occurs at mitosis^[16] • has higher affinity for ssDNA than Rad51^[106, 107] • functions in the unwinding of the SV40 origin of replication^[108], its presence on ssDNA prevents Rad51 from binding <i>in vitro</i>^[106, 107], arrives at DSB sites prior to Rad51 during the presynaptic phase^[109] and binds to ssDNA to <u>prevent secondary structure formation</u> that could potentially lead to inhibitory effects during Rad51 nucleoprotein filament formation^[106], during the isoenergetic strand exchange phase RPA ensures unidirectional heteroduplex extension by binding to the displaced ssDNA^[110, 111], has a crucial role in <u>meiosis</u>^[112], is required for the G1/S and intra-S DNA damage checkpoints^[113, 114], binds to regulatory elements in DNA repair and DNA metabolism genes so may be involved in the <u>regulation</u> of a number of DNA repair and DNA metabolism <u>genes</u>^[115]	• expressed throughout the cell cycle and is induced by DNA-damaging agents and during meiosis^[116, 117], is nuclear and forms discrete foci in response to IR and during S phase of unirradiated cells^[118] • interacts directly with RPA^[119] RAD51^[120] and Rad59 <i>in vivo</i>^[121, 122] • binds ssDNA^[123] and can anneal DNA strands that are either bare or coated with RPA^[124-126] • involved in <u>Rad51 recruitment to DNA</u>^[127] and Rad51 nucleofilament formation, mediates the <u>displacement of RPA from ssDNA</u>, mediates Rad51 strand invasion by physically associating with the Rad51 protein^[120] and allowing for highly efficient removal of RPA^[107], stimulates <u>DNA strand exchange</u> by targeting Rad51 protein to a complex of RPA with ssDNA^[128], assists in capturing the second DNA end and promotes its annealing to the D-loop^[129, 130], promotes post-invasion steps of both crossover and noncrossover pathways of <u>meiotic recombination</u>^[131], is required for <u>SSA</u>^[132] and <u>BIR</u>^[133] and has a critical role in the <u>HDR pathway</u>^[36]	<i>defective for RAD51-independent spontaneous mitotic recombination between inverted repeats</i>^[134] • interacts <i>in vivo</i> with Rad52^[121], can self-associate^[122] • binds ssDNA and dsDNA with a preference for ssDNA • can anneal complementary ssDNA^[121, 135, 136] • <i>rad59</i> mutant: modest defects in several mitotic recombination assays and moderate sensitivity to IR^[137], <i>rad59</i> mutation can be partially complemented by overexpression of RAD52, suggesting that the functions of both proteins overlap^[134] • RAD59 is dispensable in RAD51-<u>dependent homologous recombination pathways</u> but plays an <u>important role in the RAD51-independent repair pathways</u>^[134], such as SSA^[137-139], its requirement in SSA increases as the repeat length decreases^[139], can overcome the inhibitory effect of RPA on annealing of oligonucleotides but can not substitute for Rad52 <i>in vivo</i>, even in RAD51-independent recombination events such as SSA^[121], regulates association of Rad52 with DSB^[140]	<i>Shares 66% similarity and 48% identity with its human homologue</i>^[66, 83] • expression is damage inducible^[116], expression levels increase during the late G1 phase^[117, 141], RAD54 focus formation is dependent of Rad51, Rad52 and Rad55^[142] • interacts with both free Rad51 and the Rad51 ssDNA nucleoprotein filament <i>in vivo</i>^[143] and this interaction is functionally important for recombinational DNA damage repair^[144-146], interacts with Mus81-Mms4^[147] • binds ssDNA and dsDNA with similar affinities, although binding to ssDNA does not stimulate ATP hydrolysis^[46] • motor protein that translocates along dsDNA in an ATP hydrolysis dependent manner^[148] • <i>rad54</i> mutant has increased rates of spontaneous chromosome loss^[149] • stabilizes the Rad51 nucleoprotein filament but its ATPase activity is not required^[143], disassembles rad51:dsDNA filaments^[150], promotes <u>Rad51-dependent homologous DNA pairing</u> via ATP hydrolysis-driven change in DNA double helix conformation^[135], strongly stimulates the <u>Rad51-mediated strand exchange</u> and its ATPase activity is required^[48, 135], acts during the synaptic phase of DNA strand exchange and after the formation of presynaptic Rad51 protein-ssDNA filaments^[151], has an additional role in the postsynaptic phase of DNA strand exchange by <u>stimulating heteroduplex DNA extension</u> of established joint molecules in Rad51/Rpa-mediated DNA strand exchange^[152], cooperates with Mus81-Mms4 nuclease in the resolution of recombination and replication intermediates^[153], plays a relatively minor role in meiotic recombination, due to the presence of a meiosis-specific homologue, Rdh54/Tid1^[154], non required for SSA^[155]	<i>Rdh54: Rad homologue 54</i>^[154] / <i>Tid1: two-hybrid interaction with Dmc1</i>^[156], <i>structural and functional relative of Rad54</i>^[157] • forms foci at DNA damage sites in a Rad51- and Rad52-dependent manner^[142] • Rdh54 physically interacts with Rad51^[158, 159] and Dmc1^[156] • has dsDNA-activated ATPase activity which generates unconstrained negative and positive supercoils in DNA^[158], translocates on dsDNA in an ATP dependent manner and can disrupt joint molecules^[160, 161] • <i>rdh54/tid1</i> mutant: retardation of the repair of meiosis-specific DSBs and delayed formation of physical recombinants^[162], modest delay in elongation of Zip1 structures and a more pronounced delay in Zip1 disappearance, reduction of crossover interference^[163], <i>rad54 rdh54</i> mutant: fails to form viable meiotic progeny and to repair meiotic DSBs^[162] • is involved in a <u>minor pathway of mitotic recombination in the absence of Rad54</u>^[162], promotes <u>Rad51-dependent D-loop formation</u>^[158], promotes colocalization of Rad51 and Dmc1 during meiotic recombination^[157], promotes dissociation of Dmc1 from nonrecombinogenic sites on meiotic chromatin^[164], catalyzes Rad51 removal from dsDNA of nondamage-associated foci^[165, 166], controls resolution of cohesin-dependent linkages during meiosis^[167], is involved in <u>coordination of different DNA repair systems</u> including mutagenic and recombinagenic pathways as well as nucleotide excision repair^[168]

Table 1: RPA, RAD52, RAD59, RAD54 and RAD54B proteins in eukaryotes (3/4)

	spRPA = spSSB	Rad22A+ & Rad22B+	RAD59	Rhp54	Rdh54
<i>S. pombe</i>	<i>spSSB</i> = <i>S. pombe</i> single-stranded DNA-binding protein^[169] = <i>SpRPA</i>^[170] • has an important role in <u>DNA replication, repair and recombination</u> <i>in vivo</i>, is not required for the DNA damage checkpoint but may play an important role in maintaining the signal recognized by the S-phase checkpoint^[171]	<i>ScRad52</i> homologs^[172, 173] • Rad22A+ is localized to sites of DSBs <i>in vivo</i> • Rad22A+ and Rad22B+ physically interact with each other, with Rhp51 and RPA^[174] • <i>rad22A</i>+ mutation: severe sensitivity to X-rays and strong decrease in recombination (13-fold), <i>rad22B</i>+ mutation: no decrease in HR and no change in radiation sensitivity, <i>rad22A</i>–<i>rad22B</i> double mutant: enhancement of radiation sensitivity^[173] • protect dsDNA ends from nuclease digestion, is involved in <u>SSA</u> and <u>HR</u> in vegetative cells^[173, 175], Rad22B+ has an auxiliary role in the repair of DSBs^[173]		• is ubiquitylated and degraded in G1 phase^[176] • <i>rhp54</i> mutant: spontaneous minichromosome loss, increased sensitivity to X-rays and MMS, severe defects in recombination^[177], little or no reduction in meiotic recombination^[174, 178] and produces some viable spores (27%)^[178] • may be involved in the recombinational repair of UV and X-ray damage and plays a role in the processing of replication-specific lesions^[177], acts with Rdh54 to <u>repair meiotic DSBs</u>^[179]	<i>homologous to budding yeast Rdh54/Tid1</i>^[179] • protein present with appropriate timing for a meiosis specific recombination factor^[179] • interacts with Dmc1 and Rhp51^[179] • <i>rdh54</i> mutant: no effect on DNA damage repair during the haploid vegetative cell cycle, decrease of meiotic recombination and spore viability with a concomitant increase in sister chromatid exchange during meiosis^[179] • is important for <u>meiotic recombination</u>^[179]

Table 1: RPA, RAD52, RAD59, RAD54 and RAD54B proteins in eukaryotes (4/4)

	RAD51B (REC2 / RAD51L1)	RAD51D (RAD51L3)	XRCC2	RAD51C (RAD51L2)	XRCC3
H. sapiens	• expression induced by both ionizing radiation^[180] and UV radiation^[181] • physically interacts with RAD51C^[182] • binds to ssDNA, dsDNA, and 3'-tailed dsDNA <i>in vitro</i>^[183] • exhibits protein kinase activity <i>in vitro</i> and can phosphorylate P53, cyclin E, and CDK2^[184] • inhibition of RAD51B induces G2/M cell cycle arrest^[185]	• physically interacts with XRCC2^[186, 187] and RAD51C^[187] • binds to ssDNA, 3'-tailed DNA and dsDNA with different affinities (strongly to ssDNA)^[186] • has a role in telomere-length regulation^[188]	<i>XRCC2 = X-ray repair cross complementing 2</i> • physically interacts with RAD51D^[186, 187] • does not show significant DNA binding^[189] • XRCC2 depletion has no effect on XRCC3 phosphorylation in response to DSB^[190] • enhances the ATP-processing activity of RAD51 by facilitating ADP to ATP exchange though reducing the affinity for ADP^[189], stimulates RAD51 strand exchange^[189]	• accumulates at DNA damage sites concomitantly with the RAD51 recombinase and is retained after RAD51^[191], formation of foci which colocalise with γH2AX^[192] • interaction with RAD51^[192], RAD51D^[187], RAD51B^[182] and XRCC3^[193] • binds to ssDNA, dsDNA, and 3'-tailed dsDNA <i>in vitro</i> with different affinities (strongly to ssDNA)^[183] • has an apparent strand exchange activity perhaps though destabilizing dsDNA^[183] • <i>rad51c</i> mutant cells: hypersensitivity to the DNA-cross-linking agent MMC and moderately increased sensitivity to IR (in S and G2/M phases)^[194], reduction in HJ resolution activity^[195], RAD51C inhibition induced G2/M cell cycle arrest^[185], regulation of ubiquitin-mediated degradation of Rad51^[196] • facilitates checkpoint signaling by promoting CHK2 phosphorylation^[191], is essential for XRCC3 phosphorylation^[190], is required for HJ processing^[197]	<i>XRCC3 = X-ray repair cross complementing 3</i> • level of XRCC3 protein was sharply reduced in <i>rad51c</i> mutant cells^[197], is recruited to DSB early and independently of RAD51^[198] • physically interacts with RAD51^[199] and RAD51C^[193] • inhibition of XRCC3 elicits checkpoint defects^[185] and increases endoreduplication^[200] • is critical for RAD51-mediated recovery of collapsed replication forks^[190], is required for restart of HU-stalled replication forks^[201], participates in the intra-S-phase checkpoint (phosphorylation dependent) and in the G2/M checkpoint (phosphorylation indep.)^[190], is required for the production of extrachromosomal telomeric circles in human alternative lengthening of telomere cells^[202]
	BC complex	DX2 complex		CX3 complex	
	• has ssDNA binding activity^[203] • is capital for RAD51 focus formation^[192], enhances the homologous DNA pairing activity of Rad51^[203], promotes RAD51-catalyzed strand exchange <i>in vitro</i> by suppressing the inhibitory effect of RPA^[203]	• binds both ssDNA and dsDNA, forms a filamentous complex with ssDNA <i>in vitro</i> • has homologous pairing activity <i>in vitro</i>^[204] • stimulates resolution of synthetic HJs via its interaction with the BLM helicase protein^[205]		• interacts with RAD51 <i>in vitro</i>^[192] • binds DNA templates containing replication forks and HJs and forms a multimeric ring structure whose subunits are arranged into a flat disc around a central channel <i>in vitro</i>^[206] • acts downstream of RAD51 recruitment^[207], catalyzes homologous pairing^[204], is involved in HJ processing^[197]	
	BCDX2 complex				
• interacts with RAD51 <i>in vitro</i>^[192] • has a high affinity for branched DNA^[208] and can recognize nicks in DNA^[209], binds DNA templates containing replication forks and HJs and forms a multimeric ring structure whose subunits are arranged into a flat disc around a central channel <i>in vitro</i>^[206] • catalyzes strand-annealing reaction <i>in vitro</i>^[208] • acts downstream of BRCA2 recruitment but upstream of RAD51 recruitment^[207], is responsible for Rad51 recruitment or stabilization at damage sites^[207]					

Table 2 : RAD51 paralogues in eukaryotes (1/3)

	RAD51B	RAD51D	XRCC2	RAD51C	XRCC3
M. musculus	• <i>rad51b</i> mutation: embryonic lethal, but partially rescued in a p53^{-/-} background ^[210] • is essential for cell proliferation ^[210]	• interacts with RAD51C ^[211] • <i>rad51d</i> mutation: embryonic lethal ^[212] • has DNA binding activity <i>in vitro</i> ^[213] • is involved in resistance to ICL agents (ATPase motif needed) ^[211]	• <i>xrcc2</i> mutation: embryonic lethal ^[214] • has low DNA binding activity <i>in vitro</i> ^[213]	• is localized to meiotic chromosomes at pachytene/diplotene ^[215] • <i>rad51c</i> mutation: embryonic lethal ^[216] • has a DNA binding activity <i>in vitro</i> ^[213] • is required for inter-sister chromatid recombinational repair ^[217]	
				CX3 complex	
				• is associated with HJ • may play an essential role in the resolution of recombination intermediates prior to chromosome segregation ^[215]	
C. griseus		• <i>rad51d</i> mutant cells: broadly deficient in RAD51 focus formation in response to various agents, but this defect is not invariably associated with increased sensitivity ^[218]	• <i>irs1</i> mutant cells (defective in XRCC2): sensitivity to diverse DNA damaging agents such as IR, UV, monofunctional alkylating agents, camptothecin and MMC ^[219-221] • regulate the balance between short- and long-tract gene conversions between sister chromatids ^[222]	• contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability ^[223], is involved in maintaining centrosome number in mitosis ^[224]	• <i>irs1SF</i> mutant cells (defective in XRCC3): sensitivity to diverse DNA damaging agents such as IR, UV, monofunctional alkylating agents, camptothecin and MMC ^[219, 221] • is required for assembly of Rad51 complexes <i>in vivo</i> ^[225], promotes homology-directed repair of DNA damage ^[226], acts in late stages of recombination (in formation and resolution of HR intermediates), controls the fidelity of HR ^[227], regulates the balance between short- and long-tract gene conversions between sister chromatids ^[222], modulates replication fork progression on damaged chromosomes ^[228]
G. gallus	• is involved in RAD51 and RAD54 foci formation following IR and cisplatin treatments ^[229], promotes homologous recombinational repair ^[230]	• <i>rad51d</i> mutant cells: spontaneous chromosomal aberrations, high sensitivity to killing by cross-linking agents (MMC and cisplatin), mild sensitivity to gamma rays ^[231] • is involved in RAD51 and RAD54 foci formation following IR and CDDP treatments ^[229]	• <i>xrcc2</i> mutant cells: spontaneous chromosomal aberrations, high sensitivity to killing by cross-linking agents (MMC and cisplatin), mild sensitivity to gamma rays ^[231]	• <i>rad51c</i> mutant cells : spontaneous chromosomal aberrations, high sensitivity to killing by cross-linking agents (MMC and cisplatin), mild sensitivity to gamma rays ^[231]	• <i>xrcc3</i> mutant cells: spontaneous chromosomal aberrations, high sensitivity to killing by cross-linking agents (MMC and cisplatin), mild sensitivity to gamma rays ^[231] • is involved in RAD51 and RAD54 foci formation following IR and CDDP treatments ^[229]
A. thaliana	• is expressed widely and at a higher level in the floral buds than in other tissues ^[232] • <i>rad51b</i> mutant : MMC ^[233] and cisplatin hypersensitive ^[232], meiotic hyper-recombination phenotype ^[234] • is involved in somatic HR but non required for RAD51 foci formation ^[234], is involved in SSA recombination ^[235]	• is expressed widely, but at very low levels ^[236] • is involved in somatic HR but non required for RAD51 foci formation ^[234], is involved in SSA recombination ^[235], is involved in both transcription and recombination during the defense response ^[236]	• <i>xrcc2</i> mutant: meiotic hyper-recombination phenotype ^[234] • is involved in somatic HR but non required for RAD51 foci formation ^[234], is required for SSA recombination ^[235]	• <i>rad51c</i> mutant: meiotic chromosomal fragmentation and sterility, MMC-sensitive ^[233, 237, 238] • not required for SSA ^[102]	• <i>xrcc3</i> mutant: sterile due to Spo11-dependent meiotic chromosome fragmentation ^[239, 240] • mitotic hyporec and MMC hypersensitive ^[239], non required for SSA ^[102]

Table 2 : RAD51 paralogues in eukaryotes (2/3)

<i>D. melanogaster</i>	Dm CG2412	Dm CG6318	SPN-D (spindle-D)	SPN-B (spindle-B)
	CG2412 appears to be an orthologue of RAD51D (30% identity and 53% similarity with hRAD51D) ^[241, 242] • no mutations in CG2412 have been reported ^[243]	CG6318 appears to be an orthologue of XRCC2 (24% identity and 41% similarity with the core region of hXRCC2) ^[241, 242] • is expressed exclusively in the female germline ^[244] • no mutations in CG2412 have been reported ^[243]	SPN-D protein is most similar to RAD51C ^[241, 245] • is expressed exclusively in the female germline ^[244] • physically interacts with SPN-B ^[246] • <i>spn-D</i> mutant has defects in meiotic recombination, but not of repair of DSBs in somatic cells ^[245] • blocks NHEJ during meiosis ^[246]	SPN-B protein is most similar to XRCC3 ^[241, 245] • <i>spn-B</i> mutants: mildly sensitive to IR ^[244], meiotic defects ^[245], somatic defects hard to detect, because few eggs from develop into viable progeny ^[88] • physically interacts with SPN-D ^[246] • blocks NHEJ during meiosis ^[246]
<i>C. elegans</i>	RFS-1			
	Appears to be the sole RAD51 paralog in <i>C. elegans</i>, is most similar to Rad51D ^[247] • interacts with the <i>C. elegans</i> homologs of RAD51 and BRCA2 (CeBRC-2) ^[248, 249] • mutant: sterile, mildly sensitive to IR ^[250] • binds to and promotes the disassembly of RAD51 from dsDNA, but not ssDNA filaments <i>in vitro</i> ^[251] • is required for RAD51 focus formation after ICL treatment but not after IR ^[247], performs a specialized role in promoting HR-mediated repair at lesions that block replication fork progression ^[247], is involved in telomere integrity maintenance ^[250]			
<i>S. cerevisiae</i>	Rad55	Rad57		
	Rad55 appears to be an orthologue of XRCC2 ^[241] ^[252] • physically interacts with Rad51 and Rad57 both <i>in vivo</i> and <i>in vitro</i> ^[253] ^[141] • <i>rad55</i> mutant: viable ^[252], DNA damage sensitivity and DSB repair defects, which can be rescued by Rad51 overexpression and gain-of-function Rad51 mutations ^[254], SSA increased ^[255] • is phosphorylated in response to genotoxic stress and this phosphorylation event is required for an efficient DNA repair ^[256], Rad55 phosphorylation is as a sentinel for DNA damage checkpoint activation ^[257]	Rad57 would be an orthologue of XRCC3 according to <i>Lin</i> and coll. (2006) ^[253] ^[141] • physically interacts with Rad55 ^[253] ^[141] • <i>rad57</i> mutant: viable ^[252], DNA damage sensitivity and DSB repair defects, which can be rescued by Rad51 overexpression and gain-of-function Rad51 mutations ^[256] and Srs2 removal ^[127, 254]		
<i>S. pombe</i>	Rhp55	Rhp57		
	Rhp55 = <i>rad</i> homolog <i>S.pombe</i> 55 ^[260] • no direct interaction with Rhp51 ^[261], forms an heterodimeric complex with Rhp57 ^[261] • acts in one DNA repair pathway together with Rhp51 and Rhp54 proteins, but in a different pathway than the putative Rad52p, is involved in GC but not in SSA, is required for full meiotic recombination ^[260]	Rhp57 = <i>rad</i> homolog <i>S.pombe</i> 57, is phylogenetically related to XRCC3 ^[262] • strongly interacts with Rhp51 ^[261], is epistatic function with Rhp51 ^[262], forms an heterodimeric complex with Rhp55 ^[261] • is involved in GC but not in SSA		

Table 2 : RAD51 paralogues in eukaryotes (3/3)

	SHU1	SHU2	CSM2	PSY3	SWSAP1
<i>S. cerevisiae</i>	<i>Shu1</i> = suppressor of <i>Sgs1</i> HU sensitivity 1, was identified by a screen for non- <i>sgs1Δ</i> suppressors of the <i>top3Δ</i> slow-growth phenotype ^[263] , has homology with human XRCC2 , is a <i>Rad51</i> paralogue ^[264] - physically interacts with Shu2 and Psy3 ^[265] - epistatic to Mus81 for MMS sensitivity ^[266] - favours Rad51 focus formation and suppresses Srs2 focus formation ^[267], promotes both crossover and non-crossover pathways of meiotic recombination, independently of Srs2 ^[268]	<i>Shu2</i> = suppressor of <i>Sgs1</i> HU sensitivity 2, was identified by a screen for non- <i>sgs1Δ</i> suppressors of the <i>top3Δ</i> slow-growth phenotype ^[263] - physically interacts with Shu1 and Psy3 ^[265] and with Srs2 ^[269] - is epistatic to Sgs1 and Mus81 for MMS sensitivity ^[266] - favors Rad51 focus formation ^[267] and Rad52 focus formation ^[263]	<i>Csm2</i> = chromosome segregation in meiosis, was initially discovered as part of a genome-wide screen for mutations affecting chromosome segregation in meiosis ^[270] - shares similar ATP core domain architecture of proteins that belong to the Rad51 family ^[271] - physically interacts with Psy3 ^[265], with Rad51 (dependent on Rad55) and with Rad55 (independently of Rad51) ^[255] - is epistatic to Rad55 ^[255, 272] <i>csm2</i> mutant: SSA more efficient than in wt ^[255]	PSY3 = <i>platinum sensitivity</i> , was initially identified as part of a genome-wide screen for mutants sensitive to platinum ^[273] , has homology with human RAD51D , Rad51 paralogue ^[264] - shares similar ATP core domain architecture of proteins that belong to the Rad51 family ^[271] - physically interacts with Csm2, Shu1 and Shu2 ^[265] - is epistatic to Srs2 ^[265] and to Rad55 ^[272] <i>psy3</i> mutant: SSA more efficient than in wt ^[255] - functions in the same pathway as Mms2 ^[265]	
			CSM2-PSY3		
			- is a structural mimic of the Rad51-dimer ^[274] - preferentially binds synthetic forked DNA or 3'-DNA overhang substrates - recruits the Shu complex to HR substrates, needed for efficient recruitment of Rad55 to DNA repair foci after DNA damage ^[255], binds and stabilizes the Rad51-ssDNA complex independently of nucleotide cofactor <i>in vitro</i> ^[274]		
	SHU / PCSS complex				
- Shu1, Shu2, Csm2, Psy3 form a stable complex <i>in vivo</i> and <i>in vitro</i> ^[263], form a nucleoprotein filament by binding non specifically to DNA, like Rad51 ^[275] - the 4 proteins belong to the same epistatic group ^[263], are epistatic to Rad52 ^[263] and are implicated in error-free post-replicative repair (members of the Rad6 epistasis group ^[265]) - Shu complex promotes homologous recombination while suppressing error-prone DNA repair ^[263, 265, 276], inhibits the Srs2 anti-recombinase ^[267], is involved in error-free DNA-damage tolerance pathway ^[265] by recruiting the Rad55- Rad57 complex and the HR machinery to damage sites ^[272], may have specific function during S phase ^[265], functions upstream of the Sgs1-Top3-Rim1 pathway ^[265], acts to promote the formation of recombination structures in replication-associated recombinational repair, independently of Mph1 and Mms2 ^[277], binds to meiotic DSB sites and is required for formation of Rad51 complexes on meiotic chromosomes, has a critical role in meiotic recombination in mediating assembly of Rad51 nucleoprotein filaments ^[274]					
<i>S. pombe</i>	RLP1	SWS1		RDL1	
	<i>Rlp1</i> = <i>RecA</i> -like protein ^[278] , is most closely related to XRCC2 ^[264] - interacts with Rhp57 ^[278] <i>rlp1</i> mutant shows a moderate reduction of mitotic recombination ^[278], and a decrease of the frequency of intergenic recombination (crossovers), but an increase of GC recombination ^[279]	<i>Sws1</i> = <i>SWIM</i> domain-containing and <i>Srs2</i> -interacting protein 1, is related to <i>S. cerevisiae</i> <i>Shu2</i> ^[264] - physically interacts with Srs2 ^[264] - controls a very early stage of HR ^[264]		<i>Rdl1</i> = <i>Rad51D</i> -like protein ^[264] , is most closely related to RAD51D ^[264] has a more complex set of functions than Rlp1 and Sws1 ^[264]	
	RPL1-SWS1-RDL1				
RPL1, SWS1 and RDL1 form a stable complex ^[264]					

Table 3: SHU complex proteins in eukaryotes (1/2)

<i>H. sapiens</i>		SWS1		SWSAP1
		• associates with RAD51D and XRCC3^[264] • <i>sws1</i> mutant: reduction in number of RAD51 foci^[264] • has pro-recombinogenic activity^[264]		<i>SWSAP1</i> = <i>SWS</i> associated protein 1, has similarity to archeal RadA ^[280] • interacts with RAD51, RAD51B, RAD51C, RAD51D, XRCC3^[280] • preferentially binds ssDNA^[280] • is a DNA-stimulated ATPase^[280] • <i>swsap1</i> mutant: reduction in number of Rad51 foci^[280]
		SWS1-SWSAP1		
		• form a stable complex • is a non-essential HR accessory factor, which affects the efficiency and/or timing of HR^[280]		
<i>G. gallus</i>		SWS1		
		• <i>sws1</i> mutant: slight decrease in IR-induced RAD51 focus formation • its contribution to HR repair is less significant than that of BRCA1, BRCA2 and the RAD51 paralogues^[281], requires BRCA2 to contribute to HR^[280]		

Table 3: SHU complex proteins in eukaryotes (2/2)

	BRCA1	BRCA2
<i>H. sapiens</i>	<i>Breast Cancer susceptibility gene 1, tumour suppressor gene</i> - interacts with RAD51^[282] and with BRCA2^[283] in mitotic and meiotic cells, interacts <i>in vitro</i> and <i>in vivo</i> with RAD50^[284] - binds strongly to DNA and displays a preference for branched DNA structures^[285] - E3-ubiquitin ligase. exhibits a ubiquitin ligase activity <i>in vitro</i>^[286, 287], this activity is required for its tumor suppressor function^[129] - has a broader role upstream of BRCA2, participating in <u>various cellular processes in response to DNA damage</u>^[288], inhibits the nucleolytic activities of the Mre11-Rad50-Nbs1 complex^[285], BRCA1–CtIP complex promotes CtIP-mediated 5′-end resection of DSBs^[289], BRCA1–PALB2 interaction is a prerequisite for the recruitment of BRCA2 and RAD51 to the site of DNA damage and for <u>homologous recombination</u>^[290-292], is involved directly or indirectly in the repair of crosslinked DNA^[293], is involved, in complex with BARD1, in G1/S, S-phase and G2/M checkpoints activation^[294]^[295], ubiquitinates G2/M cell cycle proteins, cyclin B and Cdc25C, leading to their accelerated degradation which permits mitotic entry^[296], facilitates the ability of ATM and ATR to phosphorylate downstream substrates that directly influence cell cycle checkpoint arrest and apoptosis^[297], may regulate telomere length and stability^[298]	<i>Breast cancer susceptibility gene 2, tumour suppressor gene</i> - forms nuclear foci which colocalize with RAD51 foci after IR treatment of somatic cells^[188] - interacts <i>in vivo</i> with RAD51^[299]^[283], one BRCA2 molecule can bind 6 molecules of RAD51^[300], interacts with DMC1^[301], associates with BRCA1 through PALB2^[290-292], physically interacts with FANCD2 <i>in vivo</i>^[302] - binds ssDNA and dsDNA^[303] <i>brca2</i> mutation: impairs HR-mediated DSB repair but maintains normal NHEJ^[304] - BRCA2 mediates RAD51 filament formation and strand exchange after DNA damage by promoting the <u>assembly of RAD51 onto RPA-coated ssDNA</u>^[37, 300, 305, 306], prevents RAD51 from binding to dsDNA^[305], is essential for RAD51 foci formation after DNA damage^[307, 308], BRCA2-RAD51 interaction is critical for cellular response to DNA damage caused by MMS^[309], is a key regulator of the extension step after strand invasion at <u>replication-dependent DSBs</u> allowing Polh localization at collapsed replication forks and Polh-dependent DNA synthesis^[310]
<i>M. musculus</i>	- Most homozygous <i>brca1</i> mutations are embryonically lethal^[311-313], phenotype of <i>brca1</i> mouse embryos mimics that of <i>rad51</i> mice, <i>brca1</i> cells: telomere dysfunction, chromosome translocations, chromatid aberrations^[314-316] gene targeting defects and increase of non homologous integration, sensitive to MMC^[317, 318] - is required for RAD51 nuclear focus formation^[319], <u>promotes HDR and SSA pathways</u>^[317, 320]	- is highly expressed during spermatogenesis and detected in early meiotic prophase I^[321, 322] - most homozygous <i>brca2</i> mutations are embryonically lethal^[312, 323, 324], <i>brca2</i> cells show chromatid breaks and aberrant chromatid exchanges^[325], expression of a truncated BRCA2 showed a failure in spermatogenesis^[321] <u>promotes HDR and suppresses SSA</u>^[320], seems to be required for the correct localization of RAD51 and DMC1 recombinases to DSBs^[326]
<i>C. griseus</i>		- physically interacts with FANCD2 <i>in vivo</i>^[302] - protects the nascent strand from degradation at stalled replication forks^[327]
<i>G. gallus</i>	BRCA1–CtIP complex promotes CtIP-mediated 5′-end resection of DSBs^[289], BRCA1 is required for repair of DSBs by <u>homologous recombination</u> but not MMEJ^[289]	<i>Has 37% amino acid identity overall with human BRCA2</i>^[328] <i>brca2</i> cells: lethal^[329], BRCA2 +/- mutation: reduced growth rate, increased cell death, heightened sensitivity to specific DNA damaging agents and reduced RAD51 focus formation after irradiation^[329]
<i>D. rerio</i>	^[330]	Fancd1 - expressed in proliferating somatic cells and in meiotic oocytes and spermatocytes, Brca2 transcript is asymmetrically localized to the animal pole of the cytoplasm in developing wild-type oocytes^[331] <i>brca2</i> mutations: genome instability, slow growth of tissue culture cells, female-to-male sex reversal, testicular neoplasias^[331] and male infertility with meiotic arrest in spermatocytes^[332] - acts in <u>establishing or maintaining the architecture of the vertebrate oocyte nucleus</u>^[331], is involved in <u>ovarian development and tumorigenesis in reproductive tissues</u>^[332]
<i>D. melanogaster</i>	^[333]	- forms nuclear foci after DNA damage^[334] <i>brca2</i> null mutant: viable^[335] - physically interacts with SpnA (dmRAD51)^[334] - required for <u>mitotic and meiotic DNA repair</u> and efficient <u>activation of the meiotic recombination checkpoint</u>^[335]

Table 4: BRCA1 and BRCA2 proteins in eukaryotes (1/2)

	BRC-1	BRC-2
<i>C. elegans</i>	<i>Protein sequence 24% identical and 52% similar to human BRCA1 (delta-exon 11 splice variant)</i> ^[336] • <i>brc-1</i> animals: high incidence of males (result of X chromosome nondisjunction), elevated levels of p53-dependent germ cell death before and after irradiation, and impaired progeny survival and chromosome fragmentation after irradiation ^[336]	• Brc-2 is a little over a tenth the size of its human counterpart ^[337], expression higher in oocytes than in other germline cells, and barely detectable in mitotic cells ^[338], forms foci in response to DNA damage, independently of RAD51 ^[249] • <i>brc-2</i> mutant: embryonic lethal (Martin, 2005), <i>brc-2</i> cells: fail to repair meiotic or radiation-induced DSBs by HR ^[249] • interacts directly with RAD51 <i>in vivo</i> and <i>in vitro</i> ^[249] • binds preferentially to ssDNA ^[249] • stabilized of RAD51-DNA filaments ^[339], stimulates RAD51 mediated D-loop formation and promotes DNA single-strand annealing ^[340], plays an essential role in chromosome integrity of germ cells ^[338], is crucial in <u>meiotic progression</u> ^[338]
<i>A. thaliana</i>	• is expressed ubiquitously in plant tissues, at levels depending on organ type, with highest levels in flower buds and exponentially growing cell cultures ^[341], expression is induced by γ-irradiation ^[341, 342] • <i>brca1</i> mutant: fertile, no defect in development, HR defects ^[342] and mild hypersensitivity to MMC ^[343] • epistatic to BARD1 in <u>DNA crosslink repair</u> ^[343], <u>non essential for meiosis</u> ^[343]	BRCA2A = BRCA2(IV) et BRCA2B = BRCA2(V) <i>Arabidopsis</i> has two homologs of the <i>BRCA2</i> gene, 96.8% identity ^[344] • expressed in flower buds, shoot and root apices and, in the case of BRCA2A, at moderate levels in leaves ^[344, 345], expression not induced by γ-irradiation ^[344] • BRCA2 proteins interact with RAD51 and DMC1 ^[344, 346], existence of the BRCA2A-RAD51 complex <i>in planta</i> ^[345] • <i>brca2a</i> and <i>brca2b</i> mutant plants have mild hypersensitivity to genotoxic stresses ^[347], <i>brca2a/brca2b</i> double mutant shows an additive increase in sensitivity to genotoxic stresses, and altered cell cycle progression of shoot apical meristems ^[347] • essential role at meiosis in <u>mediating the function of both RAD51 and DMC1</u> ^[344, 348], important for both <u>somatic and meiotic homologous recombination</u> ^[348], function in cell proliferation and cell cycle regulation ^[347], BRCA2A, but not BRCA2B is required for salicylic acid-induced expression of pathogenesis-related proteins ^[345]
<i>S. cerevisiae</i>		
<i>S. pombe</i>		

Table 4: BRCA1 and BRCA2 proteins in eukaryotes (2/2)

References

- 1 Wold, M.S., 1997, Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu Rev Biochem.* 66: p. 61-92.
- 2 Wold, M.S. and T. Kelly, 1988, Purification and characterization of replication protein A, a cellular protein required for in vitro replication of simian virus 40 DNA. *Proceedings of the National Academy of Sciences of the United States of America.* 85(8): p. 2523-2527.
- 3 Golub, E.I., et al., 1998, Interaction of human rad51 recombination protein with single-stranded DNA binding protein, RPA. *Nucleic acids research.* 26(23): p. 5388-5393.
- 4 Raderschall, E., E.I. Golub, and T. Haaf, 1999, Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. *Proceedings of the National Academy of Sciences of the United States of America.* 96(5): p. 1921-1926.
- 5 Oliver-Bonet, M., et al., 2007, Analysis of replication protein A (RPA) in human spermatogenesis. *Mol Hum Reprod.* 13(12): p. 837-44.
- 6 Stauffer, M.E. and W.J. Chazin, 2004, Physical interaction between replication protein A and Rad51 promotes exchange on single-stranded DNA. *The Journal of biological chemistry.* 279(24): p. 25638-25645.
- 7 Choudhary, S.K. and R. Li, 2002, BRCA1 modulates ionizing radiation-induced nuclear focus formation by the replication protein A p34 subunit. *Journal of cellular biochemistry.* 84(4): p. 666-674.
- 8 Wong, J.M.S., D. Ionescu, and C.J. Ingles, 2003, Interaction between BRCA2 and replication protein A is compromised by a cancer-predisposing mutation in BRCA2. *Oncogene.* 22(1): p. 28-33.
- 9 Bessho, T., et al., 1997, Reconstitution of human excision nuclease with recombinant XPF-ERCC1 complex. *The Journal of biological chemistry.* 272(6): p. 3833-3837.
- 10 Brosh, R.M., Jr., et al., 2000, Replication protein A physically interacts with the Bloom's syndrome protein and stimulates its helicase activity. *J Biol Chem.* 275(31): p. 23500-8.
- 11 Doherty, K.M., et al., 2005, Physical and functional mapping of the replication protein a interaction domain of the werner and bloom syndrome helicases. *The Journal of biological chemistry.* 280(33): p. 29494-29505.
- 12 Shen, J.-C., et al., 2003, The N-terminal domain of the large subunit of human replication protein A binds to Werner syndrome protein and stimulates helicase activity. *Mechanisms of ageing and development.* 124(8-9): p. 921-930.
- 13 Fairman, M.P. and B. Stillman, 1988, Cellular factors required for multiple stages of SV40 DNA replication in vitro. *The EMBO journal.* 7(4): p. 1211-1218.
- 14 Erdile, L.F., M.S. Wold, and T.J. Kelly, 1990, The primary structure of the 32-kDa subunit of human replication protein A. *J Biol Chem.* 265(6): p. 3177-82.
- 15 de Laat, W.L., et al., 1998, DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. *Genes & development.* 12(16): p. 2598-2609.
- 16 Din, S., et al., 1990, Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. *Genes & development.* 4(6): p. 968-977.
- 17 Kenny, M.K., et al., 1990, The role of human single-stranded DNA binding protein and its individual subunits in simian virus 40 DNA replication. *J Biol Chem.* 265(13): p. 7693-700.
- 18 Kenny, M.K., S.H. Lee, and J. Hurwitz, 1989, Multiple functions of human single-stranded-DNA binding protein in simian virus 40 DNA replication: single-strand stabilization and stimulation of DNA polymerases alpha and delta. *Proc Natl Acad Sci U S A.* 86(24): p. 9757-61.
- 19 Coverley, D., et al., 1991, Requirement for the replication protein SSB in human DNA excision repair. *Nature.* 349(6309): p. 538-41.
- 20 Moore, S.P., et al., 1991, The human homologous pairing protein HPP-1 is specifically stimulated by the cognate single-stranded binding protein hRP-A. *Proc Natl Acad Sci U S A.* 88(20): p. 9067-71.
- 21 Liu, S., et al., 2012, Distinct roles for DNA-PK, ATM and ATR in RPA phosphorylation and checkpoint activation in response to replication stress. *Nucleic acids research.* 40(21): p. 10780-10794.
- 22 Oakley, G.G., et al., 2001, UV-induced hyperphosphorylation of replication protein a depends on DNA replication and expression of ATM protein. *Molecular biology of the cell.* 12(5): p. 1199-1213.
- 23 He, Z., et al., 1995, RPA involvement in the damage-recognition and incision steps of nucleotide excision repair. *Nature.* 374(6522): p. 566-9.
- 24 Sancar, A., et al., 2004, Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem.* 73: p. 39-85.
- 25 Matsunaga, T., et al., 1996, Replication protein A confers structure-specific endonuclease activities to the XPF-ERCC1 and XPG subunits of human DNA repair excision nuclease. *The Journal of biological chemistry.* 271(19): p. 11047-11050.
- 26 Aboussekhra, A., et al., 1995, Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell.* 80(6): p. 859-68.
- 27 DeMott, M.S., S. Zigman, and R.A. Bambara, 1998, Replication protein A stimulates long patch DNA base excision repair. *J Biol Chem.* 273(42): p. 27492-8.
- 28 Zou, L. and S.J. Elledge, 2003, Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science (New York, N.Y.).* 300(5625): p. 1542-1548.
- 29 Baumann, P. and S.C. West, 1997, The human Rad51 protein: polarity of strand transfer and stimulation by hRP-A. *EMBO J.* 16(17): p. 5198-206.
- 30 Chen, F., et al., 1997, Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double-strand break repair genes Rad51 and Rad52. *Mutation research.* 384(3): p. 205-211.
- 31 Park, M.S., et al., 1996, Physical interaction between human RAD52 and RPA is required for homologous recombination in mammalian cells. *The Journal of biological chemistry.* 271(31): p. 18996-19000.
- 32 Shen, Z., et al., 1996, Specific interactions between the human RAD51 and RAD52 proteins. *J Biol Chem.* 271(1): p. 148-52.
- 33 Baumann, P. and S.C. West, 1999, Heteroduplex formation by human Rad51 protein: effects of DNA end-structure, hRP-A and hRad52. *J Mol Biol.* 291(2): p. 363-74.
- 34 Kumar, J.K. and R.C. Gupta, 2004, Strand exchange activity of human recombination protein Rad52. *Proceedings of the National Academy of Sciences of the United States of America.* 101(26): p. 9562-9567.
- 35 Reddy, G., E.I. Golub, and C.M. Radding, 1997, Human Rad52 protein promotes single-strand DNA annealing followed by branch migration. *Mutation research.* 377(1): p. 53-59.
- 36 Singleton, M.R., et al., 2002, Structure of the single-strand annealing domain of human RAD52 protein. *Proceedings of the National Academy of Sciences of the United States of America.* 99(21): p. 13492-13497.
- 37 Symington, L.S., 2002, Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiology and molecular biology reviews : MMBR.* 66(4): p. 630-70- table of contents.
- 38 Jensen, R.B., A. Carreira, and S.C. Kowalczykowski, 2010, Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature.* 467(7316): p. 678-83.
- 39 Lok, B.H. and S.N. Powell, 2012, Molecular pathways: understanding the role of Rad52 in homologous recombination for therapeutic advancement. *Clin Cancer Res.* 18(23): p. 6400-6.
- 40 Feng, Z., et al., 2011, Rad52 inactivation is synthetically lethal with BRCA2 deficiency. *Proceedings of the National Academy of Sciences of the United States of America.* 108(2): p. 686-691.
- 41 McIlwraith, M.J. and S.C. West, 2008, DNA repair synthesis facilitates RAD52-mediated second-end capture during DSB repair. *Molecular cell.* 29(4): p. 510-516.
- 42 Kim, P.M., et al., 2001, Overexpression of human RAD51 and RAD52 reduces double-strand break-induced homologous recombination in mammalian cells. *Nucleic Acids Res.* 29(21): p. 4352-60.

43 Essers, J., et al., 2002, Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. *The EMBO journal*. 21(8): p. 2030-2037.
 44 van Veelen, L.R., et al., 2005, Ionizing radiation-induced foci formation of mammalian Rad51 and Rad54 depends on the Rad51 paralogs, but not on Rad52. *Mutation research*. 574(1-2): p. 34-49.
 45 Golub, E.I., et al., 1997, Interaction of human recombination proteins Rad51 and Rad54. *Nucleic acids research*. 25(20): p. 4106-4110.
 46 Mazina, O.M. and A.V. Mazin, 2008, Human Rad54 protein stimulates human Mus81-Eme1 endonuclease. *Proceedings of the National Academy of Sciences of the United States of America*. 105(47): p. 18249-18254.
 47 Swagemakers, S.M., et al., 1998, The human RAD54 recombinational DNA repair protein is a double-stranded DNA-dependent ATPase. *The Journal of biological chemistry*. 273(43): p. 28292-28297.
 48 Ristic, D., et al., 2001, The architecture of the human Rad54-DNA complex provides evidence for protein translocation along DNA. *Proc Natl Acad Sci U S A*. 98(15): p. 8454-60.
 49 Petukhova, G., S. Stratton, and P. Sung, 1998, Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature*. 393(6680): p. 91-94.
 50 Mazina, O.M., et al., 2007, Interactions of human rad54 protein with branched DNA molecules. *J Biol Chem*. 282(29): p. 21068-80.
 51 Tan, T.L., et al., 1999, Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. *Current biology : CB*. 9(6): p. 325-328.
 52 Zhang, Z., et al., 2007, Homology-driven chromatin remodeling by human RAD54. *Nature structural & molecular biology*. 14(5): p. 397-405.
 53 Bugreev, D.V., O.M. Mazina, and A.V. Mazin, 2006, Rad54 protein promotes branch migration of Holliday junctions. *Nature*. 442(7102): p. 590-593.
 54 Tanaka, K., et al., 2000, A novel human rad54 homologue, Rad54B, associates with Rad51. *J Biol Chem*. 275(34): p. 26316-21.
 55 Tanaka, K., et al., 2002, Human Rad54B is a double-stranded DNA-dependent ATPase and has biochemical properties different from its structural homolog in yeast, Tid1/Rdh54. *Nucleic acids research*. 30(6): p. 1346-1353.
 56 Miyagawa, K., et al., 2002, A role for RAD54B in homologous recombination in human cells. *The EMBO journal*. 21(1-2): p. 175-180.
 57 Sehorn, M.G., et al., 2004, Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. *Nature*. 429(6990): p. 433-437.
 58 Sarai, N., et al., 2006, Stimulation of Dmc1-mediated DNA strand exchange by the human Rad54B protein. *Nucleic Acids Res*. 34(16): p. 4429-37.
 59 Ceballos, S.J. and W.-D. Heyer, 2011, Functions of the Snf2/Swi2 family Rad54 motor protein in homologous recombination. *Biochimica et biophysica acta*. 1809(9): p. 509-523.
 60 Plug, A.W., et al., 1997, ATM and RPA in meiotic chromosome synapsis and recombination. *Nat Genet*. 17(4): p. 457-61.
 61 Plug, A.W., et al., 1998, Changes in protein composition of meiotic nodules during mammalian meiosis. *Journal of cell science*. 111 (Pt 4): p. 413-423.
 62 Moens, P.B., et al., 2007, Initiation and resolution of interhomolog connections: crossover and non-crossover sites along mouse synaptonemal complexes. *Journal of cell science*. 120(Pt 6): p. 1017-1027.
 63 Marcon, E. and P. Moens, 2003, MLH1p and MLH3p localize to precociously induced chiasmata of okadaic-acid-treated mouse spermatocytes. *Genetics*. 165(4): p. 2283-7.
 64 Wang, D., M.A. McDonnell, and A. Kelekar, 2005, Multi-probe RPA template sets to study RNA modulation and transcriptional control of BH3-only members of the Bcl-2 family. *Cancer Detect Prev*. 29(2): p. 189-200.
 65 Rijkers, T., et al., 1998, Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. *Mol Cell Biol*. 18(11): p. 6423-9.
 66 Thorpe, P.H., et al., 2006, Cells expressing murine RAD52 splice variants favor sister chromatid repair. *Molecular and cellular biology*. 26(10): p. 3752-3763.
 67 Kanaar, R., et al., 1996, Human and mouse homologs of the *Saccharomyces cerevisiae* RAD54 DNA repair gene: evidence for functional conservation. *Current biology : CB*. 6(7): p. 828-838.
 68 De Silva, I.U., et al., 2000, Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. *Mol Cell Biol*. 20(21): p. 7980-90.
 69 Essers, J., et al., 2000, Homologous and non-homologous recombination differentially affect DNA damage repair in mice. *The EMBO journal*. 19(7): p. 1703-1710.
 70 Essers, J., et al., 1997, Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell*. 89(2): p. 195-204.
 71 Wesoly, J., et al., 2006, Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. *Molecular and cellular biology*. 26(3): p. 976-989.
 72 Dronkert, M.L., et al., 2000, Mouse RAD54 affects DNA double-strand break repair and sister chromatid exchange. *Mol Cell Biol*. 20(9): p. 3147-56.
 73 Jaco, I., et al., 2003, Role of mammalian Rad54 in telomere length maintenance. *Molecular and cellular biology*. 23(16): p. 5572-5580.
 74 Bross, L., et al., 2003, Somatic hypermutation does not require Rad54 and Rad54B-mediated homologous recombination. *European journal of immunology*. 33(2): p. 352-357.
 75 Fisher, L.A., et al., 2011, Role of interaction of XPF with RPA in nucleotide excision repair. *Journal of molecular biology*. 413(2): p. 337-346.
 76 Bezzubova, O.Y., et al., 1993, Identification of a chicken RAD52 homologue suggests conservation of the RAD52 recombination pathway throughout the evolution of higher eukaryotes. *NAR*. 21(25): p. 5945-5949.
 77 Yamaguchi-Iwai, Y., et al., 1998, Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in RAD52. *Molecular and cellular biology*. 18(11): p. 6430-6435.
 78 Wang, H., et al., 2001, Efficient rejoining of radiation-induced DNA double-strand breaks in vertebrate cells deficient in genes of the RAD52 epistasis group. *Oncogene*. 20(18): p. 2212-24.
 79 Fujimori, A., et al., 2001, Rad52 partially substitutes for the Rad51 paralog XRCC3 in maintaining chromosomal integrity in vertebrate cells. *EMBO J*. 20(19): p. 5513-20.
 80 Bezzubova, O., et al., 1997, Reduced X-ray resistance and homologous recombination frequencies in a RAD54^{-/-} mutant of the chicken DT40 cell line. *Cell*. 89(2): p. 185-193.
 81 Takata, M., et al., 1998, Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *The EMBO journal*. 17(18): p. 5497-5508.
 82 Wang, W., et al., 2000, Possible association of BLM in decreasing DNA double strand breaks during DNA replication. *The EMBO journal*. 19(13): p. 3428-3435.
 83 Lee, J.-S. and Y.-S. Lee, 2002, Cloning and characterization of replication protein A p32 complementary DNA in zebrafish (*Danio rerio*). *Marine biotechnology (New York, N.Y.)*. 4(1): p. 1-5.
 84 Takahashi, N. and I.B. Dawid, 2005, Characterization of zebrafish Rad52 and replication protein A for oligonucleotide-mediated mutagenesis. *Nucleic acids research*. 33(13): p. e120.
 85 Liu, J., et al., 2012, Development of novel visual-plus quantitative analysis systems for studying DNA double-strand break repairs in zebrafish. *J Genet Genomics*. 39(9): p. 489-502.
 86 Thoma, N.H., et al., 2005, Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic Rad54. *Nature structural & molecular biology*. 12(4): p. 350-356.
 87 Marton, R.F., P. Thömmes, and S. Cotterill, 1994, Purification and characterisation of dRP-A: a single-stranded DNA binding protein from *Drosophila melanogaster*. *FEBS letters*. 342(2): p. 139-144.
 88 Mitsis, P.G., S.C. Kowalczykowski, and I.R. Lehman, 1993, A single-stranded DNA binding protein from *Drosophila melanogaster*: characterization of the heterotrimeric protein and its interaction with single-stranded DNA. *Biochemistry*. 32(19): p. 5257-5266.

89 Ghabrial, A., R.P. Ray, and T. Schüpbach, 1998, okra and spindle-B encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in *Drosophila* oogenesis. *Genes & development*. 12(17): p. 2711-2723.

90 Kooistra, R., et al., 1997, The *Drosophila melanogaster* RAD54 homolog, DmRAD54, is involved in the repair of radiation damage and recombination. *Molecular and cellular biology*. 17(10): p. 6097-6104.

91 Alexiadis, V. and J.T. Kadonaga, 2002, Strand pairing by Rad54 and Rad51 is enhanced by chromatin. *Genes & development*. 16(21): p. 2767-2771.

92 Alexiadis, V., A. Lusser, and J.T. Kadonaga, 2004, A conserved N-terminal motif in Rad54 is important for chromatin remodeling and homologous strand pairing. *The Journal of biological chemistry*. 279(26): p. 27824-27829.

93 Lee, S.-J., et al., 2010, The *Caenorhabditis elegans* Werner syndrome protein functions upstream of ATR and ATM in response to DNA replication inhibition and double-strand DNA breaks. *PLoS genetics*. 6(1): p. e1000801.

94 Garcia-Muse, T. and S.J. Boulton, 2005, Distinct modes of ATR activation after replication stress and DNA double-strand breaks in *Caenorhabditis elegans*. *EMBO J*. 24(24): p. 4345-55.

95 Hyun, M., et al., 2012, Physical and functional interactions of *Caenorhabditis elegans* WRN-1 helicase with RPA-1. *Biochemistry*. 51(7): p. 1336-45.

96 Kim, D.-H., et al., 2005, Enzymatic properties of the *Caenorhabditis elegans* Dna2 endonuclease/helicase and a species-specific interaction between RPA and Dna2. *Nucleic acids research*. 33(4): p. 1372-1383.

97 Ishibashi, T., S. Kimura, and K. Sakaguchi, 2006, A higher plant has three different types of RPA heterotrimeric complex. *J Biochem*. 139(1): p. 99-104.

98 Shultz, R.W., et al., 2007, Genome-wide analysis of the core DNA replication machinery in the higher plants *Arabidopsis* and rice. *Plant Physiol*. 144(4): p. 1697-714.

99 Osman, K., et al., 2009, Replication protein A (AtRPA1a) is required for class I crossover formation but is dispensable for meiotic DNA break repair. *The EMBO journal*. 28(4): p. 394-404.

100 Ishibashi, T., et al., 2005, Two types of replication protein A in seed plants. *The FEBS journal*. 272(13): p. 3270-3281.

101 Osakabe, K., et al., 2006, Isolation and characterization of the RAD54 gene from *Arabidopsis thaliana*. *The Plant journal : for cell and molecular biology*. 48(6): p. 827-842.

102 Shaked, H., N. Avivi-Ragolsky, and A.A. Levy, 2006, Involvement of the *Arabidopsis* SWI2/SNF2 chromatin remodeling gene family in DNA damage response and recombination. *Genetics*. 173(2): p. 985-94.

103 Roth, N., et al., 2012, The requirement for recombination factors differs considerably between different pathways of homologous double-strand break repair in somatic plant cells. *Plant J*. 72(5): p. 781-90.

104 Kaliappan, K., et al., 2012, A novel role for RAD54: this host protein modulates geminiviral DNA replication. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 26(3): p. 1142-1160.

105 Samach, A., et al., 2011, Identification of plant RAD52 homologs and characterization of the *Arabidopsis thaliana* RAD52-like genes. *The Plant cell*. 23(12): p. 4266-4279.

106 Gasior, S.L., et al., 1998, Rad52 associates with RPA and functions with rad55 and rad57 to assemble meiotic recombination complexes. *Genes & development*. 12(14): p. 2208-2221.

107 Sugiyama, T., E.M. Zaitseva, and S.C. Kowalczykowski, 1997, A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. *The Journal of biological chemistry*. 272(12): p. 7940-7945.

108 Sung, P., 1997, Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *The Journal of biological chemistry*. 272(45): p. 28194-28197.

109 Brill, S.J. and B. Stillman, 1989, Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication. *Nature*. 342(6245): p. 92-95.

110 Wolner, B., et al., 2003, Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. *Molecular cell*. 12(1): p. 221-232.

111 Van Komen, S., et al., 2002, Functional cross-talk among Rad51, Rad54, and replication protein A in heteroduplex DNA joint formation. *The Journal of biological chemistry*. 277(46): p. 43578-43587.

112 Egger, A.L., R.B. Inman, and M.M. Cox, 2002, The Rad51-dependent pairing of long DNA substrates is stabilized by replication protein A. *The Journal of biological chemistry*. 277(42): p. 39280-39288.

113 Soustelle, C., et al., 2002, Replication protein A is required for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics*. 161(2): p. 535-47.

114 Longhese, M.P., et al., 1996, The 70 kDa subunit of replication protein A is required for the G1/S and intra-S DNA damage checkpoints in budding yeast. *Nucleic acids research*. 24(18): p. 3533-3537.

115 Lee, S.E., et al., 1998, *Saccharomyces* Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell*. 94(3): p. 399-409.

116 Singh, K.K. and L. Samson, 1995, Replication protein A binds to regulatory elements in yeast DNA repair and DNA metabolism genes. *Proceedings of the National Academy of Sciences of the United States of America*. 92(11): p. 4907-4911.

117 Cole, G.M., et al., 1987, Regulation of RAD54- and RAD52-lacZ gene fusions in *Saccharomyces cerevisiae* in response to DNA damage. *Mol Cell Biol*. 7(3): p. 1078-84.

118 Cole, G.M., D. Schild, and R.K. Mortimer, 1989, Two DNA repair and recombination genes in *Saccharomyces cerevisiae*, RAD52 and RAD54, are induced during meiosis. *Mol Cell Biol*. 9(7): p. 3101-4.

119 Lisby, M., R. Rothstein, and U.H. Mortensen, 2001, Rad52 forms DNA repair and recombination centers during S phase. *Proc Natl Acad Sci U S A*. 98(15): p. 8276-82.

120 Hays, S.L., et al., 1998, Studies of the interaction between Rad52 protein and the yeast single-stranded DNA binding protein RPA. *Mol Cell Biol*. 18(7): p. 4400-6.

121 Milne, G.T. and D.T. Weaver, 1993, Dominant negative alleles of RAD52 reveal a DNA repair/recombination complex including Rad51 and Rad52. *Genes Dev*. 7(9): p. 1755-65.

122 Davis, A.P. and L.S. Symington, 2001, The yeast recombinational repair protein Rad59 interacts with Rad52 and stimulates single-strand annealing. *Genetics*. 159(2): p. 515-525.

123 Davis, A.P. and L.S. Symington, 2003, The Rad52-Rad59 complex interacts with Rad51 and replication protein A. *DNA repair*. 2(10): p. 1127-1134.

124 Mortensen, U.H., et al., 1996, DNA strand annealing is promoted by the yeast Rad52 protein. *Proceedings of the National Academy of Sciences of the United States of America*. 93(20): p. 10729-10734.

125 Shinohara, A., et al., 1998, Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. *Genes Cells*. 3(3): p. 145-56.

126 Sugiyama, T., J.H. New, and S.C. Kowalczykowski, 1998, DNA annealing by RAD52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. *Proceedings of the National Academy of Sciences of the United States of America*. 95(11): p. 6049-6054.

127 Sugiyama, T. and S.C. Kowalczykowski, 2002, Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation. *The Journal of biological chemistry*. 277(35): p. 31663-31672.

128 Fung, C.W., A.M. Mozlin, and L.S. Symington, 2009, Suppression of the double-strand-break-repair defect of the *Saccharomyces cerevisiae* rad57 mutant. *Genetics*. 181(4): p. 1195-206.

129 New, J.H., et al., 1998, Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature*. 391(6665): p. 407-410.

130 Wu, Y., et al., 2008, Rad51 protein controls Rad52-mediated DNA annealing. *The Journal of biological chemistry*. 283(21): p. 14883-14892.

131 Nimonkar, A.V., R.A. Sica, and S.C. Kowalczykowski, 2009, Rad52 promotes second-end DNA capture in double-stranded break repair to form complement-stabilized joint molecules. *Proceedings of the National Academy of Sciences of the United States of America*. 106(9): p. 3077-3082.

132 Lao, J.P., et al., 2008, Rad52 promotes postinvasion steps of meiotic double-strand-break repair. *Molecular cell*. 29(4): p. 517-524.

133 Sugawara, N. and J.E. Haber, 1992, Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Molecular and cellular biology*. 12(2): p. 563-575.

134 McEachern, M.J. and J.E. Haber, 2006, Break-induced replication and recombinational telomere elongation in yeast. *Annu Rev Biochem*. 75: p. 111-35.

135 Bai, Y. and L.S. Symington, 1996, A Rad52 homolog is required for RAD51-independent mitotic recombination in *Saccharomyces cerevisiae*. *Genes & development*. 10(16): p. 2025-2037.

136 Petukhova, G., S.A. Stratton, and P. Sung, 1999, Single strand DNA binding and annealing activities in the yeast recombination factor Rad59. *J Biol Chem*. 274(48): p. 33839-42.

137 Wu, Y., T. Sugiyama, and S.C. Kowalczykowski, 2006, DNA annealing mediated by Rad52 and Rad59 proteins. *The Journal of biological chemistry*. 281(22): p. 15441-15449.

138 Bai, Y., A.P. Davis, and L.S. Symington, 1999, A novel allele of RAD52 that causes severe DNA repair and recombination deficiencies only in the absence of RAD51 or RAD59. *Genetics*. 153(3): p. 1117-1130.

139 Jablonovich, Z., et al., 1999, Characterization of the role played by the RAD59 gene of *Saccharomyces cerevisiae* in ectopic recombination. *Curr Genet*. 36(1-2): p. 13-20.

140 Sugawara, N., G. Ira, and J.E. Haber, 2000, DNA length dependence of the single-strand annealing pathway and the role of *Saccharomyces cerevisiae* RAD59 in double-strand break repair. *Molecular and cellular biology*. 20(14): p. 5300-5309.

141 Pannunzio, N.R., et al., 2012, Rad59 regulates association of Rad52 with DNA double-strand breaks. *MicrobiologyOpen*. 1(3): p. 285-297.

142 Johnson, R.D. and L.S. Symington, 1995, Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57. *Mol Cell Biol*. 15(9): p. 4843-50.

143 Lisby, M., et al., 2004, Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell*. 118(6): p. 699-713.

144 Mazin, A.V., A.A. Alexeev, and S.C. Kowalczykowski, 2003, A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. *The Journal of biological chemistry*. 278(16): p. 14029-14036.

145 Jiang, H., et al., 1996, Direct association between the yeast Rad51 and Rad54 recombination proteins. *The Journal of biological chemistry*. 271(52): p. 33181-33186.

146 Clever, B., et al., 1997, Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. *The EMBO journal*. 16(9): p. 2535-2544.

147 Raschle, M., et al., 2004, Multiple interactions with the Rad51 recombinase govern the homologous recombination function of Rad54. *The Journal of biological chemistry*. 279(50): p. 51973-51980.

148 Interthal, H. and W.D. Heyer, 2000, MUS81 encodes a novel helix-hairpin-helix protein involved in the response to UV- and methylation-induced DNA damage in *Saccharomyces cerevisiae*. *Molecular & general genetics* : MGG. 263(5): p. 812-827.

149 Amitani, I., R.J. Baskin, and S.C. Kowalczykowski, 2006, Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. *Molecular cell*. 23(1): p. 143-148.

150 Schmuckli-Maurer, J., et al., 2003, Genome instability in rad54 mutants of *Saccharomyces cerevisiae*. *Nucleic Acids Res*. 31(3): p. 1013-23.

151 Solinger, J.A., K. Kiianitsa, and W.D. Heyer, 2002, Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. *Mol Cell*. 10(5): p. 1175-88.

152 Solinger, J.A., et al., 2001, Rad54 protein stimulates heteroduplex DNA formation in the synaptic phase of DNA strand exchange via specific interactions with the presynaptic Rad51 nucleoprotein filament. *Journal of molecular biology*. 307(5): p. 1207-1221.

153 Solinger, J.A. and W.D. Heyer, 2001, Rad54 protein stimulates the postsynaptic phase of Rad51 protein-mediated DNA strand exchange. *Proceedings of the National Academy of Sciences of the United States of America*. 98(15): p. 8447-8453.

154 Matulova, P., et al., 2009, Cooperativity of Mus81.Mms4 with Rad54 in the resolution of recombination and replication intermediates. *The Journal of biological chemistry*. 284(12): p. 7733-7745.

155 Klein, H.L., 1997, RDH54, a RAD54 homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. *Genetics*. 147(4): p. 1533-1543.

156 Ivanov, E.L., et al., 1996, Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. *Genetics*. 142(3): p. 693-704.

157 Dresser, M.E., et al., 1997, DMC1 functions in a *Saccharomyces cerevisiae* meiotic pathway that is largely independent of the RAD51 pathway. *Genetics*. 147(2): p. 533-544.

158 Shinohara, M., et al., 2000, Tid1/Rdh54 promotes colocalization of rad51 and dmc1 during meiotic recombination. *Proceedings of the National Academy of Sciences of the United States of America*. 97(20): p. 10814-10819.

159 Petukhova, G., P. Sung, and H. Klein, 2000, Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. *Genes & development*. 14(17): p. 2206-2215.

160 Santa Maria, S.R., et al., 2013, Characterization of the interaction between the *Saccharomyces cerevisiae* Rad51 recombinase and the DNA translocase Rdh54. *The Journal of biological chemistry*. 288(30): p. 21999-22005.

161 Nimonkar, A.V., et al., 2007, Single molecule imaging of Tid1/Rdh54, a Rad54 homolog that translocates on duplex DNA and can disrupt joint molecules. *J Biol Chem*. 282(42): p. 30776-84.

162 Prasad, T.K., et al., 2007, A DNA-translocating Snf2 molecular motor: *Saccharomyces cerevisiae* Rdh54 displays processive translocation and extrudes DNA loops. *Journal of molecular biology*. 369(4): p. 940-953.

163 Shinohara, M., et al., 1997, Characterization of the roles of the *Saccharomyces cerevisiae* RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. *Genetics*. 147(4): p. 1545-1556.

164 Shinohara, M., et al., 2003, Crossover interference in *Saccharomyces cerevisiae* requires a TID1/RDH54- and DMC1-dependent pathway. *Genetics*. 163(4): p. 1273-1286.

165 Holzen, T.M., et al., 2006, Tid1/Rdh54 promotes dissociation of Dmc1 from nonrecombinogenic sites on meiotic chromatin. *Genes & development*. 20(18): p. 2593-2604.

166 Chi, P., et al., 2006, Yeast recombination factor Rdh54 functionally interacts with the Rad51 recombinase and catalyzes Rad51 removal from DNA. *The Journal of biological chemistry*. 281(36): p. 26268-26279.

167 Shah, P.P., et al., 2010, Swi2/Snf2-related translocases prevent accumulation of toxic Rad51 complexes during mitotic growth. *Molecular cell*. 39(6): p. 862-872.

168 Kateneva, A.V., et al., 2005, Recombination protein Tid1p controls resolution of cohesin-dependent linkages in meiosis in *Saccharomyces cerevisiae*. *The Journal of cell biology*. 171(2): p. 241-253.

169 Latypov, V.F., et al., 2010, [The Rdh54 protein role in regulation of DNA repair in yeast *Saccharomyces cerevisiae*]. *Genetika*. 46(2): p. 194-202.

170 Ishiai, M., et al., 1996, Purification, gene cloning, and reconstitution of the heterotrimeric single-stranded DNA-binding protein from *Schizosaccharomyces pombe*. *J Biol Chem*. 271(34): p. 20868-78.

171 Park, J.S., et al., 1997, A DNA helicase from *Schizosaccharomyces pombe* stimulated by single-stranded DNA-binding protein at low ATP concentration. *The Journal of biological chemistry*. 272(30): p. 18910-18919.

172 Parker, A.E., et al., 1997, The *Schizosaccharomyces pombe* rad11+ gene encodes the large subunit of replication protein A. *Mol Cell Biol.* 17(5): p. 2381-90.

173 Ostermann, K., A. Lorentz, and H. Schmidt, 1993, The fission yeast rad22 gene, having a function in mating-type switching and repair of DNA damages, encodes a protein homolog to Rad52 of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 21(25): p. 5940-4.

174 van den Bosch, M., et al., 2001, Characterization of RAD52 homologs in the fission yeast *Schizosaccharomyces pombe*. *Mutat Res.* 461(4): p. 311-23.

175 van den Bosch, M., et al., 2002, Differential expression and requirements for *Schizosaccharomyces pombe* RAD52 homologs in DNA repair and recombination. *Nucleic acids research.* 30(6): p. 1316-1324.

176 Kim, W.J., et al., 2000, Rad22 protein, a rad52 homologue in *Schizosaccharomyces pombe*, binds to DNA double-strand breaks. *J Biol Chem.* 275(45): p. 35607-11.

177 Octobre, G., et al., 2008, The Rad52 homologs Rad22 and Rti1 of *Schizosaccharomyces pombe* are not essential for meiotic interhomolog recombination, but are required for meiotic intrachromosomal recombination and mating-type-related DNA repair. *Genetics.* 178(4): p. 2399-412.

178 Trickey, M., M. Grimaldi, and H. Yamano, 2008, The anaphase-promoting complex/cyclosome controls repair and recombination by ubiquitylating Rhp54 in fission yeast. *Mol Cell Biol.* 28(12): p. 3905-16.

179 Muris, D.F., et al., 1996, Isolation of the *Schizosaccharomyces pombe* RAD54 homologue, rhp54+, a gene involved in the repair of radiation damage and replication fidelity. *Journal of cell science.* 109 (Pt 1): p. 73-81.

180 Muris, D.F., et al., 1997, Homologous recombination in the fission yeast *Schizosaccharomyces pombe*: different requirements for the rhp51+, rhp54+ and rad22+ genes. *Current genetics.* 31(3): p. 248-254.

181 Catlett, M.G. and S.L. Forsburg, 2003, *Schizosaccharomyces pombe* Rdh54 (TID1) acts with Rhp54 (RAD54) to repair meiotic double-strand breaks. *Molecular biology of the cell.* 14(11): p. 4707-4720.

182 Rice, M.C., et al., 1997, Isolation of human and mouse genes based on homology to REC2, a recombinational repair gene from the fungus *Ustilago maydis*. *Proceedings of the National Academy of Sciences of the United States of America.* 94(14): p. 7417-7422.

183 Peng, L., M.C. Rice, and E.B. Kmiec, 1998, Analysis of the human RAD51L1 promoter region and its activation by UV light. *Genomics.* 54(3): p. 529-41.

184 Miller, K.A., et al., 2002, RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51. *The Journal of biological chemistry.* 277(10): p. 8406-8411.

185 Lio, Y.C., et al., 2003, Complex formation by the human Rad51B and Rad51C DNA repair proteins and their activities in vitro. *J Biol Chem.* 278(4): p. 2469-78.

186 Havre, P.A., et al., 2000, HsRec2/Rad51L1, a protein influencing cell cycle progression, has protein kinase activity. *Exp Cell Res.* 254(1): p. 33-44.

187 Rodrigue, A., et al., 2012, The RAD51 paralogs ensure cellular protection against mitotic defects and aneuploidy. *Journal of cell science.*

188 Braybrooke, J.P., et al., 2000, The RAD51 family member, RAD51L3, is a DNA-stimulated ATPase that forms a complex with XRCC2. *J Biol Chem.* 275(37): p. 29100-6.

189 Schild, D., et al., 2000, Evidence for simultaneous protein interactions between human Rad51 paralogs. *The Journal of biological chemistry.* 275(22): p. 16443-16449.

190 Tarsounas, M., et al., 2004, Telomere maintenance requires the RAD51D recombination/repair protein. *Cell.* 117(3): p. 337-47.

191 Shim, K.S., et al., 2004, hXRCC2 enhances ADP/ATP processing and strand exchange by hRAD51. *The Journal of biological chemistry.* 279(29): p. 30385-30394.

192 Somyajit, K., et al., 2013, ATM- and ATR-mediated phosphorylation of XRCC3 regulates DNA double-strand break-induced checkpoint activation and repair. *Molecular and cellular biology.* 33(9): p. 1830-1844.

193 Badie, S., et al., 2009, RAD51C facilitates checkpoint signaling by promoting CHK2 phosphorylation. *J Cell Biol.* 185(4): p. 587-600.

194 Rodrigue, A., et al., 2006, Interplay between human DNA repair proteins at a unique double-strand break in vivo. *EMBO J.* 25(1): p. 222-31.

195 Wiese, C., et al., 2002, Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells. *Nucleic acids research.* 30(4): p. 1001-1008.

196 Lio, Y.C., et al., 2004, Human Rad51C deficiency destabilizes XRCC3, impairs recombination, and radiosensitizes S/G2-phase cells. *J Biol Chem.* 279(40): p. 42313-20.

197 Kuznetsov, S., et al., 2007, RAD51C deficiency in mice results in early prophase I arrest in males and sister chromatid separation at metaphase II in females. *J Cell Biol.* 176(5): p. 581-92.

198 Bennett, B.T. and K.L. Knight, 2005, Cellular localization of human Rad51C and regulation of ubiquitin-mediated proteolysis of Rad51. *J Cell Biochem.* 96(6): p. 1095-109.

199 Liu, Y., et al., 2004, RAD51C is required for Holliday junction processing in mammalian cells. *Science.* 303(5655): p. 243-6.

200 Forget, A.L., B.T. Bennett, and K.L. Knight, 2004, Xrcc3 is recruited to DNA double strand breaks early and independent of Rad51. *Journal of cellular biochemistry.* 93(3): p. 429-436.

201 Liu, N., et al., 1998, XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol Cell.* 1(6): p. 783-93.

202 Yoshihara, T., et al., 2004, XRCC3 deficiency results in a defect in recombination and increased endoreduplication in human cells. *EMBO J.* 23(3): p. 670-80.

203 Petermann, E., et al., 2010, Hydroxyurea-stalled replication forks become progressively inactivated and require two different RAD51-mediated pathways for restart and repair. *Mol Cell.* 37(4): p. 492-502.

204 Compton, S.A., et al., 2007, Xrcc3 and Nbs1 are required for the production of extrachromosomal telomeric circles in human alternative lengthening of telomere cells. *Cancer Res.* 67(4): p. 1513-9.

205 Sigurdsson, S., et al., 2001, Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. *Genes Dev.* 15(24): p. 3308-18.

206 Kurumizaka, H., et al., 2002, Homologous pairing and ring and filament structure formation activities of the human Xrcc2*Rad51D complex. *The Journal of biological chemistry.* 277(16): p. 14315-14320.

207 Braybrooke, J.P., et al., 2003, Functional Interaction between the Bloom's Syndrome Helicase and the RAD51 Paralog, RAD51L3 (RAD51D). *jbc.org.*

208 Compton, S.A., S. Ozgür, and J.D. Griffith, 2010, Ring-shaped Rad51 paralog protein complexes bind Holliday junctions and replication forks as visualized by electron microscopy. *The Journal of biological chemistry.* 285(18): p. 13349-13356.

209 Chun, J., E.S. Buechelmaier, and S.N. Powell, 2013, Rad51 paralog complexes BCDX2 and CX3 act at different stages in the BRCA1-BRCA2-dependent homologous recombination pathway. *Molecular and cellular biology.* 33(2): p. 387-395.

210 Yokoyama, H., et al., 2004, Preferential binding to branched DNA strands and strand-annealing activity of the human Rad51B, Rad51C, Rad51D and Xrcc2 protein complex. *Nucleic Acids Res.* 32(8): p. 2556-65.

211 Masson, J.Y., et al., 2001, Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes & development.* 15(24): p. 3296-3307.

212 Shu, Z., et al., 1999, Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(-/-) background. *Molecular and cellular biology.* 19(12): p. 8686-8693.

213 Gruver, A.M., et al., 2005, The ATPase motif in RAD51D is required for resistance to DNA interstrand crosslinking agents and interaction with RAD51C. *Mutagenesis.* 20(6): p. 433-440.

214 Pittman, D.L. and J.C. Schimenti, 2000, Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis.* 26(3): p. 167-73.

215 Rajesh, C., et al., 2009, The interaction profile of homologous recombination repair proteins RAD51C, RAD51D and XRCC2 as determined by proteomic analysis. *Proteomics.* 9(16): p. 4071-86.

216 Deans, B., et al., 2000, Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *EMBO J.* 19(24): p. 6675-85.

217 Liu, Y., et al., 2007, Role of RAD51C and XRCC3 in genetic recombination and DNA repair. *The Journal of biological chemistry.* 282(3): p. 1973-1979.

218 Kuznetsov, S.G., et al., 2009, Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. *Cancer research.* 69(3): p. 863-872.

219 Suwaki, N., K. Klare, and M. Tarsounas, 2011, RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. *Semin Cell Dev Biol.* 22(8): p. 898-905.

220 Urbin, S.S., et al., 2012, Uncoupling of RAD51 focus formation and cell survival after replication fork stalling in RAD51D null CHO cells. *Environmental and molecular mutagenesis.* 53(2): p. 114-124.

221 Jones, N.J., R. Cox, and J. Thacker, 1987, Isolation and cross-sensitivity of X-ray-sensitive mutants of V79-4 hamster cells. *Mutat Res.* 183(3): p. 279-86.

222 Thacker, J. and A.N. Ganesh, 1990, DNA-break repair, radioresistance of DNA synthesis, and camptothecin sensitivity in the radiation-sensitive irs mutants: comparisons to ataxia-telangiectasia cells. *Mutat Res.* 235(2): p. 49-58.

223 Caldecott, K. and P. Jeggo, 1991, Cross-sensitivity of gamma-ray-sensitive hamster mutants to cross-linking agents. *Mutat Res.* 255(2): p. 111-21.

224 Nagaraju, G., et al., 2009, XRCC2 and XRCC3 regulate the balance between short- and long-tract gene conversions between sister chromatids. *Molecular and cellular biology.* 29(15): p. 4283-4294.

225 Godthelp, B.C., et al., 2002, Mammalian Rad51C contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability.

226 Renglin Lindh, A., et al., 2007, RAD51C (RAD51L2) is involved in maintaining centrosome number in mitosis. *Cytogenetic and genome research.* 116(1-2): p. 38-45.

227 Bishop, D.K., et al., 1998, Xrcc3 is required for assembly of Rad51 complexes in vivo. *J Biol Chem.* 273(34): p. 21482-8.

228 Pierce, A.J., et al., 1999, XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes & development.* 13(20): p. 2633-2638.

229 Breneman, M.A., et al., 2002, XRCC3 controls the fidelity of homologous recombination: roles for XRCC3 in late stages of recombination. *Molecular cell.* 10(2): p. 387-395.

230 Henry-Mowatt, J., et al., 2003, XRCC3 and Rad51 Modulate Replication Fork Progression on Damaged Vertebrate Chromosomes. *Molecular cell.* 11(4): p. 1109-1117.

231 Yonetani, Y., et al., 2005, Differential and collaborative actions of Rad51 paralog proteins in cellular response to DNA damage. *Nucleic Acids Res.* 33(14): p. 4544-52.

232 Takata, M., et al., 2000, The Rad51 Paralog Rad51B Promotes Homologous Recombinational Repair.

233 Takata, M., et al., 2001, Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. *Molecular and cellular biology.* 21(8): p. 2858-2866.

234 Osakabe, K., et al., 2005, Arabidopsis Rad51B is important for double-strand DNA breaks repair in somatic cells. *Plant molecular biology.* 57(6): p. 819-833.

235 Bleuyard, J.Y., et al., 2005, Differing requirements for the Arabidopsis Rad51 paralogs in meiosis and DNA repair. *Plant J.* 41(4): p. 533-45.

236 Da Ines, O., et al., 2013, Effects of XRCC2 and RAD51B mutations on somatic and meiotic recombination in Arabidopsis thaliana. *Plant J.* 74(6): p. 959-70.

237 Serra, H., et al., 2013, Roles of XRCC2, RAD51B and RAD51D in RAD51-Independent SSA Recombination. *PLoS genetics.* 9(11): p. e1003971.

238 Durrant, W.E., S. Wang, and X. Dong, 2007, Arabidopsis SN11 and RAD51D regulate both gene transcription and DNA recombination during the defense response. *Proc Natl Acad Sci U S A.* 104(10): p. 4223-7.

239 Li, W., et al., 2005, The AtRAD51C gene is required for normal meiotic chromosome synapsis and double-stranded break repair in Arabidopsis. *Plant physiology.* 138(2): p. 965-976.

240 Abe, K., et al., 2005, Arabidopsis RAD51C gene is important for homologous recombination in meiosis and mitosis. *Plant physiology.* 139(2): p. 896-908.

241 Bleuyard, J.-Y. and C.I. White, 2004, The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis. *The EMBO journal.* 23(2): p. 439-449.

242 Bleuyard, J.Y., M.E. Gallego, and C.I. White, 2004, Meiotic defects in the Arabidopsis rad50 mutant point to conservation of the MRX complex function in early stages of meiotic recombination. *Chromosoma.* 113(4): p. 197-203.

243 Lin, Z., et al., 2006, Origins and evolution of the recA/RAD51 gene family: evidence for ancient gene duplication and endosymbiotic gene transfer. *Proceedings of the National Academy of Sciences of the United States of America.* 103(27): p. 10328-10333.

244 Sekelsky, J.J., M.H. Brodsky, and K.C. Burtis, 2000, DNA repair in Drosophila: insights from the Drosophila genome sequence. *J Cell Biol.* 150(2): p. F31-6.

245 Radford, S.J. and J.J. Sekelsky, 2004, Taking Drosophila Rad51 for a SPiN. *Nat Struct Mol Biol.* 11(1): p. 9-10.

246 Staeva-Vieira, E., S. Yoo, and R. Lehmann, 2003, An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. *The EMBO journal.* 22(21): p. 5863-5874.

247 Abdu, U., et al., 2003, The Drosophila spn-D gene encodes a RAD51C-like protein that is required exclusively during meiosis. *Genetics.* 165(1): p. 197-204.

248 Joyce, E.F., et al., 2012, Multiple barriers to nonhomologous DNA end joining during meiosis in Drosophila. *Genetics.* 191(3): p. 739-746.

249 Ward, J.D., et al., 2007, Replication blocking lesions present a unique substrate for homologous recombination. *The EMBO journal.* 26(14): p. 3384-3396.

250 Boulton, S.J., et al., 2002, Combined functional genomic maps of the C. elegans DNA damage response. *Science.* 295(5552): p. 127-31.

251 Martin, J.S., et al., 2005, RAD-51-dependent and -independent roles of a Caenorhabditis elegans BRCA2-related protein during DNA double-strand break repair. *Mol Cell Biol.* 25(8): p. 3127-39.

252 Yanowitz, J.L., 2008, Genome integrity is regulated by the Caenorhabditis elegans Rad51D homolog rfs-1. *Genetics.* 179(1): p. 249-262.

253 Ward, J.D., et al., 2010, Overlapping mechanisms promote postsynaptic RAD-51 filament disassembly during meiotic double-strand break repair. *Molecular cell.* 37(2): p. 259-272.

254 Thacker, J., 1999, A surfeit of RAD51-like genes? *Trends in genetics* : TIG. 15(5): p. 166-168.

255 Hays, S.L., A.A. Firmenich, and P. Berg, 1995, Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. *Proc Natl Acad Sci U S A.* 92(15): p. 6925-9.

256 Fortin, G.S. and L.S. Symington, 2002, Mutations in yeast Rad51 that partially bypass the requirement for Rad55 and Rad57 in DNA repair by increasing the stability of Rad51-DNA complexes. *The EMBO journal.* 21(12): p. 3160-3170.

257 Godin, S., et al., 2013, The Shu complex interacts with Rad51 through the Rad51 paralogues Rad55-Rad57 to mediate error-free recombination. *Nucleic acids research.*

258 Herzberg, K., et al., 2006, Phosphorylation of Rad55 on serines 2, 8, and 14 is required for efficient homologous recombination in the recovery of stalled replication forks. *Mol Cell Biol.* 26(22): p. 8396-409.

259 Bashkirov, V.I., et al., 2006, DNA damage-induced phosphorylation of Rad55 protein as a sentinel for DNA damage checkpoint activation in S. cerevisiae. *Methods Enzymol.* 409: p. 166-82.

260 Sung, P., 1997, Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes & development.* 11(9): p. 1111-1121.

261 Liu, J., et al., 2011, Rad51 paralogues Rad55-Rad57 balance the antirecombinase Srs2 in Rad51 filament formation. *Nature*. 479(7372): p. 245-8.
 262 Khasanov, F.K., et al., 1999, A New Recombinational DNA Repair Gene From *Schizosaccharomyces pombe* With Homology to *Escherichia coli* RecA.
 263 Tsutsui, Y., et al., 2001, Multiple interactions among the components of the recombinational DNA repair system in *Schizosaccharomyces pombe*. *Genetics*. 159(1): p. 91-105.
 264 Tsutsui, Y., et al., 2000, A recombination repair gene of *Schizosaccharomyces pombe*, *rhp57*, is a functional homolog of the *Saccharomyces cerevisiae* RAD57 gene and is phylogenetically related to the human XRCC3 gene. *Genetics*. 154(4): p. 1451-1461.
 265 Shor, E., J. Weinstein, and R. Rothstein, 2005, A genetic screen for top3 suppressors in *Saccharomyces cerevisiae* identifies SHU1, SHU2, PSY3 and CSM2: four genes involved in error-free DNA repair. *Genetics*. 169(3): p. 1275-1289.
 266 Martín, V., et al., 2006, Sws1 is a conserved regulator of homologous recombination in eukaryotic cells. *The EMBO journal*. 25(11): p. 2564-2574.
 267 Ball, L.G., et al., 2009, The yeast Shu complex couples error-free post-replication repair to homologous recombination. *Mol Microbiol*. 73(1): p. 89-102.
 268 Li, M., et al., 2011, Epistasis analysis between homologous recombination genes in *Saccharomyces cerevisiae* identifies multiple repair pathways for Sgs1, Mus81-Mms4 and RNase H2. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 714(1-2): p. 33-43.
 269 Bernstein, K.A., et al., 2011, The Shu complex, which contains Rad51 paralogues, promotes DNA repair through inhibition of the Srs2 anti-recombinase. *Molecular biology of the cell*. 22(9): p. 1599-1607.
 270 Hong, S. and K.P. Kim, 2013, Shu1 promotes homolog bias of meiotic recombination in *Saccharomyces cerevisiae*. *Molecules and cells*. 36(5): p. 446-454.
 271 Ito, T., et al., 2001, A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci U S A*. 98(8): p. 4569-74.
 272 Rabitsch, K.P., et al., 2001, A screen for genes required for meiosis and spore formation based on whole-genome expression. *Current biology* : CB. 11(13): p. 1001-1009.
 273 She, Z., et al., 2012, Structural and SAXS analysis of the budding yeast SHU-complex proteins. *FEBS letters*. 586(16): p. 2306-2312.
 274 Xu, X., et al., 2013, The Yeast Shu Complex Utilizes Homologous Recombination Machinery for Error-free Lesion Bypass via Physical Interaction with a Rad51 Parologue. *PLoS one*. 8(12): p. e81371.
 275 Wu, H.I., et al., 2004, Genome-wide identification of genes conferring resistance to the anticancer agents cisplatin, oxaliplatin, and mitomycin C. *Cancer research*. 64(11): p. 3940-3948.
 276 Sasanuma, H., et al., 2013, A new protein complex promoting the assembly of Rad51 filaments. *Nature communications*. 4: p. 1676.
 277 Tao, Y., et al., 2012, Structural analysis of Shu proteins reveals a DNA binding role essential for resisting damage. *The Journal of biological chemistry*. 287(24): p. 20231-20239.
 278 Mankouri, H.W., H.-P. Ngo, and I.D. Hickson, 2007, Shu proteins promote the formation of homologous recombination intermediates that are processed by Sgs1-Rmi1-Top3. *Molecular biology of the cell*. 18(10): p. 4062-4073.
 279 Choi, K., et al., 2010, The Smc5/6 complex and Esc2 influence multiple replication-associated recombination processes in *Saccharomyces cerevisiae*. *Molecular biology of the cell*. 21(13): p. 2306-2314.
 280 Khasanov, F.K., et al., 2004, Identification and characterization of the *rlp1+*, the novel Rad51 paralog in the fission yeast *Schizosaccharomyces pombe*. *DNA repair*. 3(10): p. 1363-1374.
 281 Grishchuk, A.L. and J. Kohli, 2003, Five RecA-like proteins of *Schizosaccharomyces pombe* are involved in meiotic recombination. *Genetics*. 165(3): p. 1031-43.
 282 Liu, T., et al., 2011, hSWS1-SWSAP1 is an evolutionarily conserved complex required for efficient homologous recombination repair. *The Journal of biological chemistry*. 286(48): p. 41758-41766.
 283 Qing, Y., et al., 2011, The epistatic relationship between BRCA2 and the other RAD51 mediators in homologous recombination. *PLoS genetics*. 7(7): p. e1002148.
 284 Scully, R., et al., 1997, Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell*. 88(2): p. 265-275.
 285 Chen, J., et al., 1998, Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Molecular cell*. 2(3): p. 317-328.
 286 Zhong, Q., et al., 1999, Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science (New York, N.Y.)*. 285(5428): p. 747-750.
 287 Paull, T.T., et al., 2001, Direct DNA binding by Brca1. *Proc Natl Acad Sci U S A*. 98(11): p. 6086-91.
 288 Hashizume, R., et al., 2001, The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *The Journal of biological chemistry*. 276(18): p. 14537-14540.
 289 Ruffner, H., et al., 2001, Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proceedings of the National Academy of Sciences of the United States of America*. 98(9): p. 5134-5139.
 290 Gudmundsdottir, K. and A. Ashworth, 2006, The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. *Oncogene*. 25(43): p. 5864-5874.
 291 Yun, M.H. and K. Hiom, 2009, CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature*. 459(7245): p. 460-463.
 292 Sy, S.M., M.S. Huen, and J. Chen, 2009, PALB2 is an integral component of the BRCA complex required for homologous recombination repair. *Proc Natl Acad Sci U S A*. 106(17): p. 7155-60.
 293 Zhang, F., et al., 2009, PALB2 links BRCA1 and BRCA2 in the DNA-damage response. *Curr Biol*. 19(6): p. 524-9.
 294 Zhang, F., et al., 2009, PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. *Mol Cancer Res*. 7(7): p. 1110-8.
 295 Westermarck, U.K., et al., 2003, BARD1 participates with BRCA1 in homology-directed repair of chromosome breaks. *Mol Cell Biol*. 23(21): p. 7926-36.
 296 Fabbro, M., et al., 2004, BARD1 regulates BRCA1 apoptotic function by a mechanism involving nuclear retention. *Exp Cell Res*. 298(2): p. 661-73.
 297 Xu, B., S. Kim, and M.B. Kastan, 2001, Involvement of Brca1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol Cell Biol*. 21(10): p. 3445-50.
 298 Shabbeer, S., et al., 2013, BRCA1 targets G2/M cell cycle proteins for ubiquitination and proteasomal degradation. *Oncogene*. 32(42): p. 5005-5016.
 299 Foray, N., et al., 2003, A subset of ATM- and ATR-dependent phosphorylation events requires the BRCA1 protein. *EMBO J*. 22(11): p. 2860-71.
 300 Ballal, R.D., et al., 2009, BRCA1 localization to the telomere and its loss from the telomere in response to DNA damage. *The Journal of biological chemistry*. 284(52): p. 36083-36098.
 301 Wong, A.K., et al., 1997, RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*. *The Journal of biological chemistry*. 272(51): p. 31941-31944.
 302 Liu, J., et al., 2010, Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. *Nat Struct Mol Biol*. 17(10): p. 1260-2.
 303 Thorslund, T. and S.C. West, 2007, BRCA2: a universal recombinase regulator. *Oncogene*. 26(56): p. 7720-30.
 304 Hussain, S., et al., 2004, Direct interaction of FANCD2 with BRCA2 in DNA damage response pathways. *Human molecular genetics*. 13(12): p. 1241-1248.

305 Yang, H., et al., 2002, BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* (New York, N.Y.). 297(5588): p. 1837-1848.
 306 Xia, F., et al., 2001, Deficiency of human BRCA2 leads to impaired homologous recombination but maintains normal nonhomologous end joining. *Proc Natl Acad Sci U S A*. 98(15): p. 8644-9.
 307 Thorslund, T., et al., 2010, The breast cancer tumor suppressor BRCA2 promotes the specific targeting of RAD51 to single-stranded DNA. *Nat Struct Mol Biol*. 17(10): p. 1263-5.
 308 Xia, B., et al., 2006, Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell*. 22(6): p. 719-29.
 309 Sharan, S.K. and A. Bradley, 1997, Murine Brca2: sequence, map position, and expression pattern. *Genomics*. 40(2): p. 234-241.
 310 Yuan, S.S., et al., 1999, BRCA2 is required for ionizing radiation-induced assembly of Rad51 complex in vivo. *Cancer Res*. 59(15): p. 3547-51.
 311 Chen, P.L., et al., 1998, The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proceedings of the National Academy of Sciences of the United States of America*. 95(9): p. 5287-5292.
 312 Buisson, R., et al., 2014, Breast cancer proteins PALB2 and BRCA2 stimulate polymerase η in recombination-associated DNA synthesis at blocked replication forks. *Cell reports*. 6(3): p. 553-564.
 313 Hakem, R., et al., 1996, The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell*. 85(7): p. 1009-1023.
 314 Evers, B. and J. Jonkers, 2006, Mouse models of BRCA1 and BRCA2 deficiency: past lessons, current understanding and future prospects. *Oncogene*. 25(43): p. 5885-5897.
 315 Deng, C.X. and F. Scott, 2000, Role of the tumor suppressor gene Brca1 in genetic stability and mammary gland tumor formation. *Oncogene*. 19(8): p. 1059-64.
 316 Shen, S.X., et al., 1998, A targeted disruption of the murine Brca1 gene causes gamma-irradiation hypersensitivity and genetic instability. *Oncogene*. 17(24): p. 3115-24.
 317 Xu, X., et al., 1999, Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat Genet*. 22(1): p. 37-43.
 318 McPherson, J.P., et al., 2006, A role for Brca1 in chromosome end maintenance. *hmg.oxfordjournals.org*.
 319 Moynahan, M.E., et al., 1999, Brca1 controls homology-directed DNA repair. *Molecular cell*. 4(4): p. 511-518.
 320 Moynahan, M.E., T.Y. Cui, and M. Jasin, 2001, Homology-directed dna repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. *Cancer research*. 61(12): p. 4842-4850.
 321 Bhattacharyya, A., et al., 2000, The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. *The Journal of biological chemistry*. 275(31): p. 23899-23903.
 322 Stark, J.M., et al., 2004, Genetic steps of mammalian homologous repair with distinct mutagenic consequences. *Molecular and cellular biology*. 24(21): p. 9305-9316.
 323 Connor, F., et al., 1997, Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. *Nat Genet*. 17(4): p. 423-30.
 324 Blackshear, P.E., et al., 1998, Brca1 and Brca2 expression patterns in mitotic and meiotic cells of mice. *Oncogene*. 16(1): p. 61-8.
 325 Suzuki, A., et al., 1997, Brca2 is required for embryonic cellular proliferation in the mouse. *Genes Dev*. 11(10): p. 1242-52.
 326 Hakem, R., J.L. de la Pompa, and T.W. Mak, 1998, Developmental studies of Brca1 and Brca2 knock-out mice. *Journal of mammary gland biology and neoplasia*. 3(4): p. 431-445.
 327 Patel, K.J., et al., 1998, Involvement of Brca2 in DNA repair. *Molecular cell*. 1(3): p. 347-357.
 328 Sharan, S.K., et al., 2004, BRCA2 deficiency in mice leads to meiotic impairment and infertility. *Development*. 131(1): p. 131-42.
 329 Schlacher, K., et al., 2011, Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell*. 145(4): p. 529-42.
 330 Warren, M., et al., 2002, Structural analysis of the chicken BRCA2 gene facilitates identification of functional domains and disease causing mutations. *Human molecular genetics*. 11(7): p. 841-851.
 331 Warren, M., et al., 2003, Phenotypic effects of heterozygosity for a BRCA2 mutation. *Human molecular genetics*. 12(20): p. 2645-2656.
 332 Howe, K., et al., 2013, The zebrafish reference genome sequence and its relationship to the human genome. *Nature*. 496(7446): p. 498-503.
 333 Rodríguez-Mari, A., et al., 2011, Roles of brca2 (fancd1) in oocyte nuclear architecture, gametogenesis, gonad tumors, and genome stability in zebrafish. *PLoS genetics*. 7(3): p. e1001357.
 334 Shive, H.R., et al., 2010, brca2 in zebrafish ovarian development, spermatogenesis, and tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 107(45): p. 19350-19355.
 335 Meetei, A.R., Z. Yan, and W. Wang, 2004, FANCL replaces BRCA1 as the likely ubiquitin ligase responsible for FANCD2 monoubiquitination. *Cell cycle* (Georgetown, Tex.). 3(2): p. 179-181.
 336 Brough, R., et al., 2008, Functional analysis of Drosophila melanogaster BRCA2 in DNA repair. *DNA repair*. 7(1): p. 10-19.
 337 Klovstad, M., U. Abdu, and T. Schüpbach, 2008, Drosophila brca2 is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. *PLoS genetics*. 4(2): p. e31.
 338 Boulton, S.J., et al., 2004, BRCA1/BARD1 orthologs required for DNA repair in *Caenorhabditis elegans*. *Current biology : CB*. 14(1): p. 33-39.
 339 Lo, T., et al., 2003, Sequence fingerprints in BRCA2 and RAD51: implications for DNA repair and cancer. *DNA repair*. 2(9): p. 1015-1028.
 340 Ko, E., J. Lee, and H. Lee, 2008, Essential role of brc-2 in chromosome integrity of germ cells in *C. elegans*. *Mol Cells*. 26(6): p. 590-4.
 341 Petalcorin, M.I., et al., 2007, Stabilization of RAD-51-DNA filaments via an interaction domain in *Caenorhabditis elegans* BRCA2. *Proc Natl Acad Sci U S A*. 104(20): p. 8299-304.
 342 Petalcorin, M.I.R., et al., 2006, CeBRC-2 stimulates D-loop formation by RAD-51 and promotes DNA single-strand annealing. *Journal of molecular biology*. 361(2): p. 231-242.
 343 Lafarge, S. and M.-H. Montané, 2003, Characterization of Arabidopsis thaliana ortholog of the human breast cancer susceptibility gene 1: AtBRCA1, strongly induced by gamma rays. *NAR*. 31(4): p. 1148-1155.
 344 Block-Schmidt, A.S., et al., 2011, BRCC36A is epistatic to BRCA1 in DNA crosslink repair and homologous recombination in Arabidopsis thaliana. *Nucleic Acids Res*. 39(1): p. 146-54.
 345 Reidt, W., et al., 2006, A homologue of the breast cancer-associated gene BARD1 is involved in DNA repair in plants. *The EMBO journal*. 25(18): p. 4326-4337.
 346 Siaud, N., et al., 2004, Brca2 is involved in meiosis in Arabidopsis thaliana as suggested by its interaction with Dmc1. *The EMBO journal*. 23(6): p. 1392-1401.
 347 Wang, S., et al., 2010, Arabidopsis BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses. *Proc Natl Acad Sci U S A*. 107(52): p. 22716-21.
 348 Dray, E., et al., 2006, Interaction between Arabidopsis Brca2 and its partners Rad51, Dmc1, and Dss1. *Plant Physiol*. 140(3): p. 1059-69.
 349 Abe, K., et al., 2009, Inefficient double-strand DNA break repair is associated with increased fasciation in Arabidopsis BRCA2 mutants. *Journal of experimental botany*. 60(9): p. 2751-2761.
 350 Seeliger, K., et al., 2012, BRCA2 is a mediator of RAD51- and DMC1-facilitated homologous recombination in Arabidopsis thaliana. *New Phytol*. 193(2): p. 364-75.

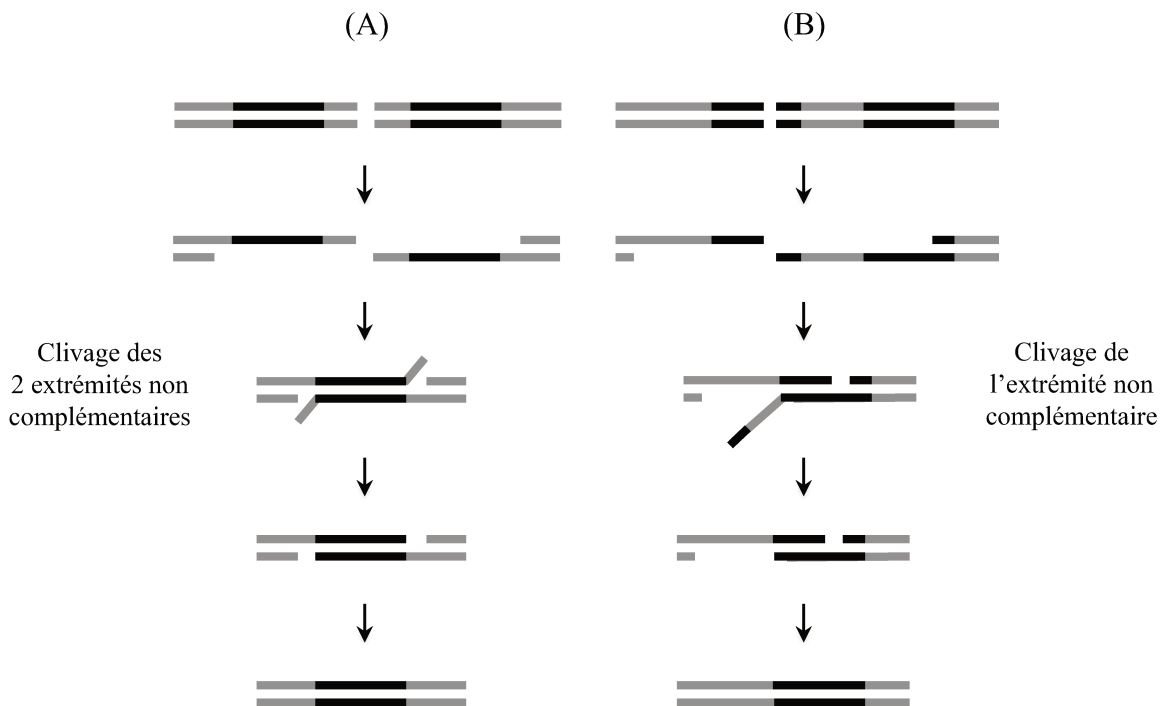


Figure 13 : La réparation d'une CDB par SSA implique l'élimination de deux (A) ou d'une (B) extrémité(s) non complémentaire(s) selon la localisation de la cassure.

- (A) Quand la CDB se produit entre deux régions homologues (traits noirs), leur appariement génère deux extrémités d'ADN simple brin 3' sortantes qui devront être clivées pour restaurer l'intégrité de la molécule d'ADN.
- (B) Quand la CDB se produit dans l'une des répétitions, une seule extrémité d'ADN doit être éliminée pour amorcer la synthèse d'ADN nécessaire à la réparation.

II.3. Les protéines impliquées dans l'élimination des extrémités non complémentaires

Au cours de la voie d'hybridation simple brin (SSA), les deux régions homologues situées de part et d'autre de la CDB s'apparient grâce à l'activité de RAD52 (et Rad59 chez la levure). Cependant, les extrémités d'ADNsb 3' sortantes ne peuvent s'apparier puisqu'elles ne sont pas complémentaires des nouvelles régions flanquantes (**Figure 13 A**). Ces extrémités doivent donc être clivées pour permettre la réparation de chacun des brins. Dans le cas où la CDB se produit dans une des séquences répétées, la réparation par SSA n'implique le clivage que d'une seule extrémité 3' (**Figure 13 B**).

La recombinaison homologue de type conversion génique (DSBR, SDSA, BIR) peut également nécessiter l'élimination d'une ou plusieurs extrémités 3' non complémentaires. Dans la voie SDSA, voie privilégiée pour la réparation des CDB somatiques (Ira *et al.*, 2006), le clivage de ces extrémités est un prérequis à l'initiation de la synthèse d'ADN à deux étapes distinctes : lors de l'invasion du brin dans la double hélice d'ADN non endommagée et lors de l'appariement du brin envahisseur à l'autre extrémité de la cassure (**Figure 14**). Il a été montré que la présence de séquences non homologues au niveau d'une ou des deux extrémités de la CDB n'affecte pas le choix de la voie de réparation et la proportion des produits de CO et de NCO (Ira *et al.*, 2006).

La majorité des études portant sur l'identification et la caractérisation des protéines impliquées dans l'élimination des extrémités non complémentaires ont été conduites chez la levure. Le clivage de ce type d'extrémités est assuré chez *S. cerevisiae* par le complexe Rad1-Rad10 (Fishman-Lobell & Haber, 1992 ; Bardwell *et al.*, 1994 ; Ivanov & Haber, 1995). Il reconnaît les transitions entre ADNsb et ADNdb et clive spécifiquement le brin contenant l'extrémité d'ADNsb 3' sortante (Bardwell *et al.*, 1994). Ce complexe a initialement été identifié comme un acteur du système de réparation par excision de nucléotide (pour revue,

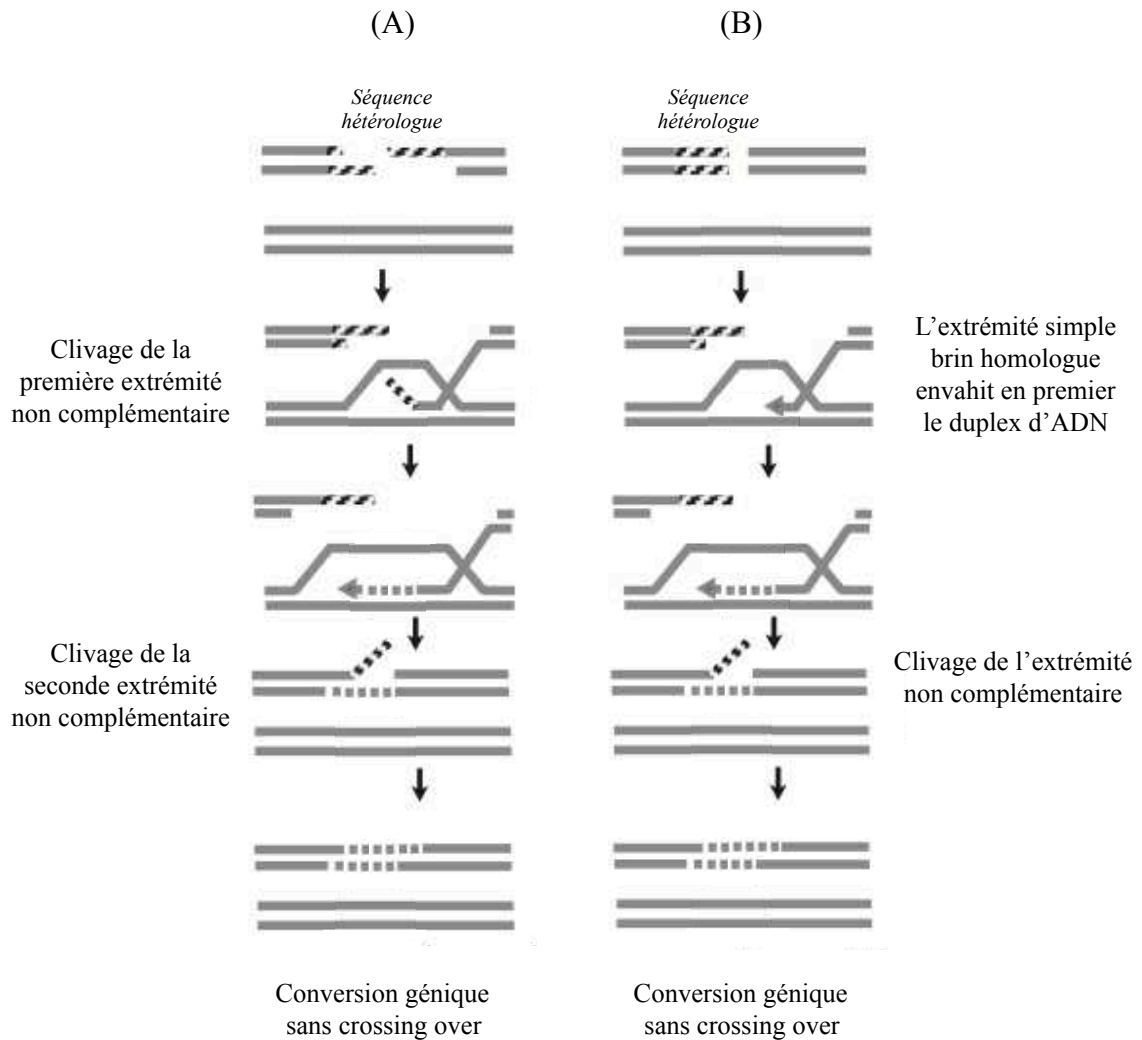


Figure 14 : La réparation par SDSA d'une CDB située dans une séquence hétérologue implique l'élimination de deux (A) ou d'une (B) extrémité(s) non homologue(s) (d'après Ira *et al.*, 2006)

- (A) Si une CDB se forme dans une séquence hétérologue (ex : site de restriction de l'enzyme I-SceI), les deux extrémités de la cassure contiennent des séquences non homologues au locus donneur (régions hachurées). L'extrémité 3' non complémentaire du brin réalisant l'invasion doit être éliminée pour initier la synthèse d'ADN utilisant comme matrice la séquence donneuse. Le brin nouvellement synthétisé peut ensuite s'apparier avec l'autre extrémité de la cassure. La non complémentarité des séquences du brin néosynthétisé et du brin d'origine nécessite l'élimination de l'extrémité d'ADNsb 3' sortante.
- (B) Si une CDB se forme à une extrémité d'une séquence hétérologue, seule une extrémité de la CDB contient une séquence non homologue au locus donneur (région hachurée). Dans ce cas, c'est principalement l'extrémité homologue qui réalise l'invasion du duplex homologue et initie la synthèse d'ADN. La suite du processus se déroule comme en (A).

voir Prakash and Prakash, 2000). Des analyses ultérieures ont montré que le complexe Rad1-Rad10 est nécessaire au clivage des séquences non homologues au cours du SSA et de la conversion génique (CG) (Fishman-Lobell & Haber, 1992 ; Ivanov & Haber, 1995). Les autres protéines du NER ne sont en revanche pas impliquées dans ce processus (Ivanov & Haber, 1995).

Les protéines Msh2 et Msh3 sont de plus requises à l'élimination des extrémités non homologues dépendante de Rad1-Rad10 (Sugawara *et al.*, 1997 ; Studamire *et al.*, 1999 ; Langston & Symington, 2005 ; Surtees & Alani, 2006). Ces deux protéines forment un complexe de reconnaissance des mésappariements d'ADN impliqué dans le système de réparation des mésappariements. Msh2-Msh3 reconnaît également des structures d'ADN branchées avec une extrémité 3' libre (Sugawara *et al.*, 1997) et se lie rapidement et spécifiquement à la jonction entre ADNsb et ADNdb (Evans *et al.*, 2000 ; Surtees & Alani, 2006 ; Lyndaker *et al.*, 2008). Cette liaison modifie la conformation du complexe à l'origine d'une légère ouverture de la jonction d'ADN (Surtees & Alani, 2006). Grâce à la stabilisation de ces jonctions, Msh2-Msh3 facilite l'élimination des extrémités non homologues dépendante de Rad1-Rad10 au cours du SSA et de la CG (Sugawara *et al.*, 1997 ; Studamire *et al.*, 1999). Aucun autre facteur du système de réparation des mésappariements n'est requis dans ce processus (Saparbaev *et al.*, 1996 ; Sugawara *et al.*, 1997 ; Langston & Symington, 2005).

Des travaux plus récents ont identifié deux nouveaux acteurs de l'élimination de ce type d'extrémités chez la levure : Slx4 (*Synthetic lethal of unknown function protein 4*) et Saw1 (*Single-strand annealing weakened 1*) (Flott *et al.*, 2007 ; Li *et al.*, 2008). Slx4 est une sous-unité du complexe Slx1-Slx4, initialement identifié comme indispensable à la survie cellulaire en absence de l'hélicase Sgs1 (Mullen *et al.*, 2001). Slx4 est phosphorylé en réponse aux dommages de l'ADN grâce aux kinases Mec1 et Tel1 (Flott & Rouse, 2005 ; Flott *et al.*, 2007). Cette activation de Slx4 joue un rôle essentiel dans la réparation par SSA mais le

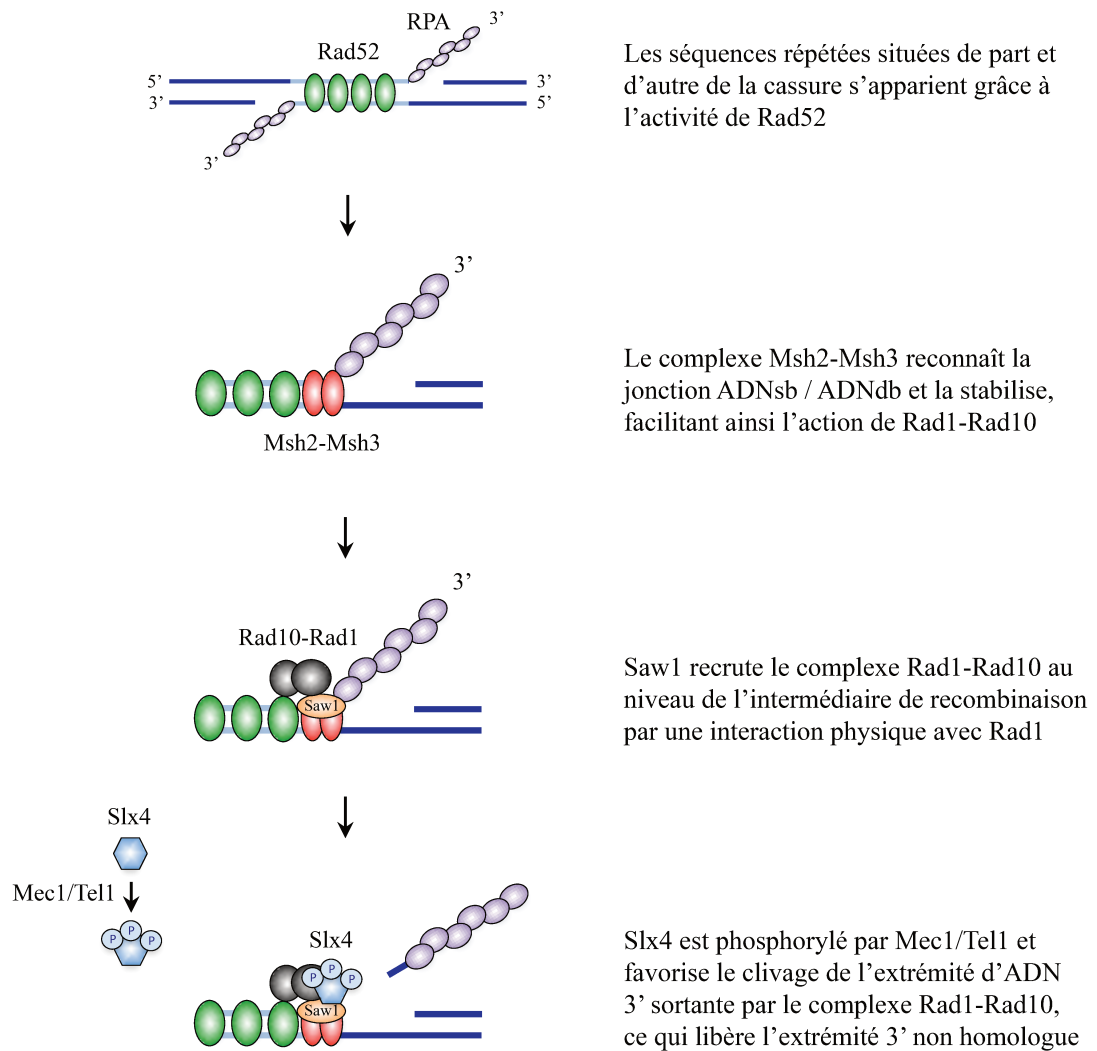
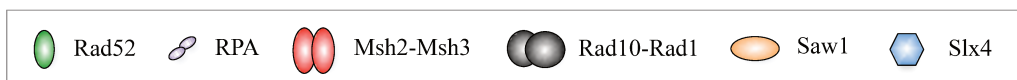


Figure 15 : Modèle d'élimination des longues extrémités 3' non homologues au cours du SSA chez *Saccharomyces cerevisiae* (d'après Lyndaker *et al.*, 2009 et Li *et al.*, 2013)



mécanisme précis n'est pas connu (Toh *et al.*, 2010). Saw1 interagit physiquement avec plusieurs acteurs du SSA : Rad1-Rad10, Msh2-Msh3 et Rad52 (Li *et al.*, 2008). La délétion de Saw1 ou l'expression d'une protéine Saw1 mutée dans son domaine d'interaction avec Rad1-Rad10 abolit la liaison de Rad1-Rad10 aux extrémités d'ADNsb 3' sortantes et bloque le SSA (Li *et al.*, 2008). Une étude récente a de plus montré que Saw1 est une protéine de liaison à l'ADN qui se lie spécifiquement et avec une haute affinité aux structures d'ADN branchées et facilite le recrutement de Rad1 au niveau des extrémités 3' *in vivo* et *in vitro* (Li *et al.*, 2013). Saw1 favorise également l'activité de clivage du complexe Rad1-Rad10 *in vitro* (Li *et al.*, 2013). L'ensemble de ces données indique que Saw1 joue (au minimum) un rôle essentiel dans le recrutement de Rad1-Rad10 au niveau des intermédiaires de recombinaison présentant une extrémité d'ADNsb 3' sortante. Le modèle actuel d'élimination des longues extrémités 3' non homologues au cours du SSA est présenté **figure 15**.

Cependant, l'implication de Rad1, Rad10, Msh2, Msh3 et Saw1 dans le SSA et la CG dépend du nombre et de la longueur des extrémités non homologues (Pâques & Haber, 1997 ; Colaiácovo *et al.*, 1999). En effet, quand ces extrémités ont une longueur inférieure à 30 nucléotides, la CG reste efficace en absence de Rad1, Msh2 ou Msh3 (Pâques & Haber, 1997). Ces protéines sont donc essentielles à la CG uniquement quand les deux extrémités de la CDB contiennent des séquences non homologues de plus de 30 nucléotides. En accord avec ces résultats, il a été montré récemment que l'affinité de Saw1 pour les structures d'ADN branchées dépend de la longueur de l'extrémité 3' sortante : Saw1 ne se lie pas aux extrémités dont la taille est inférieure à 10 nucléotides, puis l'affinité de la protéine augmente avec la longueur de l'extrémité (Li *et al.*, 2013). Les extrémités courtes sont prises en charge par un mécanisme indépendant de Rad1-Rad10 et Msh2-Msh3 qui dépend de l'activité de relecture 3'-5' de la polymérase δ (Pâques & Haber, 1997). De plus, Colaiacovo *et al.* ont montré que dans les mutants *rad1* ou *msh2*, quand les deux extrémités de la CDB présentent de longues

séquences non homologues, l'efficacité de la CG est réduite d'un facteur 90 comparé à une CDB sans extrémité non homologue. En revanche, quand une seule longue extrémité est présente, l'efficacité de la réparation n'est réduite que d'un facteur 5 (Colaiácovo *et al.*, 1999). Il existe donc chez la levure une voie alternative d'élimination des extrémités non homologues mais celle-ci est moins efficace que le mécanisme dépendant de Rad1-Rad10. Msh2 inhiberait cette voie alternative (Manthey *et al.*, 2009). L'hypothèse proposée pour expliquer que Rad1-Rad10 est indispensable seulement quand deux extrémités non homologues sont présentes est la suivante. Lors de l'invasion initiale de l'ADNsb dans le duplexe homologue, la présence d'une extrémité non complémentaire rend la jonction particulièrement instable et serait un bon substrat pour Rad1-Rad10 (Sugawara *et al.*, 1997). Si une seule extrémité de CDB contient une séquence non homologue, c'est l'autre extrémité qui assurera l'invasion de brin (**Figure 14 A**). La jonction formée ensuite au niveau de l'extrémité non invasive serait une structure plus stable (puisque une partie de la molécule est correctement appariée), un substrat moins efficace pour Rad1-Rad10.

Chez les Mammifères, XPF et ERCC1 (les homologues de Rad1 et Rad10, respectivement) forment également un hétérodimère capable de cliver spécifiquement les extrémités d'ADNsb 3' sortantes à la jonction entre ADNsb et ADNdb (Biggerstaff *et al.*, 1993). Alors que ERCC1 est catalytiquement inactif, XPF fournit l'activité endonucléasique structure spécifique du complexe. Chacun d'eux est instable en absence de son partenaire (Gaillard & Wood, 2001). En plus de son rôle essentiel dans le NER, il a été démontré que le complexe XPF-ERCC1 est requis dans les voies du SSA et de la CG dans les cellules de Mammifères (Adair, 2000 ; Sargent *et al.*, 2000 ; Al-Minawi *et al.*, 2008). Ce rôle est cependant dépendant de la longueur des extrémités non homologues : XPF-ERCC1 est indispensable au clivage des longues extrémités (> 270 nucléotides), alors que les extrémités courtes (11 et 18 nucléotides) sont efficacement éliminées en absence de ERCC1 (Adair, 2000 ; Sargent *et al.*, 2000). Ces résultats mettent donc en évidence une excellente conservation des

fonctions de ce complexe entre la levure et les Mammifères. Motycka *et al.* ont de plus démontré que la protéine XPF humaine interagit directement avec la protéine RAD52. Cette interaction stimule d'une part l'activité endonucléasique de XPF-ERCC1 et atténue d'autre part l'activité d'hybridation de brins de RAD52 (Motycka *et al.*, 2004), ce qui favorise par conséquent l'élimination des extrémités 3' des intermédiaires de recombinaison.

Chez *Arabidopsis*, le rôle de AtXPF et AtERCC1 dans l'élimination de longues extrémités non homologues (> 230 nucléotides) au cours du SSA a été vérifié (Dubest *et al.*, 2002 ; Dubest *et al.*, 2004). Dubest *et al.* (2004) ont également montré que AtERCC1 est impliqué dans la réparation de substrats de recombinaison présentant des séquences répétées en orientation inverse. L'utilisation de ce type de cassettes permet de quantifier les événements de CG chromosomiques. La position de la CDB dans le substrat étant aléatoire, sa réparation implique dans la majorité des cas l'élimination de longues extrémités non homologues. Le complexe AtXPF-AtERCC1 est donc également requis au clivage des longues extrémités non complémentaires au cours de la CG chez les plantes (Dubest *et al.*, 2004).

II.4. Les protéines impliquées dans la dissociation des intermédiaires de recombinaison

Au cours des voies de RH dépendantes de RAD51, l'invasion d'une région homologe par une extrémité d'ADNsb 3' sortante aboutit à formation de la boucle D. Suite à la synthèse d'ADN initiée à partir de l'extrémité 3', les deux molécules jointes doivent être dissociées pour achever le processus. Dans le cas d'une réparation par SDSA, le brin nouvellement synthétisé est dissocié du duplexe d'ADN par l'action d'hélicases, telles que Srs2, Sgs1 et Mph1 chez *S.cerevisiae* (Ira *et al.*, 2003 ; Dupaigne *et al.*, 2008 ; Muira *et al.*, 2012 ; Mitchel *et al.*, 2013). La situation est plus complexe dans le cas d'une réparation de type DSBR puisque la capture de l'extrémité non invasive (par une liaison covalente avec le brin néosynthétisé)

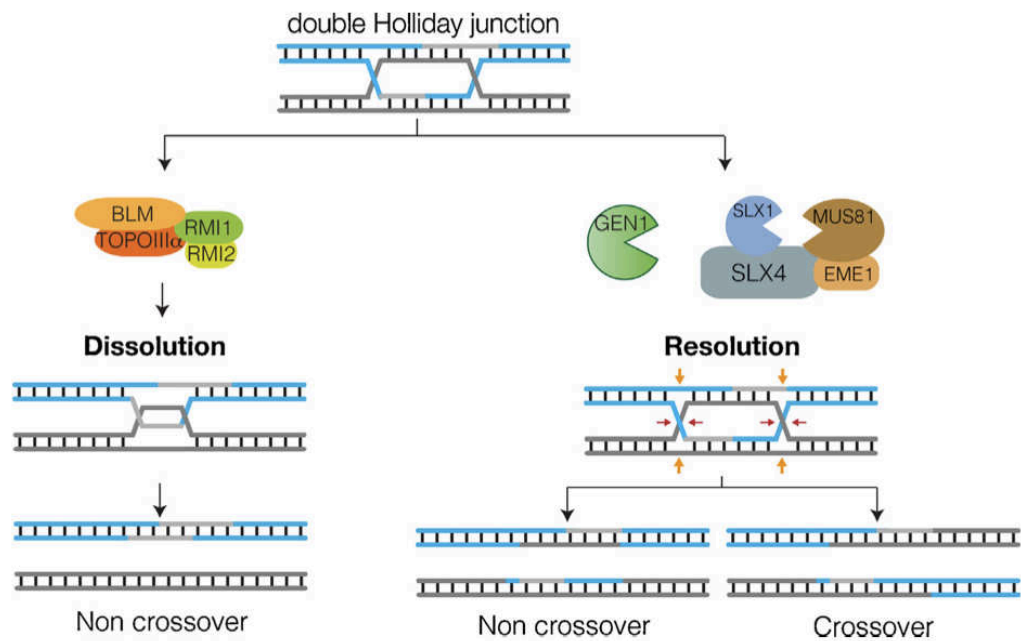


Figure 16 : Voies de dissolution et de résolution des doubles jonctions de Holliday chez les Mammifères (d'après Matos *et al.*, 2014)

Le complexe BLM-TOPOIII α -RMI1-RMI2 désengage la double JH par un mécanisme de « dissolution » et génère exclusivement des produits de non crossing-over. MUS81-EME1 et SLX1-SLX4 interagissent pour former un complexe qui résout les JH par clivage endonucléasique et peut générer des produits de crossing-over ou non crossing-over selon l'orientation des clivages. GEN1 constitue une voie parallèle de résolution des JH.

conduit à la formation d'une double jonction de Holliday (JH) (Szostak *et al.*, 1983) (**Figure 8**). La séparation des deux molécules d'ADN implique soit la dissolution, soit la résolution de cette double jonction, chacun de ces processus nécessitant l'action de protéines différentes.

II.4.1. Les protéines de la dissolution des jonctions de Holliday

La dissolution des dJH est assurée par l'action combinée d'une hélicase, qui permet le rapprochement des JH par migration des branches, et d'une topoisomérase, indispensable au relâchement des surenroulements générés. La rencontre des deux JH forme une structure hémicaténaire, qui sera prise en charge par la topoisomérase pour séparer les deux doubles hélices d'ADN (**Figure 16**). Chez *S. cerevisiae*, la dissolution des JH est assurée par l'hélicase Sgs1 associée en complexe avec la topoisomérase Top3 et la protéine Rmi1 (*RecQ-mediated genome instability*) (Chang *et al.*, 2005 ; Mullen *et al.*, 2005). Rmi1, grâce à sa haute affinité pour les structures d'ADN cruciformes, permettrait le recrutement de Sgs1-Top3 au niveau des JH (Mullen *et al.*, 2005 ; Cejka *et al.*, 2010b).

Chez les Mammifères comme chez les plantes, des homologues des différentes protéines de levure ont été identifiés. La protéine BLM (*Bloom syndrom protein*) humaine et la protéine AtRECQ4A d'*Arabidopsis* sont les homologues de Sgs1 ; TOPOIII α et AtTOP3a, les homologues de Top3 et enfin RMI1, RMI2 et AtRMI1, les homologues de Rmi1. Les fonctions de chacune de ces protéines sont extrêmement bien conservées au sein des Eucaryotes (Hartung *et al.*, 2008 ; Bernstein *et al.*, 2010).

Dans les cellules mitotiques, la dissolution des JH semble être la voie privilégiée de réparation des intermédiaires de recombinaison (pour revue, voir Heyer *et al.*, 2010). La réaction de dissolution des JH, qui aboutit exclusivement à des produits de NCO, est essentielle pour éviter les CO, comme le montre l'augmentation de la fréquence des CO dans les cellules déficientes pour BLM (Wu & Hickson, 2003).

II.4.2. Les protéines de la résolution des jonctions de Holliday

Une activité résolvasse des JH a pour la première fois été observée dans des extraits préparés à partir de tissus de thymus de veau (Elborough & West, 1990) puis à partir de cellules en culture (Hyde *et al.*, 1994 ; Constantinou *et al.*, 2001). L'existence de plusieurs résolvasse chez les Eucaryotes a compliqué la recherche de simples mutants déficients pour la résolution des JH. D'abord, l'hétérodimère Mus81-Mms4 (MUS81-EME1 chez les Mammifères) a été identifié chez la levure *S. pombe* (Boddy *et al.*, 2001) et chez l'Homme (Chen *et al.*, 2001) comme une endonucléase capable de cliver les JH, ainsi qu'une large gamme de structures branchées. La protéine MUS81 (une protéine homologue de XPF) possède l'activité endonucléasique du complexe, EME1 est en revanche une sous-unité non catalytique. Cependant, la capacité du complexe à cliver les JH intactes est très limitée par rapport aux autres types de structures, ce qui suggère que Mus81-Mms4 pourrait prendre en charge des JH déjà maturées (dont un brin est déjà clivé par exemple) (Bastin-Shanower *et al.*, 2003 ; Ciccio *et al.*, 2003).

Un deuxième hétérodimère à activité endonucléasique structure spécifique a été identifié chez plusieurs organismes dont la levure et l'Homme, il s'agit de SLX1-SLX4. Ce complexe est également capable de cliver les JH (Fekairi *et al.*, 2009 ; Muñoz *et al.*, 2009 ; Svendsen *et al.*, 2009). SLX1 possède le domaine endonucléase alors que SLX4 réalise les interactions avec de multiples nucléases impliquées dans le clivage (symétrique ou asymétrique) des JH (pour revue, voir Svendsen & Harper, 2010). Il a été montré récemment que SLX1-SLX4 interagit directement avec MUS81-EME1 et que ce complexe multiprotéique agit de manière plus efficace que les hétérodimères individuels (Wyatt *et al.*, 2013). L'augmentation de la résolution des JH requière l'action coordonnée des activités nucléases de MUS81 et SLX1 (Wyatt *et al.*, 2013).

Des travaux récents, menés au sein du laboratoire de Stephen West, ont permis l'identification de la protéine humaine GEN1 - une nouvelle résolvasse des JH - et son orthologue Yen1 chez la levure (Ip *et al.*, 2008 ; Rass *et al.*, 2010). GEN1 lie spécifiquement les JH et permet leur résolution par un mécanisme de double incision dans lequel chacun des brins d'ADN de la jonction est clivé symétriquement et simultanément (Rass *et al.*, 2010). Bien que GEN1 soit une endonucléase monomérique, deux monomères GEN1 sont capables de s'associer au niveau de la JH. Cette dimérisation est nécessaire à la résolution efficace des JH, puisqu'elle fournit les deux sites actifs requis au double clivage (Rass *et al.*, 2010). Des analyses d'épistasie ont révélé que GEN1 agit dans une voie de résolution des JH distincte de la voie dépendante de MUS81-EME1 et SLX1-SLX4 dans les cellules humaines (Wyatt *et al.*, 2013). Il existe donc chez les Mammifères comme chez la levure, deux voies de résolution des JH (**Figure 16**). Selon l'orientation des clivages, ces voies peuvent aboutir à des produits de CO ou de NCO (**Figures 8 et 16**).

Les mécanismes de résolution des JH chez les plantes n'ont pas encore été élucidés. Un orthologue de MUS81 a été identifié chez *Arabidopsis* (AtMUS81) ainsi que deux orthologues de EME1 (AtEME1a et b). Des analyses biochimiques ont montré que les deux complexes AtMUS81-AtEME1a et AtMUS81-AtEME1b sont capables de cliver les JH (Hartung *et al.*, 2006 ; Geuting *et al.*, 2009). De plus, le génome d'*Arabidopsis* contient deux orthologues de GEN1 (Osman *et al.*, 2011) mais leurs fonctions respectives restent à déterminer.

Projet de thèse

La réparation des cassures double brin de l'ADN par recombinaison est un processus fondamental au maintien de l'intégrité du génome. L'absence ou la mauvaise réparation d'une seule de ces lésions peut en effet avoir des conséquences dramatiques à l'échelle de la cellule, voire de l'organisme (mort cellulaire, tumorigenèse...). Dans une cellule sauvage, les CDB de l'ADN sont prises en charge par les voies de recombinaison homologue et non homologue. Chacune d'elle implique l'action coordonnée de nombreuses protéines, dont les fonctions sont extrêmement bien conservées.

La plante *Arabidopsis thaliana* constitue un modèle de choix pour l'étude des voies de recombinaison puisque des mutations dans les protéines clés n'affectent pas le développement végétatif de la plante, alors que ces mêmes mutations peuvent être extrêmement délétères chez les autres organismes. *Arabidopsis* permet donc l'étude des acteurs de la réparation des CDB à l'échelle de l'organisme, mais aussi les analyses d'épistasie puisqu'il est possible de générer des mutants multiples. Le génome d'*Arabidopsis* est de plus entièrement séquencé et de petite taille (environ 125 Mb répartis sur cinq chromosomes) (Arabidopsis Genome Initiative, 2000). Son cycle de vie relativement court et sa petite taille sont également des avantages pratiques intéressants. Enfin des banques de mutants d'insertion ont été générées et sont à la disposition de la communauté scientifique.

Les études précédemment menées au laboratoire ont consisté en l'analyse des protéines et des voies de la réparation des CDB induites par irradiation. Bien que les rayonnements ionisants produisent de multiples types de lésions au niveau de la molécule d'ADN, la réparation spécifique des CDB a pu être suivie grâce au marquage du variant d'histone γ H2AX - un marqueur spécifique des CDB (Charbonnel *et al.*, 2010 ; Charbonnel *et al.*, 2011). L'analyse plus précise des protéines impliquées dans la réparation d'une CDB dans un contexte donné (séquence nucléotidique bordant la cassure, nature des extrémités d'ADN...) nécessite de simplifier le modèle d'étude en générant une lésion unique et localisée. Dans ce

contexte, l'objectif de ma thèse était d'analyser la contribution relative des différentes voies de recombinaison dans la réparation d'une CDB unique induite par une enzyme de restriction. Deux approches ont été menées en parallèle : la génération de mutants multiples de recombinaison portant des substrats chromosomiques rapporteurs de recombinaison et la mise au point d'une analyse en protoplastes grâce à l'expression transitoire de protéines fluorescentes, marqueurs des différentes voies de recombinaison.

L'analyse des lignées multiples mutantes a d'abord suggéré un rôle de la protéine XRCC2, un paralogue de RAD51, dans la voie d'hybridation simple brin (SSA) indépendante de RAD51. Or, seules des fonctions de cette protéine dans les voies de recombinaison homologue dépendantes de RAD51 avaient été décrites jusqu'alors chez les Eucaryotes (Shim *et al.*, 2004 ; Nagaraju *et al.*, 2009 ; Da Ines *et al.*, 2013a). Mes travaux se sont donc focalisés sur cette protéine, ainsi que sur deux autres homologues de RAD51, RAD51B et RAD51D, et ont permis de démontrer leur implication dans la voie SSA (Article 1). Cette étude nous a mené à développer une analyse par Southern blot permettant de visualiser directement les produits de la recombinaison.

La seconde partie de ma thèse s'est focalisée sur le rôle du complexe XPF-ERCC1 dans les voies de recombinaison homologue, SSA et SDSA. Chez la levure comme chez les Mammifères, ce complexe est impliqué dans le clivage des extrémités d'ADN 3' non homologues des intermédiaires de recombinaison, mais cette fonction semble dépendante de la longueur des extrémités (Fishman-Lobell & Haber, 1992 ; Bardwell *et al.*, 1994 ; Ivanov & Haber, 1995 ; Pâques & Haber, 1997 ; Adair, 2000 ; Sargent *et al.*, 2000 ; Al-Minawi *et al.*, 2008). Nos analyses ont d'abord mis en évidence un rôle inhibiteur inattendu du complexe XPF-ERCC1 sur la recombinaison de type SDSA, et ont de plus confirmé la conservation des fonctions de ce complexe dans la recombinaison homologue chez la plante (Article 2).

Résultats

Chapitre I

Article 1: "Roles of XRCC2, RAD51B and RAD51D in RAD51-Independent SSA Recombination"

Heidi Serra, Olivier Da Ines, Fabienne Degroote,
Maria E. Gallego & Charles I. White

PLoS Genetics (2013) - 9(11): e1003971

Roles of XRCC2, RAD51B and RAD51D in RAD51-Independent SSA Recombination

Heïdi Serra, Olivier Da Ines, Fabienne Degroote, Maria E. Gallego, Charles I. White*

Génétique, Reproduction et Développement, UMR CNRS 6293 - Clermont Université - INSERM U1103, Aubière, France

Abstract

The repair of DNA double-strand breaks by recombination is key to the maintenance of genome integrity in all living organisms. Recombination can however generate mutations and chromosomal rearrangements, making the regulation and the choice of specific pathways of great importance. In addition to end-joining through non-homologous recombination pathways, DNA breaks are repaired by two homology-dependent pathways that can be distinguished by their dependence or not on strand invasion catalysed by the RAD51 recombinase. Working with the plant *Arabidopsis thaliana*, we present here an unexpected role in recombination for the Arabidopsis RAD51 paralogues XRCC2, RAD51B and RAD51D in the RAD51-independent single-strand annealing pathway. The roles of these proteins are seen in spontaneous and in DSB-induced recombination at a tandem direct repeat recombination tester locus, both of which are unaffected by the absence of RAD51. Individual roles of these proteins are suggested by the strikingly different severities of the phenotypes of the individual mutants, with the *xrcc2* mutant being the most affected, and this is confirmed by epistasis analyses using multiple knockouts. Notwithstanding their clearly established importance for RAD51-dependent homologous recombination, XRCC2, RAD51B and RAD51D thus also participate in Single-Strand Annealing recombination.

Citation: Serra H, Da Ines O, Degroote F, Gallego ME, White CI (2013) Roles of XRCC2, RAD51B and RAD51D in RAD51-Independent SSA Recombination. PLoS Genet 9(11): e1003971. doi:10.1371/journal.pgen.1003971

Editor: Holger Puchta, Karlsruhe Institute of Technology, Germany

Received: June 26, 2013; **Accepted:** October 7, 2013; **Published:** November 21, 2013

Copyright: © 2013 Serra et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: HS was the recipient of a PhD fellowship financed by Institut National des Sciences Biologiques (INSB/CNRS) and the Conseil régional d'Auvergne (PAVIRMA project). This work was financed by a European Union research grant (FP7-KBBE-2008-227190), the Centre National de la Recherche Scientifique, the Université Blaise Pascal, the Université d'Auvergne, and the Institut National de la Santé et de la Recherche Médicale. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: chwhite@univ-bpclermont.fr

Introduction

DNA double-strand breaks (DSB) are produced by ionizing radiation, free radicals derived from metabolism, DNA cross-linking reagents and during DNA replication [1,2]. DSB can lead to mutations and rearrangements and/or loss of chromosomes, causing tumorigenesis or cell death. DSB must be repaired to maintain genome integrity, and this is carried out by end-joining through non-homologous recombination or by homologous recombination, which implicates DNA sequence homology of the recombining molecules (for reviews, see [3,4]). The pathways that utilize homology for repair can be distinguished by their dependence or not on strand-invasion catalysed by the RAD51 recombinase (or DMC1 in meiosis): gene conversion homologous recombination (HR) is RAD51-dependent while single-strand annealing (SSA) is RAD51-independent [3].

RAD51-dependent HR is an error-free DSB repair mechanism involving the use of a homologous template for restoration of the original sequence. It involves resection of the 5'-ended DNA strands at the DSB, generating 3' single-stranded DNA overhangs that are bound by replication protein A (RPA). Assisted by mediator proteins, RAD51 displaces RPA and forms a helical nucleofilament on the exposed single-stranded DNA (ssDNA) flanking the DSB. This nucleofilament performs the homology search and catalyses invasion of the homologous template DNA, following which the invading 3' ends are extended through DNA synthesis. The joint recombination intermediate is resolved to

separate the recombining DNA molecules and thus restore chromosome integrity (for a review, see [3]).

In addition to RAD51 and the meiosis-specific DMC1, a number of RAD51 paralogue proteins have been described in a variety of organisms. These share 20% to 30% homology with RAD51 and presumably arose by gene duplication and evolved new functions [5]. They clearly play key roles in DNA repair through HR, but their exact functions are not fully understood (for reviews, see [6–8]).

Two *S. cerevisiae* RAD51 paralogues, RAD55 and RAD57, form a heterodimeric complex which associates with the RAD51 nucleoprotein filament, stabilising it against disruption by the SRS2 antirecombinase [9]. Recent work has characterized novel yeast RAD51 paralogues: Shu1, Shu2, Csm2 and Psy3, components of the “suppresses *sgs1* hydroxyurea sensitivity” (SHU or PCSS) complex which also promotes RAD51 filament assembly and its stability through counteracting the antirecombination activity of the SRS2 and SGS1 helicases [10–17]. Fission yeast has homologues of Shu1, Shu2 and Psy3 (Rlp1, Sws1 and Rdl1) and SWS1 and SWSAP1 are members of a human SHU complex [11,12,18].

Five RAD51 paralogues have been identified in animals and plants: RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 (for reviews, see [7,19,20]). Animal cells defective in any of the RAD51 paralogues are hypersensitive to DNA cross-linking agents, such as Cisplatin and Mitomycin C, and show spontaneous chromosomal aberrations [21–27]. Mouse *xrcc2*, *rad51b*, *rad51c* and *rad51d* mutants are embryonic lethal [28–31]. In contrast, all five RAD51 paralogues Arabidopsis mutants grow and develop normally and

Author Summary

The repair of DNA double-strand breaks by recombination is key to the maintenance of genome integrity in all living organisms. Recombination can however generate mutations and chromosomal rearrangements, making the regulation and the choice of specific pathways of great importance. Through modulation of the activity of the recombinase RAD51, the RAD51 paralogue proteins play key roles in the regulation of recombination. Considerable advances have been made in understanding of the RAD51 paralogue proteins and their roles in mediating RAD51-mediated homologous recombination, however very little is known of possible roles that they may have in other recombination pathways. Working with the plant, *Arabidopsis thaliana*, we show here major roles for three RAD51 paralogues in RAD51-independent single-strand annealing recombination. Notwithstanding their clearly established importance for RAD51-dependent homologous recombination, XRCC2, RAD51B and RAD51D thus also participate in Single-Strand Annealing recombination.

rad51c and *xrcc3* mutant plants are sterile due to recombination defects [32,33].

Two-hybrid and co-immunoprecipitation studies have shown that the five RAD51 paralogues form two major complexes: RAD51B-RAD51C-RAD51D-XRCC2 (BCDX2) and RAD51C-XRCC3 (CX3), as well as RAD51B-RAD51C (BC) and RAD51D-XRCC2 (DX2) sub-complexes [5,7,8,34–40]. RAD51 paralogue complexes act at both early and late stages of the recombinational repair process, although their exact roles remain to be identified [32,41–48]. The early role of RAD51 paralogues in HR is to promote formation and stabilization of RAD51 nucleoprotein filament (reviewed by [6–8,19]), very probably through counter-acting disruption of the filament by helicases [9–14]. Recent work shows that the BCDX2 complex, and not the CX3 complex, is responsible for RAD51 recruitment at DNA damage sites in human cells [43]. After RAD51-mediated strand invasion, the RAD51 paralogues influence gene conversion tract length [42,47] and have been linked to Holliday junction (HJ) resolvase activity [45,46]. In addition, RAD51 paralogues can bind Y-shaped replication-like intermediates and synthetic HJ, in accordance with a role for RAD51 paralogues in repair during DNA replication and in resolution of HR intermediary structures [49,50].

The second main pathway using homology for repair, single-strand annealing (SSA), promotes recombination between tandemly repeated DNA sequences flanking a DSB. SSA does not involve DNA-strand invasion and has been shown to be independent of RAD51 [51–54]. After bidirectional 5′-3′ resection of the DSB ends, the exposed complementary sequences anneal. Subsequent removal of non-homologous 3′-ended ssDNA tails, filling-in of any single-strand gaps and ligation completes the process. The SSA recombination pathway thus leads to deletion of the interstitial DNA sequence lying between the repeats and one of the repeated homologous sequences (for reviews, see [3,55]).

Little is known about possible involvement of the RAD51 paralogues in RAD51-independent SSA. Yeast Rad55 and Rad57 are not required for SSA in a plasmid assay [51] or spontaneous direct repeat recombination [56,57] and a recent study has shown that absence of Rad55, Csm2 or Psy3 result in increased SSA recombination at a direct repeat chromosomal locus in yeast [15]. In *Arabidopsis*, RAD51, RAD51C and XRCC3 are not required for SSA, although a mild reduction in the efficiency of SSA was reported in the *rad51c* mutant [53].

In this study, we describe an unexpected role in the SSA pathway for *Arabidopsis* XRCC2, RAD51B and RAD51D, highlighting for the first time a function of these three RAD51 paralogues in RAD51-independent SSA recombination.

Results

XRCC2 is required for SSA recombination

Although XRCC2 is known to be involved in RAD51-dependent homologous recombination in both vertebrates and in plants [6,7,19,43,44], its potential role in RAD51-independent SSA has not been tested.

SSA recombination was monitored in *xrcc2* mutant *Arabidopsis thaliana* plants using the well-characterised DGU.US recombination reporter locus - consisting of an I-SceI restriction site flanked by 3′ and 5′ truncated copies of the β-glucuronidase gene (GUS) in direct orientation and with an overlap of 557 bp (Figure 1A; [58]). Cleavage of the I-SceI site induces recombination between the flanking GUS sequences and the resulting functional GUS gene is scored histochemically as blue somatic spots. I-SceI induced recombination at this tester locus has been shown not to depend upon RAD51 [53].

We introduced the GUS recombination reporter locus into *xrcc2* mutant and wild-type (WT) plants through crossing and transformed these DGU.US lines with an inducible I-SceI expression cassette (Materials and Methods). Three independent transformants (T2 lines) were selected for each genotype, each with a single insertion site of the I-SceI cassette. Seeds of these lines were plated onto medium containing hygromycin (in order to select plants carrying the I-SceI cassette), in the presence or absence of I-SceI expression inducer (β-estradiol), and numbers of blue GUS+ spots counted after 14 days of growth (Figure 1). Induction of I-SceI expression by β-estradiol treatment in WT plants resulted in a considerable increase of numbers of recombinant blue spots/sectors (Figure 1B and C). In contrast, expression of I-SceI had very little effect on numbers of blue spots in *xrcc2* mutant plants, with means of 5.9 spots per plant in the presence of β-estradiol, and 4.1 in its absence. Repetition of these analyses with two other independent I-SceI transformant lines yielded similar results (Figure 1D). XRCC2 thus clearly plays an important role in the SSA recombination pathway.

The histochemical GUS assay is an indirect measure of somatic recombination and we thus carried out Southern analyses to demonstrate directly that the decrease of number of GUS+ spots in *xrcc2* mutant plants is due to a failure of restoration of the GUS gene. Southern analysis was carried out on SacI-digested genomic DNA of WT and *xrcc2* mutant plants (induced or not by β-estradiol). In DGU.US lines, restoration of the GUS gene results in deletion of the repeated sequence, including the inserted I-SceI site. In DNA of WT plants, the reconstituted GUS gene is clearly visible as a band at the expected size (2.5 kb) after induction of I-SceI expression, but not in its absence (Figure 2, lanes 3 and 7). Treatment of the genomic DNA samples with I-SceI *in vitro* prior to electrophoresis confirms that the 2.5 kb fragment has lost its I-SceI site (Figure 2, lane 9), consistent with elimination of the I-SceI restriction site during the restoration of the marker gene. No restored GUS gene was detected in the *xrcc2* mutant line (Figure 2, lanes 8 and 10). This molecular analysis is thus fully consistent with the results of the β-glucuronidase assay and confirms the implication of the XRCC2 protein in the SSA recombination pathway.

We note also the presence of a 3.2 kb band in the SacI+I-SceI digested DNA from WT plants (Figure 2, lane 9). That this I-SceI resistant band is due to *in planta* rejoining of I-SceI breaks through

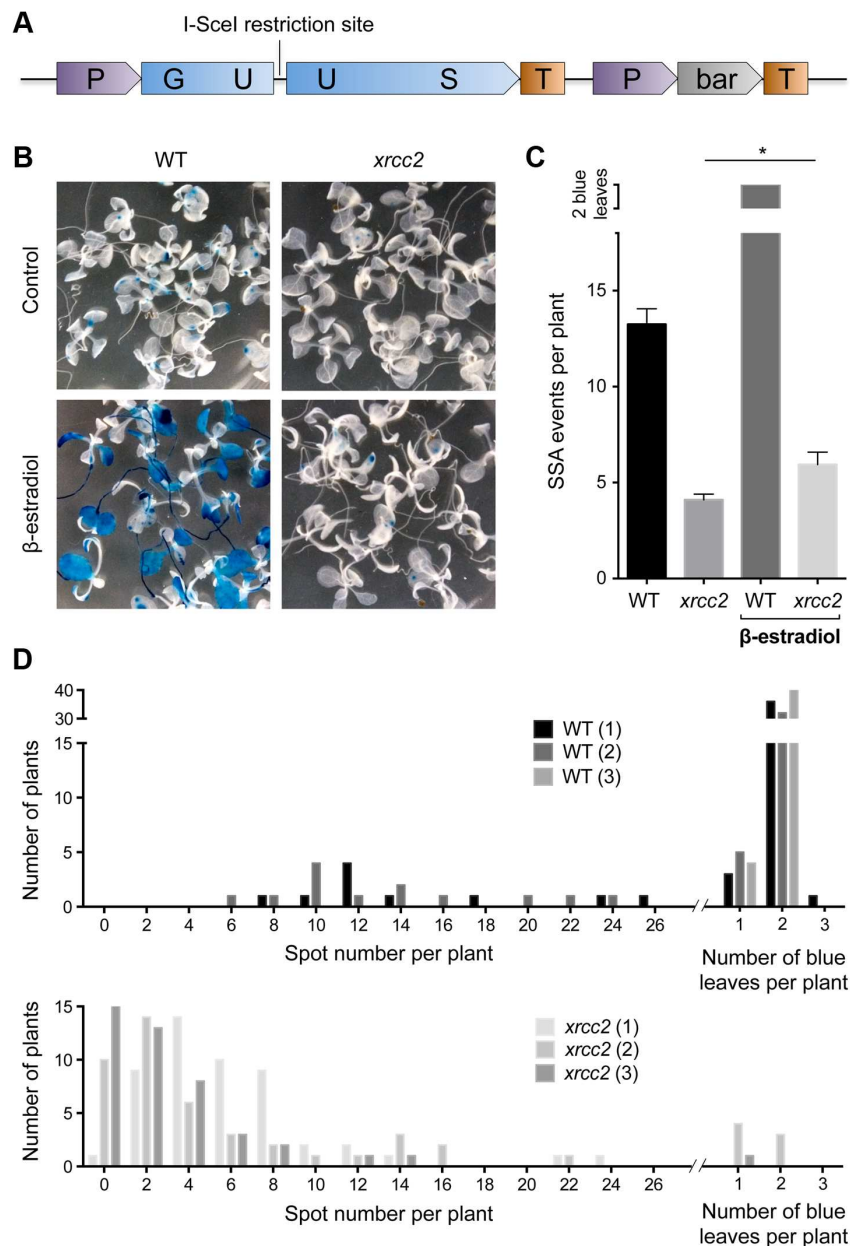


Figure 1. I-SceI induced DGU.US recombination depends upon XRCC2. (A) Schematic map of the recombination substrate DGU.US. (B) β-glucuronidase assay of 14 day-old seedlings grown with or without induction of I-SceI by β-estradiol clearly shows reduced numbers of blue recombinant GUS⁺ sectors in the *xrcc2* mutant. (C) Quantification of recombination events confirms the role of XRCC2. Bars are mean values ± standard errors. * Significant difference ($p=0.036$, Mann-Whitney test). (D) Frequency distributions of recombinant spot numbers per plant of 3 independent WT and *xrcc2* T2 lines grown in the presence of β-estradiol.
doi:10.1371/journal.pgen.1003971.g001

end-joining recombination was verified by PCR amplification and DNA sequencing. Approximately 10% of the sequences carried a mutation at the I-SceI restriction site. DNA sequencing showed that these result mostly from small deletions (Figure S1). As previously described [59–63], these events can be ascribed to end-joining exploiting the presence of microhomologies either side of the I-SceI cleavage site.

XRCC2 function in spontaneous DGU.US recombination does not depend upon RAD51 activity

Although minor, the DGU.US recombination analyses shown in Figure 1C also showed a difference in numbers of blue spots between WT and *xrcc2* plants in the absence of β-estradiol. To check whether this is due to differences in spontaneous recombination rates or to leakiness of the inducible I-SceI cassette (or

RAD51 Paralogues Act in SSA Recombination

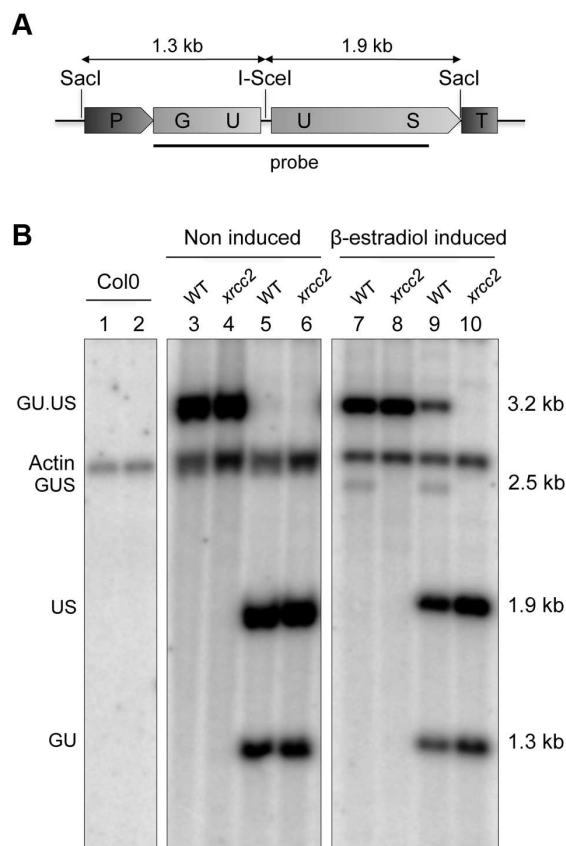


Figure 2. Molecular confirmation of recombination in WT, but not *xrc2* mutant plants. Schematic representation of the GU.US recombination tester locus (A) and Southern analysis (B) of DNA from plants grown in the absence (lanes 1 to 6) or presence of β -estradiol (lanes 7 to 10), digested with *SacI* (lanes 1,3,4,7,8) or *SacI* plus *I-SceI* (lanes 2,5,6,9,10). The blot was hybridized with a GUS-specific probe as indicated in panel (A). The recombined GUS gene has lost its *I-SceI* site and is seen as a single 2.5 kb *SacI* fragment only in DNA from WT plants grown in presence of β -estradiol (lanes 7 and 9). Col0: WT plants of Columbia ecotype.
doi:10.1371/journal.pgen.1003971.g002

both), we monitored recombination in *xrc2* mutant and WT plants with the same DGU.US locus (at the same location in genome), but which do not carry *I-SceI* (Figure 3). This analysis showed a reduction of number of recombinant spots in the absence of the *I-SceI* cassette, for both WT and *xrc2* plants (from 13.24 to 5.46 and 4.10 to 0.36 spots per plant, respectively; Figures 1C and 3A), confirming the presence of some leakiness in expression of the *I-SceI* inducible promoter in the absence of β -estradiol. In the absence of the *I-SceI* cassette, mean numbers of blue spots per plant were however still significantly (15-fold) reduced in *xrc2* mutants (0.36 ; s.e.m = 0.08) compared with WT controls (5.46 ; s.e.m = 0.34 ; Figure 3). An independent repetition of this experiment confirmed these results (Table 1). XRCC2 is thus clearly involved in spontaneous recombination of the DGU.US substrate.

As mentioned above, *I-SceI* induced recombination at the DGU.US locus has been shown to be RAD51-independent [53]. This has not however been confirmed for spontaneous recombination, for which different mechanisms can be envisaged - single-strand annealing, intermolecular synthesis-dependent strand annealing,

break-induced replication [62,64,65]. We thus tested the RAD51-dependence of spontaneous recombination at DGU.US by expressing the dominant-negative RAD51-GFP fusion protein [66]. Plants were transformed with the RAD51-GFP fusion protein construct and three T2 lines each with a single insertion (RAD51-GFP plants) were selected and their *rad51* mutant phenotype tested by verification of sensitivity to the cross-linking agent, Mitomycin C (MMC) (Figure S2). Wild-type plants are not sensitive to the MMC dose used (2% sensitive plants), in contrast to the segregating RAD51-GFP population, in which 76.9% are sensitive. PCR genotyping confirmed that all of the MMC-sensitive and none of the MMC-resistant T2 plants carry RAD51-GFP. Presence of RAD51-GFP is thus perfectly correlated with MMC-sensitivity, confirming the dominant-negative inhibition of RAD51 by the fusion protein [66]. We then tested spontaneous DGU.US recombination in the RAD51-GFP plants (Figure 4). No significant difference was observed in numbers of GUS+ recombinant spots between control and RAD51-GFP plants (Mann-Whitney test) clearly confirming that spontaneous recombination of the DGU.US substrate does not depend upon RAD51 activity.

XRCC2, RAD51B and RAD51D have non-epistatic functions in the SSA pathway

XRCC2 is one of five RAD51 paralogue proteins, all of which play important roles in recombination [7]. Given the function of XRCC2 in the RAD51-independent SSA pathway presented above, we also tested for evidence of roles of the other RAD51 paralogues, RAD51B and RAD51D, in this pathway. We thus crossed the DGU.US recombination reporter locus into *rad51b* and *rad51d* mutant plants and monitored spontaneous SSA recombination at DGU.US in *rad51b* and *rad51d* mutants. Although less pronounced than the 15-fold reduction observed in *xrc2* plants, numbers of spontaneous recombination events are also reduced in *rad51b* and *rad51d* mutants (respectively 4.6-fold and 3.4-fold; Figure 5; Table 2) clearly establishing roles for RAD51B and RAD51D in the SSA pathway.

Epistasis relationships in SSA recombination between the three RAD51 paralogue genes were tested in *xrc2 rad51b* double and *xrc2 rad51b rad51d* triple mutants. Spontaneous SSA recombination was significantly less efficient in *xrc2 rad51b* double mutants than in the corresponding single mutants ($p < 0.02$) (Figure 5). A slight further reduction in numbers of blue spots per plant was observed in the triple *xrc2 rad51b rad51d* mutants with respect to the double *xrc2 rad51b* mutant, but the difference is not significant.

To confirm at the molecular level the results of the GUS assays, we transformed *rad51b*, *rad51d*, *xrc2 rad51b* and *xrc2 rad51b rad51d* mutant plants with the inducible *I-SceI* expression cassette. Southern analysis of recombination was carried out on β -estradiol induced T2 plants. As expected, the 2.5 kb fragment of the recombination product is only detected in the WT, confirming the GUS assay data (Figure 6).

Arabidopsis XRCC2, RAD51B and RAD51D thus play roles in SSA recombination pathway and these roles are non-epistatic, at least for XRCC2 and RAD51B.

Discussion

The roles of RAD51 paralogues in RAD51-dependent recombination have been the subject of considerable interest in recent years [6–8,19]. Little is known however of possible roles in RAD51-independent SSA recombination. In Arabidopsis, no effect was found on SSA in *xrc3* mutants and a barely statistically significant reduction observed in *rad51c* plants [53]. We show here the involvement of three RAD51 paralogues, XRCC2, RAD51B

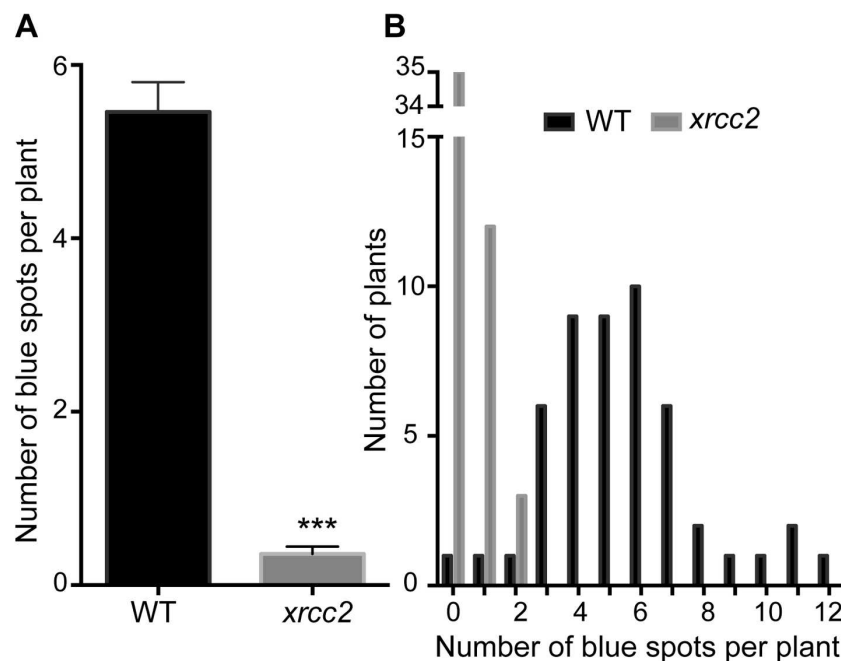


Figure 3. Spontaneous DGU.US recombination is reduced in the *xrcc2* mutant. A significant reduction in spontaneous recombination rate is observed in *xrcc2* mutant compared to WT plants. (A) Mean values $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test). (B) Frequency distributions.

doi:10.1371/journal.pgen.1003971.g003

and RAD51D, in RAD51-independent single-strand annealing in *Arabidopsis thaliana*. XRCC2 plays a major role in this pathway with a striking reduction of I-SceI induced recombination and a 15-fold reduction in the number of spontaneous SSA events in its absence (Figures 1 and 3). Spontaneous SSA is also clearly reduced in *rad51b* and *rad51d* mutants (4.6-fold and 3.4-fold reduction respectively; Figure 5; Table 2), although less strongly than in *xrcc2* mutants. The differing severity of the phenotypes of the three mutants is suggestive of individual roles for these proteins, and this is supported by epistasis analyses of double and triple mutant plants (Figure 5, Table 2). An alternative to a direct role of these proteins is that the presence of non-functional RAD51 nucleofilaments in these mutants which might block SSA. The lack of effect on SSA of RAD51-GFP (which forms foci at DSBs and is dominant-negative for GC/SSA recombination) however argues

against this interpretation. Data suggesting differing roles for individual RAD51 paralogues, or sub-complexes, can be found in a number of reports. Individual paralogue mutants in DT-40 cells show non-epistatic phenotypes [67] and biochemical analyses show specific roles for the sub-complexes [68–70]. In *Arabidopsis*, absence of XRCC2 and RAD51B, but not RAD51D, increases rates of meiotic crossing-over [44] and RAD51D appears to be the only RAD51 paralogue to be essential for telomere integrity in human cells [71]. A recent report shows opposing effects on cell-cycle regulation of the inhibition of XRCC3 and RAD51C in HeLa cells, with inhibition of XRCC3 eliciting checkpoint defects and inhibition of RAD51C inducing G2/M cell cycle arrest [48].

What can the roles of XRCC2, RAD51B and RAD51D be in the SSA pathway? The main steps of SSA are (1) bidirectional 5' to 3' resection of the DSB ends flanking a DSB, (2) annealing of exposed complementary sequences, (3) excision of non-homologous 3'-ended overhangs, (4) DNA synthesis and (5) ligation which restores two continuous strands [72,73]. A role in the annealing step is suggested by the capacity of the human BCDX2 complex to catalyse annealing between single-strand DNAs *in vitro* [50]. This study also showed a high affinity of the BCDX2 complex for branched DNA structures, such as Y-shaped DNA, that result from this annealing between tandem repeats during single-strand annealing. Taken together, these results strongly suggest a role of XRCC2, RAD51B and RAD51D in the annealing of the two exposed repeat sequences on either side of the DSB.

Biochemical studies have identified two main complexes of the five RAD51 paralogue proteins in animal and plant cells: RAD51B-RAD51C-RAD51D-XRCC2 and RAD51C-XRCC3 [5,7,8,34–40]. No self-assembly of individual RAD51 paralogues have been detected. Analysis of epistasis relationships of RAD51 paralogues in chicken DT-40 cells show that *rad51b* and *rad51d* are

Table 1. Spontaneous DGU.US recombination in *xrcc2* mutant and in wild-type plants.

Experiment		n	N	m $\pm$ SEM	Ratio <i>xrcc2</i> /WT
1	WT	50	273	5.46 $\pm$ 0.34	
	<i>xrcc2</i>	50	18	0.36 $\pm$ 0.08	0.066
2	WT	50	310	6.20 $\pm$ 0.51	
	<i>xrcc2</i>	50	19	0.38 $\pm$ 0.09	0.061

Recombination in the mutants and WT were compared using non-parametric statistical analysis (Mann-Whitney test). Differences between *xrcc2* and WT are highly significant ($p < 0.0001$) in both cases. n, the number of plantlets screened; N, the total number of blue spots (recombination events); m $\pm$ SEM, the mean number of recombination events per plant $\pm$ standard error of the mean.

doi:10.1371/journal.pgen.1003971.t001

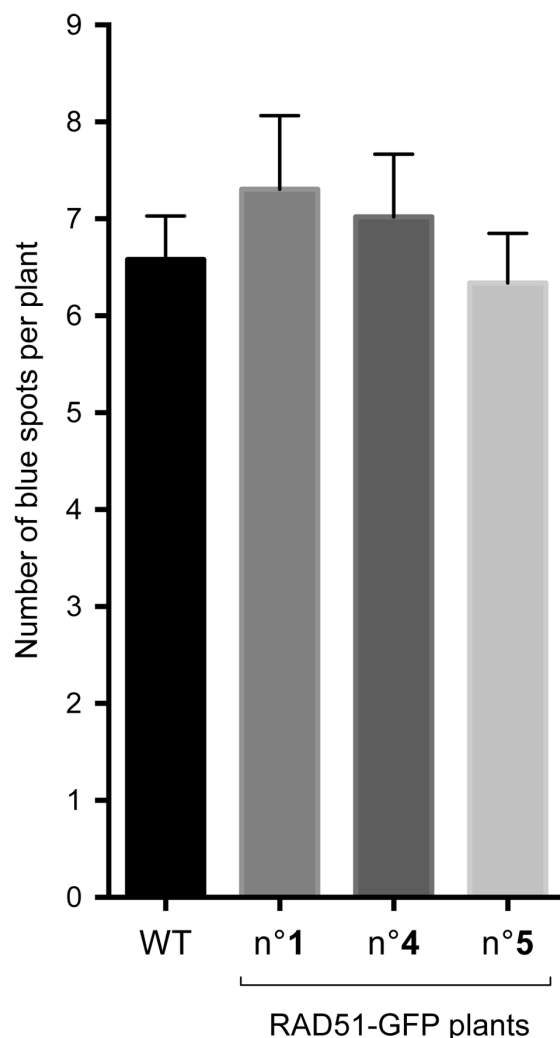


Figure 4. Spontaneous DGU.US recombination is RAD51-independent. No significant effect on spontaneous recombination rate was observed in three independent transformants carrying the dominant-negative RAD51-GFP construct. Bars are mean values $\pm$ standard errors.

doi:10.1371/journal.pgen.1003971.g004

epistatic while *xrcc3 rad51d* double mutant cells exhibit an additive sensitivity to ionizing radiation [67], consistent with differential actions of two major complexes in cellular response to DNA damage. That the three RAD51 paralogues involved in SSA are components of the BCDX2 complex suggests this complex is the active species in SSA. However, the differing severity of the phenotypes of the *xrcc2*, *rad51b* and *rad51d* (and *rad51c*; [53]) mutants argues against the implication of the BCDX2 complex as such. The proposed structure of the complex also argues against being the active form in SSA, with protein-protein interaction studies showing that the four proteins are linked in the order: RAD51B-RAD51C-RAD51D-XRCC2 [35]. Absence of RAD51D should thus exclude XRCC2 from the complex, yet SSA in the *xrcc2* mutants is significantly more affected than in *rad51d* (and similarly for *rad51b* versus *rad51c*). This argument also applies to the RAD51B-RAD51C and RAD51D-XRCC2 sub-complexes (for

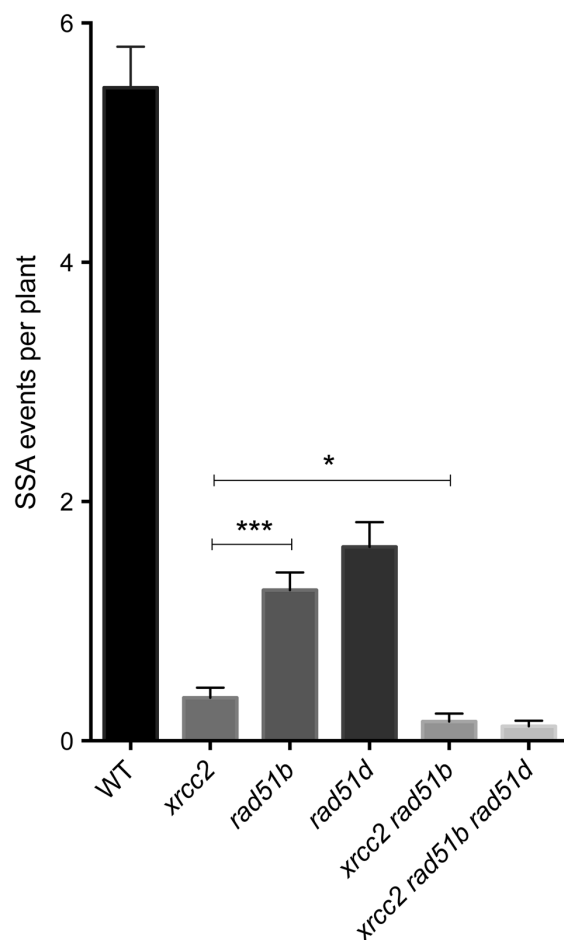


Figure 5. Individual and combined effects of *xrcc2*, *rad51b* and *rad51d* on spontaneous DGU.US recombination. Significant reductions in spontaneous recombination rate are observed in *xrcc2*, *rad51b* and *rad51d* mutants, and the severities of the reductions differ between these single mutants. A further significant reduction is seen in *xrcc2 rad51b* mutant plants. The triple *xrcc2 rad51b rad51d* mutant shows a further reduction, but this does not differ significantly from that observed in the *xrcc2 rad51b* plants. Bars are mean values $\pm$ standard errors. * $0.05 < p < 0.0001$; *** $p < 0.0001$ (Mann-Whitney test). doi:10.1371/journal.pgen.1003971.g005

reviews, [7,8]). Our data thus favour individual roles of XRCC2, RAD51B and RAD51D in single-strand annealing recombination.

The yeast RAD51 paralogues Rad55 and Rad57 are not required in SSA recombination in a plasmid-based assay [51] and a chromosomal assay shows that absence of Csm2, Psy3 (also RAD51 paralogues) or Rad55 favours SSA with respect to gene conversion recombination [15]. The description here of roles for XRCC2, RAD51B and RAD51D in the RAD51-independent SSA pathway thus highlights a difference in the roles of Arabidopsis and yeast RAD51 paralogues in the SSA pathway. Such a difference is also seen in the roles of RAD51 paralogues in meiotic recombination with *psy3* mutants exhibiting a strong hypo-recombination in yeast [12], while absence of XRCC2 or RAD51B increases meiotic crossing-over in Arabidopsis [44].

In conclusion, we describe here an unexpected role in recombination for the Arabidopsis RAD51 paralogues XRCC2, RAD51B and RAD51D. The roles of these proteins are seen in

Table 2. Spontaneous DGU.US recombination in wild-type, *rad51b*, *rad51d*, double and triple mutants.

Experiment		<i>n</i>	<i>N</i>	<i>m</i> ± SEM	Ratio mutant/WT
1	WT	50	273	5.46 ± 0.34	
	<i>rad51b</i>	50	63	1.26 ± 0.15	0.231
	<i>rad51d</i>	50	81	1.62 ± 0.21	0.297
	<i>xrcc2, rad51b</i>	50	8	0.16 ± 0.07	0.023
	<i>xrcc2, rad51b, rad51d</i>	50	6	0.12 ± 0.05	0.022
2	WT	50	310	6.20 ± 0.51	
	<i>rad51b</i>	50	62	1.24 ± 0.18	0.200
	<i>rad51d</i>	50	92	1.84 ± 0.23	0.297
	<i>xrcc2, rad51b</i>	50	6	0.12 ± 0.05	0.019
	<i>xrcc2, rad51b, rad51d</i>	50	1	0.02 ± 0.02	0.003

Recombination in the mutants and WT was compared using non-parametric statistical analysis (Mann-Whitney test). Differences between each mutant and corresponding WT are highly significant ($p < 0.0001$). *n*, the number of plantlets screened; *N*, the total number of blue spots (recombination events); *m* ± SEM, the mean number of recombination events per plant ± standard error of the mean.

doi:10.1371/journal.pgen.1003971.t002

spontaneous and in DSB-induced recombination at a tandem direct repeat recombination tester locus, both of which are unaffected by the absence of RAD51. Notwithstanding their clearly established importance for RAD51-dependent homologous recombination, these proteins thus also participate in RAD51-independent Single-Strand Annealing recombination.

Materials and Methods

Plant material

The *Arabidopsis thaliana* *xrcc2*, *rad51b* [74] and *rad51d* [44] mutants used in this work have been previously described. A triple *xrcc2/xrcc2 rad51b/rad51b rad51d/rad51d* mutant was crossed with the recombination tester DGU.US-1 line [58] and single, double and triple mutants homozygous for the DGU.US substrate were identified in the F2. Wild-type control plants come from the same crosses.

The I-SceI coding sequence [75] was placed under control of β -estradiol in the plasmid pMDC7 [76] by Gateway cloning. The resulting vector was transferred into *Agrobacterium tumefaciens*, and used to transform the plant lines utilising the floral dip method [77].

Growth conditions

Surface-sterilized seeds were stratified at 4°C for 2 days and grown *in vitro* on germination medium (0.8% w/v agar, 1% w/v sucrose and half-strength Murashige & Skoog salts (M0255; Duchefa

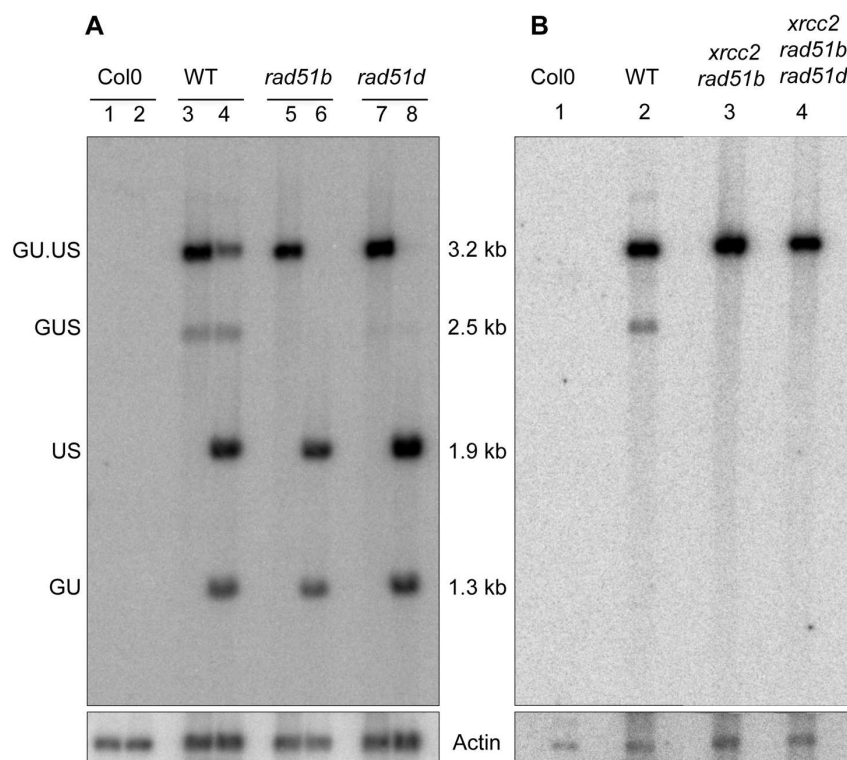
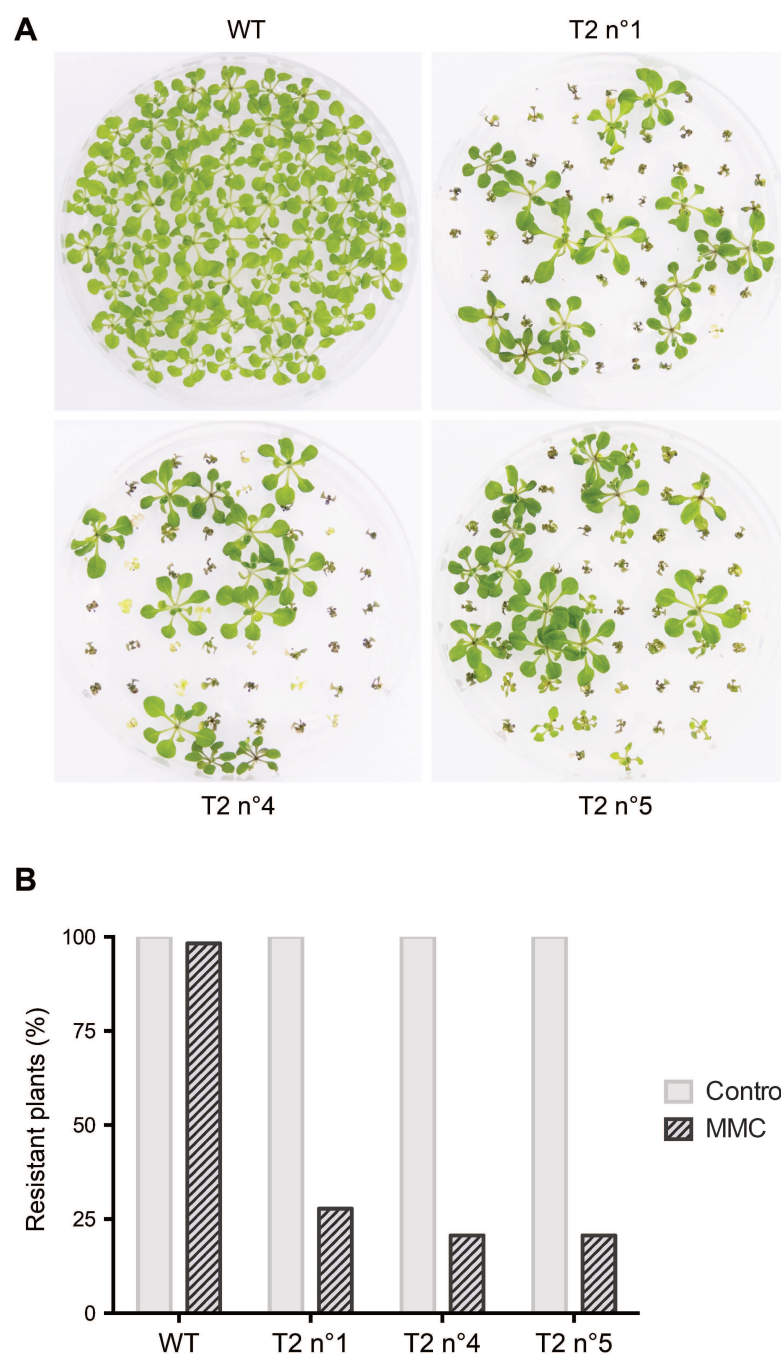


Figure 6. Molecular confirmation of recombination defects in *rad51b*, *rad51d*, *xrcc2 rad51b* and *xrcc2 rad51b rad51d* mutants. (A) Southern analysis of DNA from *rad51b* and *rad51d* mutant plants grown in the presence of β -estradiol, digested with *SacI* (lanes 1,3,5,7) or *SacI* plus *I-SceI* (lanes 2,4,6,8). (B) Southern analysis of DNA from *xrcc2 rad51b* and *xrcc2 rad51b rad51d* mutant plants grown in the presence of β -estradiol, digested with *SacI*. The blots were hybridized with a GUS-specific probe. The recombined GUS gene has lost its *I-SceI* site and is seen as a 2.5 kb band only in DNA from WT plants grown in presence of β -estradiol (A, lanes 3 and 4; B, lane 2). Col0: WT plants of Columbia ecotype.

RAD51 Paralogues Act in SSA Recombination

24. Johnson RD, Liu N, Jasin M (1999) Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. *Nature* 401: 397–399.
25. Pierce AJ, Johnson RD, Thompson LH, Jasin M (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes & development* 13: 2633–2638.
26. Liu N, Lamerding JE, Tebbs RS, Schild D, Tucker JD, et al. (1998) XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Molecular cell* 1: 783–793.
27. Tebbs RS, Zhao Y, Tucker JD, Scheerer JB, Siciliano MJ, et al. (1995) Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene. *Proceedings of the National Academy of Sciences of the United States of America* 92: 6354–6358.
28. Deans B, Griffin CS, Maconochie M, Thacker J (2000) Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *The EMBO journal* 19: 6675–6685.
29. Kuznetsov SG, Haines DC, Martin BK, Sharan SK (2009) Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. *Cancer Res* 69: 863–872.
30. Pittman DL, Schimenti JC (2000) Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis* 26: 167–173.
31. Shu Z, Smith S, Wang L, Rice MC, Kmiec EB (1999) Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(-/-) background. *Molecular and cellular biology* 19: 8686–8693.
32. Bleuyard J-Y, White CI (2004) The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis. *The EMBO journal* 23: 439–449.
33. Li W, Yang X, Lin Z, Timofejeva L, Xiao R, et al. (2005) The AtRAD51C gene is required for normal meiotic chromosome synapsis and double-stranded break repair in Arabidopsis. *Plant physiology* 138: 965–976.
34. Liu N, Schild D, Thelen MP, Thompson LH (2002) Involvement of Rad51C in two distinct protein complexes of Rad51 paralogs in human cells. *Nucleic acids research* 30: 1009–1015.
35. Miller KA, Sawicka D, Barsky D, Albala JS (2004) Domain mapping of the Rad51 paralog protein complexes. *Nucleic acids research* 32: 169–178.
36. Miller KA, Yoshikawa DM, McConnell IR, Clark R, Schild D, et al. (2002) RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51. *The Journal of biological chemistry* 277: 8406–8411.
37. Osakabe K, Abe K, Yamanouchi H, Takyuu T, Yoshioka T, et al. (2005) Arabidopsis Rad51B is important for double-strand DNA breaks repair in somatic cells. *Plant molecular biology* 57: 819–833.
38. Osakabe K, Yoshioka T, Ichikawa H, Toki S (2002) Molecular cloning and characterization of RAD51-like genes from Arabidopsis thaliana. *Plant molecular biology* 50: 71–81.
39. Schild D, Lio YC, Collins DW, Tsomondo T, Chen DJ (2000) Evidence for simultaneous protein interactions between human Rad51 paralogs. *The Journal of biological chemistry* 275: 16443–16449.
40. Wiese C, Collins DW, Albala JS, Thompson LH, Kronenberg A, et al. (2002) Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells. *Nucleic acids research* 30: 1001–1008.
41. Badie S, Liao C, Thanassoula M, Barber P, Hill MA, et al. (2009) RAD51C facilitates checkpoint signaling by promoting CHK2 phosphorylation. *The Journal of cell biology* 185: 587–600.
42. Brennenman MA, Wagener BM, Miller CA, Allen C, Nickoloff JA (2002) XRCC3 controls the fidelity of homologous recombination: roles for XRCC3 in late stages of recombination. *Molecular cell* 10: 387–395.
43. Chun J, Buechelmaier ES, Powell SN (2013) BRCA1 paralog complexes BCDX2 and CX3 act at different stages in the BRCA1-BRCA2-dependent homologous recombination pathway. *Molecular and cellular biology* 33: 387–395.
44. Da Ines O, Degroote F, Amiard S, Goubely C, Gallego ME, et al. (2013) Effects of XRCC2 and RAD51B mutations on somatic and meiotic recombination in Arabidopsis thaliana. *The Plant Journal* 74: 959–970.
45. Liu Y, Masson J-Y, Shah R, O'Regan P, West SC (2004) RAD51C is required for Holliday junction processing in mammalian cells. *Science* 303: 243–246.
46. Liu Y, Tarsounas M, O'Regan P, West SC (2007) Role of RAD51C and XRCC3 in genetic recombination and DNA repair. *The Journal of biological chemistry* 282: 1973–1979.
47. Nagaraju G, Hartlerode A, Kwok A, Chandramouly G, Scully R (2009) XRCC2 and XRCC3 regulate the balance between short- and long-tract gene conversions between sister chromatids. *Molecular and cellular biology* 29: 4283–4294.
48. Rodrigue A, Coulombe Y, Jacquet K, Gagne JP, Roques C, et al. (2013) The RAD51 paralogs ensure cellular protection against mitotic defects and aneuploidy. *J Cell Sci* 126: 348–359.
49. Compton SA, Ozgur S, Griffith JD (2010) Ring-shaped Rad51 paralog protein complexes bind Holliday junctions and replication forks as visualized by electron microscopy. *J Biol Chem* 285: 13349–13356.
50. Yokoyama H, Sarai N, Kagawa W, Enomoto R, Shibata T, et al. (2004) Preferential binding to branched DNA strands and strand-annealing activity of the human Rad51B, Rad51C, Rad51D and Xrcc2 protein complex. *Nucleic acids research* 32: 2556–2565.
51. Ivanov EL, Sugawara N, Fishman-Lobell J, Haber JE (1996) Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. *Genetics* 142: 693–704.
52. Mansour WY, Schumacher S, Rosskopf R, Rhein T, Schmidt-Petersen F, et al. (2008) Hierarchy of nonhomologous end-joining, single-strand annealing and gene conversion at site-directed DNA double-strand breaks. *Nucleic Acids Res* 36: 4088–4098.
53. Roth N, Klimesch J, Dukowicz-Schulze S, Pacher M, Mannuss A, et al. (2012) The requirement for recombination factors differs considerably between different pathways of homologous double-strand break repair in somatic plant cells. *The Plant journal* 72: 781–790.
54. Stark JM, Pierce AJ, Oh J, Pastink A, Jasin M (2004) Genetic steps of mammalian homologous repair with distinct mutagenic consequences. *Mol Cell Biol* 24: 9305–9316.
55. Krogh BO, Symington LS (2004) Recombination proteins in yeast. *Annual review of genetics* 38: 233–271.
56. McDonald JP, Rothstein R (1994) Unrepaired heteroduplex DNA in *Saccharomyces cerevisiae* is decreased in RAD1 RAD52-independent recombination. *Genetics* 137: 393–405.
57. Mozin AM, Fung CW, Symington LS (2008) Role of the *Saccharomyces cerevisiae* Rad51 paralogs in sister chromatid recombination. *Genetics* 178: 113–126.
58. Orel N, Kyryk A, Puchta H (2003) Different pathways of homologous recombination are used for the repair of double-strand breaks within tandemly arranged sequences in the plant genome. *The Plant Journal* 35: 604–612.
59. Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, et al. (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic acids research* 39: e82.
60. Heacock M, Spangler E, Riha K, Puizina J, Shippen DE (2004) Molecular analysis of telomere fusions in Arabidopsis: multiple pathways for chromosome end-joining. *The EMBO journal* 23: 2304–2313.
61. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, et al. (2011) A TALE nuclease architecture for efficient genome editing. *Nature biotechnology* 29: 143–148.
62. Puchta H (2005) The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *Journal of experimental botany* 56: 1–14.
63. Salomon S, Puchta H (1998) Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. *The EMBO journal* 17: 6086–6095.
64. Malkova A, Ivanov EL, Haber JE (1996) Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. *Proc Natl Acad Sci U S A* 93: 7131–7136.
65. Watanabe K, Pacher M, Dukowicz S, Schubert V, Puchta H, et al. (2009) The Structural Maintenance of Chromosomes 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in Arabidopsis thaliana. *Plant Cell* 21: 2688–2699.
66. Da Ines O, Degroote F, Goubely C, Amiard S, Gallego ME, et al. (2013) Meiotic Recombination in Arabidopsis Is Catalysed by DMC1, with RAD51 Playing a Supporting Role. *PLoS genetics* 9: e1003787.
67. Yonetani Y, Hochegger H, Sonoda E, Shinya S, Yoshikawa H, et al. (2005) Differential and collaborative actions of Rad51 paralog proteins in cellular response to DNA damage. *Nucleic acids research* 33: 4544–4552.
68. Lio YC, Mazin AV, Kowalczykowski SC, Chen DJ (2003) Complex formation by the human Rad51B and Rad51C DNA repair proteins and their activities in vitro. *J Biol Chem* 278: 2469–2478.
69. Kurumizaka H, Ikawa S, Nakada M, Enomoto R, Kagawa W, et al. (2002) Homologous pairing and ring and filament structure formation activities of the human Xrcc2*Rad51D complex. *J Biol Chem* 277: 14315–14320.
70. Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, et al. (2001) Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. *Genes Dev* 15: 3308–3318.
71. Tarsounas M, Munoz P, Claas A, Smiraldi PG, Pittman DL, et al. (2004) Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* 117: 337–347.
72. Lin FL, Sperle K, Sternberg N (1984) Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. *Molec Cell Biol* 4: 1020–1034.
73. Prado F, Aguilera A (1995) Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the RAD1, RAD10, and RAD52 genes. *Genetics* 139: 109–123.
74. Bleuyard J-Y, Gallego ME, Savigny F, White CI (2005) Differing requirements for the Arabidopsis Rad51 paralogs in meiosis and DNA repair. *The Plant Journal* 41: 533–545.
75. Fauser F, Roth N, Pacher M, Ilg G, S  nchez-Fern  ndez R, et al. (2012) In planta gene targeting. *Proceedings of the National Academy of Sciences of the United States of America* 109: 7533–7540.
76. Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant physiology* 133: 462–469.
77. Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *The Plant Journal* 16: 735–743.
78. Gallego ME, White CI (2001) RAD50 function is essential for telomere maintenance in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* 98: 1711–1716.

Figure S2



Chapitre I

Résultats complémentaires

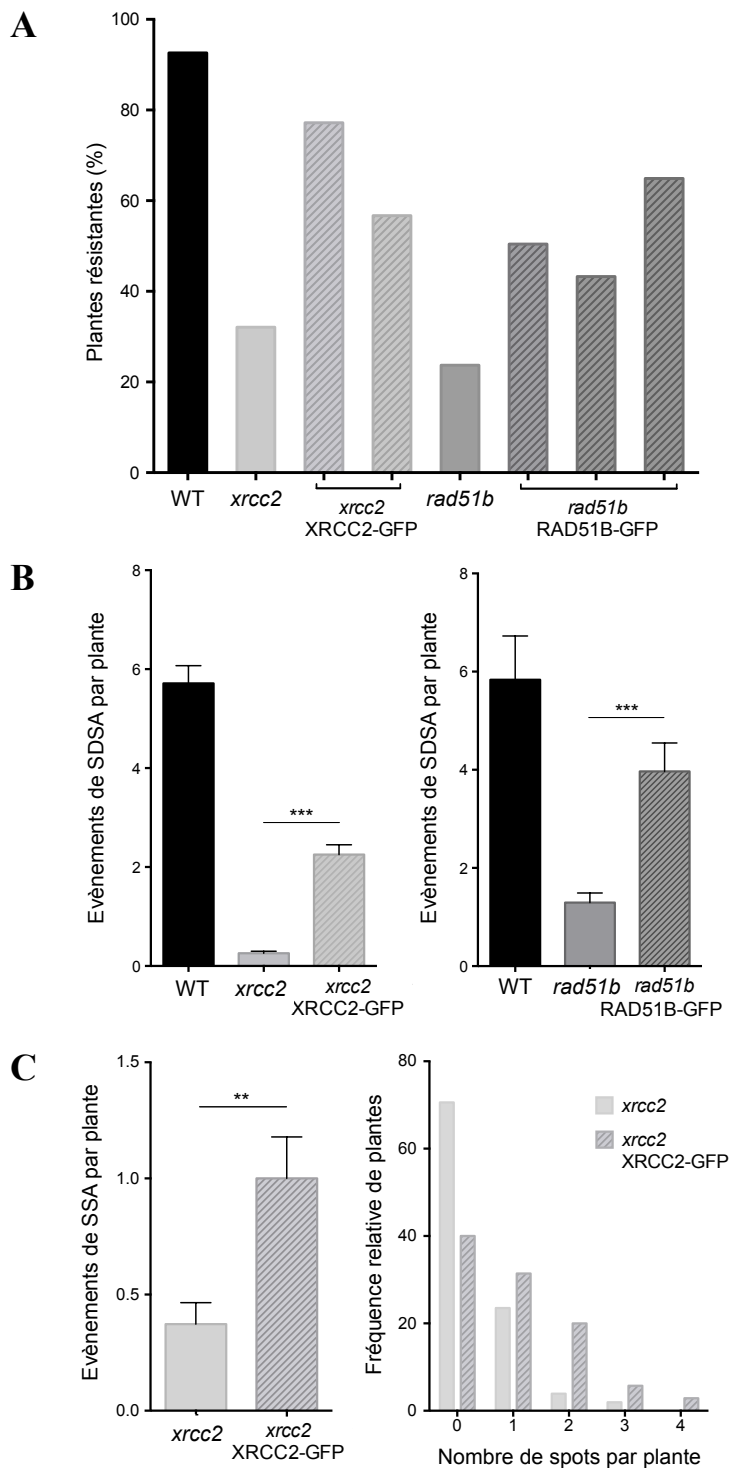


Figure 17 : Les protéines de fusion XRCC2-GFP et RAD51B-GFP complètent la sensibilité à la Mitomycine C et les défauts de recombinaison des mutants *xrc2* et *rad51b*, respectivement.

- (A) Sensibilité à la MMC (40 μ M) mesurée à 14 jours. Plusieurs lignées exprimant les protéines de fusion sont analysées. Une plantule avec au minimum 3 vraies feuilles est considérée résistante.
- (B-C) Efficacité de la recombinaison de type SDSA au locus IU.GUS ou de la recombinaison de type SSA au locus DGU.US (cf Matériel et Méthodes, Article 1). Moyennes du nombre de spots recombinants par plante $\pm$ erreur standard. *** $p < 0,0001$; ** $p = 0,0015$ (test Mann-Whitney).

Dans cet article, nous avons mis en évidence un rôle de XRCC2, RAD51B et RAD51D - trois des cinq paralogues de RAD51 - dans la voie de recombinaison d'hybridation simple brin (SSA) chez la plante modèle *Arabidopsis thaliana*. Ce résultat était totalement inattendu puisque la recombinaison de type SSA est indépendante de RAD51 et seules des fonctions de ces paralogues dans la médiation de la stabilité et de l'activité de RAD51 ont été décrites jusqu'à présent (Brenneman *et al.*, 2002 ; Bleuyard & White, 2004 ; Liu *et al.*, 2004 ; Liu *et al.*, 2007 ; Nagaraju *et al.*, 2009 ; Rodrigue *et al.*, 2012 ; Chun *et al.*, 2013 ; Da Ines *et al.*, 2013a). En complément des résultats publiés dans l'Article 1, nous avons mené différentes expériences afin d'étayer et d'étendre les conclusions. Les résultats de ces expériences complémentaires sont présentés ci-dessous.

I. Validation du matériel d'étude

I.1. Vérification des mutants par complémentation

Afin de confirmer que les effets observés dans l'Article 1 sont strictement liés aux mutations des gènes des paralogues de RAD51, nous avons réalisé des expériences de complémentation des mutants avec des protéines de fusion générées au laboratoire. Les séquences génomiques de XRCC2 et RAD51B ainsi que leurs régions promotrices ont été amplifiées par PCR à partir de l'ADN d'une plante sauvage (d'écotype Columbia), puis fusionnées avec la séquence codante de la GFP. Les constructions XRCC2-GFP et RAD51B-GFP ont ensuite été introduites dans les plantes mutantes *xrcc2* ou *rad51b*, respectivement, par transformation selon la méthode d'immersion florale (Clough & Bent, 1998). L'analyse de la sensibilité de ces plantes à la Mitomycine C (MMC), un agent générant des ponts inter-brins dans l'ADN, a révélé que les protéines de fusion sont capables de compléter la sensibilité des mutants correspondants (**Figure 17 A**). Dans les deux cas, la présence de la

protéine de fusion (XRCC2-GFP ou RAD51B-GFP) sauvage restaure le phénotype sauvage des plantes mutantes (bien que partiellement dans certaines lignées).

Les lignées mutantes exprimant les protéines de fusion (*xrcc2* XRCC2-GFP et *rad51b* RAD51B-GFP) ont de plus été croisées avec des plantes mutantes possédant l'un des loci rapporteurs de recombinaison DGU.US ou IU.GUS (Orel *et al.*, 2003). La construction DGU.US est décrite dans la figure 1 de l'Article 1 et la construction IU.GUS (spécifique des événements de CG/SDSA) sera présentée dans la figure 4 de l'Article 2. La quantification du nombre de spots recombinants par plante montre clairement que la présence des protéines XRCC2-GFP et RAD51B-GFP augmente l'efficacité de la recombinaison de type CG/SDSA (**Figure 17 B**) mais aussi du SSA (**Figure 17 C**) dans les plantes mutantes.

L'ensemble de ces résultats montre que la présence des protéines fonctionnelles complémente les phénotypes des mutants *xrcc2* et *rad51b* (sensibilité à la MMC et efficacité de la recombinaison). La restauration des phénotypes n'est cependant pas totale et varie dans les différentes lignées analysées. Ceci est probablement dû à la présence de l'étiquette GFP (27 kDa) et/ou aux différents niveaux d'expression des transgènes. Bien que la complémentation des phénotypes mutants avec les protéines sauvages soit dans certains cas partielle, ces expériences confirment que les taux faibles de recombinaison de type SSA dans les mutants *xrcc2* et *rad51b* sont liés à la mutation des gènes correspondants.

I.2. Vérification de l'induction des cassures double brin dans les plantes mutantes

L'efficacité de la recombinaison dans les plantes sauvages et mutantes pour XRCC2 a dans un premier temps été mesurée grâce à l'induction d'une CDB par l'enzyme de restriction I-SceI dans le locus DGU.US (Orel *et al.*, 2003). La cassette d'expression inductible d'I-SceI a été introduite dans chacune des lignées par transformation (*via Agrobacterium tumefaciens*) et les plantes présentant un seul site d'insertion ont été sélectionnées. Etant donné que le

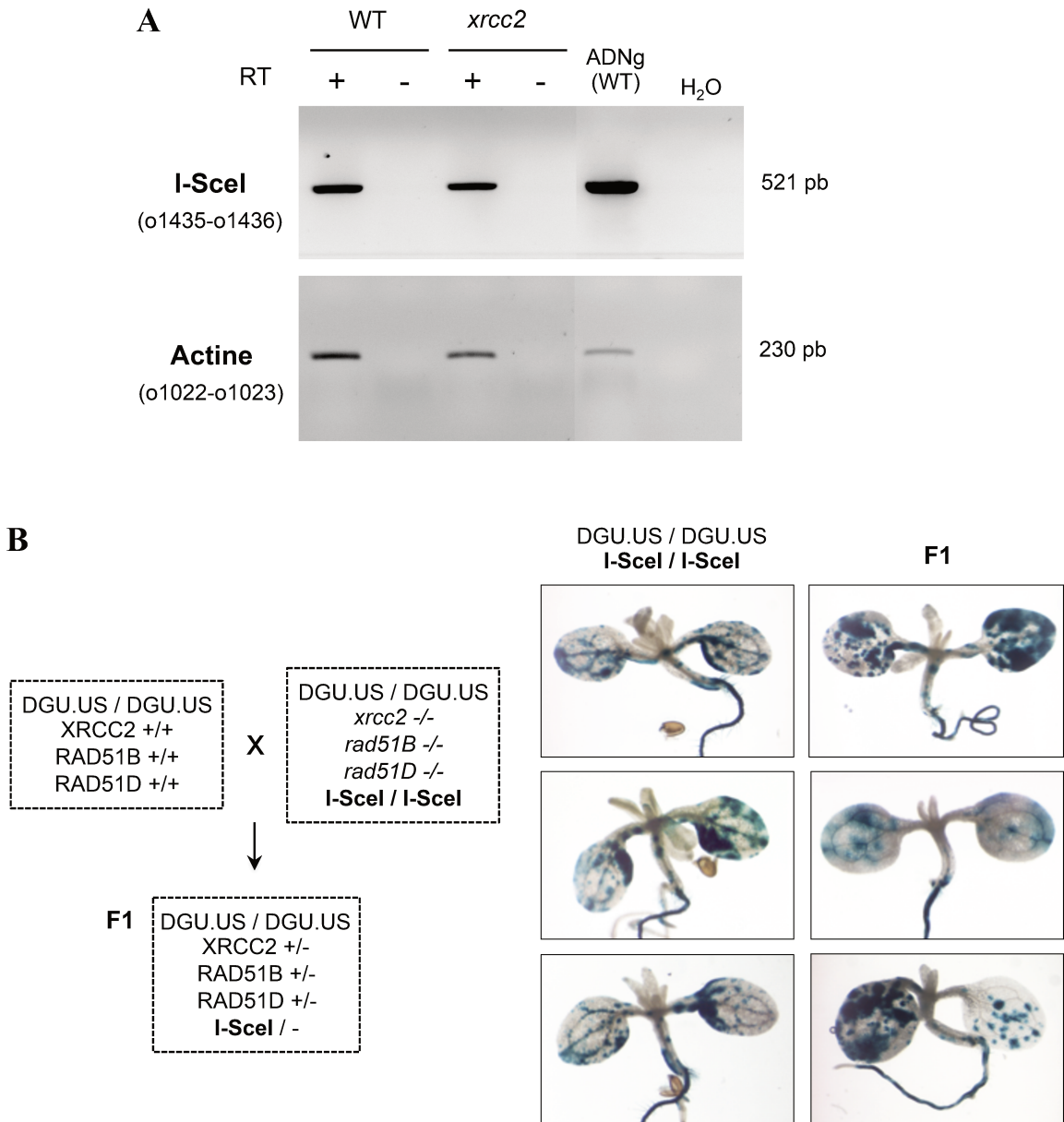


Figure 18 : La cassette d'expression inducible de l'enzyme I-SceI s'exprime dans les plantes mutantes *xrcc2* en présence d'inducteur et l'enzyme est fonctionnelle dans les mutants *xrcc2 rad51b rad51d*.

- (A) Analyse de l'expression du gène I-SceI par RT-PCR à partir d'ARN extraits de plantes cultivées en présence d'œstradiol (l'inducteur de l'expression d'I-SceI). RT (*Reverse Transcription*), ADNg (WT) : ADN génomique extrait de plantes sauvages possédant la cassette d'expression inducible d'I-SceI.
- (B) Croisement de plantes sauvages avec des plantes mutantes *xrcc2 rad51b rad51d* exprimant I-SceI et mesure de l'efficacité de recombinaison des plantules F1 de 5 jours cultivées en présence d'œstradiol par révélation GUS (cf Matériel et Méthodes, Article 1).

transgène s'intègre de manière aléatoire dans le génome, le site d'insertion de la construction - et le contexte chromatinien - sont différents dans chacune des lignées sélectionnées. L'analyse de trois lignées indépendantes a donné des résultats similaires (**Article 1, figure 1 D**), ce qui suggère que le faible nombre de spots recombinants observés dans les mutants *xrcc2* n'est pas lié au site d'insertion de la cassette d'expression d'I-SceI. Afin d'étayer cette conclusion, deux analyses supplémentaires ont été menées pour vérifier l'expression du transgène et l'activité de clivage de l'enzyme I-SceI dans ces lignées (**Figure 18**). D'une part, l'expression de la construction dans une lignée de plantes sauvages et une lignée mutante *xrcc2* a été analysée par RT-PCR grâce à des amorces spécifiques de la séquence codante d'I-SceI. Cette analyse a confirmé que la quantité d'ARN messagers d'I-SceI est équivalente dans les deux lignées (**Figure 18 A**). D'autre part, pour confirmer que l'activité endonucléase d'I-SceI (au niveau de son site de clivage dans le locus DGU.US) est similaire dans les plantes sauvages et les plantes mutantes analysées, des mutants *xrcc2 rad51b rad51d* exprimant de manière inducible I-SceI ont été croisés avec des plantes sauvages (**Figure 18 B**). Ces deux lignées possèdent le substrat DGU.US à l'état homozygote. L'objectif est de vérifier l'activité de l'enzyme I-SceI du triple mutant en restaurant un contexte sauvage en F1 (un des allèles de chaque gène permet la production de chacun des paralogues de RAD51). Suite à l'induction de l'expression d'I-SceI, les plantules F1 présentent de nombreux spots et secteurs recombinants (**Figure 18 B**), contrairement aux plantules *xrcc2 rad51b rad51d* (où seuls quelques spots par plantes sont observés - donnée non montrée). La présence des paralogues de RAD51 fonctionnels est donc suffisante à la restauration du taux de recombinaison de plantules sauvages exprimant l'enzyme I-SceI (**Figure 18 B**). Ceci implique que le nombre de CDB générées dans le substrat DGU.US est équivalent dans les plantules sauvages et celles de la génération F1. Puisque le gène codant pour l'endonucléase responsable des cassures est

apporté par le génome des plantes *xrcc2 rad51b rad51d*, l'efficacité de clivage de l'enzyme I-SceI des plantules triples mutantes est donc équivalente à celle des plantules sauvages.

II. XRCC2 n'est pas impliqué dans la jonction des extrémités d'ADN

Pour confirmer à l'échelle moléculaire le rôle de XRCC2, RAD51B et RAD51D dans la recombinaison de type SSA, nous avons mis au point une analyse par Southern blot permettant de visualiser directement le produit de la recombinaison. Cette analyse a permis de démontrer que l'absence d'un ou plusieurs de ces trois paralogues de RAD51 abolit la capacité des cellules végétales à générer le produit de la recombinaison (**Article 1, figures 2 et 6**). L'ensemble des blots réalisés ont de plus mis en évidence l'existence d'une bande de 3,2 kb dans l'ADN des plantes sauvages préalablement digérées par I-SceI (**Article 1, figure 2, colonne 9 et figure 6 A, colonne 4**). Cette bande n'est visible que dans l'ADN des plantes cultivées en présence d'œstradiol (donc dans lesquelles l'enzyme I-SceI est exprimée) et est résistante à la digestion par I-SceI *in vitro*. L'amplification par PCR et le séquençage de ces fragments a révélé qu'une partie d'entre eux porte des mutations dans le site de restriction d'I-SceI (au niveau du substrat DGU.US), principalement des délétions de quelques nucléotides (**Article 1, figure supplémentaire 1**). Ces produits peuvent être attribués à des événements de jonction des extrémités d'ADN (recombinaison non homologue), comme précédemment décrit dans la littérature (Salomon & Puchta, 1998 ; Heacock *et al.*, 2004 ; Puchta, 2005 ; Cermak *et al.*, 2011 ; Miller *et al.*, 2011). Ces fragments ne sont en revanche jamais détectés dans l'ADN de plantes mutantes *xrcc2* (**Article 1, figure 2**). Ceci suggère que la protéine XRCC2 pourrait jouer un rôle dans la jonction des deux extrémités d'ADN issues de la digestion par l'enzyme I-SceI (extrémités cohésives 3' sortantes).

Afin de tester cette hypothèse, nous avons utilisé un test *in vivo* de recircularisation d'un plasmide mesurant la capacité des cellules végétales à réparer une CDB générée par

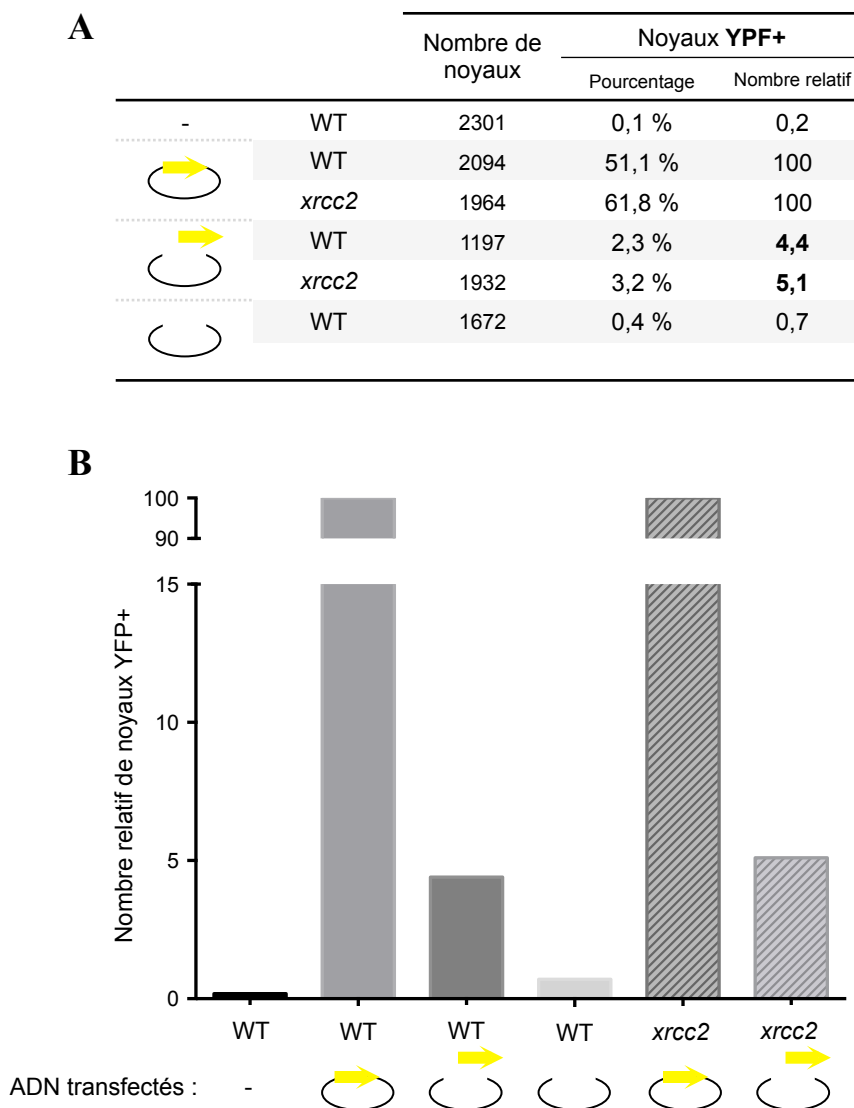


Figure 19 : XRCC2 n'est pas impliqué dans la jonction des extrémités d'ADN.

Un plasmide contenant le gène de fusion H2B-YFP sous le contrôle du promoteur 35S (préalablement digéré ou non par l'enzyme I-SceI) est transfecté dans des protoplastes d'*Arabidopsis* sauvages ou mutants pour XRCC2. Le plasmide linéaire sans le fragment H2B-YFP- T_{NOS} (représenté par un rectangle jaune) est également transfecté dans des protoplastes sauvages comme contrôle négatif. 24h après transfection, les noyaux sont extraits des protoplastes et la fluorescence jaune analysée par cytométrie en flux.

l'enzyme de restriction I-SceI. Des tests similaires ont précédemment été utilisés dans des protoplastes ou des feuilles de tabac et d'*Arabidopsis thaliana* (Gorbunova & Levy, 1997 ; Gorbunova *et al.*, 2000 ; Gallego *et al.*, 2003). Le plasmide utilisé contient la séquence codante de l'histone H2B fusionnée à celle du gène rapporteur YFP, sous le contrôle du promoteur 35S. Un site de restriction de l'enzyme I-SceI est situé entre le promoteur 35S et la séquence codante H2B et un autre se situe en aval du terminateur du gène de fusion (terminateur de la nopaline synthétase, T_{NOS}). La digestion du plasmide par I-SceI excise donc le fragment H2B-YFP-T_{NOS}. Des protoplastes issus de feuilles de plantules d'*Arabidopsis* sauvages et mutantes *xrcc2* sont transfectés avec le plasmide circulaire (contrôle de transfection) ou le plasmide digéré *in vitro* par I-SceI (plasmide linéarisé + fragment H2B-YFP-T_{NOS}). La réintégration du fragment H2B-YFP-T_{NOS} dans le plasmide linéarisé au sein des protoplastes permet l'expression du gène de fusion. La présence de la protéine nucléaire H2B-YFP dans les protoplastes est détectée par cytométrie en flux après extraction des noyaux (24h après transfection). Davantage de détails concernant le plasmide utilisé, l'obtention et la transfection des protoplastes ainsi que l'analyse par cytométrie en flux sont présentés dans le chapitre III.

Les résultats présentés dans la **figure 19** indiquent que l'efficacité de transfection des protoplastes sauvages et *xrcc2* est relativement similaire (51,1 % et 61,8 % de protoplastes fluorescents YFP⁺, respectivement). La capacité des cellules végétales à réparer les CDB générées par I-SceI est mesurée par le nombre de protoplastes YFP⁺ après transfection du plasmide digéré, normalisé au nombre obtenu avec le plasmide circulaire. Nos résultats montrent que cette capacité est équivalente dans les deux fonds génétiques (**Figure 19**), indiquant que la protéine XRCC2 n'est pas requise à la jonction des extrémités d'ADN dans ce système plasmidique. Ces analyses suggèrent donc que XRCC2 n'est pas impliqué dans les

voies de jonctions des extrémités d'ADN chez *Arabidopsis*, comme c'est le cas chez les Mammifères (Johnson *et al.*, 1999).

III. XRCC2 joue un rôle indépendant de RAD51 dans la voie SSA

Les analyses menées dans cet article ont démontré que les protéines XRCC2, RAD51B et RAD51D sont impliquées dans la voie de recombinaison d'hybridation simple brin (SSA). La capacité du complexe BCDX2 humain à catalyser l'hybridation de fragments d'ADNsb *in vitro* suggère un rôle direct de ces protéines dans l'étape d'hybridation des deux séquences répétées (Yokoyama *et al.*, 2004). Cette étude a de plus montré que le complexe BCDX2 présente une haute affinité pour les structures d'ADN branchées, telles que les structures d'ADN en Y qui résultent de l'hybridation des séquences répétées au cours du SSA. Bien que ces résultats soutiennent l'hypothèse d'un rôle direct de XRCC2, RAD51B et RAD51D dans la voie SSA, une action indirecte de ces protéines ne peut pas être exclue. Etant donné que les paralogues de RAD51 sont impliqués dans la formation et la stabilisation des filaments nucléoprotéiques RAD51-ADNsb (pour revue, voir Suwaki *et al.*, 2011), il est possible que l'absence d'un paraglogue conduise à la formation d'un filament non-fonctionnel. La présence de ce filament pourrait bloquer l'hybridation des séquences répétées situées de part et d'autre de la cassure par encombrement stérique. Ainsi, la voie SSA serait inhibée de manière indirecte en absence d'un ou de plusieurs paralogues de RAD51.

Afin de tester ces hypothèses, nous avons généré des plantes mutantes pour XRCC2 et RAD51 possédant le locus rapporteur de recombinaison DGU.US (Orel *et al.*, 2003). Ces plantes sont issues du croisement d'une lignée homozygote pour le substrat DGU.US et pour la mutation *xrcc2* avec une lignée homozygote pour le même substrat, pour un allèle KO *rad51* et pour la construction RAD51-GFP. La protéine de fusion RAD51-GFP restaure la fertilité du mutant *rad51* indispensable à la réalisation des croisements (Da Ines *et al.*, 2013b).

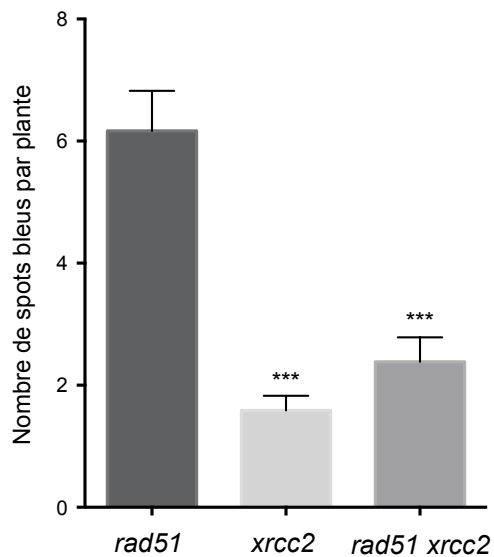


Figure 20 : La réduction de l'efficacité du SSA observée en absence de XRCC2 est indépendante de RAD51

L'efficacité de la recombinaison de type SSA au locus DGU.US est mesurée comme décrit dans l'Article 1 (Matériel et Méthodes). Moyennes du nombre de spots recombinants par plante $\pm$ erreur standard. Le nombre moyen de spots recombinants dans les lignées *xrcc2* et *rad51 xrcc2* n'est pas significativement différent. *** $p \leq 0,0001$ (test Mann-Whitney).

L'efficacité de la recombinaison de type SSA est mesurée dans des plantes *xrcc2*, *rad51* et *xrcc2 rad51* qui ne possèdent pas la construction RAD51-GFP (**Figure 20**). Comme attendu, le nombre moyen de spots recombinants par plante est significativement réduit dans les mutants *xrcc2* par rapport aux mutants *rad51* - l'efficacité du SSA étant équivalente dans des plantes sauvages et des plantes dont RAD51 est non-fonctionnel (**Article 1, Figure 4**). L'efficacité de la réparation dans le double mutant *xrcc2 rad51* n'est en revanche pas significativement différente de celle du simple mutant *xrcc2*, indiquant que l'inhibition du SSA en absence de la protéine XRCC2 est indépendante de la présence de la protéine RAD51. Ces résultats démontrent donc que XRCC2 joue un rôle indépendant de RAD51 - très probablement direct - dans la voie SSA. Cette étude constitue, à notre connaissance, la première démonstration d'une fonction indépendante de RAD51 d'un paralogue de RAD51. Des études biochimiques seront nécessaires à l'identification de son rôle précis.

Chapitre II

Article 2: "A role for XPF-ERCC1 in inhibiting homologous recombination"

Heïdi Serra, Fabienne Degroote, Olivier Da Ines,
Maria E. Gallego & Charles I. White

(article soumis)

ABSTRACT

In addition to its well-characterized role in nucleotide excision repair, the XPF-ERCC1 (Rad1-Rad10 in yeast) structure-specific endonuclease plays an important role in recombination. Cleaving DNA at double-strand to single-strand junctions, XPF-ERCC1 acts to remove non-homologous single-stranded 3' tails from homologous recombination intermediates. The need for this activity however depends on both the length and the number of the non-homologous tails. Using recombination substrates which generate only short non-homologous tails, and thus should not require the action of XPF-ERCC1, we have taken advantage of this to further study the roles of this endonuclease in recombination in the plant, *Arabidopsis thaliana*.

Using a specific reporter for RAD51-dependent recombination we show that both spontaneous and I-SceI induced recombination are significantly enhanced (more than 2-fold) in *xpf* mutants relative to the WT controls. Expression of a wild-type XPF-GFP fusion protein in *xpf* mutant plants suppresses the hyper-recombination phenotype, confirming that it specifically results from the absence of XPF. In contrast, expressing the nuclease-dead XPF_{D757A}-GFP protein in *xpf* mutant plants does not affect the hyper-recombination effect of the absence of XPF.

The endonuclease activity of XPF thus acts to inhibit RAD51-dependent homologous recombination and we propose that this results from effects on homology search *in cis*, versus *in trans*, in a repair pathway competition model.

AUTHOR SUMMARY

XPF-ERCC1 is a key actor in the repair of DNA through nucleotide excision and the importance of its role is seen in patients with the cancer-prone Xeroderma Pigmentosum syndrome. In addition to this role in nucleotide excision repair, the XPF-ERCC1 structure-specific endonuclease plays a well-characterized role in recombination and the repair of DNA double-strand breaks. In order to search for further roles of this endonuclease, we present here a study involving specific tester loci which do not require the action of XPF-ERCC1 for recombination. Absence of XPF endonuclease activity results in an enhancement of RAD51-dependent homologous recombination in *Arabidopsis thaliana*. XPF thus acts to inhibit homologous recombination and we propose that it does so by favouring the use of local micro-homologies and thus restricting the search for more distant homologous donor sequences.

INTRODUCTION

The efficient repair of damaged DNA is essential for the maintenance of genomic integrity of all living organisms. Living cells have evolved multiple ways of repairing the many types of DNA damage thanks to the coordinated action of numerous proteins (for reviews, see [1,2]). This work concerns one of these proteins - the XPF (*xeroderma pigmentosum complementation group F*) protein. Disruption of XPF gene has dramatic consequences in mammals: XPF-deficient mice show growth retardation and die approximately 3 weeks after birth [3] and mutations of human XPF result in the UV sensitive cancer-prone syndrome, *Xeroderma Pigmentosum* [4]. Consistent with its essential role in DNA damage repair, XPF is very well conserved in eukaryotes, with well-studied orthologues in many organisms including Rad1 in *Saccharomyces cerevisiae* [5], Rad16 in *Schizosaccharomyces pombe* [6], Xpf-1 in *Caenorhabditis elegans* [7], Mei-9 in *Drosophila melanogaster* [8] and UVH1/RAD1/XPF in *Arabidopsis thaliana* [9-12].

The XPF protein forms a heterodimeric complex with ERCC1 (*excision repair cross-complementation 1*) protein *in vitro* and *in vivo* [4] and formation of this complex stabilises the two proteins [13-15]. The XPF-ERCC1 complex and its yeast homologue Rad1-Rad10 are structure- specific endonucleases which cleave DNA at double strand (ds) to single strand (ss) junctions, nicking the dsDNA on the 5'-3' strand, two nucleotides from the junction [4,16-18]. *In vitro* endonuclease assays revealed that XPF-ERCC1 cleaves a variety of substrates, including stem- loops and structures with 3' overhangs [16]. XPF provides the endonuclease activity of the complex, while ERCC1 is catalytically inactive but instead regulates DNA- and protein-protein interactions [19-21].

XPF-ERCC1 was first identified as a factor required for nucleotide excision repair (NER) of helix-distorting DNA lesions, especially ultra-violet (UV)-induced damage [4,11,15]. The complex is required for 5' cleavage of the DNA lesion in DNA near sites of

damage (for reviews, see [22,23]). XPF-ERCC1 is also involved in the initial step of interstrand crosslinking (ICL) repair pathway, making the incisions on both sides of an ICL placed near a dsDNA-ssDNA junction [24]. Moreover, the XPF-ERCC1 complex has been found to function in homology-directed double-strand break (DSB) repair, through single-strand annealing (SSA) and gene conversion (GC) [17,25-31].

SSA is a major recombination pathway for repairing DSB that arise between repeated sequences [32,33]. After bidirectional 5'-3' resection of the DSB ends, RAD52 (and Rad59 in budding yeast) promotes the annealing of the exposed complementary sequences. The 3' ssDNA ends are non-homologous with the new flanking regions, and must be cleaved in order to complete repair by filling-in of any single-strand gaps and strand ligation (for reviews, see [32,34]). The removal of the non-homologous 3' tails is ensured by XPF-ERCC1 (Rad1-Rad10) endonuclease in association with MSH2-MSH3 mismatch recognition complex [17,26,27,30,31,35,36]. Two additional actors have been identified in yeast: Slx4 (*Synthetic lethal of unknown function protein 4*) and Saw1 (*Single-strand annealing weakened 1*) [37,38]. Saw1 facilitates targeting of Rad1 at 3' tailed substrates and enhances the cleavage activity of Rad1-Rad10 [39], and Slx4 is a scaffold for the binding of a number of DNA repair proteins, including XPF-ERCC1 [40-42].

Homologous recombination by gene conversion (GC) can also involve the removal of 3' non-homologous tails. The most common models of GC are the synthesis dependent strand annealing (SDSA) [43], the double strand break repair (DSBR) [44] and the break induced replication (BIR) models [45,46]. Most mitotic GC events are thought to occur by the SDSA mechanism [32,47,48]. In this model, the 5'-3' resection of the DSB ends generates 3' ssDNA overhangs that are bound by RAD51 and the resulting nucleofilament is the active molecular species in searching for and invading a homologous donor DNA sequence. Before an invading 3' DNA end can prime new DNA synthesis to copy the donor template, any non-

homologous 3' tail must however be removed. Subsequent unwinding of the extended invading strand from the donor DNA duplex allows it to then anneal back to the non-invading DSB end. Removal of the second non-homologous 3' tail is then potentially required to permit gap filling synthesis and ligation, which complete the process (for reviews, see [32,34,35]). If only one side of the DSB ends with non-homologous sequence, the 3' end that shares homology with the donor sequence can perform the strand invasion and there is no barrier to the initiation of DNA synthesis. Numerous studies have demonstrated the involvement of XPF-ERCC1 (Rad1-Rad10) complex in the removal of non-homologous 3' tails during GC in yeasts, animals and plants [17,25,26,28-31,35]. The requirement for XPF-ERCC1 (Rad1-Rad10) during both SSA and GC processes depends not only on the presence of non-homologous 3' tails, but also on their length. Neither Rad1-Rad10 in yeast, nor XPF-ERCC1 in mammals, is required for GC or SSA if non-homologous 3' tails are less than approximately 30 nucleotides long [25,27,30,31,49]. In plants, the roles of Arabidopsis XPF and ERCC1 in SSA and GC involving the removal of long non-homologous tails have been confirmed using plasmid and chromosomal substrates [28,29] and in this work we confirm that Arabidopsis XPF is not required for the removal of short non-homologous 3' tails in both SSA and SDSA recombination, as is the case for recombination in yeasts and animals.

The use of recombination substrates with only short non-homologous overhangs has permitted us to bring to light an unexpected hyper-recombination phenotype in *xpf* mutant plants. We show that this is due to a role of the nuclease activity of XPF in inhibiting SDSA recombination and propose that it results from effects on homology search in a repair pathway competition model.

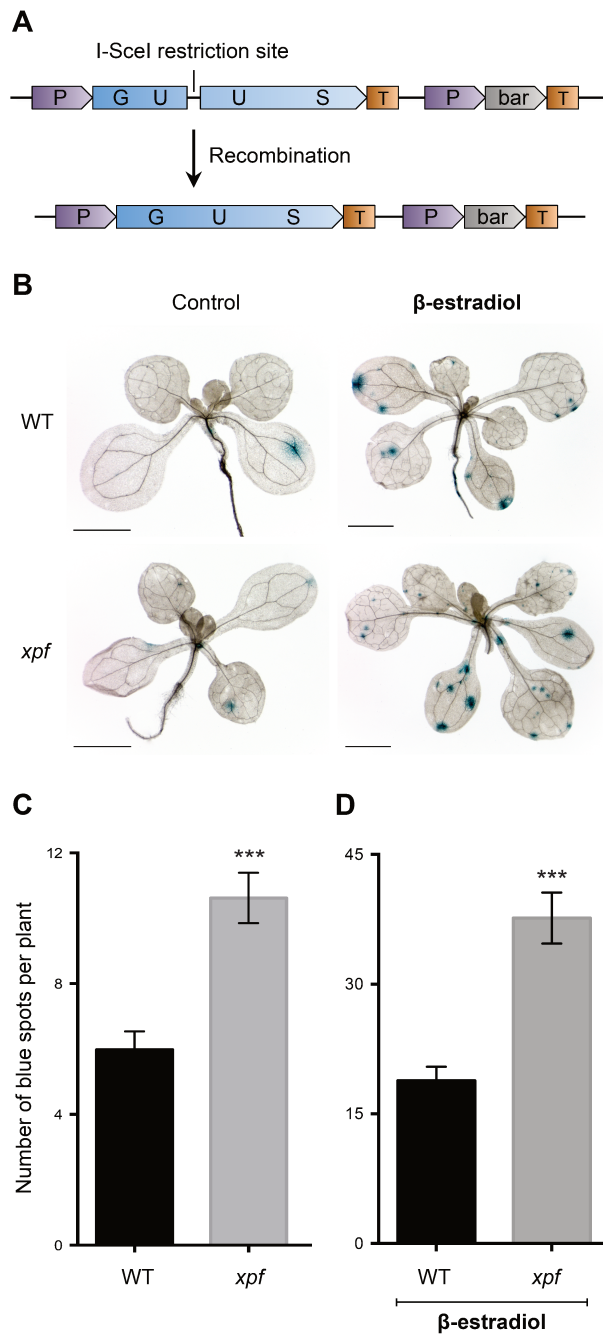


Figure 1

RESULTS

XPF is not required for the removal of short non-homologous ends during SSA

Neither yeast Rad1-Rad10, nor mammalian XPF-ERCC1 is required for removal of short non-homologous tails during homologous recombination [25,27,30,31,49]. In the course of our studies of the roles of structure-specific endonucleases in recombination, we tested in *Arabidopsis* this XPF-independence of recombination involving short non-homologous overhangs using the well-characterised chromosomal DGU.US recombination reporter locus. This consists of an I-SceI restriction site flanked by 3' and 5' truncated copies of the β -glucuronidase gene (GUS) in direct orientation and with a sequence homology overlap of 557 bp (**Figure 1**) [50]. DNA cleavage by I-SceI expression *in planta* induces recombination between the flanking GUS sequences and the resulting functional GUS gene is scored histochemically as blue spots. In the case of I-SceI induced DSB, repair by SSA involves the removal of two 3' single stranded DNA ends of 21 nucleotides. The situation is more complex in the case of spontaneous DSB: if the break occurs between the two repeated sequences, SSA repair would involve the removal of two short tails, however repair of a break in one of the repeated sequences would involve the removal of only one 3' tail.

The GUS reporter locus was introduced into *xpf* mutant and wild-type (WT) plants through crossing, followed by further crosses to introduce the inducible I-SceI expression cassette (Materials and Methods). Recombination efficiency was monitored in 14 day-old DGU.US WT and *xpf* mutant plants carrying, or not, the I-SceI expression cassette (**Figure 1B, C, D**). The data show clearly that the absence of XPF causes no reduction in efficiencies of spontaneous nor of I-SceI induced DGU.US recombination - to the contrary, *xpf* mutant plants are significantly hyper-recombinant at this locus. An independent repetition of this experiment gave similar results (**Table 1**). These data thus confirm that XPF is not required

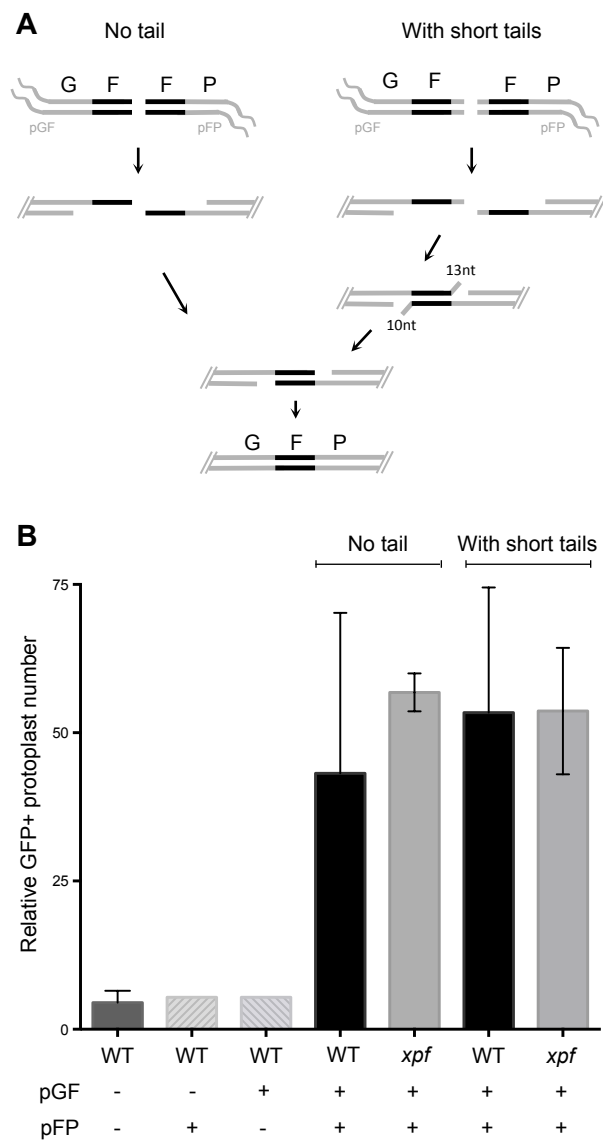


Figure 2

for removal of short non-homologous ends during SSA recombination *in planta*, and bring to light an unexpected inhibitory role for XPF on homologous recombination in this context.

The XPF-independence of SSA recombination in Arabidopsis involving short non-homologous tails was further confirmed by monitoring inter-molecular recombination between linearized plasmids co-transfected into protoplasts from WT and *xpf* mutant plants (**Figure 2**). The pGF plasmid contains a 35S promoter followed by 3' truncated GFP gene encoding the N-terminal region of the GFP protein. The pFP plasmid contains the 5' truncated GFP gene encoding the C-terminal region of the GFP protein, and the nopaline synthetase (NOS) terminator. The overlapping common homology region of the truncated GFP genes in the two plasmids is 607 bp long. Linearization of pGF and pFP with HpaI and SnaBI respectively test SSA recombination in the absence of non-homologous ends ("No tail"). Digestion of pGF and pFP by SnaBI and HpaI (respectively) generates short non-homologous tails (of 13 and 10 nucleotides, respectively) which must be removed for completion of recombination between the two genes (**Figure 2A**). The digested plasmids were co-transfected into WT and *xpf* mutant Arabidopsis protoplasts and GFP⁺ cells quantified by flow cytometry (Materials and Methods). As shown in **Figure 2B**, frequencies of SSA events are equivalent in the two lines in the presence or absence of short non-homologous tails. XPF is thus not required for removal of short non-homologous tails (< 13 nucleotides) during SSA processing in Arabidopsis cells.

XPF inhibits SDSA recombination

DGU.US → GUS recombination is thus more efficient in *xpf* mutant than in WT plants. Given that the absence of RAD51 does not reduce the efficiency of DGU.US → GUS recombination [51,52], productive (GUS⁺) recombination at the DGU.US locus occurs majoritarily through RAD51-independent SSA. RAD51-dependent recombination can

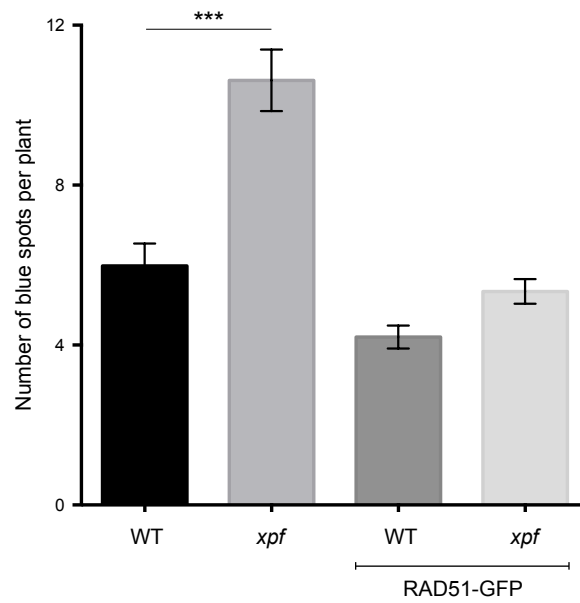


Figure 3

however also yield GUS+ [53,54] and it was thus necessary to clarify the question of the relative contributions of RAD51-dependent (SDSA/GC) and -independent (SSA) recombination in the hyper-recombination phenotype of the *xpf* mutant plants. To test this we knocked-out RAD51 activity in the DGU.US lines by expressing the dominant-negative RAD51-GFP fusion protein [55]. Knocking-out RAD51 strand-exchange activity almost completely eliminates the hyper-recombination phenotype of *xpf* mutant plants, while having little effect on spontaneous DGU.US recombination in control WT plants as expected (**Figure 3**). RAD51-dependent SDSA/GC thus accounts for the observed increase in spontaneous DGU.US → GUS recombination in the absence of XPF.

To confirm and extend this conclusion, the analysis was carried out with a different recombination tester locus which specifically scores RAD51-dependent SDSA/GC recombination. Productive (GUS+) recombination at the IU.GUS locus involves the use of an internal fragment of the GUS gene (5' and 3' deleted) as donor sequence to repair DSB in an interrupted (by an inserted I-SceI site), but otherwise complete GUS gene [50]. Repair of an I-SceI induced DSB at the IU.GUS involves the removal of two short (16 and 18 nt) non-homologous tails and would thus be expected to give similar results to those observed for the DGU.US locus (above).

Spontaneous SDSA/GC recombination at IU.GUS was monitored in WT and *xpf* mutant plants (**Figure 4B**) and I-SceI induced SDSA recombination was tested in plants carrying the I-SceI expression cassette (homozygotes) and grown in the presence of the inductor β-estradiol (**Figure 4C**). As seen for the DGU.US → GUS recombination, these analyses show a significant increase (more than 2-fold) of SDSA/GC events in *xpf* mutants compared to WT plants (**Figure 4**). This increase is observed for both spontaneous and DSB-induced recombination. Control experiments with the double *xpfxrcc2* mutant confirmed the dependence of this effect on the RAD51 paralogue XRCC2, which we have previously shown

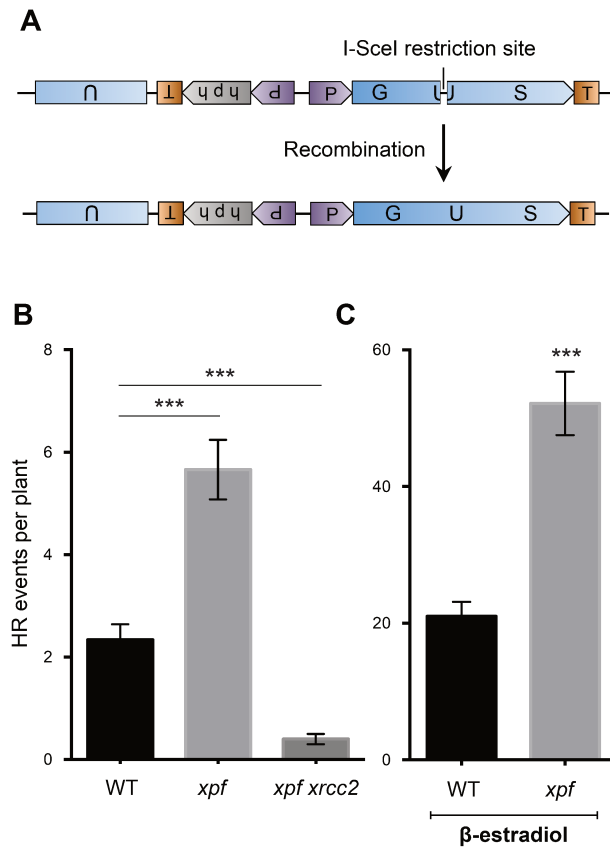


Figure 4

to be required for SDSA and SSA recombination in Arabidopsis [52]. Given that the structure of this locus precludes productive GUS⁺ recombination through RAD51-independent SSA, the increased recombination observed in *xpf* mutant plants results from RAD51-dependent SDSA/GC homologous recombination. An independent repetition of this experiment confirmed these results (**Table 2**) and as expected a similar effect was observed in *ercc1* mutant plants (**Figure S1**). Taken together, these data clearly demonstrate an inhibitory action of the XPF-ERCC1 complex on SDSA recombination.

The nuclease activity of XPF is required for inhibition of SDSA

The catalytic activity of the XPF-ERCC1 site-specific endonuclease is carried on the XPF subunit [19,20]. The amino acids essential for the catalytic activity have been identified in the human protein using site-directed mutagenesis and are highly conserved among eukaryotes [20]. To determine whether the inhibition of SDSA by XPF-ERCC1 is due to its endonuclease activity or to some other role of the complex, we constructed nuclease-minus (and WT) XPF-GFP translational fusions driven by the XPF promoter region (**Supplementary Figures 2A and 3A**; Materials and Methods). The nuclease-minus XPF protein, XPF_{D757A}, carries the equivalent amino acid substitution which in the human protein disrupts XPF catalytic activity but has no influence in DNA binding activity [20]. XPF-GFP and XPF_{D757A}-GFP were introduced independently into *xpf* mutant plants, transformants selected and expression of the fusion protein confirmed by visual examination of GFP fluorescence. The presence of XPF-GFP fully complements XPF-deficient plants for repair of UV- and MMC-induced DNA damage, while the XPF_{D757A}-GFP does not, confirming that XPF-GFP is active and functional in DNA repair, while the nuclease-minus XPF_{D757A}-GFP protein is inactive (**Supplementary Figures 2 and 3**).

We introduced the IU.GUS recombination reporter locus into these lines and

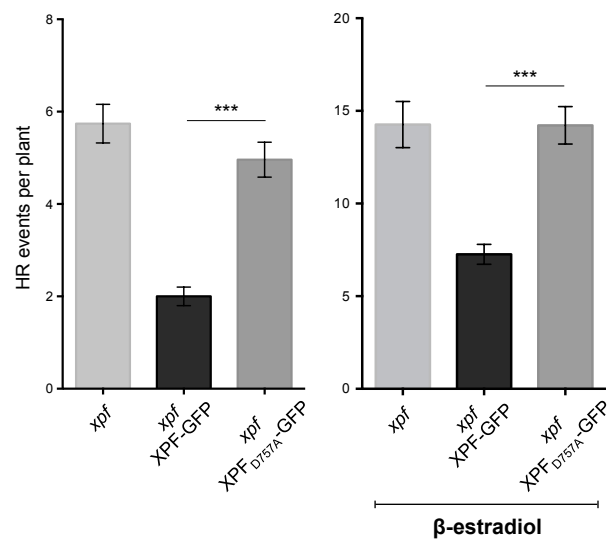


Figure 5

monitored spontaneous SDSA recombination at IU.GUS in *xpf*XPF-GFP and *xpf* XPF_{D757A}-GFP plants (**Figure 5**). As expected, complementation of *xpf* mutants with the XPF-GFP fusion protein gives SDSA recombination rates equivalent to WT plants, however this is not the case with the nuclease-minus XPF_{D757A}-GFP expressing line, which has a similar phenotype to the *xpf* mutant. The inhibitory role of XPF on SDSA recombination in Arabidopsis is thus due to its nuclease activity.

DISCUSSION

In addition to its well-characterized role in NER, the role of the XPF-ERCC1 complex in homology-directed DSB repair is well established (for reviews, see [56,57]) and has been shown to be due to cleavage of non-homologous 3' tails of recombination intermediates produced by both Single Strand Annealing and Synthesis Dependent Strand Annealing pathways [17,25-31]. The need for this activity however depends on both the length of the non-homologous tails, and their number (reviewed by [35]). The results presented here confirm that, as is the case in yeast and in animals, this requirement for Arabidopsis XPF-ERCC1 endonuclease in both SSA and SDSA pathways depends on the length of non-homologous 3' tails: the complex is needed to cleave long non-homologous 3' tails but is dispensable for the removal of short tails (< 21 nucleotides) *in planta* (this work and [28,29]).

Using a specific reporter for SDSA recombination (the IU.GUS substrate), we show that recombination efficiency is significantly increased (more than 2-fold) in *xpf* mutants relative to the WT controls (**Figure 4, Table 2**). This effect is seen in both spontaneous and I-SceI induced recombination (**Figure 4, Table 2**) and as expected, absence of ERCC1 shows the same effect (**Figure S1**). Complementation of *xpf* mutant plants with the XPF-GFP protein confirms that the increase of SDSA efficiency is specifically due to the absence of XPF (**Figure 5**). In contrast to the control XPF-GFP fusion protein, the presence of XPF_{D757A}-GFP has no effect on SDSA recombination rate in *xpf* mutant plants (**Figure 5**), confirming that the inhibitory role of XPF on SDSA is dependent on its ability to cleave DNA. A similar hyper-recombination effect of the absence of XPF is seen with the DGU.US recombination tester locus (**Figure 1, Table 1**). DGU.US → GUS recombination can in principle occur through SSA and SDSA recombination [54], although its independence of RAD51 strongly suggests that it occurs principally *via* SSA [51,52]. The increased recombination in *xpf* mutant

plants is however fully RAD51-dependent, as seen in the wild-type phenotype of *xpf* plants lacking RAD51 strand-exchange activity (expressing the dominant-negative RAD51-GFP) (**Figure 3**).

Our results thus show increased numbers of recombinant GUS⁺ spots in the absence of XPF endonuclease activity. This effect is observed in both the DGU.US and IU.GUS recombination tester loci and thus concerns both SDSA (IU.GUS and possibly DGU.US) and SSA (DGU.US) recombination. In both cases this increase is RAD51-dependent and thus absence of XPF results in an additional pool of SDSA events at these loci. It is possible that this reflects increased initiation of recombination (eg DSB) in *xpf* mutants, however the fact that *xpf* mutants are not general hyper-rec. and that we see similar effects on spontaneous and I-SceI induced recombination would argue against this. Taking this reasoning further, there must exist a pool of events which are repaired by non-productive (-> gus-) recombination in the presence of XPF, and by productive SDSA (-> GUS+) recombination in *xpf* mutants.

Numerous studies in yeasts, animals and plants have reported that repair of DSB arising between tandem direct repeated sequences mostly results in deletion events ascribed to the SSA pathway [32,33,50,58,59]. In *S. cerevisiae*, SSA is nearly 100% efficient when homologous regions flanking the DSB are at least 400 bp long [60]. Decreasing length of the repeated sequences and increasing sequence divergence both reduce the efficiency of SSA (3 % divergence between 205 bp repeats reduced SSA by a factor of 5) - as does to a lesser extent increased distance between repeats [36,59,60]. Similarly, introduction of DSB into one of two chromosomal repeats in hamster cells leads more frequently to deletion events than GC events (approximately 3:1 ratio) [33]. Consistent with these results, Orel and colleagues showed that recombination associated with deletions is the most efficient pathway of homologous DSB repair in *Arabidopsis* [50].

A model based on homology search competition has been proposed to explain the

DGU.US



IU.GUS



MEMEJ

preference for intra-chromosomal donors for homologous repair [59]. Following formation of the DSB and concomitantly with resection of the DSB ends, base pairing is examined in order to find homology between the resected strands (*in cis*), while the ends search for inter- or intra-chromosomal homology (*in trans*). Homology search *in cis* and *in trans* are simultaneous, and the choice of repair pathway depends on the first region of homology identified. According to this model, base pairing during homology search *in cis* generates ssDNA/dsDNA junctions, substrates of Rad1-Rad10 (XPF-ERCC1) endonuclease. The cleavage of 3' single strand tails by the complex would thus limit homology search *in trans* and, in turn, SDSA/GC recombination. XPF-ERCC1 would thus promote deletion-associated repair by inhibiting SDSA/GC recombination and favouring SSA.

In Figure 6 we present a model to explain our results based on this action of XPF-ERCC1 in limiting homology search through cleavage of a substrate generated by annealing of microhomologies during resection. By analogy with the model presented above [59], we propose that the XPF-ERCC1 complex recognizes the ssDNA/dsDNA junctions formed by annealing of microhomologies (during homology search *in cis*) and cleaves the 3' single strand tails (**Figure 6**). XPF-ERCC1 cleavage would thus disfavour homology search *in trans* and result in non-productive microhomology-mediated end-joining (MMEJ) events (*gus*⁻). In the absence of XPF-ERCC1 endonuclease activity, the enhanced stability of long 3' single-strand ends would promote homology search *in trans*, hence favouring SDSA/GC recombination (**Figure 4**, **Table 2**, **Figure S1**). Thus, we propose that the observed hyper-recombination phenotype in the absence of XPF nuclease activity is the result of competition between homology research *in cis* and *trans*.

MATERIALS AND METHODS

Plant material and growth conditions

The *Arabidopsis thaliana* *xpf* (*uvh1-1*) (Harlow *et al.*, 1994), *ercc1* (Dubest *et al.*, 2004) and *xrcc2* (Bleuyard *et al.*, 2005) mutants used in this work have been described previously. Single and double mutants were crossed with the recombination tester DGU.US-1 or IU.GUS-8 lines (Orel *et al.*, 2003) and mutants homozygous for the DGU.US or IU.GUS substrate loci were identified in the F₂, then used for spontaneous recombination tests. Wild-type lines homozygous for DGU.US or IU.GUS substrates were transformed with the inducible I-SceI expression cassette previously described (I-SceI coding sequence under control of β -estradiol) (Serra *et al.*, 2013) utilising the floral dip method (Clough & Bent, 1998). T₂ plants homozygotes for the I-SceI expression cassette (and DGU.US or IU.GUS substrate) were subsequently crossed with the mutant lines homozygous for DGU.US or IU.GUS substrate previously generated. F₂ homozygotes for each construct were used for I-SceI induced recombination tests. Wild-type control plants come from the same crosses.

Plants were grown under standard conditions: surface-sterilized seeds were stratified at 4°C for 2 days and grown *in vitro* on germination medium (0.8% w/v agar, 1% w/v sucrose and half-strength Murashige & Skoog salts (M0255; Duchefa Biochemie, Netherlands)) in a growth cabinet with a 16-h light/8-h dark cycle, at 23°C with 45–60% relative humidity. The growth medium was supplemented with 170 μ M 17- β -estradiol (E2758; Sigma-Aldrich) for induction of I-SceI expression.

Plasmid constructs

As control for transformation efficiency, we used the pGFP plasmid containing the cauliflower mosaic virus (CaMV) 35S promoter, the GFP gene and the nopaline synthetase

(NOS) transcription terminator. The 35S promoter sequence and the GFP open reading frame without the last 49 bp were amplified from pGFP by PCR (forward primer GATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTC and reverse primer TACGTATCGCGAAGTTAACTCCAGCAGGACC) and inserted into the pGEM-T Easy (Promega) to produce the pGF plasmid. The GFP open reading frame without the first 64 bp and the NOS terminator was amplified from pGFP by PCR (forward primer GTTAACTCGCGAATACGTAAACGGCCACAAGTTCAG and reverse primer AGCGCCCAATACGCAAACCGCCTCTCCCC) and inserted as above into the pGEM-T Easy to produce the pFP plasmid. Both generated plasmids were verified by sequencing.

pGF and pFP plasmids were linearized with *HpaI* or *SnaBI*, respectively, to prepare the "No tail" recombination substrate. For the "With short tails" recombination substrate, pGF and pFP plasmids were digested by *SnaBI* and *HpaI*, respectively. In both cases, the overlapping homology region is 607 bp to which pGF/*SnaBI* adds a non-homologous tail of 13 nucleotides and pFP/*HpaI* a tail of 10 nucleotides. Digested plasmids were purified and concentrated with QiaexII kit (Qiagen) as recommended by the manufacturer. DNA concentrations were adjusted to 1 µg/µl before protoplast transfection.

Protoplast isolation and transfection

The Arabidopsis protoplasts were isolated based on the methods reported by Sheen laboratory (Yoo *et al.*, 2007) with minor modifications. Briefly, leaves from 3-4 week old WT or *xpf* mutant plants were cut into 0.5-1 mm wide strips with fresh razor blades. The leaf strips were quickly transferred into 8 ml of digestion solution (0.25% w/v Macerozyme Onozuka R-10, 1% w/v Cellulase Onozuka R-10 (Yakult, Japan), 0.4 M mannitol, 20 mM 4-morpholineethanesulfonic acid (MES) pH 5.7, 20 mM KCl, 10 mM CaCl₂, 5 mM β-mercaptoethanol and 0.1% bovine serum albumin (BSA)) and vacuum applied for 30 min.

The vacuum was released gently and samples incubated for 3-h without shaking in the dark at 23°C. After incubation, the digestion solution was filtered through 30 µm nylon mesh to eliminate leaf debris. The Petri dish was rinsed with 8 ml of W5 solution (125 mM CaCl₂, 154 mM NaCl, 5 mM KCl, 2 mM MES pH 5.7) with shaking to release the remaining protoplasts followed by filtration. The pooled protoplast suspensions were then centrifuged at 100g for 2 min. The collected protoplasts were gently washed once with cold W5 solution and incubated 30 min on ice in 2 ml of W5 solution. The protoplast solution was subsequently centrifuged and the pellet resuspended in room temperature MaMg solution (0.4 M mannitol, 15 mM MgCl₂ and 4 mM MES pH 5.7). Protoplast concentrations were quantified by microscopy using Malassez cytometer slide and the protoplast concentration adjusted to 1.5×10^6 /ml.

All steps of protoplast transfection were carried out at 23°C. 100 µl (1.5×10^5) of protoplasts were gently mixed with 10 µl (10 µg) of each plasmid (digested pGF and pFP) and then 110 µl of 40% v/v polyethylene glycol solution (PEG-4000 (Fluka #81240), 0.8M mannitol, 1M CaCl₂ and 1M KCl). The transfection mixture was incubated for 30 min, then diluted in 15 ml of W5 solution and centrifuged at 100g for 2 min. The protoplasts were gently resuspended in 1 ml of WI solution (0.5 M mannitol, 0.5 M KCl and 4 mM MES pH 5.7) and then incubated for 24-h at 23°C in the dark without shaking. For each preparation, 100µl of WT and *xpf* mutant protoplasts were transfected in parallel with 10µl (10 µg) of pGFP to quantify the transfection efficiency in each transformation experiment.

GFP fluorescence analyses by flow cytometer

Transfected protoplasts were filtered through 30 µm nylon mesh and analysed directly in WI solution. Flow cytometric analyses were performed with an Attune Acoustic Focusing Cytometer (Life Technologies). GFP fluorescence excited with a 488 nm diode laser was detected using a 530/30 nm bandpass filter in the BL1 channel.

Histochemical staining of GUS expression

Fourteen-day old seedlings grown under standard conditions (supplemented or not with 17- β -estradiol) were harvested and incubated in staining buffer (0.2% Triton X-100, 50 mM sodium phosphate buffer (pH 7.2), 2 mM X-Gluc (5-bromo-4-chloro-3-indolyl- β -D-glucuronic acid; Biosynth), dissolved in N,N-dimethylformamide). Plants were infiltrated under vacuum for 15 min and incubated at 37°C overnight. The staining solution was then replaced with 70% ethanol to remove leaf pigments and the blue spots were counted under a binocular microscope.

Cloning of XPF, mutagenesis and plant transformation

To analyse the role of the XPF nuclease activity, the genomic region without stop codon (3873 bp) and a 1410 bp 5' upstream sequence of XPF was amplified (forward primer TGATTAGCATTTAGCGTCAAG and reverse primer ATCCTTGCAATCTGTTACACC), inserted into pDONR221 and verified by sequencing. This pDONR221_pXPF::XPF_{wt} was then used as a template for site-directed mutagenesis. Mutagenesis was performed using the QuikChange Lightning Site-Directed Mutagenesis kit (Stratagene) following the manufacturer's instructions. To generate a plasmid carrying the a3089c mutation in the XPF active site, equivalent to that described by Enzlin and Schärer (2002), two oligonucleotide primers complementary to opposite strands of the target sequence and both containing the mutation (forward primer TACTACTAGAGGTCGGCGcCTATATTCTATCTCCTTC and reverse primer GAAGGAGATAGAATATAGgCGCCGACCTCTAGTGTA) were synthesized and extended by thermal cycling using *PfuUltra* HF DNA polymerase. This generates the pDONR221_pXPF::XPF_{D757A} carrying the a3089c mutation in the XPF genomic sequence. The reaction was then treated with *DpnI* enzyme to digest methylated and hemimethylated parental plasmid DNA. The remaining extended plasmid DNA containing the

a3089c mutation was subsequently transformed into XL10-Gold ultracompetent cells. The pDONR221_pXPF::XPF_{wt} and the pDONR221_pXPF::XPF_{D757A} were verified by sequencing and cloned into the GATEWAY destination vector pB7FWG2 from which the 35S promoter had been removed with a *SacI/SpeI* digest (Karimi *et al.*, 2007). This generates a translational fusion of eGFP to the C-terminus of the XPF. The pXPF-GFP and pXPF_{D757A}-GFP plasmids were transformed into the *Agrobacterium tumefaciens* C58C1 strain and used to transform *xpf* mutant plants (Harlow *et al.*, 1994) by the floral dip method (Clough & Bent, 1998).

GFP fluorescence observation of XPF-GFP fusion proteins

Expression of the XPF-GFP and XPF_{D757A}-GFP fusion proteins in transformed plants was analysed by observation of GFP fluorescence in roots of five day-old seedlings using a motorised Zeiss AxioImager.Z1 epifluorescence microscope (Carl Zeiss AG, Germany). Photographs were taken with an AxioCam Mrm camera and Zeiss filter set 38HE. Images were further treated using the AxioVision 4.6.2 software (Carl Zeiss AG, Germany).

UV-light and Mitomycin C sensitivity assays

For UV sensitivity assays, seeds were surface-sterilised and germinated on vertical plates containing 0.8% w/v agar, 1% w/v sucrose and half-strength Murashige and Skoog salts medium. Five-day-old seedlings were then treated with 300 J.m⁻² UV-C radiation (GS Gene linker; Bio-Rad, Hercules, CA, USA), allowed to grow for 5 days in the dark to avoid photoreactivation and final root length was measured.

For the Mitomycin C sensitivity assay, seeds were sown on plates containing fresh solid germination medium supplemented with 40 µM Mitomycin C (M0503; Sigma-Aldrich). The plates were then incubated for 17 days (16-h light, 23°C) and growth/death of plantlets scored visually.

FIGURE LEGENDS

Figure 1. Spontaneous and I-SceI induced DGU.US recombination is increased in the *xpf* mutant.

(A) Schematic map of the DGU.US chromosomal recombination reporter locus.

(B) Examples of WT and *xpf* mutant plants after GUS staining. Scale bar = 2 mm.

(C) Counting shows that *xpf* mutant plants have significantly more blue recombinant GUS⁺ spots than wild-type controls. Means $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test) N= 50

(D) The increased numbers of recombinant GUS⁺ spots are also seen after induction of GUS recombination by I-SceI cleavage using a β -estradiol-inducible I-SceI expression cassette. Means $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test) N= 50

Figure 2. XPF is not required for the removal of short non-homologous tails during SSA recombination.

(A) Recombination assay. Linear recombination substrates were prepared from pGF and pFP plasmids by restriction with enzymes that leave (with tails) or not (no tail) non-homologous DNA overhangs. The "no tail" test was carried out with pGF/*HpaI* and pFP/*SnaBI* and the "with short tails" test with pGF/*SnaBI* and pFP/*HpaI*. The short tails are 13 and 10 nucleotides respectively. Resection of the linear molecules permits annealing of the exposed complementary sequences. The annealing of the 3'-ended strands from "with short tails" plasmids generates unpaired overhangs, which must be removed to permit re-synthesis and strand ligation to restore a complete functional GFP gene.

(B) The linearized plasmids were co-transfected into WT and *xpf* mutant protoplasts either alone or together and restoration of the GFP gene was scored by flow cytometry 24h after transfection. The relative numbers of WT and *xpf* mutant GFP⁺ protoplasts are presented

relative to the transfection with a circular GFP containing plasmid (100%). Median with range, N > 1800 protoplasts, 2 repeats.

Figure 3. The increase of spontaneous DGU.US recombination in *xpf* mutant plants depends upon RAD51.

The significant increase in spontaneous recombination rate in *xpf* mutant plants is absent in the presence of the dominant-negative RAD51-GFP. Mean numbers of recombinant GUS+ spots per plant $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test). N=50

Figure 4. Spontaneous and I-SceI induced IU.GUS recombination is increased in the *xpf* mutant.

(A) Schematic map of the IU.GUS chromosomal recombination reporter locus. Both spontaneous (B) and I-SceI-induced (C) IU.GUS recombination rates are significantly increased in *xpf* mutant plants. Absence of XRCC2 completely eliminates the *xpf* hyper-rec phenotype, confirming that it is the result of increased homologous recombination. Mean numbers of recombinant GUS+ spots per plant $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test).

Figure 5. XFP-GFP but not XPF_{D757A}-GFP restores the wild-type IU.GUS recombination rate in the *xpf* mutant.

Spontaneous IU.GUS $\rightarrow$ GUS recombination was monitored in two F1 populations from independent crosses for each genotype. *xpf* mutant plants expressing the nuclease-minus XPF_{D757A}-GFP retain the hyper-rec phenotype of the *xpf* mutant, while those complemented by the wild-type XPF-GFP fusion protein are not hyper-rec. *xpf/xpf* plants are hemizygous for the IU.GUS locus and the XPF- GFP/XPF_{D757A}-GFP construct. Mean numbers of recombinant GUS+ spots per plant $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test).

Figure 6. A competition model to explain the increased homologous recombination in the *xpf* mutant.

By analogy with the model proposed by Agmon et al (2009), we propose that the hyper-rec phenotype of *xpf* mutants is due to shifting of the balance between productive HR and non-productive MMEJ recombination pathways.

(A) The tandem direct repeat DGU.US locus can generate a functional GUS gene *via* SSA or SDSA recombination. (B) To generate a functional GUS gene from the IU.GUS substrate, the interrupted GUS copy must recombine with the 5' and 3' deleted donor through RAD51-dependent recombination (SDSA). (A and B) Following I-SceI induced DSB formation, resection of both sides of the break generates 3'-ended ssDNA which will participate in the search for homology. Concomitantly with the resection, exposed complementary microhomologies can anneal and these present a substrate for the XPF-ERCC1 endonuclease. Cleavage of the ssDNA tails of the microhomology-annealing intermediate by XPF-ERCC1 would block the search for homology, and repair of the DSB through microhomology-mediated end-joining (MMEJ) will not generate a functional GUS gene. In the absence of XPF or ERCC1, these long 3' single-strand ends would promote homology search *in trans*, thus favouring RAD51-dependent SDSA recombination and producing the observed increase in RAD51-dependent recombination in *xpf* mutant plants.

SUPPORTING INFORMATION LEGENDS

Figure S1. I-SceI induced IU.GUS recombination is increased in the *ercc1* mutant.

ercc1 mutant plants have significantly more blue recombinant GUS+ spots than wild-type controls after induction of GUS recombination by I-SceI cleavage using a β -estradiol-inducible I-SceI expression cassette. Means $\pm$ standard errors of the means. *** $p < 0.0001$ (Mann-Whitney test). $N \geq 28$

Figure S2. XPF-GFP restores UV-resistance of the *xpf* mutant plants.

(A) Schematic representation of the XPF-GFP fusion construct.

(B-C) Sensitivity of *xpf* plants expressing or not XPF-GFP to DNA damage induced by ultraviolet (UV) radiation. Six T3 lines from *xpf* plants transformed with XPF-GFP construct were analysed. Five day-old seedlings were treated with 300 J.m^{-2} UV radiations, incubated for 5 days in the dark and length of the roots measured. Mean root lengths $\pm$ standard errors of the means.

Figure S3. XPF_{D757A}-GFP does not restore UV- and MMC resistance of the *xpf* mutant plants.

(A) Schematic representation of the XPF_{D757A}-GFP fusion construct. The adenine 3089 of the XPF genomic sequence was replaced by cytosine using site-directed mutagenesis (Materials and Methods). This mutation induces the substitution of the aspartic acid to alanine at position 757 of the XPF protein.

(B) XPF_{D757A}-GFP is expressed in nuclei of root meristematic cells. Differential interference contrast (DIC) + GFP (upper panel) and GFP fluorescence (lower panel) images are shown. Scale bar = $50 \mu\text{m}$.

(C) Sensitivity of *xpf* plants expressing or not XPF_{D75A}-GFP to DNA damage induced by UV radiation. Four T3 lines from *xpf* plants transformed with XPF_{D75A}-GFP construct and expressing the fusion protein were analysed. Five day-old seedlings were treated with 300 J.m⁻² UV radiations, incubated for 5 days in the dark and length of the roots measured. Mean root lengths $\pm$ standard errors of the means.

(D) Sensitivity of *xpf* plants expressing XFP-GFP or XPF_{D757A}-GFP to DNA crosslinking agent Mitomycin C (MMC). Expression of the XPF-GFP protein restores MMC resistance to *xpf* plants, while the XPF_{D757A}-GFP does not. Seventeen day-old seedlings grown in presence of 40 μ M of MMC are shown.

REFERENCES

1. **Hoeijmakers JH** (2009) DNA damage, aging, and cancer. *The New England journal of medicine* 361: 1475-1485.
2. **Iyama T, Wilson DM** (2013) DNA repair mechanisms in dividing and non-dividing cells. *DNA Repair (Amst)* 12: 620-636.
3. **Tian M, Shinkura R, Shinkura N, Alt FW** (2004) Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. *Molecular and cellular biology* 24: 1200-1205.
4. **Sijbers AM, de Laat WL, Ariza RR, Biggerstaff M, Wei YF, et al.** (1996) Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. *Cell* 86: 811-822.
5. **Higgins DR, Prakash S, Reynolds P, Prakash L** (1983) Molecular cloning and characterization of the RAD1 gene of *Saccharomyces cerevisiae*. *Gene* 26: 119-126.
6. **Carr AM, Schmidt H, Kirchhoff S, Muriel WJ, Sheldrick KS, et al.** (1994) The rad16 gene of *Schizosaccharomyces pombe*: a homolog of the RAD1 gene of *Saccharomyces cerevisiae*. *Molecular and cellular biology* 14: 2029-2040.
7. **Park HK, Suh D, Hyun M, Koo H-S, Ahn B** (2004) A DNA repair gene of *Caenorhabditis elegans*: a homolog of human XPF. *DNA repair* 3: 1375-1383.
8. **Sekelsky JJ, McKim KS, Chin GM, Hawley RS** (1995) The *Drosophila* meiotic recombination gene mei-9 encodes a homologue of the yeast excision repair protein Rad1. *Genetics* 141: 619-627.
9. **Fidantsef AL, Mitchell DL, Britt AB** (2000) The *Arabidopsis* UVH1 gene is a homolog of the yeast repair endonuclease RAD1. *Plant physiology* 124: 579-586.
10. **Gallego F, Fleck O, Li A, Wyrzykowska J, Tinland B** (2000) AtRAD1, a plant homologue of human and yeast nucleotide excision repair endonucleases, is involved in dark repair of UV damages and recombination. *The Plant Journal* 21: 507-518.

- 11. Liu Z, Hossain GS, Islas-Osuna MA, Mitchell DL, Mount DW (2000)** Repair of UV damage in plants by nucleotide excision repair: Arabidopsis UVH1 DNA repair gene is a homolog of *Saccharomyces cerevisiae* Rad1. *The Plant Journal* 21: 519-528.
- 12. Harlow GR, Jenkins ME, Pittalwala TS, Mount DW (1994)** Isolation of *uvh1*, an Arabidopsis mutant hypersensitive to ultraviolet light and ionizing radiation. *The Plant cell* 6: 227- 235.
- 13. Gaillard PH, Wood RD (2001)** Activity of individual ERCC1 and XPF subunits in DNA nucleotide excision repair. *Nucleic Acids Res* 29: 872-879.
- 14. Yagi T, Wood RD, Takebe H (1997)** A low content of ERCC1 and a 120 kDa protein is a frequent feature of group F xeroderma pigmentosum fibroblast cells. *Mutagenesis* 12: 41-44.
- 15. Biggerstaff M, Szymkowski DE, Wood RD (1993)** Co-correction of the ERCC1, ERCC4 and xeroderma pigmentosum group F DNA repair defects in vitro. *The EMBO journal* 12: 3685-3692.
- 16. De Laat WL, Appeldoorn E, Jaspers NG, Hoeijmakers JH (1998)** DNA structural elements required for ERCC1-XPF endonuclease activity. *The Journal of biological chemistry* 273: 7835-7842.
- 17. Bardwell AJ, Bardwell L, Tomkinson AE, Friedberg EC (1994)** Specific cleavage of model recombination and repair intermediates by the yeast Rad1-Rad10 DNA endonuclease. *Science (New York, NY)* 265: 2082-2085.
- 18. Park CH, Bessho T, Matsunaga T, Sancar A (1995)** Purification and characterization of the XPF-ERCC1 complex of human DNA repair excision nuclease. *The Journal of biological chemistry* 270: 22657-22660.
- 19. Tripsianes K, Folkers G, Ab E, Das D, Odijk H, et al. (2005)** The structure of the human ERCC1/XPF interaction domains reveals a complementary role for the two proteins in nucleotide excision repair. *Structure* 13: 1849-1858.
- 20. Enzlin JH, Schärer OD (2002)** The active site of the DNA repair endonuclease XPF-ERCC1 forms a highly conserved nuclease motif. *The EMBO journal* 21: 2045-2053.

- 21. Tsodikov OV, Enzlin JH, Schärer OD, Ellenberger T** (2005) Crystal structure and DNA binding functions of ERCC1, a subunit of the DNA structure-specific endonuclease XPF- ERCC1. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11236-11241.
- 22. Ciccia A, McDonald N, West SC** (2008) Structural and functional relationships of the XPF/MUS81 family of proteins. *Annual review of biochemistry* 77: 259-287.
- 23. Prakash S, Prakash L** (2000) Nucleotide excision repair in yeast. *Mutation research* 451: 13-24.
- 24. Kuraoka I, Kobertz WR, Ariza RR, Biggerstaff M, Essigmann JM, et al.** (2000) Repair of an interstrand DNA cross-link initiated by ERCC1-XPF repair/recombination nuclease. *The Journal of biological chemistry* 275: 26632-26636.
- 25. Adair GM** (2000) Role of ERCC1 in removal of long non-homologous tails during targeted homologous recombination. *The EMBO journal* 19: 5552-5561.
- 26. Al-Minawi AZ, Saleh-Gohari N, Helleday T** (2008) The ERCC1/XPF endonuclease is required for efficient single-strand annealing and gene conversion in mammalian cells. *Nucleic acids research* 36: 1-9.
- 27. Sargent RG, Meservy JL, Perkins BD, Kilburn AE, Intody Z, et al.** (2000) Role of the nucleotide excision repair gene ERCC1 in formation of recombination-dependent rearrangements in mammalian cells. *Nucleic acids research* 28: 3771-3778.
- 28. Dubest S, Gallego ME, White CI** (2002) Role of the AtRad1p endonuclease in homologous recombination in plants. *EMBO reports* 3: 1049-1054.
- 29. Dubest S, Gallego ME, White CI** (2004) Roles of the AtErcc1 protein in recombination. *The Plant Journal* 39: 334-342.
- 30. Fishman-Lobell J, Haber JE** (1992) Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene RAD1. *Science (New York, NY)* 258: 480-484.

- 31. Ivanov EL, Haber JE** (1995) RAD1 and RAD10, but not other excision repair genes, are required for double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 15: 2245-2251.
- 32. Pâques F, Haber JE** (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiology and molecular biology reviews* 63: 349-404.
- 33. Liang F, Han M, Romanienko PJ, Jasin M** (1998) Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America* 95: 5172-5177.
- 34. Krogh BO, Symington LS** (2004) Recombination proteins in yeast. *Annual review of genetics* 38: 233-271.
- 35. Lyndaker AM, Alani E** (2009) A tale of tails: insights into the coordination of 3' end processing during homologous recombination. *Bioessays* 31: 315-321.
- 36. Sugawara N, Pâques F, Colaiácovo M, Haber JE** (1997) Role of *Saccharomyces cerevisiae* Msh2 and Msh3 repair proteins in double-strand break-induced recombination. *Proceedings of the National Academy of Sciences of the United States of America* 94: 9214-9219.
- 37. Flott S, Alabert C, Toh GW, Toth R, Sugawara N, et al.** (2007) Phosphorylation of Slx4 by Mec1 and Tel1 regulates the single-strand annealing mode of DNA repair in budding yeast. *Molecular and cellular biology* 27: 6433-6445.
- 38. Li F, Dong J, Pan X, Oum J-H, Boeke JD, et al.** (2008) Microarray-based genetic screen defines SAW1, a gene required for Rad1/Rad10-dependent processing of recombination intermediates. *Molecular cell* 30: 325-335.
- 39. Li F, Dong J, Eichmiller R, Holland C, Minca E, et al.** (2013) Role of Saw1 in Rad1/Rad10 complex assembly at recombination intermediates in budding yeast. *The EMBO journal* 32: 461-472.
- 40. Fekairi S, Scaglione S, Chahwan C, Taylor ER, Tissier A, et al.** (2009) Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. *Cell* 138: 78-89.

- 41. Muñoz IM, Hain K, Déclais A-C, Gardiner M, Toh GW, et al. (2009)** Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. *Molecular cell* 35: 116-127.
- 42. Sarbajna S, Davies D, West SC (2014)** Roles of SLX1–SLX4, MUS81–EME1, and GEN1 in avoiding genome instability and mitotic catastrophe. *Genes & Development* 28: 1124- 1136.
- 43. Nassif N, Penney J, Pal S, Engels WR, Gloor GB (1994)** Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Molecular and cellular biology* 14: 1613-1625.
- 44. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983)** The double-strand-break repair model for recombination. *Cell* 33: 25-35.
- 45. Lundblad V, Blackburn EH (1993)** An alternative pathway for yeast telomere maintenance rescues est1- senescence. *Cell* 73: 347-360.
- 46. Lydeard JR, Jain S, Yamaguchi M, Haber JE (2007)** Break-induced replication and telomerase-independent telomere maintenance require Pol32. *Nature* 448: 820-823.
- 47. Symington LS (2002)** Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiology and molecular biology reviews* : MMBR 66: 630-670.
- 48. Ira G, Satory D, Haber JE (2006)** Conservative inheritance of newly synthesized DNA in double-strand break-induced gene conversion. *Molecular and cellular biology* 26: 9424-9429.
- 49. Pâques F, Haber JE (1997)** Two pathways for removal of nonhomologous DNA ends during double-strand break repair in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 17: 6765-6771.
- 50. Orel N, Kyryk A, Puchta H (2003)** Different pathways of homologous recombination are used for the repair of double-strand breaks within tandemly arranged sequences in the plant genome. *The Plant Journal* 35: 604-612.

- 51. Roth N, Klimesch J, Dukowic-Schulze S, Pacher M, Mannuss A, et al. (2012)** The requirement for recombination factors differs considerably between different pathways of homologous double-strand break repair in somatic plant cells. *Plant J* 72: 781-790.
- 52. Serra H, Da Ines O, Degroote F, Gallego ME, White CI (2013)** Roles of XRCC2, RAD51B and RAD51D in RAD51-independent SSA recombination. *PLoS Genet* 9: e1003971.
- 53. Puchta H (2005)** The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *Journal of experimental botany* 56: 1-14.
- 54. Watanabe K, Pacher M, Dukowic S, Schubert V, Puchta H, et al. (2009)** The Structural maintenance of chromosomes 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. *The Plant cell* 21: 2688-2699.
- 55. Da Ines O, Degroote F, Goubely C, Amiard S, Gallego ME, et al. (2013)** Meiotic recombination in *Arabidopsis* is catalysed by DMC1, with RAD51 playing a supporting role. *PLoS genetics* 9: e1003787.
- 56. Kirschner K, Melton DW (2010)** Multiple Roles of the ERCC1-XPF Endonuclease in DNA Repair and Resistance to Anticancer Drugs. *ANTICANCER RESEARCH* 30: 3223-3232.
- 57. McNeil EM, Melton DW (2012)** DNA repair endonuclease ERCC1–XPF as a novel therapeutic target to overcome chemoresistance in cancer therapy. *Nucleic Acids Research* 10: 1-15.
- 58. Elliott B, Richardson C, Jasin M (2005)** Chromosomal translocation mechanisms at intronic alu elements in mammalian cells. *Molecular cell* 17: 885-894.
- 59. Agmon N, Pur S, Liefshitz B, Kupiec M (2009)** Analysis of repair mechanism choice during homologous recombination. *Nucleic acids research* 37: 5081-5092.
- 60. Sugawara N, Haber JE (1992)** Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Molecular and cellular biology* 12: 563-575.

- 61. Bleuyard J-Y, Gallego ME, Savigny F, White CI** (2005) Differing requirements for the Arabidopsis Rad51 paralogs in meiosis and DNA repair. *The Plant Journal* 41: 533-545.
- 62. Clough SJ, Bent AF** (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *The Plant Journal* 16: 735-743.
- 63. Yoo SD, Cho YH, Sheen J** (2007) Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nature protocols* 2: 1565-1572.
- 64. Karimi M, Depicker A, Hilson P** (2007) Recombinational cloning with plant gateway vectors. *Plant physiology* 145: 1144-1154.

Table 1. Spontaneous and I-SceI induced DGU.US recombination in *xpf* mutant and in wild-type plants.

Experiment		Spontaneous DGU.US recombination				I-SceI induced DGU.US recombination			
		<i>n</i>	<i>N</i>	<i>m</i> ± SEM	Ratio	<i>n</i>	<i>N</i>	<i>m</i> ± SEM	Ratio
1	WT	49	293	5.98 ± 0.56		50	941	18.82 ± 1.63	
	<i>xpf</i>	50	531	10.62 ± 0.77	1.776***	50	1882	37.64 ± 2.95	2.000***
2	WT	50	412	8.24 ± 0.50		50	2102	42.04 ± 2.77	
	<i>xpf</i>	50	601	12.02 ± 0.60	1.459***	51	2766	54.24 ± 3.24	1.291**

Recombination in the mutants and wild-type was compared using non-parametric statistical analysis (Mann-Whitney test). Differences between *xpf* and wild-type are highly significant in all cases (** $p < 0.005$; *** $p < 0.0001$). *n*, the number of plantlets screened; *N*, the total number of blue spots (recombination events); *m* ± SEM, the mean number of recombination events per plant ± standard error of the mean.

Table 2. Spontaneous and I-SceI induced IU.GUS recombination in *xpf* mutant and in wild-type plants.

Experiment		Spontaneous IU.GUS recombination				I-SceI induced IU.GUS recombination			
		<i>n</i>	<i>N</i>	<i>m</i> ± SEM	Ratio	<i>n</i>	<i>N</i>	<i>m</i> ± SEM	Ratio
1	WT	50	117	2.34 ± 0.30		50	1050	21.00 ± 2.12	
	<i>xpf</i>	50	283	5.66 ± 0.58	2.419	50	2608	52.16 ± 4.64	2.484
	<i>xpf xrcc2</i>	50	20	0.40 ± 0.10	0.171				
2	WT	50	180	3.60 ± 0.36		49	1159	23.65 ± 3.27	
	<i>xpf</i>	50	373	7.46 ± 0.88	2.072	51	2923	57.31 ± 4.52	2.423
	<i>xpf xrcc2</i>	50	27	0.54 ± 0.12	0.150				

Recombination in the mutants and wild-type were compared using non-parametric statistical analysis (Mann-Whitney test). Differences between *xpf* and wild-type are highly significant in all cases ($p < 0.0001$). *n*, the number of plantlets screened; *N*, the total number of blue spots (recombination events); *m* ± SEM, the mean number of recombination events per plant ± standard error of the mean.

Table 3. Spontaneous and I-SceI induced IU.GUS recombination in *xpf* mutants expressing or not XPF-GFP or XPF_{D757A}-GFP.

Experiment		Spontaneous IU.GUS recombination			I-SceI induced IU.GUS recombination		
		<i>n</i>	<i>N</i>	<i>m</i> ± <i>SEM</i>	<i>n</i>	<i>N</i>	<i>m</i> ± <i>SEM</i>
1	<i>xpf</i>	50	287	5.74 ± 0.42	50	713	14.26 ± 1.24
	<i>xpf</i> XPF-GFP	50	100	2.00 ± 0.20	50	363	7.26 ± 0.54
	<i>xpf</i> XPF _{D757A} -GFP	50	248	4.96 ± 0.38	50	711	14.22 ± 1.01
2	<i>xpf</i>	50	339	6.78 ± 0.44	54	554	10.26 ± 0.55
	<i>xpf</i> XPF-GFP	50	149	2.98 ± 0.21	53	319	6.02 ± 0.46
	<i>xpf</i> XPF _{D757A} -GFP	50	283	5.66 ± 0.38	53	595	11.23 ± 0.94

n, the number of plantlets screened; *N*, the total number of blue spots (recombination events); *m* ± *SEM*, the mean number of recombination events per plant ± standard error of the mean.

Figure S1

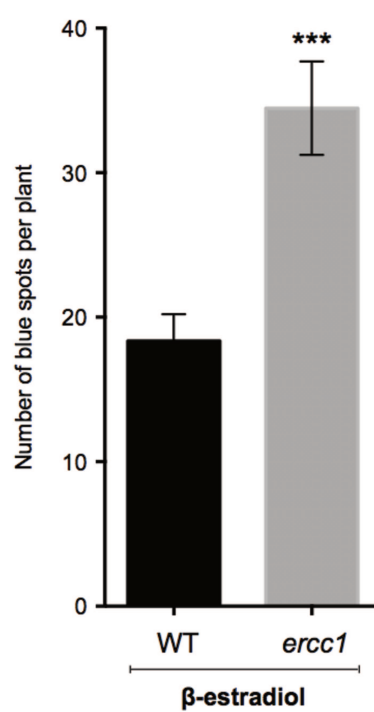


Figure S2

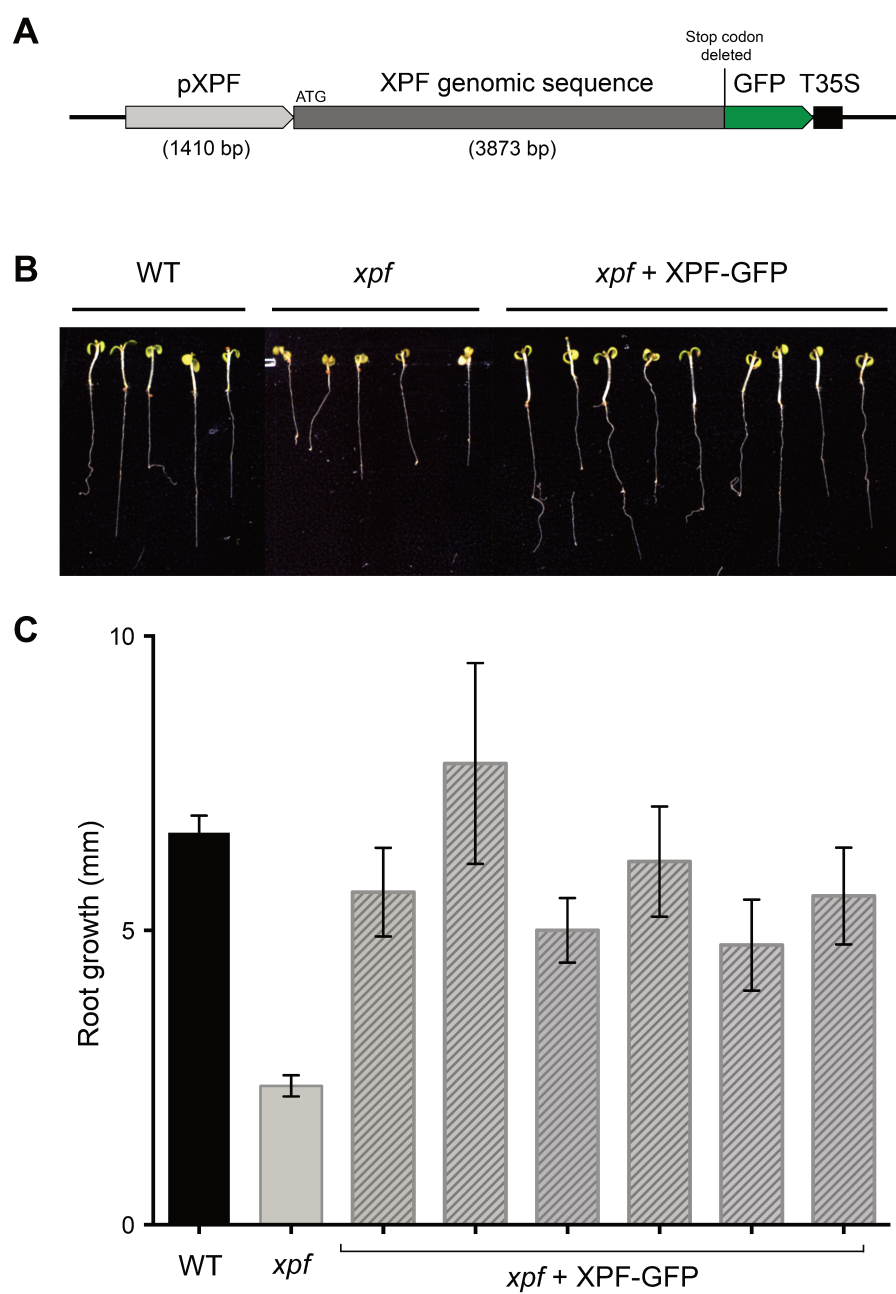
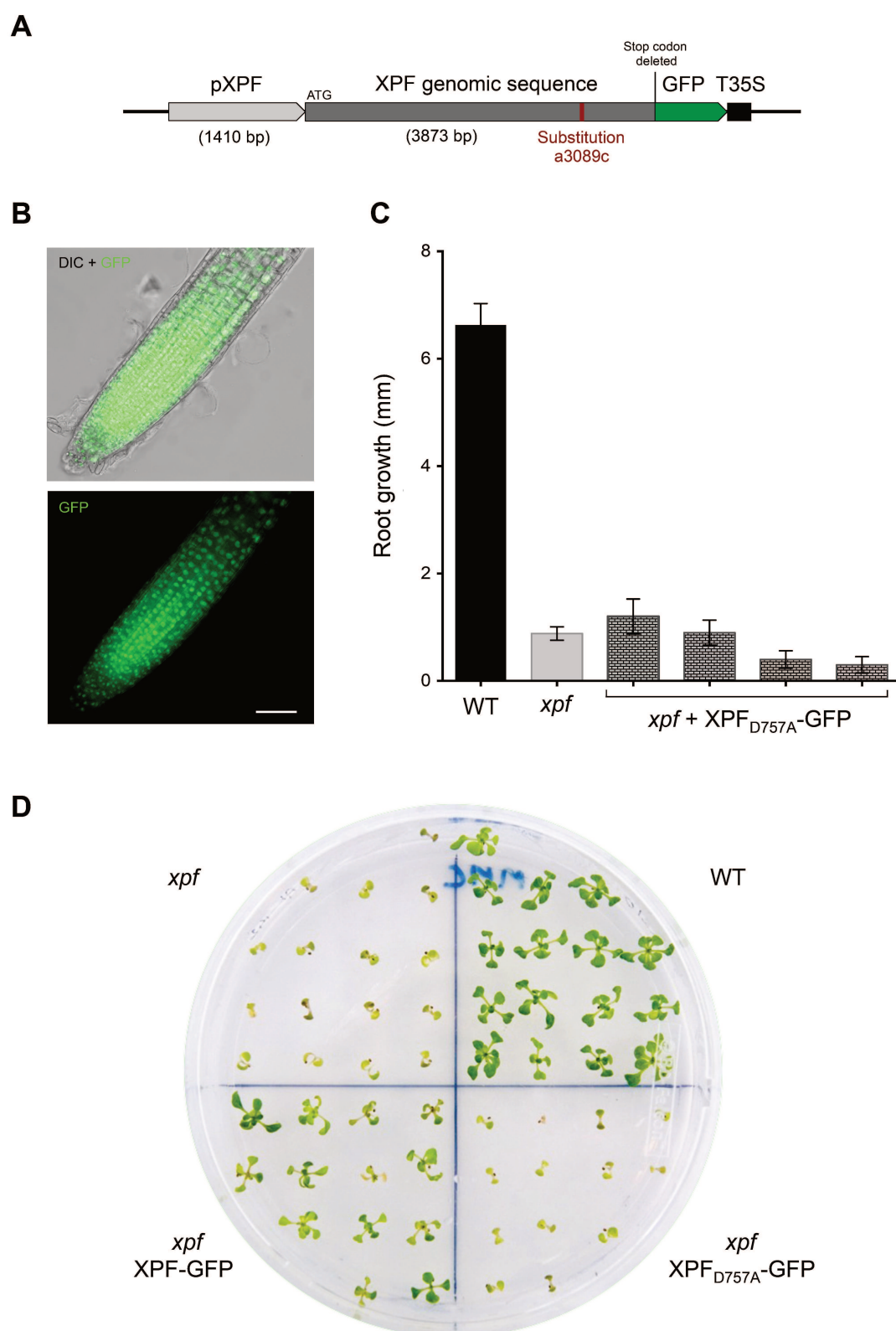


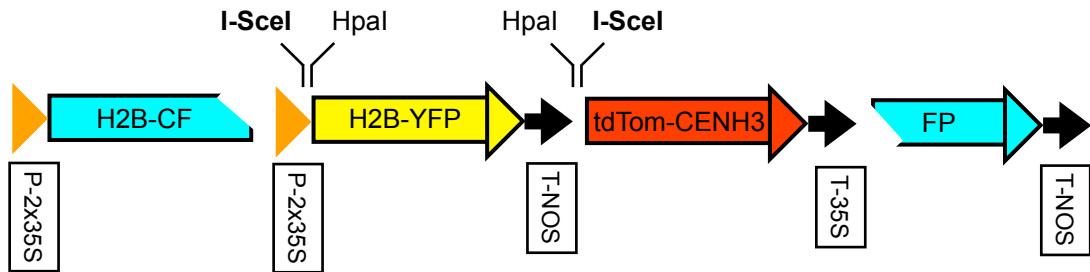
Figure S3



Chapitre III

**Mise au point d'un système rapporteur
d'évènements de jonction d'extrémités d'ADN
en protoplastes d'*Arabidopsis thaliana***

A



B

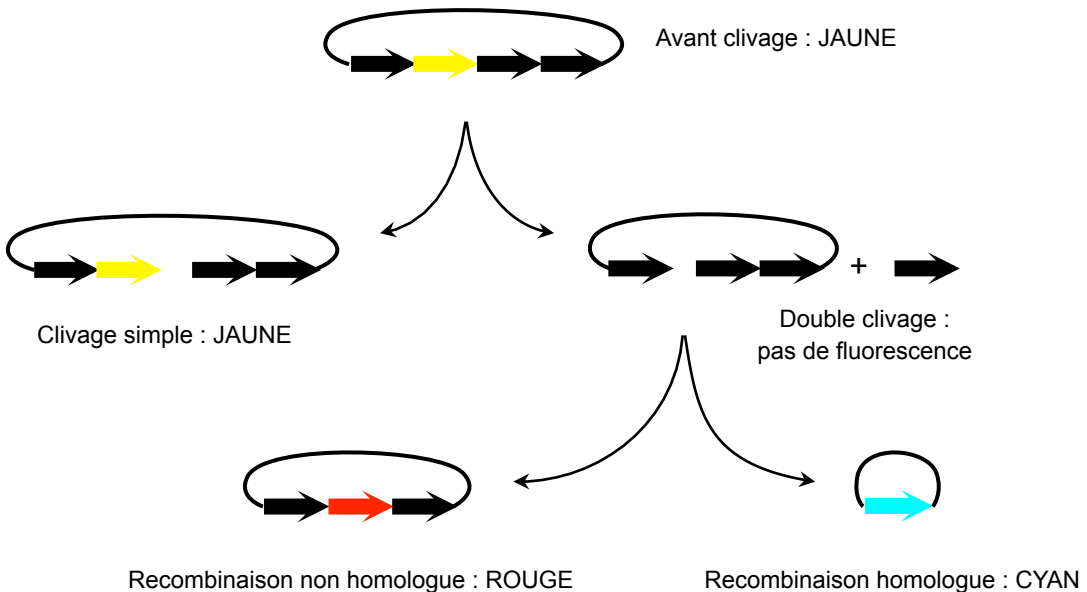


Figure 21 : Le plasmide rapporteur de recombinaison

(A) Carte schématique de la construction rapportrice de recombinaison. Elle est composée de deux copies tronquées du gène de fusion H2B-CFP (rectangles cyans) portant une séquence commune de 564 pb, et des gènes de fusion H2B-YFP (rectangle jaune) et tdTomato-CENH3 (rectangle rouge). Les triangles oranges représentent les promoteurs et les flèches noires, les terminateurs. P-2x35S : double promoteur 35S ; T-NOS : terminateur de la nopaline synthase ; T-35S : terminateur 35S ; I-SceI et HpaI : sites de restriction des enzymes I-SceI et HpaI, respectivement.

(B) Principe de la mise en évidence des événements de recombinaison homologue et non homologue selon la couleur de la fluorescence (associée à la reconstitution d'un gène de fusion).

Afin d'étudier les interactions entre les voies de recombinaison, nous avons développé au laboratoire un système plasmidique permettant de distinguer les événements de recombinaison non homologue des événements de recombinaison homologue. Le plasmide initial a été construit par P. Fargier sur le même principe que les systèmes rapporteurs développés par plusieurs laboratoires, dont ceux de H. Puchta et B. Lopez (Salomon & Puchta, 1998 ; Siebert & Puchta, 2002 ; Guirouilh-Barbat *et al.*, 2004 ; Rass *et al.*, 2009). Notre système a pour but de quantifier *in vivo* l'efficacité de réparation de CDB générées par une enzyme de restriction, par les voies de jonction des extrémités d'ADN vs les voies de recombinaison homologue. Basé sur la reconstruction par recombinaison de gènes codant des protéines de fusion fluorescentes, ce système rapporteur peut être directement utilisé en protoplastes. Ceci simplifie considérablement les analyses dans différents contextes mutants, puisqu'il n'est pas nécessaire de réaliser des croisements avec chacune des lignées mutantes comme c'est le cas avec une construction chromosomique.

I. Présentation du plasmide rapporteur de recombinaison

Le plasmide rapporteur de recombinaison a été construit par clonage à partir de plusieurs gènes de fusion. Le choix s'est porté sur des protéines nucléaires afin de s'affranchir de la fragilité des protoplastes au moment de l'analyse. Ce plasmide est constitué de deux copies tronquées du gène de fusion H2B-CFP (séquence codante de l'histone H2B fusionnée avec le gène rapporteur CFP (Boisnard-Lorig *et al.*, 2001)) en orientation directe et présentant une séquence répétée de 564 pb (**Figure 21 A**). Entre ces régions, deux autres gènes de fusion ont été introduits : H2B-YFP sous le contrôle d'un double promoteur 35S (P-2x35S) et du terminateur de la nopaline synthétase (T-NOS) (Boisnard-Lorig *et al.*, 2001), suivi du gène tdTomato-CenH3 (séquence codante du gène rapporteur tdTomato fusionnée à celle de l'histone spécifique des centromères CENH3 (Kurihara *et al.*, 2008)) et du terminateur 35S.

Matériel

Feuilles de 1 à 2 cm de plantules d'environ 3 semaines cultivées sur terreau dans des conditions standards (16h jour/ 8h nuit, à 23°C avec une humidité relative de 45-60%).

Préparation des protoplastes

- Prélever une vingtaine de feuilles (sans le pétiole)
 - Couper des bandes de 0,5 à 1 mm de largeur avec une lame de rasoir neuve dans une boîte de Pétri
 - Ajouter 8 ml de solution de digestion et infiltrer sous vide pendant 30 min (à l'obscurité)
 - Incuber 3h à 23°C à l'obscurité et sans agitation
 - Agiter soigneusement la solution de digestion, la filtrer (filtre de 30 µm) et la recueillir dans un tube de 15 ml à bout arrondi
 - Laver les fragments de feuilles avec environ 7 ml de W5 (à température ambiante) et filtrer la solution
 - Centrifuger la solution de protoplastes à 100g pendant 2 min
- Les protoplastes forment un culot vert bien visible
- Jeter le surnageant, resuspendre le culot dans 10 ml de solution W5
 - Centrifuger à 100g pendant 2 min
 - Jeter le surnageant, resuspendre le culot dans 2 ml de solution W5 et incuber 30 min sur de la glace
 - Centrifuger à 100g pendant 2 min
 - Jeter le surnageant, resuspendre le culot dans une solution MaMg
 - Quantifier la concentration des protoplastes au microscope grâce à une cellule de Malassez et l'ajuster à $1,5 \times 10^6/\text{ml}$ avec la solution MaMg

Transfection des protoplastes

Toutes les étapes sont effectuées à 23°C.

- Prélever 100 µl ($1,5 \times 10^5$) de protoplastes avec une pipette dont le cône a été sectionné et les placer dans un nouveau tube de 15 ml à bout arrondi
- Ajouter 10 µl (10 µg) d'ADN purifié et mélanger soigneusement
- Ajouter 110 µl de polyéthylène-glycol (PEG) et mélanger soigneusement
- Incuber le mélange de transfection pendant 30 min
- Ajouter soigneusement 15 ml de solution W5 (au goutte à goutte pour les premiers millilitres)
- Centrifuger à 100g pendant 2 min
- Jeter le surnageant et remettre délicatement en suspension les protoplastes dans 1 ml de solution WI
- Incuber la solution pendant 24h à 23°C dans l'obscurité en inclinant le tube (augmentation de la surface solution/air)

Solutions

- **Solution de digestion** : 0,25% p/v Macerozyme Onozuka R-10, 1% p/v Cellulase Onozuka R-10 (Yakult, Japon), 0,4 M mannitol, 20 mM acide 4-morpholineethanesulfonique (MES) pH 5,7, 20 mM KCl, 10 mM CaCl_2 , 5 mM β -mercaptoéthanol, 0,1% d'albumine de sérum bovin (BSA). Chauffer à 55°C pendant 10 min et laisser refroidir avant l'ajout du CaCl_2 , du β -mercaptoéthanol et de la BSA.
- **Solution W5** : 125 mM CaCl_2 , 154 mM NaCl, 5 mM KCl, 2 mM MES pH 5,7
- **Solution WI (Washing and Incubation)** : 0,5 M mannitol, 0,5 M KCl, 4 mM MES pH 5,7
- **Solution MaMg** : 0,4 M mannitol, 15 mM MgCl_2 , 4 mM MES pH 5,7
- **Solution PEG** : 40% v/v PEG-4000 (Fluka # 81240), 0,8 M mannitol, 1 M CaCl_2 , 1 M KCl

Figure 22 : Protocole de transfection des protoplastes d'*Arabidopsis thaliana*

Un site de restriction de l'endonuclease I-SceI a été placé entre le double promoteur 35S et la séquence codante d'H2B-YFP, et un second en aval de son terminateur NOS (**Figure 21 A**).

En absence de clivage, la protéine de fusion H2B-YFP est exprimée et confère une fluorescence jaune nucléaire à la cellule (**Figure 21 B**). La double digestion par l'enzyme I-SceI excise le fragment H2B-YFP-T_{NOS} du plasmide, ce qui empêche la production de la protéine. Les deux extrémités d'ADN ainsi générées peuvent être reliées entre elles par recombinaison non homologue (voies de jonction des extrémités) ou par recombinaison homologue (voie SSA ou SDSA) entre les deux séquences répétées situées de part et d'autre de la cassure. Dans le premier cas, le double promoteur 35S permet la production de la protéine de fusion tdTomato-CENH3 qui confère une fluorescence rouge à la cellule. Les événements de SSA ou SDSA en revanche reconstituent le gène de fusion H2B-CFP dont la protéine est responsable d'une fluorescence cyan (**Figure 21 B**). L'efficacité relative des deux processus peut donc être quantifiée par la couleur de la fluorescence.

II. Analyses de la recombinaison en microscopie à fluorescence

La première étape a consisté en l'optimisation du protocole de transfection des protoplastes d'*Arabidopsis* par le polyéthylène glycol (PEG), après leur préparation à partir de jeunes feuilles. Les principales difficultés rencontrées ont été la mort d'une grande partie des protoplastes au cours des différentes étapes du protocole de transfection, ainsi que la variabilité de l'efficacité de transfection. Nos expériences ont mené à l'établissement d'un protocole optimisé de préparation et de transfection des protoplastes d'*Arabidopsis*, à partir du protocole de J. Sheen (Yoo *et al.*, 2007) (**Figure 22**).

Les protoplastes sauvages transformés avec le plasmide rapporteur de recombinaison circulaire (non digéré par I-SceI) ont d'abord été analysés au microscope à fluorescence. Jusqu'à 70 % d'entre eux présentent un marquage jaune nucléaire relativement homogène

Toutes les étapes sont effectuées à température ambiante.

- Prélever 100 µl de solution de protoplastes avec une pipette dont le cône a été sectionné et les placer dans un tube de 2 ml
- Ajouter 100 µl de Buffer Chopping contenant du triton à 0,6 %
- Agiter soigneusement et incubé 20 min sans agitation
- Ajouter 400 µl de solution GB et mélanger par retournement du tube
- Ajouter le marqueur d'ADN : DAPI (concentration finale : 5 µg/ml) ou iodure de propidium (concentration finale : 10 µg/ml).

Solutions

- **Buffer Chopping** : 45 mM MgCl₂, 30 mM Na citrate, 20 mM MOPS pH 7.0, 0,6 % p/v Triton X-100, 1% PVP, 1% d'albumine de sérum bovin (BSA)
- **Solution GB** : 45 mM MgCl₂, 30 mM Na citrate, 20 mM MOPS pH 7.0, 0,1 % p/v Triton X-100, 1% BSA

Figure 23 : Protocole d'isolation des noyaux de protoplastes d'*Arabidopsis thaliana*

(colocalisation de la fluorescence YFP et du marquage DAPI), 24h après transfection. Comme attendu, aucun protoplaste ne présente de marquage rouge ou cyan. La transfection simultanée des protoplastes avec le plasmide rapporteur de recombinaison et un plasmide d'expression de l'enzyme I-SceI (sous le contrôle d'un promoteur 35S) réduit par deux la quantité de protoplastes fluorescents en jaune (32 % de protoplastes YFP+). Des protoplastes présentant un marquage cyan (CFP+) ou rouge (Tomato+) ont également été observés, mais en quantité très faible (3 et 1%, respectivement). Il est intéressant de noter que ces protoplastes sont également YFP+. Deux hypothèses peuvent être émises : plusieurs copies du plasmide sont présentes dans ces protoplastes et seulement certains d'entre eux ont subi un évènement de recombinaison et/ou des protéines H2B-YFP sont produites avant la digestion par I-SceI et persistent dans le noyau des protoplastes.

III. Analyses de la recombinaison en cytométrie en flux

Face à la faible fréquence des protoplastes CFP+ et Tomato+, une analyse par cytométrie en flux de la fluorescence des protoplastes transfectés avec le plasmide rapporteur de recombinaison a été mise au point. Les avantages du cytomètre par rapport au microscope à fluorescence sont principalement la vitesse d'acquisition et l'analyse simultanée de plusieurs paramètres (fluorescences YFP, CFP, Tomato et du marqueur d'ADN). L'observation des protoplastes au microscope a révélé que les chloroplastes gênent souvent la détection de la fluorescence (noyau situé derrière les chloroplastes par rapport à l'expérimentateur). Afin d'évaluer précisément la fluorescence nucléaire, les noyaux sont isolés à partir des protoplastes en solution, 24h après transfection. Le protocole d'isolation des noyaux utilisé a été établi à partir du protocole de D. Galbraith (Galbraith, 2009) et des recommandations de S. Brown (CNRS, Gif-sur-Yvette) (**Figure 23**).

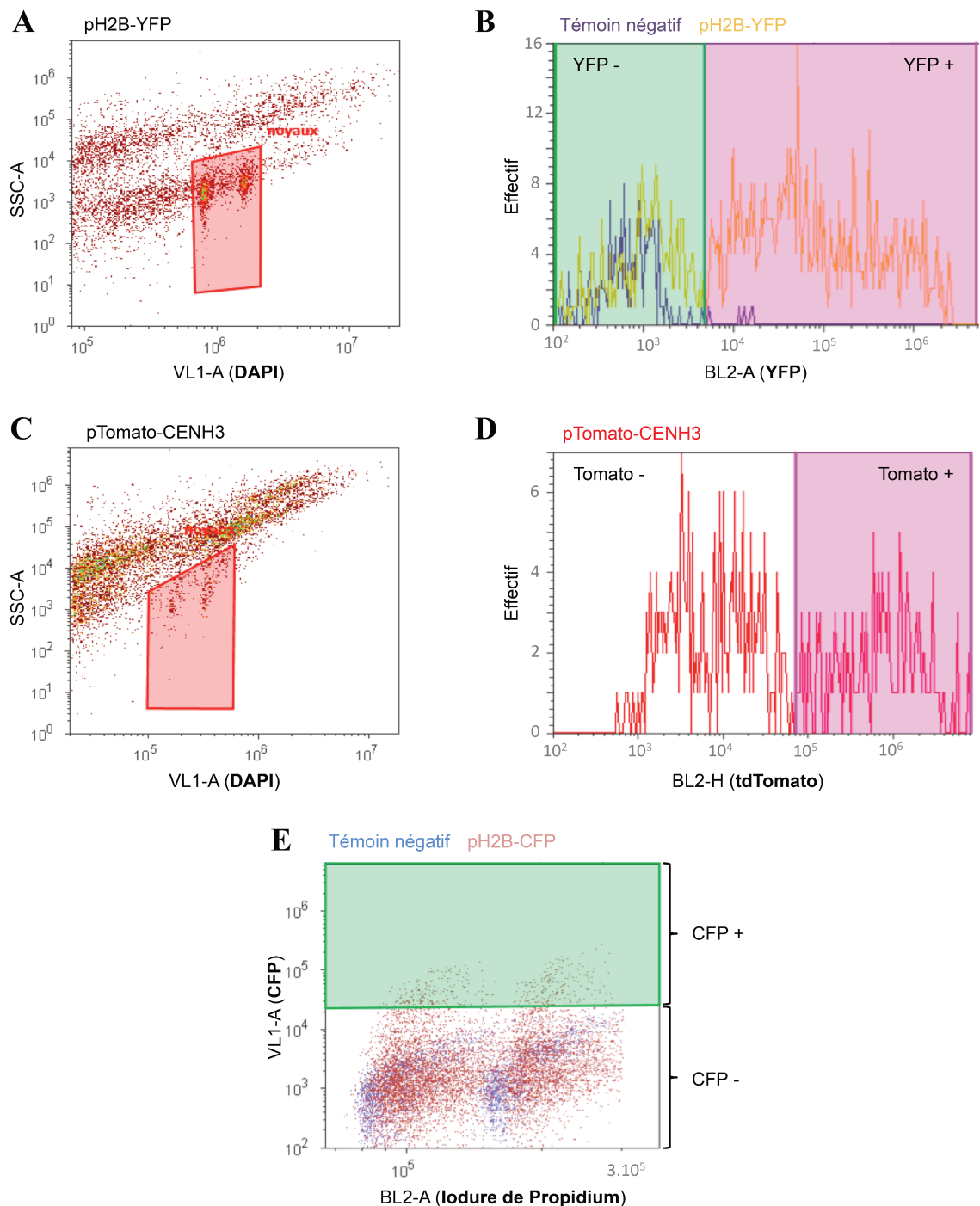


Figure 24 : Détection des protéines de fusion H2B-YFP, tdTomato-CENH3 et H2B-CFP par cytométrie en flux.

- (A-B) Noyaux de protoplastes transfectés avec le plasmide pH2B-YFP et marqués au DAPI. SSC (*side scatter*) ; Rectangle rouge : noyaux (les deux populations correspondent aux noyaux 4C et 8C, C étant la quantité d'ADN d'une cellule haploïde en G1). (B) Courbe bleue : témoin négatif (noyaux de protoplastes ayant subi le protocole de transfection mais sans ajout d'ADN plasmidique) ; courbe jaune/orange : noyaux de protoplastes transfectés avec pH2B-YFP.
- (C-D) Noyaux de protoplastes transfectés avec le plasmide pTomato-CENH3 et marqués au DAPI.
- (E) Noyaux de protoplastes transfectés avec le plasmide pH2B-CFP et marqués à l'iode de propidium.

La quantification de la fluorescence nucléaire nécessite au préalable l'identification de la population de noyaux grâce à un marquage de l'ADN. Afin de limiter les chevauchements des spectres d'excitation et d'émission du marqueur d'ADN et des protéines fluorescentes étudiées, la stratégie suivante a été adoptée. Les noyaux des protoplastes sont isolés 24h après transfection puis séparés en deux lots : le premier est marqué au DAPI et permettra la détection de la fluorescence YFP et Tomato (événements de jonction des extrémités), le second est marqué à l'Iodure de Propidium pour la détection de la fluorescence CFP (événements de recombinaison homologue).

La détection de chacune des protéines de fusion par le cytomètre en flux a d'abord été vérifiée. Pour cela, des protoplastes sauvages ont été transfectés avec les plasmides d'expression des protéines de fusion individuelles : pH2B-YFP, pTomato-CENH3 ou pH2B-CFP. L'analyse par cytométrie en flux a permis dans chacun des cas de mettre en évidence deux populations de noyaux selon leur niveau de fluorescence, démontrant que le cytomètre détecte les noyaux exprimant chacune des protéines de fusion (**Figure 24**). Avec le plasmide rapporteur de recombinaison, des résultats similaires à ceux observés par microscopie ont été obtenus : la digestion *in vivo* du plasmide par l'enzyme I-SceI n'aboutit qu'à un très faible nombre de noyaux Tomato⁺ ou CFP⁺ (< 3%). Afin de garantir la digestion du plasmide par I-SceI, cette étape a été réalisée *in vitro* et vérifiée par électrophorèse, avant la transfection des protoplastes. Pourtant, ceci ne favorise pas la reconstitution des gènes de fusion P35S-tdTomato-CENH3 et P35S-H2B-CFP par recombinaison puisque la proportion des noyaux marqués reste inférieure à 3%. La quantification de l'efficacité relative des deux modes de réparation dans les cellules sauvages est donc compromise par la rareté de ces événements dans notre système d'étude.

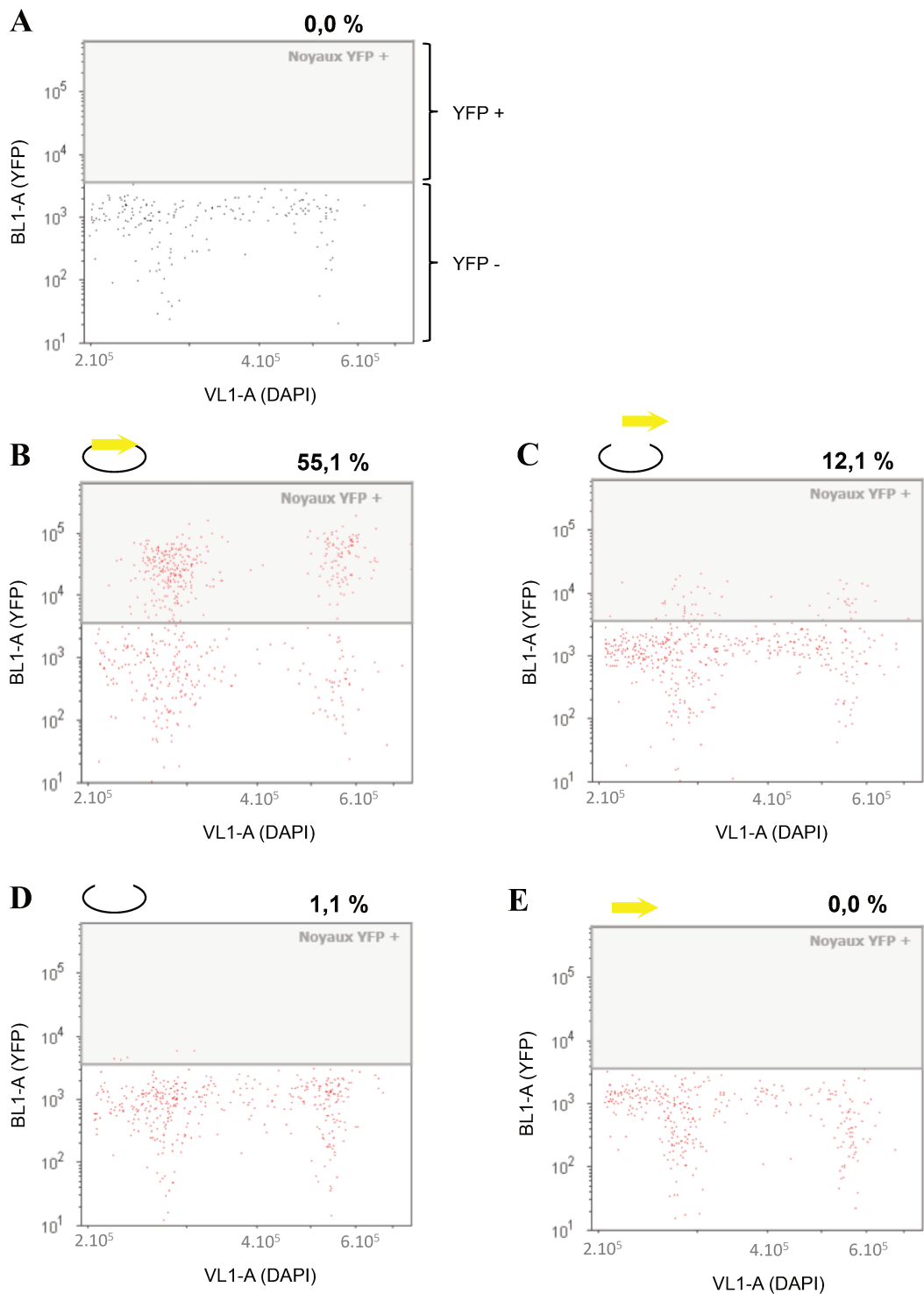


Figure 25 : Détection de la fluorescence YFP de protoplastes transfectés avec le plasmide rapporteur de recombinaison par cytométrie en flux.

Des protoplastes issus de jeunes feuilles d'*Arabidopsis* sauvages (écotype Columbia) sont soit non transfectés (A), soit transfectés avec le plasmide rapporteur de recombinaison circulaire (B) ou préalablement digéré par I-SceI (C) (cf protocole figure 22). Deux autres lots de protoplastes sont transfectés soit avec le plasmide sans le gène de fusion H2B-YFP (D), soit uniquement le fragment H2B-YFP (flèche jaune) (E). Les noyaux des protoplastes sont extraits puis marqués avec du DAPI (5 µg/ml). Les chiffres en gras correspondent aux pourcentages de noyaux fluorescents YFP+.

IV. Un système rapporteur de l'efficacité de jonction d'extrémités d'ADN

Bien que la reconstitution du gène P35S-tdTomato-CENH3 après digestion *in vitro* du plasmide soit un évènement rare, la réinsertion du fragment H2B-YFP semble plus fréquente. En effet, la transfection du produit de digestion (plasmide linéaire + fragment H2B-YFP) aboutit à plus de 12 % de noyaux YFP+ (**Figure 25 C**) - contre environ 1% de noyaux Tomato+. L'efficacité de la digestion *in vitro* par I-SceI a été vérifiée par transfection du plasmide linéaire (sans H2B-YFP) seul ou du fragment H2B-YFP seul après extraction des deux produits de digestion sur gel (**Figure 25 D-E**). Comme la recircularisation du plasmide, la réintégration du fragment excisé nécessite au minimum un évènement de jonction des extrémités d'ADN (entre le double promoteur 35S et la séquence codante d'HB2-YFP). Deux hypothèses peuvent être émises pour expliquer la surprenante préférence du substrat pour la recombinaison bimoléculaire (réintégration du fragment excisé) plutôt que unimoléculaire (recircularisation). Il est d'abord probable que la concentration de plasmides linéaires et d'inserts dans les noyaux des protoplastes transfectés soit très élevée, donc que la probabilité de rencontre entre ces deux molécules soit importante. De plus, les deux sites de restriction de l'enzyme I-SceI dans le plasmide utilisé sont en orientation inversée ; la recircularisation du plasmide nécessite donc une étape de maturation des extrémités d'ADN avant ligature. En revanche, les extrémités du plasmide linéaire et de l'insert sont compatibles (complémentarité des extrémités 3' sortantes), ce qui pourrait favoriser la réintégration du fragment H2B-YFP par simple ligature. Un variant du plasmide rapporteur de recombinaison portant les deux sites de restriction d'I-SceI dans la même orientation a également été construit au laboratoire. La digestion *in vitro* puis la transfection de ce plasmide dans des protoplastes sauvages permettrait de tester cette hypothèse.

Au même titre que la recircularisation du plasmide linéaire, la réintégration du fragment H2B-YFP constitue (au minimum) un événement de jonction d'extrémités d'ADN. Suite à la transfection du plasmide digéré *in vitro*, les noyaux Tomato+ mais aussi YFP+ sont donc des marqueurs d'évènements de recombinaison non homologue. Ainsi, environ 13 % des protoplastes ont subi au moins un événement de recombinaison non homologue (1 % de noyaux Tomato+ et 12 % YFP+), contre 3 % ayant subi un événement de recombinaison homologue (CFP+). Ces premières analyses suggèrent donc que dans les cellules sauvages, les voies de jonction des extrémités d'ADN sont plus efficaces que les voies de recombinaison homologue pour la réparation de CDB induites par I-SceI dans notre système d'étude. La quantification précise des événements de recombinaison homologue reste cependant délicate à cause de la faible fréquence de ces événements (< 3%).

L'ensemble de ces travaux a permis de développer un outil permettant d'évaluer la capacité des cellules végétales à réaliser la jonction d'extrémités d'ADN *in vivo*, reflétant l'efficacité des voies de recombinaison non homologue. Cet outil, maintenant disponible au laboratoire, a été utilisé pour tester le rôle de la protéine XRCC2 dans la jonction des extrémités d'ADN (Résultats, Chapitre I, Résultats complémentaires, II). Il ouvre de plus la possibilité de caractériser les voies de recombinaison non homologue en étudiant notamment les substrats préférentiels de ces différentes voies ainsi que le type de jonctions qu'elles produisent au cours de la réparation. Dans ce sens, plusieurs types d'extrémités peuvent être générées : extrémités franches (*via* HpaI, **Figure 21 A**), cohésives (*via* I-SceI), compatibles ou non-compatibles (selon l'orientation des sites de restriction d'I-SceI). La modification chimique des extrémités (absence de phosphate en 5' ou de groupement OH en 3', sucres ou bases modifiées...) peut également être envisagée.

*Discussion générale
& Perspectives*

Mes travaux de thèse se sont principalement focalisés sur l'étude des acteurs et des interactions entre les voies de recombinaison homologue chez la plante modèle *Arabidopsis thaliana*. Dans les cellules somatiques, les deux voies majeures de réparation des CDB par recombinaison homologue sont la voie SSA (*single strand annealing*) et la voie SDSA (*synthesis-dependant strand-annealing*) (Pâques & Haber, 1999 ; Symington, 2002b ; Ira *et al.*, 2006). Dans un souci de simplification du modèle d'étude - par rapport à l'éventail de lésions générées par l'irradiation - mon travail a porté sur l'analyse de la réparation d'une CDB unique et localisée, induite par la méganucléase I-SceI.

Notre intérêt s'est d'abord porté sur trois acteurs de la recombinaison homologue, XRCC2, RAD51B et RAD51D, des paralogues de RAD51. Chez les animaux comme chez les plantes, ces protéines jouent le rôle de médiateur de l'activité de la recombinase RAD51 (pour revue, voir Suwaki *et al.*, 2011). Bien que leurs rôles exacts restent à identifier, il a été montré qu'ils agissent au cours de plusieurs étapes des voies de recombinaison homologue dépendantes de RAD51 : la formation et la stabilisation du nucléofilament RAD51-ADNsb (Takata *et al.*, 2000 ; Takata *et al.*, 2001 ; Chun *et al.*, 2013 ; Da Ines *et al.*, 2013a), ainsi que l'échange de brin catalysé par RAD51 (Sigurdsson *et al.*, 2001).

Grâce à l'induction d'une CDB dans le locus DGU.US (Orel *et al.*, 2003) par l'enzyme I-SceI, nous avons montré que l'efficacité de reconstitution du gène GUS par recombinaison est nettement diminuée dans les plantes *xrcc2* par rapport aux plantes sauvages (**Article 1, figure 1**). Etant donné que cette construction est rapportrice d'évènements de recombinaison homologue indépendants de RAD51 (Roth *et al.*, 2012), ces données démontrent que XRCC2 est impliqué dans la voie SSA. Afin de vérifier ce résultat totalement inattendu à l'échelle moléculaire, nous avons développé une analyse par Southern blot permettant de visualiser et de quantifier précisément les produits de la recombinaison. Bien que le substrat DGU.US soit

largement utilisé dans la littérature, cette analyse n'avait à notre connaissance jamais été menée sur plante entière. La confirmation du rôle de XRCC2 dans la voie SSA (**Article 1, figure 2**) nous a conduit à rechercher les fonctions potentielles de RAD51B et RAD51D, deux autres paralogues de RAD51. Puisque l'efficacité du SSA est clairement réduite dans les mutants *rad51b* et *rad51d* par rapport à des plantes sauvages, ces deux protéines sont également impliquées dans la voie SSA (**Article 1, figures 5 et 6**). L'analyse du double mutant *xrcc2 rad51b* et du triple mutant *xrcc2 rad51b rad51d* a de plus révélé que ces trois paralogues de RAD51 jouent des fonctions non-épistatiques (au moins pour XRCC2 et RAD51B) dans cette voie de recombinaison indépendante de RAD51 (**Article 1, figure 5**). Afin de confirmer que le rôle de la protéine XRCC2 dans le SSA ne dépend pas de RAD51, le double mutant *xrcc2 rad51* a été généré. Des résultats similaires au simple mutant *xrcc2* ont été obtenus (**Figure 20**), ce qui confirme que la fonction de la protéine XRCC2 dans le SSA est bien indépendante de RAD51. A notre connaissance, ces travaux constituent la première démonstration de l'existence d'une fonction d'un paralogue de RAD51 dans la recombinaison indépendante de RAD51, chez les Eucaryotes.

Quels sont les rôles de XRCC2, RAD51B et RAD51D dans la voie SSA ? Des études *in vitro* ont montré que le complexe BCDX2 présente une grande affinité pour les structures d'ADN branchées, telles que les structures en Y issues de l'hybridation des séquences répétées au cours du SSA (Yokoyama *et al.*, 2004). Les mêmes auteurs ont démontré que ce complexe est capable de catalyser l'hybridation de fragments d'ADNs *in vitro*. Ceci suggère très fortement que ces paralogues soient impliqués dans l'étape d'hybridation des séquences complémentaires situées de part et d'autre de la cassure. En revanche, l'absence de RAD51C (un membre du complexe BCDX2) n'induit qu'une très faible diminution de l'efficacité du SSA chez *Arabidopsis* (Roth *et al.*, 2012). La différence de sévérité des phénotypes des simples mutants (*xrcc2*, *rad51b*, *rad 51c* et *rad51d*), ainsi que l'existence de fonctions non-

épistatiques suggèrent des rôles individuels de ces protéines, plutôt que sous la forme d'un complexe. La vérification de ces hypothèses nécessite d'entreprendre des études biochimiques des paralogues de RAD51 d'*Arabidopsis*. Dans ce sens, nous envisageons de produire ces protéines dans un système bactérien puis de réaliser des tests d'activité *in vitro* des protéines individuelles ou sous forme de complexe.

Une alternative à ce rôle direct dans l'étape d'hybridation des séquences répétées est une action au cours de la résection des extrémités de la cassure. Cette étape est en effet commune aux différentes voies dans lesquelles XRCC2, RAD51B et RAD51D interviennent : les voies de recombinaison homologue dépendantes de RAD51 (pour revue, voir Suwaki *et al.*, 2011) et la voie SSA (Article 1). De plus, il a été montré que ces protéines agissent en amont du recrutement de RAD51 (Chun *et al.*, 2013), soit à une étape très précoce de la recombinaison, ce qui serait compatible avec un rôle dans la résection. Il est possible de suivre la résection des extrémités des CDB *in vivo* grâce à un marquage immunohistochimique de la protéine RPA, qui se lie à l'ADN simple brin (Osman *et al.*, 2009). Ce type d'analyse nécessite de connaître la cinétique de formation puis de réparation de la CDB dans une cellule sauvage. Dans cette optique, nous avons quantifié les foci du variant d'histone H2AX phosphorylé (γ H2AX), un marqueur spécifique des CDB, à différents temps après l'induction de l'expression d'I-SceI dans des plantules d'*Arabidopsis*. Les résultats préliminaires sont encourageants mais la rapidité de la réparation des CDB (Charbonnel *et al.*, 2011) et l'asynchronie des événements dans les différentes cellules compliquent actuellement l'analyse.

La seconde partie de mes travaux de thèse s'est focalisée sur l'analyse des fonctions du complexe XPF-ERCC1 dans les voies de recombinaison homologue (RH). Ce complexe présente une activité endonucléasique spécifique de la structure de l'ADN, responsable du clivage des extrémités d'ADN non homologues des intermédiaires de recombinaison (Fishman-Lobell & Haber, 1992 ; Bardwell *et al.*, 1994 ; Ivanov & Haber, 1995 ; Adair, 2000 ; Sargent *et al.*, 2000 ; Dubest *et al.*, 2002 ; Dubest *et al.*, 2004 ; Al-Minawi *et al.*, 2008). Chez *Arabidopsis*, l'inactivation de XPF ou ERCC1 inhibe d'une part la voie SSA - quand celle-ci implique le clivage de longues extrémités 3' non homologues - et d'autre part, les voies de RH dépendantes de RAD51 (Dubest *et al.*, 2002 ; Dubest *et al.*, 2004). Cependant, en utilisant les loci rapporteurs de recombinaison DGU.US et IU.GUS (Orel *et al.*, 2003), nous avons observé que l'efficacité de reconstruction du gène GUS n'est pas réduite dans les plantes mutantes *xpf* (**Article 2, figures 2 et 4**). Contrairement au substrat U'G'.US utilisé par Dubest *et al.* (2004), la réparation de ces loci par RH nécessite le clivage d'extrémités non homologues de petite taille (< 21 nucléotides) (**Article 2, figure supplémentaire 1**). Grâce à un système plasmidique analogue à celui utilisé par Dubest *et al.* (2002, 2004) - mais produisant des extrémités non homologues de petite taille - nous avons démontré que le complexe XPF-ERCC1 n'est pas nécessaire au clivage des courtes extrémités 3' sortantes au cours de la RH chez *Arabidopsis* (**Article 2, figure 1**). Ces résultats sont parfaitement en accord avec les expériences menées chez la levure et les Mammifères. En effet, la réparation par RH d'une CDB dont les extrémités contiennent des séquences non homologues de moins de 30 nucléotides est indépendante du complexe Rad1-Rad10 (l'homologue de XPF-ERCC1) chez *S. cerevisiae* (Pâques & Haber, 1997). Dans les cellules CHO, les extrémités non homologues dont la taille est inférieure à 18 nucléotides sont efficacement éliminées en absence de la protéine ERCC1 (Adair, 2000 ; Sargent *et al.*, 2000). Il a de plus été rapporté

que la protéine ERCC1 humaine présente une faible affinité pour les extrémités d'ADNsb de moins de 15 nucléotides (Tsodikov *et al.*, 2005).

Afin de poursuivre cette analyse, il serait d'abord intéressant de préciser la taille limite à partir de laquelle le complexe XPF-ERCC1 est requis pour l'élimination des séquences non homologues. Pour cela, différentes digestions enzymatiques des plasmides pGF et pFP (Article 2) permettraient la génération de substrats avec des séquences non homologues de taille variable. L'efficacité de la recombinaison pourra être testée en protoplastes sauvages ou mutants pour XPF (ou ERCC1), selon la méthode décrite dans l'article 2. L'initiation de la synthèse d'ADN puis la ligature des brins au cours des voies SSA et SDSA nécessitent l'élimination de toutes les extrémités non homologues, quelle que soit leur longueur. Puisque l'élimination des courtes extrémités est indépendante du complexe XPF-ERCC1, quelle activité enzymatique réalise cette fonction ? Chez *S. cerevisiae*, les extrémités de 10 ou 20 nucléotides sont dégradées par un mécanisme qui dépend au moins en partie de l'activité de relecture 3'-5' de la polymérase δ (Pâques & Haber, 1997). Sachant qu'un homologue de la polymérase δ a été identifié chez *Arabidopsis* (Schuermann *et al.*, 2009), cette enzyme pourrait être un bon candidat.

Dans un contexte où XPF-ERCC1 n'est pas requis à la recombinaison SDSA (taille des extrémités non homologues inférieure à 18 nucléotides), une augmentation inattendue de la recombinaison au locus IU.GUS a été observée dans les plantes mutantes *xpf* (**Article 2, figure 4, tableau 2**). La complémentation de ces mutants avec la protéine de fusion XPF-GFP a permis de confirmer que l'augmentation de l'efficacité du SDSA est strictement liée à l'absence de la protéine XPF (**Article 2, figure 5**). XPF, et probablement le complexe XPF-ERCC1, joue donc un rôle inhibiteur sur la recombinaison SDSA. Ce résultat rappelle le rôle anti-recombinogène de ce complexe dans la protection des télomères courts chez *Arabidopsis* (Vannier *et al.*, 2009) et les Mammifères (Zhu *et al.*, 2003). En effet l'inactivation de XPF ou

ERCC1 dans un contexte de télomères déprotégés stimule fortement l'instabilité chromosomique. Ceci semble être le résultat de l'invasion d'extrémités 3' télomériques dans des régions interstitielles de répétitions télomériques dégénérées. Le complexe XPF-ERCC1 serait impliqué dans le clivage de ces extrémités 3' sortantes et inhiberait ainsi l'invasion de séquences ectopiques par les extrémités télomériques (Zhu *et al.*, 2003 ; Vannier *et al.*, 2009).

Nous proposons une explication analogue à l'effet recombino-gène observé au locus IU.GUS en absence d'XPF. Afin de vérifier que l'activité nucléase d'XPF est spécifiquement responsable de l'inhibition de la recombinaison SDSA, nous avons construit la protéine XPF_{D757A}-GFP mutée dans le site actif d'XPF (par analogie avec la protéine humaine (Enzlin & Schärer, 2002)). Contrairement à la protéine fonctionnelle (XPF-GFP), la présence d'XPF_{D757A}-GFP ne réduit pas l'efficacité du SDSA dans les plantes *xpf* (**Article 2, figure 5**). Le rôle inhibiteur d'XPF sur la recombinaison SDSA est donc dépendant de sa capacité à cliver l'ADN.

Des études biochimiques ont révélé que le complexe XPF-ERCC1 est capable de cliver différents types de substrats *in vitro*, et notamment les extrémités d'ADNsb 3' sortantes de fragments d'ADNdb ou de molécules d'ADN branchées (De Laat *et al.*, 1998a). Une première hypothèse pour expliquer le rôle inhibiteur du complexe sur la recombinaison SDSA est qu'il clive les extrémités d'ADN 3' sortantes générées au cours de la résection. Cependant, ce clivage aurait un effet hypo-recombino-gène général en limitant à la fois les voies SSA et SDSA, puisque les deux processus nécessitent la présence d'extrémités d'ADNsb. Une augmentation de l'efficacité de la recombinaison DGU.US -> GUS devrait donc être observée en absence de la protéine XPF (réparation par SSA et/ou SDSA), mais aussi en absence d'XPF et de RAD51 fonctionnel (réparation par SSA). Puisque l'efficacité de la réparation est similaire dans les plantes sauvages et les plantes *xpf*RAD51-GFP (**Article 2, figure 3**), XPF n'a pas d'effet sur la voie SSA (dans le cas où les extrémités non homologues sont courtes), ce

qui infirme cette hypothèse. Selon le modèle de Agmon *et al.*, la recherche d'homologie puis l'appariement de quelques bases (au niveau de microhomologies) entre les deux extrémités d'ADNsb de la cassure forme une structure d'ADN branchée avec des extrémités d'ADN 3' sortantes (Agmon *et al.*, 2009). Une seconde hypothèse serait que le complexe XPF-ERCC1 reconnaisse ces structures et clive les extrémités 3' sortantes, limitant ainsi la voie SDSA (puisque les extrémités réalisant l'invasion ont été éliminées), sans affecter *a priori* la voie SSA.

Dans ce contexte, nous proposons un modèle expliquant le phénotype hyper-recombinogène des plantes mutantes *xpf* par la modulation de la balance entre les voies de RH (associées à la reconstitution du gène GUS) et la voie MMEJ (non productive) (**Article 2, figure 6**). Les CDB induites par I-SceI dans le substrat DGU.US seraient réparées soit par la voie SSA, soit par la voie MMEJ suite à l'appariement de courtes régions (microhomologies) entre les deux extrémités simple brin de la cassure. En absence de la protéine XPF, le clivage des longues extrémités 3' sortantes nécessaire à la réparation de la CDB par MMEJ est inhibé. Ces intermédiaires de recombinaison seraient alors redirigés vers la voie SDSA *via* l'invasion puis la copie d'une séquence homologue par l'une des extrémités non clivées. Ceci explique d'une part l'augmentation globale du nombre de spots recombinants observée dans les mutants *xpf* par rapport aux plantes sauvages (**Article 2, figures 2 et 3, tableau 1**), et d'autre part que cette augmentation soit dépendante de RAD51 (**Article 2, figure 3**). Dans le cas du substrat IU.GUS, les CDB induites par I-SceI seraient prises en charge soit par la voie SDSA, soit par la voie MMEJ (**Article 2, figure 6**). Comme précédemment, l'augmentation du nombre de spots recombinants en absence de la protéine XPF serait liée à la redirection des intermédiaires de recombinaison présentant de longues extrémités d'ADN 3' sortantes, vers la voie SDSA.

Afin de tester ce modèle, nous envisageons de séquencer les produits de réparation des substrats DGU.US et IU.GUS dans des plantes sauvages et des plantes *xpf* après induction de l'expression de l'enzyme I-SceI. La quantification relative des différents types de produits permettra d'abord de vérifier l'importance de la voie MMEJ dans la réparation des CDB induites par I-SceI et de confirmer à l'échelle moléculaire la contribution relative des différentes voies mises en jeu.

L'ensemble de ces travaux a permis de préciser les fonctions des protéines XRCC2, RAD51B, RAD51D, XPF et ERCC1 dans les voies de recombinaison homologue en contexte somatique chez *Arabidopsis thaliana*. Nous avons démontré un rôle inattendu des protéines XRCC2, RAD51B et RAD51D dans la voie SSA indépendante de RAD51 (Article 1). Ces travaux ont mis en évidence pour la première fois que certains paralogues de RAD51 possèdent également des fonctions indépendantes de la recombinaison. Nous avons de plus démontré que le complexe XPF-ERCC1 joue un rôle inhibiteur sur la voie de recombinaison SDSA (Article 2). Grâce à son activité nucléase, celui-ci cliverait les extrémités d'ADN 3' sortantes des intermédiaires de recombinaison, limitant ainsi la recherche d'homologie par les extrémités.

Références bibliographiques

- Abdu U, González-Reyes A, Ghabrial A, Schüpbach T** (2003) The *Drosophila* spn-D gene encodes a RAD51C-like protein that is required exclusively during meiosis. *Genetics* 165: 197-204
- Abe K, Osakabe K, Nakayama S, Endo M, Tagiri A, Todoriki S, Ichikawa H, Toki S** (2005) Arabidopsis RAD51C gene is important for homologous recombination in meiosis and mitosis. *Plant physiology* 139: 896-908
- Aboussekhra A, Chanet R, Adjiri A, Fabre F** (1992) Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the RAD51 gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins. *Molecular and cellular biology* 12: 3224-3234
- Adair GM** (2000) Role of ERCC1 in removal of long non-homologous tails during targeted homologous recombination. *The EMBO journal* 19: 5552-5561
- Adzuma K, Ogawa T, Ogawa H** (1984) Primary structure of the RAD52 gene in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 4: 2735-2744
- Agmon N, Pur S, Liefshitz B, Kupiec M** (2009) Analysis of repair mechanism choice during homologous recombination. *Nucleic Acids Res* 37: 5081-5092
- Ahmad A, Robinson AR, Duensing A, van Drunen E, Beverloo HB, Weisberg DB, Hasty P, Hoeijmakers JHJ, Niedernhofer LJ** (2008) ERCC1-XPF endonuclease facilitates DNA double-strand break repair. *Molecular and cellular biology* 28: 5082-5092
- Ahnesorg P, Smith P, Jackson SP** (2006) XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* 124: 301-313
- Al-Minawi AZ, Saleh-Gohari N, Helleday T** (2008) The ERCC1/XPF endonuclease is required for efficient single-strand annealing and gene conversion in mammalian cells. *Nucleic acids research* 36: 1-9
- Albala JS, Thelen MP, Prange C, Fan W, Christensen M, Thompson LH, Lennon GG** (1997) Identification of a novel human RAD51 homolog, RAD51B. *Genomics* 46: 476-479
- Alexiadis V, Kadonaga JT** (2002) Strand pairing by Rad54 and Rad51 is enhanced by chromatin. *Genes & development* 16: 2767-2771
- Allers T, Lichten M** (2001) Differential timing and control of noncrossover and crossover recombination during meiosis. *Cell* 106: 47-57
- Alpi A, Pasierbek P, Gartner A, Loidl J** (2003) Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. *Chromosoma* 112: 6-16
- Amitani I, Baskin RJ, Kowalczykowski SC** (2006) Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. *Molecular cell* 23: 143-148
- Andersen SL, Sekelsky J** (2010) Meiotic versus mitotic recombination: two different routes for double-strand break repair: the different functions of meiotic versus mitotic DSB repair

are reflected in different pathway usage and different outcomes. *BioEssays : news and reviews in molecular, cellular and developmental biology* 32: 1058-1066

Andrade P, Martín MJ, Juárez R, López de Saro F, Blanco L (2009) Limited terminal transferase in human DNA polymerase μ defines the required balance between accuracy and efficiency in NHEJ. *Proceedings of the National Academy of Sciences of the United States of America* 106: 16203-16208

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796-815

Audebert M, Salles B, Calsou P (2004) Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. *The Journal of biological chemistry* 279: 55117-55126

Babiychuk E, Cottrill PB, Storozhenko S, Fuangthong M, Chen Y, O'Farrell MK, Van Montagu M, Inzé D, Kushnir S (1998) Higher plants possess two structurally different poly(ADP-ribose) polymerases. *The Plant journal : for cell and molecular biology* 15: 635-645

Badie S, Liao C, Thanasoula M, Barber P, Hill MA, Tarsounas M (2009) RAD51C facilitates checkpoint signaling by promoting CHK2 phosphorylation. *J Cell Biol* 185: 587-600

Bagherieh-Najjar MB, de Vries OMH, Hille J, Dijkwel PP (2005) *Arabidopsis* RecQI4A suppresses homologous recombination and modulates DNA damage responses. *The Plant journal : for cell and molecular biology* 43: 789-798

Bagherieh-Najjar MB, de Vries OMH, Kroon JTM, Wright EL, Elborough KM, Hille J, Dijkwel PP (2003) *Arabidopsis* RecQsim, a plant-specific member of the RecQ helicase family, can suppress the MMS hypersensitivity of the yeast *sgs1* mutant. *Plant molecular biology* 52: 273-284

Bai Y, Davis AP, Symington LS (1999) A novel allele of RAD52 that causes severe DNA repair and recombination deficiencies only in the absence of RAD51 or RAD59. *Genetics* 153: 1117-1130

Bai Y, Symington LS (1996) A Rad52 homolog is required for RAD51-independent mitotic recombination in *Saccharomyces cerevisiae*. *Genes & development* 10: 2025-2037

Baldeyron C, Jacquemin E, Smith J, Jacquemont C, De Oliveira I, Gad S, Feunteun J, Stoppa-Lyonnet D, Papadopoulos D (2002) A single mutated BRCA1 allele leads to impaired fidelity of double strand break end-joining. *Oncogene* 21: 1401-1410

Ball LG, Zhang K, Cobb JA, Boone C, Xiao W (2009) The yeast Shu complex couples error-free post-replication repair to homologous recombination. *Molecular microbiology* 73: 89-102

- Ballal RD, Saha T, Fan S, Haddad BR, Rosen EM** (2009) BRCA1 localization to the telomere and its loss from the telomere in response to DNA damage. *The Journal of biological chemistry* 284: 36083-36098
- Bardwell AJ, Bardwell L, Tomkinson AE, Friedberg EC** (1994) Specific cleavage of model recombination and repair intermediates by the yeast Rad1-Rad10 DNA endonuclease. *Science (New York, NY)* 265: 2082-2085
- Basile G, Aker M, Mortimer RK** (1992) Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene RAD51. *Mol Cell Biol* 12: 3235-3246
- Bastin-Shanower SA, Fricke WM, Mullen JR, Brill SJ** (2003) The mechanism of Mus81-Mms4 cleavage site selection distinguishes it from the homologous endonuclease Rad1-Rad10. *Molecular and cellular biology* 23: 3487-3496
- Baudat F, de Massy B** (2004) [SPO11: an activity that promotes DNA breaks required for meiosis]. *Médecine sciences : M/S* 20: 213-218
- Baumann P, Benson FE, West SC** (1996) Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell* 87: 757-766
- Baumann P, West SC** (1997) The human Rad51 protein: polarity of strand transfer and stimulation by hRP-A. *EMBO J* 16: 5198-5206
- Benjamin RC, Gill DM** (1980) Poly(ADP-ribose) synthesis in vitro programmed by damaged DNA. A comparison of DNA molecules containing different types of strand breaks. *The Journal of biological chemistry* 255: 10502-10508
- Benson FE, Stasiak A, West SC** (1994) Purification and characterization of the human Rad51 protein, an analogue of E. coli RecA. *The EMBO journal* 13: 5764-5771
- Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P** (1997) An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* 386: 414-417
- Bernstein KA, Gangloff S, Rothstein R** (2010) The RecQ DNA helicases in DNA repair. *Annual review of genetics* 44: 393-417
- Bernstein KA, Reid RJD, Sunjevaric I, Demuth K, Burgess RC, Rothstein R** (2011) The Shu complex, which contains Rad51 paralogues, promotes DNA repair through inhibition of the Srs2 anti-recombinase. *Molecular biology of the cell* 22: 1599-1607
- Bezzubova O, Silbergleit A, Yamaguchi-Iwai Y, Takeda S, Buerstedde JM** (1997) Reduced X-ray resistance and homologous recombination frequencies in a RAD54^{-/-} mutant of the chicken DT40 cell line. *Cell* 89: 185-193
- Bezzubova OY, Schmidt H, Ostermann K, Heyer WD, Buerstedde JM** (1993) Identification of a chicken RAD52 homologue suggests conservation of the RAD52 recombination pathway throughout the evolution of higher eukaryotes. *Nucleic acids research* 21: 5945-5949

Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK (2000) The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. *The Journal of biological chemistry* 275: 23899-23903

Bianco PR, Tracy RB, Kowalczykowski SC (1998) DNA strand exchange proteins: a biochemical and physical comparison. *Frontiers in bioscience : a journal and virtual library* 3: D570-603

Bishop DK (2012) Rad51, the lead in mitotic recombinational DNA repair, plays a supporting role in budding yeast meiosis. *Cell cycle (Georgetown, Tex)* 11: 4105-4106

Bishop DK, Ear U, Bhattacharyya A, Calderone C, Beckett M, Weichselbaum RR, Shinohara A (1998) Xrcc3 is required for assembly of Rad51 complexes in vivo. *J Biol Chem* 273: 21482-21488

Bishop DK, Park D, Xu L, Kleckner N (1992) DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell* 69: 439-456

Bleuyard J-Y, White CI (2004) The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis. *The EMBO journal* 23: 439-449

Bleuyard JY, Gallego ME, Savigny F, White CI (2005) Differing requirements for the Arabidopsis Rad51 paralogs in meiosis and DNA repair. *Plant J* 41: 533-545

Block-Schmidt AS, Dukowic-Schulze S, Wanieck K, Reidt W, Puchta H (2011) BRCC36A is epistatic to BRCA1 in DNA crosslink repair and homologous recombination in Arabidopsis thaliana. *Nucleic Acids Res* 39: 146-154

Boboila C, Oksenysh V, Gostissa M, Wang JH, Zha S, Zhang Y, Chai H, Lee C-S, Jankovic M, Saez L-MA *et al.* (2012) Robust chromosomal DNA repair via alternative end-joining in the absence of X-ray repair cross-complementing protein 1 (XRCC1). *Proceedings of the National Academy of Sciences of the United States of America* 109: 2473-2478

Boddy MN, Gaillard PH, McDonald WH, Shanahan P, Yates JR, Russell P (2001) Mus81-Eme1 are essential components of a Holliday junction resolvase. *Cell* 107: 537-548

Bohgaki T, Bohgaki M, Hakem R (2010) DNA double-strand break signaling and human disorders. *Genome integrity* 1: 15

Boisnard-Lorig C, Colon-Carmona A, Bauch M, Hodge S, Doerner P, Bancharel E, Dumas C, Haseloff J, Berger F (2001) Dynamic analyses of the expression of the HISTONE::YFP fusion protein in arabidopsis show that syncytial endosperm is divided in mitotic domains. *The Plant cell* 13: 495-509

Borde V, de Massy B (2013) Programmed induction of DNA double strand breaks during meiosis: setting up communication between DNA and the chromosome structure. *Genetics & Development* 23: 147-155

- Borde V, Robine N, Lin W, Bonfils S, Geli V, Nicolas A** (2009) Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. *EMBO J* 28: 99-111
- Börner GV, Kleckner N, Hunter N** (2004) Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. *Cell* 117: 29-45
- Boulton S, Kyle S, Durkacz BW** (1999) Interactive effects of inhibitors of poly(ADP-ribose) polymerase and DNA-dependent protein kinase on cellular responses to DNA damage. *Carcinogenesis* 20: 199-203
- Boulton SJ, Martin JS, Polanowska J, Hill DE, Gartner A, Vidal M** (2004) BRCA1/BARD1 orthologs required for DNA repair in *Caenorhabditis elegans*. *Current biology : CB* 14: 33-39
- Branzei D, Foiani M** (2007) Interplay of replication checkpoints and repair proteins at stalled replication forks. *DNA Repair (Amst)* 6: 994-1003
- Brenneman MA, Wagener BM, Miller CA, Allen C, Nickoloff JA** (2002) XRCC3 controls the fidelity of homologous recombination: roles for XRCC3 in late stages of recombination. *Molecular cell* 10: 387-395
- Brough R, Wei D, Leulier S, Lord CJ, Rong YS, Ashworth A** (2008) Functional analysis of *Drosophila melanogaster* BRCA2 in DNA repair. *DNA repair* 7: 10-19
- Brugmans L, Kanaar R, Essers J** (2007) Analysis of DNA double-strand break repair pathways in mice. *Mutation research* 614: 95-108
- Bugreev DV, Golub EI, Stasiak AZ, Stasiak A, Mazin AV** (2005) Activation of human meiosis-specific recombinase Dmc1 by Ca²⁺. *The Journal of biological chemistry* 280: 26886-26895
- Bugreev DV, Mazina OM, Mazin AV** (2006) Rad54 protein promotes branch migration of Holliday junctions. *Nature* 442: 590-593
- Buisson R, Niraj J, Pauty J, Maity R, Zhao W, Coulombe Y, Sung P, Masson J-Y** (2014) Breast cancer proteins PALB2 and BRCA2 stimulate polymerase η in recombination-associated DNA synthesis at blocked replication forks. *Cell reports* 6: 553-564
- Bundock P, Hooykaas P** (2002) Severe developmental defects, hypersensitivity to DNA-damaging agents, and lengthened telomeres in *Arabidopsis* MRE11 mutants. *The Plant cell* 14: 2451-2462
- Caestecker KW, Van de Walle GR** (2013) The role of BRCA1 in DNA double-strand repair: past and present. *Experimental cell research* 319: 575-587
- Caldecott KW** (2003) XRCC1 and DNA strand break repair. *DNA repair* 2: 955-969

Caldecott KW (2008) Single-strand break repair and genetic disease. *Nature reviews Genetics* 9: 619-631

Caldecott KW, McKeown CK, Tucker JD, Ljungquist S, Thompson LH (1994) An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. *Molecular and cellular biology* 14: 68-76

Cao L, Alani E, Kleckner N (1990) A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell* 61: 1089-1101

Capp J-P, Boudsocq F, Bertrand P, Laroche-Clary A, Pourquier P, Lopez BS, Cazaux C, Hoffmann J-S, Canitrot Y (2006) The DNA polymerase lambda is required for the repair of non-compatible DNA double strand breaks by NHEJ in mammalian cells. *Nucleic acids research* 34: 2998-3007

Cartwright R, Dunn AM, Simpson PJ, Tambini CE, Thacker J (1998a) Isolation of novel human and mouse genes of the recA/RAD51 recombination-repair gene family. *Nucleic Acids Res* 26: 1653-1659

Cartwright R, Tambini CE, Simpson PJ, Thacker J (1998b) The XRCC2 DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family. *Nucleic Acids Res* 26: 3084-3089

Catlett MG, Forsburg SL (2003) *Schizosaccharomyces pombe* Rdh54 (TID1) acts with Rhp54 (RAD54) to repair meiotic double-strand breaks. *Molecular biology of the cell* 14: 4707-4720

Ceballos SJ, Heyer W-D (2011) Functions of the Snf2/Swi2 family Rad54 motor protein in homologous recombination. *Biochimica et biophysica acta* 1809: 509-523

Cejka P, Cannavo E, Polaczek P, Masuda-Sasa T, Pokharel S, Campbell JL, Kowalczykowski SC (2010a) DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. *Nature* 467: 112-116

Cejka P, Plank JL, Bachrati CZ, Hickson ID, Kowalczykowski SC (2010b) Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. *Nature structural & molecular biology* 17: 1377-1382

Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic acids research* 39: e82

Chang M, Bellaoui M, Zhang C, Desai R, Morozov P, Delgado-Cruzata L, Rothstein R, Freyer GA, Boone C, Brown GW (2005) RMI1/NCE4, a suppressor of genome instability, encodes a member of the RecQ helicase/Topo III complex. *The EMBO journal* 24: 2024-2033

Chappell C, Hanakahi LA, Karimi-Busheri F, Weinfeld M, West SC (2002) Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining. *The EMBO journal* 21: 2827-2832

Charbonnel C, Allain E, Gallego ME, White CI (2011) Kinetic analysis of DNA double-strand break repair pathways in Arabidopsis. *DNA repair* 10: 611-619

Charbonnel C, Gallego ME, White CI (2010) Xrcc1-dependent and Ku-dependent DNA double-strand break repair kinetics in Arabidopsis plants. *The Plant journal : for cell and molecular biology* 64: 280-290

Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM et al. (1998a) Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Molecular cell* 2: 317-328

Chen PL, Chen CF, Chen Y, Xiao J, Sharp ZD, Lee WH (1998b) The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proceedings of the National Academy of Sciences of the United States of America* 95: 5287-5292

Chen XB, Melchionna R, Denis CM, Gaillard PH, Blasina A, Van de Weyer I, Boddy MN, Russell P, Vialard J, McGowan CH (2001) Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. *Molecular cell* 8: 1117-1127

Chi P, Kwon Y, Seong C, Epshtein A, Lam I, Sung P, Klein HL (2006a) Yeast recombination factor Rdh54 functionally interacts with the Rad51 recombinase and catalyzes Rad51 removal from DNA. *The Journal of biological chemistry* 281: 26268-26279

Chi P, Van Komen S, Sehorn MG, Sigurdsson S, Sung P (2006b) Roles of ATP binding and ATP hydrolysis in human Rad51 recombinase function. *DNA repair* 5: 381-391

Choi K, Szakal B, Chen Y-H, Branzei D, Zhao X (2010) The Smc5/6 complex and Esc2 influence multiple replication-associated recombination processes in *Saccharomyces cerevisiae*. *Molecular biology of the cell* 21: 2306-2314

Chun J, Buechelmaier ES, Powell SN (2013) Rad51 paralog complexes BCDX2 and CX3 act at different stages in the BRCA1-BRCA2-dependent homologous recombination pathway. *Molecular and cellular biology* 33: 387-395

Ciccia A, Constantinou A, West SC (2003) Identification and characterization of the human mus81-eme1 endonuclease. *The Journal of biological chemistry* 278: 25172-25178

Ciccia A, Elledge SJ (2010) The DNA damage response: making it safe to play with knives. *Molecular cell* 40: 179-204

Clerici M, Mantiero D, Lucchini G, Longhese MP (2005) The *Saccharomyces cerevisiae* Sae2 protein promotes resection and bridging of double strand break ends. *The Journal of biological chemistry* 280: 38631-38638

Clever B, Interthal H, Schmuckli-Maurer J, King J, Sigrist M, Heyer WD (1997) Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. *The EMBO journal* 16: 2535-2544

Cloud V, Chan Y-L, Grubb J, Budke B, Bishop DK (2012) Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. *Science (New York, NY)* 337: 1222-1225

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant journal : for cell and molecular biology* 16: 735-743

Colaiácovo MP, Pâques F, Haber JE (1999) Removal of one nonhomologous DNA end during gene conversion by a RAD1- and MSH2-independent pathway. *Genetics* 151: 1409-1423

Compton SA, Ozgür S, Griffith JD (2010) Ring-shaped Rad51 paralog protein complexes bind Holliday junctions and replication forks as visualized by electron microscopy. *The Journal of biological chemistry* 285: 13349-13356

Connor F, Bertwistle D, Mee PJ, Ross GM, Swift S, Grigorieva E, Tybulewicz VL, Ashworth A (1997) Tumorigenesis and a DNA repair defect in mice with a truncating *Breca2* mutation. *Nature genetics* 17: 423-430

Constantinou A, Davies AA, West SC (2001) Branch migration and Holliday junction resolution catalyzed by activities from mammalian cells. *Cell* 104: 259-268

Conway AB, Lynch TW, Zhang Y, Fortin GS, Fung CW, Symington LS, Rice PA (2004) Crystal structure of a Rad51 filament. *Nature structural & molecular biology* 11: 791-796

Corneo B, Wendland RL, Deriano L, Cui X, Klein IA, Wong S-Y, Arnal S, Holub AJ, Weller GR, Pancake BA et al. (2007) Rag mutations reveal robust alternative end joining. *Nature* 449: 483-486

Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Mariani KJ (2000) The importance of repairing stalled replication forks. *Nature* 404: 37-41

Critchlow SE, Bowater RP, Jackson SP (1997) Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. *Current biology : CB* 7: 588-598

Cupp JD, Nielsen BL (2014) Minireview: DNA replication in plant mitochondria. *Mitochondrion* In press

Da Ines O, Abe K, Goubely C, Gallego ME, White CI (2012) Differing requirements for RAD51 and DMC1 in meiotic pairing of centromeres and chromosome arms in *Arabidopsis thaliana*. *PLoS genetics* 8: e1002636

Da Ines O, Degroote F, Amiard S, Goubely C, Gallego ME, White CI (2013a) Effects of XRCC2 and RAD51B mutations on somatic and meiotic recombination in *Arabidopsis thaliana*. *Plant J* 74: 959-970

Da Ines O, Degroote F, Goubely C, Amiard S, Gallego ME, White CI (2013b) Meiotic recombination in *Arabidopsis* is catalysed by DMC1, with RAD51 playing a supporting role. *PLoS Genet* 9: e1003787

- Davis AP, Symington LS** (2001) The yeast recombinational repair protein Rad59 interacts with Rad52 and stimulates single-strand annealing. *Genetics* 159: 515-525
- De Jager M, van Noort J, van Gent DC, Dekker C, Kanaar R, Wyman C** (2001) Human Rad50/Mre11 is a flexible complex that can tether DNA ends. *Molecular cell* 8: 1129-1135
- De Laat WL, Appeldoorn E, Jaspers NG, Hoeijmakers JH** (1998a) DNA structural elements required for ERCC1-XPF endonuclease activity. *J Biol Chem* 273: 7835-7842
- De Laat WL, Appeldoorn E, Sugawara K, Weterings E, Jaspers NG, Hoeijmakers JH** (1998b) DNA-binding polarity of human replication protein A positions nucleases in nucleotide excision repair. *Genes & development* 12: 2598-2609
- De Silva IU, McHugh PJ, Clingen PH, Hartley JA** (2000) Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. *Mol Cell Biol* 20: 7980-7990
- Deans B, Griffin CS, Maconochie M, Thacker J** (2000) Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *EMBO J* 19: 6675-6685
- Delacôte F, Lopez BS** (2008) Importance of the cell cycle phase for the choice of the appropriate DSB repair pathway, for genome stability maintenance: the trans-S double-strand break repair model. *Cell cycle (Georgetown, Tex)* 7: 33-38
- Deng CX, Scott F** (2000) Role of the tumor suppressor gene Brca1 in genetic stability and mammary gland tumor formation. *Oncogene* 19: 1059-1064
- Deriano L, Stracker TH, Baker A, Petrini JHJ, Roth DB** (2009) Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. *Molecular cell* 34: 13-25
- Dianov GL, Hübscher U** (2013) Mammalian base excision repair: the forgotten archangel. *Nucleic acids research* 41: 3483-3490
- Dianov GL, O'Neill P, Goodhead DT** (2001) Securing genome stability by orchestrating DNA repair: removal of radiation-induced clustered lesions in DNA. *Bioessays* 23: 745-749
- Donnianni RA, Symington LS** (2013) Break-induced replication occurs by conservative DNA synthesis. *PNAS* 110: 13475-13480
- Dosanjh MK, Collins DW, Fan W, Lennon GG, Albala JS, Shen Z, Schild D** (1998) Isolation and characterization of RAD51C, a new human member of the RAD51 family of related genes. *Nucleic Acids Res* 26: 1179-1184
- Dresser ME, Ewing DJ, Conrad MN, Dominguez AM, Barstead R, Jiang H, Kodadek T** (1997) DMC1 functions in a *Saccharomyces cerevisiae* meiotic pathway that is largely independent of the RAD51 pathway. *Genetics* 147: 533-544

Dubest S, Gallego ME, White CI (2002) Role of the AtRad1p endonuclease in homologous recombination in plants. *EMBO Rep* 3: 1049-1054

Dubest S, Gallego ME, White CI (2004) Roles of the AtErcc1 protein in recombination. *Plant J* 39: 334-342

Dupaigne P, Le Breton C, Fabre F, Gangloff S, Le Cam E, Veaute X (2008) The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: implications for crossover incidence during mitotic recombination. *Mol Cell* 29: 243-254

Durrant WE, Wang S, Dong X (2007) Arabidopsis SNI1 and RAD51D regulate both gene transcription and DNA recombination during the defense response. *Proc Natl Acad Sci U S A* 104: 4223-4227

Eggler AL, Inman RB, Cox MM (2002) The Rad51-dependent pairing of long DNA substrates is stabilized by replication protein A. *The Journal of biological chemistry* 277: 39280-39288

Elborough KM, West SC (1990) Resolution of synthetic Holliday junctions in DNA by an endonuclease activity from calf thymus. *The EMBO journal* 9: 2931-2936

Enzlin JH, Schärer OD (2002) The active site of the DNA repair endonuclease XPF-ERCC1 forms a highly conserved nuclease motif. *The EMBO journal* 21: 2045-2053

Erdile LF, Wold MS, Kelly TJ (1990) The primary structure of the 32-kDa subunit of human replication protein A. *J Biol Chem* 265: 3177-3182

Esposito MS (1978) Evidence that spontaneous mitotic recombination occurs at the two-strand stage. *Proceedings of the National Academy of Sciences of the United States of America* 75: 4436-4440

Essers J, Hendriks RW, Swagemakers SM, Troelstra C, de Wit J, Bootsma D, Hoeijmakers JH, Kanaar R (1997) Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* 89: 195-204

Essers J, Houtsmuller AB, van Veelen L, Paulusma C, Nigg AL, Pastink A, Vermeulen W, Hoeijmakers JHJ, Kanaar R (2002) Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. *The EMBO journal* 21: 2030-2037

Essers J, van Steeg H, de Wit J, Swagemakers SM, Vermeij M, Hoeijmakers JH, Kanaar R (2000) Homologous and non-homologous recombination differentially affect DNA damage repair in mice. *The EMBO journal* 19: 1703-1710

Evans E, Sugawara N, Haber JE, Alani E (2000) The *Saccharomyces cerevisiae* Msh2 mismatch repair protein localizes to recombination intermediates in vivo. *Molecular cell* 5: 789-799

Evers B, Jonkers J (2006) Mouse models of BRCA1 and BRCA2 deficiency: past lessons, current understanding and future prospects. *Oncogene* 25: 5885-5897

Fairman MP, Stillman B (1988) Cellular factors required for multiple stages of SV40 DNA replication in vitro. *The EMBO journal* 7: 1211-1218

Fekairi S, Scaglione S, Chahwan C, Taylor ER, Tissier A, Coulon S, Dong M-Q, Ruse C, Yates JR, Russell P et al. (2009) Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. *Cell* 138: 78-89

Feldmann E, Schmiemann V, Goedecke W, Reichenberger S, Pfeiffer P (2000) DNA double-strand break repair in cell-free extracts from Ku80-deficient cells: implications for Ku serving as an alignment factor in non-homologous DNA end joining. *Nucleic acids research* 28: 2585-2596

Feng Q, During L, de Mayolo AA, Lettier G, Lisby M, Erdeniz N, Mortensen UH, Rothstein R (2007) Rad52 and Rad59 exhibit both overlapping and distinct functions. *DNA Repair (Amst)* 6: 27-37

Feng Z, Scott SP, Bussen W, Sharma GG, Guo G, Pandita TK, Powell SN (2011) Rad52 inactivation is synthetically lethal with BRCA2 deficiency. *Proceedings of the National Academy of Sciences of the United States of America* 108: 686-691

Ferguson DO, Holloman WK (1996) Recombinational repair of gaps in DNA is asymmetric in *Ustilago maydis* and can be explained by a migrating D-loop model. *Proceedings of the National Academy of Sciences of the United States of America* 93: 5419-5424

Fidantsef AL, Mitchell DL, Britt AB (2000) The Arabidopsis UVH1 gene is a homolog of the yeast repair endonuclease RAD1. *Plant physiology* 124: 579-586

Fishman-Lobell J, Haber JE (1992) Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene RAD1. *Science (New York, NY)* 258: 480-484

Fishman-Lobell J, Rudin N, Haber JE (1992) Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. *Molecular and cellular biology* 12: 1292-1303

Flaus A, Martin DMA, Barton GJ, Owen-Hughes T (2006) Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic acids research* 34: 2887-2905

Flott S, Alabert C, Toh GW, Toth R, Sugawara N, Campbell DG, Haber JE, Pasero P, Rouse J (2007) Phosphorylation of Slx4 by Mec1 and Tel1 regulates the single-strand annealing mode of DNA repair in budding yeast. *Molecular and cellular biology* 27: 6433-6445

Flott S, Rouse J (2005) Slx4 becomes phosphorylated after DNA damage in a Mec1/Tel1-dependent manner and is required for repair of DNA alkylation damage. *The Biochemical journal* 391: 325-333

Fujimori A, Tachiiri S, Sonoda E, Thompson LH, Dhar PK, Hiraoka M, Takeda S, Zhang Y, Reth M, Takata M (2001) Rad52 partially substitutes for the Rad51 paralog XRCC3 in maintaining chromosomal integrity in vertebrate cells. *EMBO J* 20: 5513-5520

Fung CW, Mozlin AM, Symington LS (2009) Suppression of the double-strand-break-repair defect of the *Saccharomyces cerevisiae* rad57 mutant. *Genetics* 181: 1195-1206

Furukawa T, Imamura T, Kitamoto HK, Shimada H (2008) Rice exonuclease-1 homologue, OsEXO1, that interacts with DNA polymerase lambda and RPA subunit proteins, is involved in cell proliferation. *Plant molecular biology* 66: 519-531

Gaillard PH, Wood RD (2001) Activity of individual ERCC1 and XPF subunits in DNA nucleotide excision repair. *Nucleic acids research* 29: 872-879

Galbraith DW (2009) Simultaneous flow cytometric quantification of plant nuclear DNA contents over the full range of described angiosperm 2C values. *Cytometry Part A : the journal of the International Society for Analytical Cytology* 75: 692-698

Gallego ME, Bleuyard J-Y, Daoudal-Cotterell S, Jallut N, White CI (2003) Ku80 plays a role in non-homologous recombination but is not required for T-DNA integration in Arabidopsis. *The Plant journal : for cell and molecular biology* 35: 557-565

Gallego ME, Jeanneau M, Granier F, Bouchez D, Bechtold N, White CI (2001) Disruption of the Arabidopsis RAD50 gene leads to plant sterility and MMS sensitivity. *The Plant journal : for cell and molecular biology* 25: 31-41

Gao Y, Katyal S, Lee Y, Zhao J, Rehg JE, Russell HR, McKinnon PJ (2011) DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair. *Nature* 471: 240-244

Gasior SL, Wong AK, Kora Y, Shinohara A, Bishop DK (1998) Rad52 associates with RPA and functions with rad55 and rad57 to assemble meiotic recombination complexes. *Genes & development* 12: 2208-2221

Getts RC, Stamato TD (1994) Absence of a Ku-like DNA end binding activity in the xrs double-strand DNA repair-deficient mutant. *The Journal of biological chemistry* 269: 15981-15984

Geuting V, Kobbe D, Hartung F, Dürr J, Focke M, Puchta H (2009) Two distinct MUS81-EME1 complexes from Arabidopsis process Holliday junctions. *Plant physiology* 150: 1062-1071

Ghabrial A, Ray RP, Schüpbach T (1998) okra and spindle-B encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in *Drosophila* oogenesis. *Genes & development* 12: 2711-2723

Ghosal G, Chen J (2013) DNA damage tolerance: a double-edged sword guarding the genome. *Translational cancer research* 2: 107-129

- Gilfillan S, Benoist C, Mathis D** (1995) Mice lacking terminal deoxynucleotidyl transferase: adult mice with a fetal antigen receptor repertoire. *Immunological reviews* 148: 201-219
- Gloor GB, Nassif NA, Johnson-Schlitz DM, Preston CR, Engels WR** (1991) Targeted gene replacement in *Drosophila* via P element-induced gap repair. *Science (New York, NY)* 253: 1110-1117
- Godin S, Wier A, Kabbinavar F, Bratton-Palmer DS, Ghodke H, Van Houten B, Vandemark AP, Bernstein KA** (2013) The Shu complex interacts with Rad51 through the Rad51 paralogues Rad55-Rad57 to mediate error-free recombination. *Nucleic acids research*: 1-10
- Godthelp BC, Wiegant WW, van Duijn-Goedhart A, Schärer OD, van Buul PPW, Kanaar R, Zdzienicka MZ** (2002) Mammalian Rad51C contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability. 30: 2767-2771
- Golub EI, Kovalenko OV, Gupta RC, Ward DC, Radding CM** (1997) Interaction of human recombination proteins Rad51 and Rad54. *Nucleic acids research* 25: 4106-4110
- Gorbunova V, Avivi-Ragolski N, Shalev G, Kovalchuk I, Abbo S, Hohn B, Levy AA** (2000) A new hyperrecombinogenic mutant of *Nicotiana tabacum*. *The Plant journal : for cell and molecular biology* 24: 601-611
- Gorbunova V, Levy AA** (1997) Non-homologous DNA end joining in plant cells is associated with deletions and filler DNA insertions. *Nucleic acids research* 25: 4650-4657
- Gostissa M, Alt FW, Chiarle R** (2011) Mechanisms that promote and suppress chromosomal translocations in lymphocytes. *Annual review of immunology* 29: 319-350
- Gottlieb TM, Jackson SP** (1993) The DNA-dependent protein kinase: Requirement for DNA ends and association with Ku antigen. *Cell* 72: 131-142
- Grawunder U, Wilm M, Wu X, Kulesza P, Wilson TE, Mann M, Lieber MR** (1997) Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. *Nature* 388: 492-495
- Grawunder U, Zimmer D, Fugmann S, Schwarz K, Lieber MR** (1998) DNA ligase IV is essential for V(D)J recombination and DNA double-strand break repair in human precursor lymphocytes. *Molecular cell* 2: 477-484
- Gudmundsdottir K, Ashworth A** (2006) The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. *Oncogene* 25: 5864-5874
- Guirouilh-Barbat J, Huck S, Bertrand P, Pirzio L, Desmaze C, Sabatier L, Lopez BS** (2004) Impact of the KU80 pathway on NHEJ-induced genome rearrangements in mammalian cells. *Molecular cell* 14: 611-623
- Gupta RC, Folta-Stogniew E, O'Malley S, Takahashi M, Radding CM** (1999) Rapid exchange of A:T base pairs is essential for recognition of DNA homology by human Rad51 recombination protein. *Molecular cell* 4: 705-714

Haber JE (2012) Mating-type genes and MAT switching in *Saccharomyces cerevisiae*. *Genetics* 191: 33-64

Haber JE, Ira G, Malkova A, Sugawara N (2004) Repairing a double-strand chromosome break by homologous recombination: revisiting Robin Holliday's model. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 359: 79-86

Hakem R, de la Pompa JL, Mak TW (1998) Developmental studies of Brca1 and Brca2 knock-out mice. *Journal of mammary gland biology and neoplasia* 3: 431-445

Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmaier A, Billia F et al. (1996) The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* 85: 1009-1023

Han L, Mao W, Yu K (2012) X-ray repair cross-complementing protein 1 (XRCC1) deficiency enhances class switch recombination and is permissive for alternative end joining. *Proceedings of the National Academy of Sciences of the United States of America* 109: 4604-4608

Hartung F, Suer S, Bergmann T, Puchta H (2006) The role of AtMUS81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. *Nucleic acids research* 34: 4438-4448

Hartung F, Suer S, Knoll A, Wurzel-Wildersinn R, Puchta H (2008) Topoisomerase 3alpha and RMI1 suppress somatic crossovers and are essential for resolution of meiotic recombination intermediates in *Arabidopsis thaliana*. *PLoS genetics* 4: e1000285

Haruta N, Kurokawa Y, Murayama Y, Akamatsu Y, Unzai S, Tsutsui Y, Iwasaki H (2006) The Swi5-Sfr1 complex stimulates Rhp51/Rad51- and Dmc1-mediated DNA strand exchange in vitro. *Nature structural & molecular biology* 13: 823-830

Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ogata H, Ohta T (2001) The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *The Journal of biological chemistry* 276: 14537-14540

Hays SL, Firmenich AA, Massey P, Banerjee R, Berg P (1998) Studies of the interaction between Rad52 protein and the yeast single-stranded DNA binding protein RPA. *Mol Cell Biol* 18: 4400-4406

Heacock M, Spangler E, Riha K, Puizina J, Shippen DE (2004) Molecular analysis of telomere fusions in *Arabidopsis*: multiple pathways for chromosome end-joining. *The EMBO journal* 23: 2304-2313

Helleday T, Lo J, van Gent DC, Engelward BP (2007) DNA double-strand break repair: from mechanistic understanding to cancer treatment. *DNA repair* 6: 923-935

Heyer WD, Ehmsen KT, Liu J (2010) Regulation of homologous recombination in eukaryotes. *Annual review of genetics* 44: 113-139

Heyer WD, Li X, Rolfsmeier M, Zhang XP (2006) Rad54: the Swiss Army knife of homologous recombination? *Nucleic Acids Res* 34: 4115-4125

Hoeijmakers JH (2001) Genome maintenance mechanisms for preventing cancer. *Nature* 411: 366-374

Holzen TM, Shah PP, Olivares HA, Bishop DK (2006) Tid1/Rdh54 promotes dissociation of Dmcl from nonrecombinogenic sites on meiotic chromatin. *Genes & development* 20: 2593-2604

Hong EL, Shinohara A, Bishop DK (2001) *Saccharomyces cerevisiae* Dmcl protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous super-coiled duplex DNA. *The Journal of biological chemistry* 276: 41906-41912

Hong S, Kim KP (2013) Shu1 promotes homolog bias of meiotic recombination in *Saccharomyces cerevisiae*. *Molecules and cells* 36: 446-454

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L et al. (2013) The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 496: 498-503

Hu Y, Raynard S, Sehorn MG, Lu X, Bussen W, Zheng L, Stark JM, Barnes EL, Chi P, Janscak P et al. (2007) RECQL5/Recql5 helicase regulates homologous recombination and suppresses tumor formation via disruption of Rad51 presynaptic filaments. *Genes & development* 21: 3073-3084

Huen MSY, Sy SMH, Chen J (2010) BRCA1 and its toolbox for the maintenance of genome integrity. *Nature reviews Molecular cell biology* 11: 138-148

Hunter N, Kleckner N (2001) The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. *Cell* 106: 59-70

Hyde H, Davies AA, Benson FE, West SC (1994) Resolution of recombination intermediates by a mammalian activity functionally analogous to *Escherichia coli* RuvC resolvase. *The Journal of biological chemistry* 269: 5202-5209

Hyun M, Park S, Kim E, Kim DH, Lee SJ, Koo HS, Seo YS, Ahn B (2012) Physical and functional interactions of *Caenorhabditis elegans* WRN-1 helicase with RPA-1. *Biochemistry* 51: 1336-1345

Iliakis G (2009) Backup pathways of NHEJ in cells of higher eukaryotes: cell cycle dependence. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* 92: 310-315

Iliakis G, Wang H, Perrault AR, Boecker W, Rosidi B, Windhofer F, Wu W, Guan J, Terzoudi G, Pantelias G (2004) Mechanisms of DNA double strand break repair and chromosome aberration formation. *Cytogenetic and genome research* 104: 14-20

- Iliakis G, Wu W, Wang M, Terzoudi GI, Pantelias GE** (2007) Backup Pathways of Nonhomologous End Joining May Have a Dominant Role in the Formation of Chromosome Aberrations. pp 67-85. Berlin, Heidelberg: Springer Berlin Heidelberg
- Ip SCY, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC** (2008) Identification of Holliday junction resolvases from humans and yeast. *Nature* 456: 357-361
- Ira G, Malkova A, Liberi G, Foiani M, Haber JE** (2003) Srs2 and Sgs1–Top3 suppress crossovers during double-strand break repair in yeast *Cell* 115: 401–411
- Ira G, Satory D, Haber JE** (2006) Conservative inheritance of newly synthesized DNA in double-strand break-induced gene conversion. *Molecular and cellular biology* 26: 9424-9429
- Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y** (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci U S A* 98: 4569-4574
- Ivanov EL, Haber JE** (1995) RAD1 and RAD10, but not other excision repair genes, are required for double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 15: 2245-2251
- Ivanov EL, Sugawara N, Fishman-Lobell J, Haber JE** (1996) Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. *Genetics* 142: 693-704
- Iyer LM, Koonin EV, Aravind L** (2002) Classification and evolutionary history of the single-strand annealing proteins, RecT, Redbeta, ERF and RAD52. *BMC genomics* 3: 8
- Jablonovich Z, Liefshitz B, Steinlauf R, Kupiec M** (1999) Characterization of the role played by the RAD59 gene of *Saccharomyces cerevisiae* in ectopic recombination. *Curr Genet* 36: 13-20
- Jackson D, Dhar K, Wahl JK, Wold MS, Borgstahl GE** (2002) Analysis of the human replication protein A:Rad52 complex: evidence for crosstalk between RPA32, RPA70, Rad52 and DNA. *J Mol Biol* 321: 133-148
- Jackson SP, Bartek J** (2009) The DNA-damage response in human biology and disease. *Nature* 461: 1071-1078
- Jaskelioff M, Van Komen S, Krebs JE, Sung P, Peterson CL** (2003) Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. *The Journal of biological chemistry* 278: 9212-9218
- Jensen RB, Carreira A, Kowalczykowski SC** (2010) Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature* 467: 678-683
- Jiang H, Xie Y, Houston P, Stemke-Hale K, Mortensen UH, Rothstein R, Kodadek T** (1996) Direct association between the yeast Rad51 and Rad54 recombination proteins. *The Journal of biological chemistry* 271: 33181-33186

- Jin Y, Xu XL, Yang MC, Wei F, Ayi TC, Bowcock AM, Baer R** (1997) Cell cycle-dependent colocalization of BARD1 and BRCA1 proteins in discrete nuclear domains. *Proc Natl Acad Sci U S A* 94: 12075-12080
- Johnson RD, Liu N, Jasin M** (1999) Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. *Nature* 401: 397-399
- Joyce EF, Paul A, Chen KE, Tanneti N, McKim KS** (2012) Multiple barriers to nonhomologous DNA end joining during meiosis in *Drosophila*. *Genetics* 191: 739-746
- Kabotyanski EB, Gomelsky L, Han JO, Stamato TD, Roth DB** (1998) Double-strand break repair in Ku86- and XRCC4-deficient cells. *Nucleic acids research* 26: 5333-5342
- Kaliappan K, Choudhury NR, Suyal G, Mukherjee SK** (2012) A novel role for RAD54: this host protein modulates geminiviral DNA replication. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 26: 1142-1160
- Kamileri I, Karakasilioti I, Garinis GA** (2012) Nucleotide excision repair: new tricks with old bricks. *Trends in genetics : TIG* 28: 566-573
- Kanaar R, Troelstra C, Swagemakers SM, Essers J, Smit B, Franssen JH, Pastink A, Bezzubova OY, Buerstedde JM, Clever B *et al.*** (1996) Human and mouse homologs of the *Saccharomyces cerevisiae* RAD54 DNA repair gene: evidence for functional conservation. *Current biology : CB* 6: 828-838
- Kans JA, Mortimer RK** (1991) Nucleotide sequence of the RAD57 gene of *Saccharomyces cerevisiae*. *Gene* 105: 139-140
- Kateneva AV, Konovchenko AA, Guacci V, Dresser ME** (2005) Recombination protein Tid1p controls resolution of cohesin-dependent linkages in meiosis in *Saccharomyces cerevisiae*. *The Journal of cell biology* 171: 241-253
- Keeney S** (2008) Spo11 and the Formation of DNA Double-Strand Breaks in Meiosis. *Genome dynamics and stability* 2: 81-123
- Keeney S, Giroux CN, Kleckner N** (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* 88: 375-384
- Kegel A, Sjöstrand JO, Åström SU** (2001) Nej1p, a cell type-specific regulator of nonhomologous end joining in yeast. *Current biology : CB* 11: 1611-1617
- Khasanov FK, Salakhova AF, Chepurnaja OV, Korolev VG, Bashkirov VI** (2004) Identification and characterization of the rlp1+, the novel Rad51 paralog in the fission yeast *Schizosaccharomyces pombe*. *DNA repair* 3: 1363-1374
- Khasanov FK, Savchenko GV, Bashkirova EV, Korolev VG, Heyer W-D, Bashkirov VI** (1999) A New Recombinational DNA Repair Gene From *Schizosaccharomyces pombe* With Homology to *Escherichia coli* RecA. *Genetics* 152: 1557-1572

- Kiianitsa K, Solinger JA, Heyer WD** (2002) Rad54 protein exerts diverse modes of ATPase activity on duplex DNA partially and fully covered with Rad51 protein. *J Biol Chem* 277: 46205-46215
- Kim WJ, Lee S, Park MS, Jang YK, Kim JB, Park SD** (2000) Rad22 protein, a rad52 homologue in *Schizosaccharomyces pombe*, binds to DNA double-strand breaks. *J Biol Chem* 275: 35607-35611
- Klein HL** (1997) RDH54, a RAD54 homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. *Genetics* 147: 1533-1543
- Klovstad M, Abdu U, Schüpbach T** (2008) *Drosophila* brca2 is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. *PLoS genetics* 4: e31
- Klutstein M, Shaked H, Sherman A, Avivi-Ragolsky N, Shema E, Zenvirth D, Levy AA, Simchen G** (2008) Functional conservation of the yeast and *Arabidopsis* RAD54-like genes. *Genetics* 178: 2389-2397
- Ko E, Lee J, Lee H** (2008) Essential role of brc-2 in chromosome integrity of germ cells in *C. elegans*. *Mol Cells* 26: 590-594
- Ko HL, Ren EC** (2012) Functional Aspects of PARP1 in DNA Repair and Transcription. *Biomolecules* 2: 524-548
- Kolpashchikov DM, Khodyreva SN, Lavrik OI** (2000) Replicative protein A exhibits a specific polarity in binding single-stranded DNA. *Doklady biochemistry : proceedings of the Academy of Sciences of the USSR, Biochemistry section* 372: 106-108
- Kooistra R, Vreeken K, Zonneveld JB, de Jong A, Eeken JC, Osgood CJ, Buerstedde JM, Lohman PH, Pastink A** (1997) The *Drosophila melanogaster* RAD54 homolog, DmRAD54, is involved in the repair of radiation damage and recombination. *Molecular and cellular biology* 17: 6097-6104
- Kostriken R, Strathern JN, Klar AJ, Hicks JB, Heffron F** (1983) A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. *Cell* 35: 167-174
- Kowalczykowski SC** (1991) Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. *Annual review of biophysics and biophysical chemistry* 20: 539-575
- Kramer KM, Brock JA, Bloom K, Moore JK, Haber JE** (1994) Two different types of double-strand breaks in *Saccharomyces cerevisiae* are repaired by similar RAD52-independent, nonhomologous recombination events. *Molecular and cellular biology* 14: 1293-1301
- Krejci L, Song B, Bussen W, Rothstein R, Mortensen UH, Sung P** (2002) Interaction with Rad51 is indispensable for recombination mediator function of Rad52. *J Biol Chem* 277: 40132-40141

- Krogh BO, Symington LS** (2004) Recombination proteins in yeast. *Annual review of genetics* 38: 233-271
- Kurihara D, Matsunaga S, Uchiyama S, Fukui K** (2008) Live cell imaging reveals plant aurora kinase has dual roles during mitosis. *Plant & cell physiology* 49: 1256-1261
- Kurumizaka H, Ikawa S, Nakada M, Eda K, Kagawa W, Takata M, Takeda S, Yokoyama S, Shibata T** (2001) Homologous-pairing activity of the human DNA-repair proteins Xrcc3.Rad51C. *Proceedings of the National Academy of Sciences of the United States of America* 98: 5538-5543
- Kurumizaka H, Ikawa S, Nakada M, Enomoto R, Kagawa W, Kinebuchi T, Yamazoe M, Yokoyama S, Shibata T** (2002) Homologous pairing and ring and filament structure formation activities of the human Xrcc2*Rad51D complex. *The Journal of biological chemistry* 277: 14315-14320
- Kuznetsov S, Pellegrini M, Shuda K, Fernandez-Capetillo O, Liu Y, Martin BK, Burkett S, Southon E, Pati D, Tessarollo L et al.** (2007) RAD51C deficiency in mice results in early prophase I arrest in males and sister chromatid separation at metaphase II in females. *J Cell Biol* 176: 581-592
- Kuznetsov SG, Haines DC, Martin BK, Sharan SK** (2009) Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. *Cancer research* 69: 863-872
- Lafarge S, Montané M-H** (2003) Characterization of Arabidopsis thaliana ortholog of the human breast cancer susceptibility gene 1: AtBRCA1, strongly induced by gamma rays. *Nucleic acids research* 31: 1148-1155
- Lamarche BJ, Orazio NI, Weitzman MD** (2010) The MRN complex in double-strand break repair and telomere maintenance. *FEBS letters* 584: 3682-3695
- Langston LD, Symington LS** (2005) Opposing roles for DNA structure-specific proteins Rad1, Msh2, Msh3, and Sgs1 in yeast gene targeting. *The EMBO journal* 24: 2214-2223
- Latypov VF, Kozhina TN, Kozhin SA, Korolev VG** (2010) [The Rdh54 protein role in regulation of DNA repair in yeast *Saccharomyces cerevisiae*]. *Genetika* 46: 194-202
- Lee SE, Moore JK, Holmes A, Umez K, Kolodner RD, Haber JE** (1998) *Saccharomyces* Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* 94: 399-409
- Lee SE, Pellicioli A, Malkova A, Foiani M, Haber JE** (2001) The *Saccharomyces* recombination protein Tid1p is required for adaptation from G2/M arrest induced by a double-strand break. *Current biology : CB* 11: 1053-1057
- Lengsfeld BM, Rattray AJ, Bhaskara V, Ghirlando R, Paull TT** (2007) Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. *Molecular cell* 28: 638-651

Li F, Dong J, Eichmiller R, Holland C, Minca E, Prakash R, Sung P, Yong Shim E, Surtees JA, Eun Lee S (2013) Role of Saw1 in Rad1/Rad10 complex assembly at recombination intermediates in budding yeast. *EMBO J* 32: 461-472

Li F, Dong J, Pan X, Oum J-H, Boeke JD, Lee SE (2008) Microarray-based genetic screen defines SAW1, a gene required for Rad1/Rad10-dependent processing of recombination intermediates. *Molecular cell* 30: 325-335

Li W, Chen C, Markmann-Mulisch U, Timofejeva L, Schmelzer E, Ma H, Reiss B (2004) The Arabidopsis AtRAD51 gene is dispensable for vegetative development but required for meiosis. *Proceedings of the National Academy of Sciences of the United States of America* 101: 10596-10601

Li W, Yang X, Lin Z, Timofejeva L, Xiao R, Makaroff CA, Ma H (2005) The AtRAD51C gene is required for normal meiotic chromosome synapsis and double-stranded break repair in Arabidopsis. *Plant physiology* 138: 965-976

Li X, Zhang XP, Solinger JA, Kiianitsa K, Yu X, Egelman EH, Heyer WD (2007) Rad51 and Rad54 ATPase activities are both required to modulate Rad51-dsDNA filament dynamics. *Nucleic Acids Res* 35: 4124-4140

Li Z, Golub EI, Gupta R, Radding CM (1997) Recombination activities of HsDmc1 protein, the meiotic human homolog of RecA protein. *Proceedings of the National Academy of Sciences of the United States of America* 94: 11221-11226

Liang F, Jasin M (1996) Ku80-deficient cells exhibit excess degradation of extrachromosomal DNA. *The Journal of biological chemistry* 271: 14405-14411

Liang F, Romanienko PJ, Weaver DT, Jeggo PA, Jasin M (1996) Chromosomal double-strand break repair in Ku80-deficient cells. *Proceedings of the National Academy of Sciences of the United States of America* 93: 8929-8933

Lieber MR (2010) NHEJ and its backup pathways in chromosomal translocations. *Nature structural & molecular biology* 17: 393-395

Lin FL, Sperle K, Sternberg N (1984) Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. *Molecular and cellular biology* 4: 1020-1034

Lin Z, Kong H, Nei M, Ma H (2006) Origins and evolution of the recA/RAD51 gene family: Evidence for ancient gene duplication and endosymbiotic gene transfer. *PNAS* 103: 10328-10333

Lindahl T, Barnes DE (2000) Repair of endogenous DNA damage. *Cold Spring Harbor symposia on quantitative biology* 65: 127-133

Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* 118: 699-713

Lisby M, Rothstein R (2009) Choreography of recombination proteins during the DNA damage response. *DNA repair* 8: 1068-1076

Lisby M, Rothstein R, Mortensen UH (2001) Rad52 forms DNA repair and recombination centers during S phase. *Proc Natl Acad Sci U S A* 98: 8276-8282

Liu J, Doty T, Gibson B, Heyer WD (2010) Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. *Nature structural & molecular biology* 17: 1260-1262

Liu J, Gong L, Chang C, Liu C, Peng J, Chen J (2012) Development of novel visual-plus quantitative analysis systems for studying DNA double-strand break repairs in zebrafish. *Journal of genetics and genomics = Yi chuan xue bao* 39: 489-502

Liu J, Heyer W-D (2011) Who's who in human recombination: BRCA2 and RAD52. *Proceedings of the National Academy of Sciences of the United States of America* 108: 441-442

Liu J, Renault L, Veaute X, Fabre F, Stahlberg H, Heyer WD (2011a) Rad51 paralogues Rad55-Rad57 balance the antirecombinase Srs2 in Rad51 filament formation. *Nature* 479: 245-248

Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR, Brookman KW, Siciliano MJ, Walter CA, Fan W *et al.* (1998) XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol Cell* 1: 783-793

Liu N, Schild D, Thelen MP, Thompson LH (2002) Involvement of Rad51C in two distinct protein complexes of Rad51 paralogs in human cells. *Nucleic acids research* 30: 1009-1015

Liu T, Wan L, Wu Y, Chen J, Huang J (2011b) hSWS1-SWSAP1 is an evolutionarily conserved complex required for efficient homologous recombination repair. *The Journal of biological chemistry* 286: 41758-41766

Liu Y, Li M, Lee EY, Maizels N (1999) Localization and dynamic relocation of mammalian Rad52 during the cell cycle and in response to DNA damage. *Current biology : CB* 9: 975-978

Liu Y, Maizels N (2000) Coordinated response of mammalian Rad51 and Rad52 to DNA damage. *EMBO reports* 1: 85-90

Liu Y, Masson JY, Shah R, O'Regan P, West SC (2004) RAD51C is required for Holliday junction processing in mammalian cells. *Science* 303: 243-246

Liu Y, Tarsounas M, O'Regan P, West SC (2007) Role of RAD51C and XRCC3 in genetic recombination and DNA repair. *The Journal of biological chemistry* 282: 1973-1979

Llorente B, Smith CE, Symington LS (2008) Break-induced replication: what is it and what is it for? *Cell cycle (Georgetown, Tex)* 7: 859-864

Lo T, Pellegrini L, Venkitaraman AR, Blundell TL (2003) Sequence fingerprints in BRCA2 and RAD51: implications for DNA repair and cancer. *DNA repair* 2: 1015-1028

Lok BH, Powell SN (2012) Molecular pathways: understanding the role of Rad52 in homologous recombination for therapeutic advancement. *Clinical Cancer Research* 18: 6400-6406

Lovett ST (1994) Sequence of the RAD55 gene of *Saccharomyces cerevisiae*: similarity of RAD55 to prokaryotic RecA and other RecA-like proteins. *Gene* 142: 103-106

Lu H, Pannicke U, Schwarz K, Lieber MR (2007) Length-dependent binding of human XLF to DNA and stimulation of XRCC4.DNA ligase IV activity. *The Journal of biological chemistry* 282: 11155-11162

Lydeard JR, Jain S, Yamaguchi M, Haber JE (2007) Break-induced replication and telomerase-independent telomere maintenance require Pol32. *Nature* 448: 820-823

Lyndaker AM, Alani E (2009) A tale of tails: insights into the coordination of 3' end processing during homologous recombination. *BioEssays : news and reviews in molecular, cellular and developmental biology* 31: 315-321

Lyndaker AM, Goldfarb T, Alani E (2008) Mutants defective in Rad1-Rad10-Slx4 exhibit a unique pattern of viability during mating-type switching in *Saccharomyces cerevisiae*. *Genetics* 179: 1807-1821

Ma J-L, Kim EM, Haber JE, Lee SE (2003) Yeast Mre11 and Rad1 proteins define a Ku-independent mechanism to repair double-strand breaks lacking overlapping end sequences. *Molecular and cellular biology* 23: 8820-8828

Ma Y, Pannicke U, Schwarz K, Lieber MR (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 108: 781-794

Malik S-B, Pightling AW, Stefaniak LM, Schurko AM, Logsdon JM (2008) An expanded inventory of conserved meiotic genes provides evidence for sex in *Trichomonas vaginalis*. *PloS one* 3: e2879

Malkova A, Ira G (2013) Break-induced replication: functions and molecular mechanism. *Current opinion in genetics & development* 23: 271-279

Malkova A, Ivanov EL, Haber JE (1996) Double-strand break repair in the absence of RAD51 in yeast: a possible role for break-induced DNA replication. *Proceedings of the National Academy of Sciences of the United States of America* 93: 7131-7136

Mankouri HW, Ngo H-P, Hickson ID (2007) Shu proteins promote the formation of homologous recombination intermediates that are processed by Sgs1-Rmi1-Top3. *Molecular biology of the cell* 18: 4062-4073

Mansour WY, Schumacher S, Roskopf R, Rhein T, Schmidt-Petersen F, Gatzemeier F, Haag F, Borgmann K, Willers H, Dahm-Daphi J (2008) Hierarchy of nonhomologous end-joining, single-strand annealing and gene conversion at site-directed DNA double-strand breaks. *Nucleic acids research* 36: 4088-4098

Manthey GM, Bailis AM (2010) Rad51 inhibits translocation formation by non-conservative homologous recombination in *Saccharomyces cerevisiae*. *PLoS One* 5: e11889

Manthey GM, Naik N, Bailis AM (2009) Msh2 blocks an alternative mechanism for non-homologous tail removal during single-strand annealing in *Saccharomyces cerevisiae*. *PloS one* 4: e7488

Mari P-O, Florea BI, Persengiev SP, Verkaik NS, Brüggewirth HT, Modesti M, Giglia-Mari G, Bezstarosti K, Demmers JAA, Luider TM *et al.* (2006) Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. *Proceedings of the National Academy of Sciences of the United States of America* 103: 18597-18602

Martin JS, Winkelmann N, Petalcorin MI, McIlwraith MJ, Boulton SJ (2005) RAD-51-dependent and -independent roles of a *Caenorhabditis elegans* BRCA2-related protein during DNA double-strand break repair. *Mol Cell Biol* 25: 3127-3139

Martín V, Chahwan C, Gao H, Blais V, Wohlschlegel J, Yates JR, McGowan CH, Russell P (2006) Sws1 is a conserved regulator of homologous recombination in eukaryotic cells. *The EMBO journal* 25: 2564-2574

Marton RF, Thömmes P, Cotterill S (1994) Purification and characterisation of dRP-A: a single-stranded DNA binding protein from *Drosophila melanogaster*. *FEBS letters* 342: 139-144

Maryon E, Carroll D (1991) Characterization of recombination intermediates from DNA injected into *Xenopus laevis* oocytes: evidence for a nonconservative mechanism of homologous recombination. *Molecular and cellular biology* 11: 3278-3287

Masson JY, Davies AA, Hajibagheri N, Van Dyck E, Benson FE, Stasiak AZ, Stasiak A, West SC (1999) The meiosis-specific recombinase hDmc1 forms ring structures and interacts with hRad51. *The EMBO journal* 18: 6552-6560

Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC (2001) Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes & development* 15: 3296-3307

Matos J, West SC (2014) Holliday junction resolution: Regulation in space and time. *DNA repair* In press

Matulova P, Marini V, Burgess RC, Sisakova A, Kwon Y, Rothstein R, Sung P, Krejci L (2009) Cooperativity of Mus81.Mms4 with Rad54 in the resolution of recombination and replication intermediates. *The Journal of biological chemistry* 284: 7733-7745

- Mazin AV, Alexeev AA, Kowalczykowski SC** (2003) A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. *The Journal of biological chemistry* 278: 14029-14036
- Mazin AV, Bornarth CJ, Solinger JA, Heyer WD, Kowalczykowski SC** (2000) Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. *Mol Cell* 6: 583-592
- Mazin AV, Mazina OM, Bugreev DV, Rossi MJ** (2010) Rad54, the motor of homologous recombination. *DNA repair* 9: 286-302
- Mazina OM, Mazin AV** (2004) Human Rad54 protein stimulates DNA strand exchange activity of hRad51 protein in the presence of Ca²⁺. *The Journal of biological chemistry* 279: 52042-52051
- Mazina OM, Mazin AV** (2008) Human Rad54 protein stimulates human Mus81-Eme1 endonuclease. *Proceedings of the National Academy of Sciences of the United States of America* 105: 18249-18254
- Mazina OM, Rossi MJ, Thoma NH, Mazin AV** (2007) Interactions of human rad54 protein with branched DNA molecules. *J Biol Chem* 282: 21068-21080
- McEachern MJ, Haber JE** (2006) Break-induced replication and recombinational telomere elongation in yeast. *Annu Rev Biochem* 75: 111-135
- McGill C, Shafer B, Strathern J** (1989) Coconversion of flanking sequences with homothallic switching. *Cell* 57: 459-467
- Meetei AR, Yan Z, Wang W** (2004) FANCL replaces BRCA1 as the likely ubiquitin ligase responsible for FANCD2 monoubiquitination. *Cell cycle (Georgetown, Tex)* 3: 179-181
- Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ et al.** (2011) A TALE nuclease architecture for efficient genome editing. *Nature biotechnology* 29: 143-148
- Miller KA, Sawicka D, Barsky D, Albala JS** (2004) Domain mapping of the Rad51 paralog protein complexes. *Nucleic acids research* 32: 169-178
- Miller KA, Yoshikawa DM, McConnell IR, Clark R, Schild D, Albala JS** (2002) RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51. *The Journal of biological chemistry* 277: 8406-8411
- Milne GT, Weaver DT** (1993) Dominant negative alleles of RAD52 reveal a DNA repair/recombination complex including Rad51 and Rad52. *Genes & development* 7: 1755-1765
- Mimitou EP, Symington LS** (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455: 770-774
- Mimori T, Hardin JA** (1986) Mechanism of interaction between Ku protein and DNA. *The Journal of biological chemistry* 261: 10375-10379

- Miné J, Disseau L, Takahashi M, Cappello G, Dutreix M, Viovy J-L** (2007) Real-time measurements of the nucleation, growth and dissociation of single Rad51-DNA nucleoprotein filaments. *Nucleic acids research* 35: 7171-7187
- Mitchel K, Lehner K, Jinks-Robertson S** (2013) Heteroduplex DNA position defines the roles of the Sgs1, Srs2, and Mph1 helicases in promoting distinct recombination outcomes. *PLoS Genet* 9: e1003340
- Miura T, Yamana Y, Usui T, Ogawa HI, Yamamoto MT, Kusano K** (2012) Homologous recombination via Synthesis-Dependent Strand Annealing in yeast requires the Irc20 and Srs2 DNA helicases *Genetics* 191: 65–78
- Miyazaki T, Bressan DA, Shinohara M, Haber JE, Shinohara A** (2004) In vivo assembly and disassembly of Rad51 and Rad52 complexes during double-strand break repair. *The EMBO journal* 23: 939-949
- Mladenov E, Iliakis G** (2011) Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. *Mutation research* 711: 61-72
- Moldovan G-L, Dejsuphong D, Petalcorin MIR, Hofmann K, Takeda S, Boulton SJ, D'Andrea AD** (2012) Inhibition of Homologous Recombination by the PCNA-Interacting Protein PARI. *Molecular cell* 45: 75-86
- Moore JK, Haber JE** (1996) Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 16: 2164-2173
- Morrow DM, Connelly C, Hieter P** (1997) "Break copy" duplication: a model for chromosome fragment formation in *Saccharomyces cerevisiae*. *Genetics* 147: 371-382
- Mortensen UH, Bendixen C, Sunjevaric I, Rothstein R** (1996) DNA strand annealing is promoted by the yeast Rad52 protein. *Proceedings of the National Academy of Sciences of the United States of America* 93: 10729-10734
- Mortensen UH, Lisby M, Rothstein R** (2009) Rad52. *Current biology : CB* 19: R676-677
- Motycka TA, Bessho T, Post SM, Sung P, Tomkinson AE** (2004) Physical and functional interaction between the XPF/ERCC1 endonuclease and hRad52. *The Journal of biological chemistry* 279: 13634-13639
- Moynahan ME, Chiu JW, Koller BH, Jasin M** (1999) Brca1 controls homology-directed DNA repair. *Molecular cell* 4: 511-518
- Moynahan ME, Cui TY, Jasin M** (2001) Homology-directed dna repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. *Cancer research* 61: 4842-4850
- Mozlin AM, Fung CW, Symington LS** (2008) Role of the *Saccharomyces cerevisiae* Rad51 paralogs in sister chromatid recombination. *Genetics* 178: 113-126

Mullen JR, Kaliraman V, Ibrahim SS, Brill SJ (2001) Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics* 157: 103-118

Mullen JR, Nallasetth FS, Lan YQ, Slagle CE, Brill SJ (2005) Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1-Top3 complex. *Molecular and cellular biology* 25: 4476-4487

Muñoz IM, Hain K, Déclais A-C, Gardiner M, Toh GW, Sanchez-Pulido L, Heuckmann JM, Toth R, Macartney T, Eppink B *et al.* (2009) Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. *Molecular cell* 35: 116-127

Murayama Y, Kurokawa Y, Mayanagi K, Iwasaki H (2008) Formation and branch migration of Holliday junctions mediated by eukaryotic recombinases. *Nature* 451: 1018-1021

Murayama Y, Tsutsui Y, Iwasaki H (2011) The fission yeast meiosis-specific Dmc1 recombinase mediates formation and branch migration of Holliday junctions by preferentially promoting strand exchange in a direction opposite to that of Rad51. *Genes & development* 25: 516-527

Muris DF, Bezzubova O, Buerstedde JM, Vreeken K, Balajee AS, Osgood CJ, Troelstra C, Hoeijmakers JH, Ostermann K, Schmidt H (1994) Cloning of human and mouse genes homologous to RAD52, a yeast gene involved in DNA repair and recombination. *Mutation research* 315: 295-305

Muris DF, Vreeken K, Carr AM, Murray JM, Smit C, Lohman PH, Pastink A (1996) Isolation of the *Schizosaccharomyces pombe* RAD54 homologue, *rhp54+*, a gene involved in the repair of radiation damage and replication fidelity. *Journal of cell science* 109 (Pt 1): 73-81

Muris DF, Vreeken K, Schmidt H, Ostermann K, Clever B, Lohman PH, Pastink A (1997) Homologous recombination in the fission yeast *Schizosaccharomyces pombe*: different requirements for the *rhp51+*, *rhp54+* and *rad22+* genes. *Current genetics* 31: 248-254

Nagaraju G, Hartlerode A, Kwok A, Chandramouly G, Scully R (2009) XRCC2 and XRCC3 regulate the balance between short- and long-tract gene conversions between sister chromatids. *Molecular and cellular biology* 29: 4283-4294

Nasmyth K (1993) Regulating the HO endonuclease in yeast. *Current opinion in genetics & development* 3: 286-294

Nasmyth KA (1982) Molecular genetics of yeast mating type. *Annual review of genetics* 16: 439-500

Nassif N, Penney J, Pal S, Engels WR, Gloor GB (1994) Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Molecular and cellular biology* 14: 1613-1625

Neale MJ, Keeney S (2006) Clarifying the mechanics of DNA strand exchange in meiotic recombination. *Nature* 442: 153-158

New JH, Sugiyama T, Zaitseva E, Kowalczykowski SC (1998) Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature* 391: 407-410

Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C, Modrich P, Kowalczykowski SC (2011) BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes & development* 25: 350-362

Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC (2008) Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proceedings of the National Academy of Sciences of the United States of America* 105: 16906-16911

Nimonkar AV, Sica RA, Kowalczykowski SC (2009) Rad52 promotes second-end DNA capture in double-stranded break repair to form complement-stabilized joint molecules. *Proceedings of the National Academy of Sciences of the United States of America* 106: 3077-3082

Nishana M, Raghavan SC (2012) Role of recombination activating genes in the generation of antigen receptor diversity and beyond. *Immunology* 137: 271-281

Niu H, Chung W-H, Zhu Z, Kwon Y, Zhao W, Chi P, Prakash R, Seong C, Liu D, Lu L et al. (2010) Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature* 467: 108-111

Oettinger MA, Schatz DG, Gorka C, Baltimore D (1990) RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science (New York, NY)* 248: 1517-1523

Ogawa T, Yu X, Shinohara A, Egelman EH (1993) Similarity of the yeast RAD51 filament to the bacterial RecA filament. *Science (New York, NY)* 259: 1896-1899

Orel N, Kyryk A, Puchta H (2003) Different pathways of homologous recombination are used for the repair of double-strand breaks within tandemly arranged sequences in the plant genome. *Plant J* 35: 604-612

Orr-Weaver TL, Szostak JW (1983) Yeast recombination: the association between double-strand gap repair and crossing-over. *Proceedings of the National Academy of Sciences of the United States of America* 80: 4417-4421

Osakabe K, Abe K, Yamanouchi H, Takyuu T, Yoshioka T, Ito Y, Kato T, Tabata S, Kurei S, Yoshioka Y et al. (2005) Arabidopsis Rad51B is important for double-strand DNA breaks repair in somatic cells. *Plant molecular biology* 57: 819-833

Octobre G, Lorenz A, Loidl J, Kohli J (2008) The Rad52 homologs Rad22 and Rtl1 of *Schizosaccharomyces pombe* are not essential for meiotic interhomolog recombination, but are required for meiotic intrachromosomal recombination and mating-type-related DNA repair. *Genetics* 178: 2399-412

Osakabe K, Abe K, Yoshioka T, Osakabe Y, Todoriki S, Ichikawa H, Hohn B, Toki S (2006) Isolation and characterization of the RAD54 gene from *Arabidopsis thaliana*. *The Plant journal : for cell and molecular biology* 48: 827-842

Osakabe K, Yoshioka T, Ichikawa H, Toki S (2002) Molecular cloning and characterization of RAD51-like genes from *Arabidopsis thaliana*. *Plant molecular biology* 50: 71-81

Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FC (2011) Pathways to meiotic recombination in *Arabidopsis thaliana*. *New Phytol* 190: 523-544

Osman K, Sanchez-Moran E, Mann SC, Jones GH, Franklin FCH (2009) Replication protein A (AtRPA1a) is required for class I crossover formation but is dispensable for meiotic DNA break repair. *The EMBO journal* 28: 394-404

Ostermann K, Lorentz A, Schmidt H (1993) The fission yeast rad22 gene, having a function in mating-type switching and repair of DNA damages, encodes a protein homolog to Rad52 of *Saccharomyces cerevisiae*. *Nucleic Acids Res* 21: 5940-5944

Page SL, Hawley RS (2003) Chromosome choreography: the meiotic ballet. *Science (New York, NY)* 301: 785-789

Paillard S, Strauss F (1991) Analysis of the mechanism of interaction of simian Ku protein with DNA. *Nucleic acids research* 19: 5619-5624

Pan J, Sasaki M, Kniewel R, Murakami H, Blitzblau HG, Tischfield SE, Zhu X, Neale MJ, Jasin M, Socci ND et al. (2011) A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. *Cell* 144: 719-731

Pankotai T, Bonhomme C, Chen D, Soutoglou E (2012) DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks. *Nature structural & molecular biology* 19: 276-282

Pannunzio NR, Manthey GM, Liddell LC, Fu BXH, Roberts CM, Bailis AM (2012) Rad59 regulates association of Rad52 with DNA double-strand breaks. *MicrobiologyOpen* 1: 285-297

Pâques F, Haber JE (1997) Two pathways for removal of nonhomologous DNA ends during double-strand break repair in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 17: 6765-6771

Pâques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiology and molecular biology reviews : MMBR* 63: 349-404

Pâques F, Leung WY, Haber JE (1998) Expansions and contractions in a tandem repeat induced by double-strand break repair. *Molecular and cellular biology* 18: 2045-2054

- Passy SI, Yu X, Li Z, Radding CM, Masson JY, West SC, Egelman EH** (1999) Human Dmc1 protein binds DNA as an octameric ring. *Proceedings of the National Academy of Sciences of the United States of America* 96: 10684-10688
- Paul TT, Gellert M** (1998) The 3' to 5' exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. *Molecular cell* 1: 969-979
- Pawelczak KS, Turchi JJ** (2010) Purification and characterization of exonuclease-free Artemis: Implications for DNA-PK-dependent processing of DNA termini in NHEJ-catalyzed DSB repair. *DNA Repair (Amst)* 9: 670-677
- Perrotti D, Jamieson C, Goldman J, Skorski T** (2010) Chronic myeloid leukemia: mechanisms of blastic transformation. *The Journal of clinical investigation* 120: 2254-2264
- Petalcorin MI, Galkin VE, Yu X, Egelman EH, Boulton SJ** (2007) Stabilization of RAD-51-DNA filaments via an interaction domain in *Caenorhabditis elegans* BRCA2. *Proc Natl Acad Sci U S A* 104: 8299-8304
- Petalcorin MIR, Sandall J, Wigley DB, Boulton SJ** (2006) CeBRC-2 stimulates D-loop formation by RAD-51 and promotes DNA single-strand annealing. *Journal of molecular biology* 361: 231-242
- Petermann E, Helleday T** (2010) Pathways of mammalian replication fork restart. *Nat Rev Mol Cell Biol* 11: 683-687
- Petukhova G, Stratton S, Sung P** (1998) Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature* 393: 91-94
- Petukhova G, Stratton SA, Sung P** (1999a) Single strand DNA binding and annealing activities in the yeast recombination factor Rad59. *J Biol Chem* 274: 33839-33842
- Petukhova G, Sung P, Klein H** (2000) Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1. *Genes & development* 14: 2206-2215
- Petukhova G, Van Komen S, Vergano S, Klein H, Sung P** (1999b) Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. *J Biol Chem* 274: 29453-29462
- Pierce AJ, Johnson RD, Thompson LH, Jasin M** (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes & development* 13: 2633-2638
- Pittman DL, Schimenti JC** (2000) Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis* 26: 167-173
- Pittman DL, Weinberg LR, Schimenti JC** (1998) Identification, characterization, and genetic mapping of Rad51d, a new mouse and human RAD51/RecA-related gene. *Genomics* 49: 103-111

- Povirk LF, Zhou T, Zhou R, Cowan MJ, Yannone SM** (2007) Processing of 3'-phosphoglycolate-terminated DNA double strand breaks by Artemis nuclease. *The Journal of biological chemistry* 282: 3547-3558
- Powell SN, Kachnic LA** (2003) Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. *Oncogene* 22: 5784-5791
- Puchta H** (2005) The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *Journal of experimental botany* 56: 1-14
- Qing Y, Yamazoe M, Hirota K, Dejsuphong D, Sakai W, Yamamoto KN, Bishop DK, Wu X, Takeda S** (2011) The epistatic relationship between BRCA2 and the other RAD51 mediators in homologous recombination. *PLoS genetics* 7: e1002148
- Rabitsch KP, Tóth A, Gálová M, Schleiffer A, Schaffner G, Aigner E, Rupp C, Penkner AM, Moreno-Borchart AC, Primig M *et al.*** (2001) A screen for genes required for meiosis and spore formation based on whole-genome expression. *Current biology : CB* 11: 1001-1009
- Radford SJ, Sekelsky JJ** (2004) Taking Drosophila Rad51 for a SPiN. *Nature structural & molecular biology* 11: 9-10
- Radhakrishnan SK, Jette N, Lees-Miller SP** (2014) Non-homologous end joining: emerging themes and unanswered questions. *DNA Repair (Amst)* 17: 2-8
- Rahal EA, Henricksen LA, Li Y, Williams RS, Tainer JA, Dixon K** (2010) ATM regulates Mre11-dependent DNA end-degradation and microhomology-mediated end joining. *Cell cycle (Georgetown, Tex)* 9: 2866-2877
- Ramesh MA, Malik S-B, Logsdon JM** (2005) A phylogenomic inventory of meiotic genes; evidence for sex in Giardia and an early eukaryotic origin of meiosis. *Current biology : CB* 15: 185-191
- Ranatunga W, Jackson D, Lloyd JA, Forget AL, Knight KL, Borgstahl GE** (2001) Human RAD52 exhibits two modes of self-association. *J Biol Chem* 276: 15876-15880
- Raschle M, Van Komen S, Chi P, Ellenberger T, Sung P** (2004) Multiple interactions with the Rad51 recombinase govern the homologous recombination function of Rad54. *The Journal of biological chemistry* 279: 51973-51980
- Rass E, Grabarz A, Plo I, Gautier J, Bertrand P, Lopez BS** (2009) Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. *Nature structural & molecular biology* 16: 819-824
- Rass U, Compton SA, Matos J, Singleton MR, Ip SCY, Blanco MG, Griffith JD, West SC** (2010) Mechanism of Holliday junction resolution by the human GEN1 protein. *Genes & development* 24: 1559-1569
- Rathmell WK, Chu G** (1994) A DNA end-binding factor involved in double-strand break repair and V(D)J recombination. *Molecular and cellular biology* 14: 4741-4748

Reddy G, Golub EI, Radding CM (1997) Human Rad52 protein promotes single-strand DNA annealing followed by branch migration. *Mutation research* 377: 53-59

Reidt W, Wurz R, Wanieck K, Chu HH, Puchta H (2006) A homologue of the breast cancer-associated gene BARD1 is involved in DNA repair in plants. *The EMBO journal* 25: 4326-4337

Riballo E, Woodbine L, Stiff T, Walker SA, Goodarzi AA, Jeggo PA (2009) XLF-Cernunnos promotes DNA ligase IV-XRCC4 re-adenylation following ligation. *Nucleic acids research* 37: 482-492

Rice MC, Smith ST, Bullrich F, Havre P, Kmiec EB (1997) Isolation of human and mouse genes based on homology to REC2, a recombinational repair gene from the fungus *Ustilago maydis*. *Proceedings of the National Academy of Sciences of the United States of America* 94: 7417-7422

Richardson C, Jasin M (2000) Frequent chromosomal translocations induced by DNA double-strand breaks. *Nature* 405: 697-700

Richardson C, Moynahan ME, Jasin M (1998) Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. *Genes & development* 12: 3831-3842

Rijkers T, Van Den Ouweland J, Morolli B, Rolink AG, Baarends WM, Van Sloun PP, Lohman PH, Pastink A (1998) Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. *Mol Cell Biol* 18: 6423-6429

Ristic D, Wyman C, Paulusma C, Kanaar R (2001) The architecture of the human Rad54-DNA complex provides evidence for protein translocation along DNA. *Proc Natl Acad Sci U S A* 98: 8454-8460

Roberts SA, Strande N, Burkhalter MD, Strom C, Havener JM, Hasty P, Ramsden DA (2010) Ku is a 5'-dRP/AP lyase that excises nucleotide damage near broken ends. *Nature* 464: 1214-1217

Rodrigue A, Coulombe Y, Jacquet K, Gagné J-P, Roques C, Gobeil S, Poirier G, Masson J-Y (2012) The RAD51 paralogs ensure cellular protection against mitotic defects and aneuploidy. *Journal of cell science* 126: 348-359

Rossi MJ, Mazina OM, Bugreev DV, Mazin AV (2011) The RecA/RAD51 protein drives migration of Holliday junctions via polymerization on DNA. *Proceedings of the National Academy of Sciences of the United States of America* 108: 6432-6437

Roth N, Klimesch J, Dukowic-Schulze S, Pacher M, Mannuss A, Puchta H (2012) The requirement for recombination factors differs considerably between different pathways of homologous double-strand break repair in somatic plant cells. *Plant J* 72: 781-790

Roy R, Chun J, Powell SN (2012) BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nature reviews Cancer* 12: 68-78

- Roy S, Choudhury SR, Sengupta DN, Das KP** (2013) Involvement of AtPol in the Repair of High Salt- and DNA Cross-Linking Agent-Induced Double Strand Breaks in Arabidopsis. *Plant physiology* 162: 1195-1210
- Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM** (2001) Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proceedings of the National Academy of Sciences of the United States of America* 98: 5134-5139
- Saini N, Ramakrishnan S, Elango R, Ayyar S, Zhang Y, Deem A, Ira G, Haber JE, Lobachev KS, Malkova A** (2013) Migrating bubble during break-induced replication drives conservative DNA synthesis. *Nature* 502: 389-392
- Salomon S, Puchta H** (1998) Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. *The EMBO journal* 17: 6086-6095
- Samach A, Melamed-Bessudo C, Avivi-Ragolski N, Pietrokovski S, Levy AA** (2011) Identification of plant RAD52 homologs and characterization of the Arabidopsis thaliana RAD52-like genes. *The Plant cell* 23: 4266-4279
- San Filippo J, Sung P, Klein H** (2008) Mechanism of eukaryotic homologous recombination. *Annual review of biochemistry* 77: 229-257
- Santa Maria SR, Kwon Y, Sung P, Klein HL** (2013) Characterization of the interaction between the Saccharomyces cerevisiae Rad51 recombinase and the DNA translocase Rdh54. *The Journal of biological chemistry* 288: 21999-22005
- Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, Kouzarides T** (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* 419: 407-411
- Saparbaev M, Prakash L, Prakash S** (1996) Requirement of mismatch repair genes MSH2 and MSH3 in the RAD1-RAD10 pathway of mitotic recombination in Saccharomyces cerevisiae. *Genetics* 142: 727-736
- Sarai N, Kagawa W, Kinebuchi T, Kagawa A, Tanaka K, Miyagawa K, Ikawa S, Shibata T, Kurumizaka H, Yokoyama S** (2006) Stimulation of Dmcl-mediated DNA strand exchange by the human Rad54B protein. *Nucleic Acids Res* 34: 4429-4437
- Sargent RG, Meservy JL, Perkins BD, Kilburn AE, Intody Z, Adair GM, Nairn RS, Wilson JH** (2000) Role of the nucleotide excision repair gene ERCC1 in formation of recombination-dependent rearrangements in mammalian cells. *Nucleic acids research* 28: 3771-3778
- Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP** (2007) Human CtIP promotes DNA end resection. *Nature* 450: 509-514

Sasanuma H, Tawaramoto MS, Lao JP, Hosaka H, Sanda E, Suzuki M, Yamashita E, Hunter N, Shinohara M, Nakagawa A *et al.* (2013) A new protein complex promoting the assembly of Rad51 filaments. *Nature communications* 4: 1676

Sato S, Hotta Y, Tabata S (1995) Structural analysis of a recA-like gene in the genome of *Arabidopsis thaliana*. *DNA research* 2: 89-93

Sauvageau S, Stasiak AZ, Banville I, Ploquin M, Stasiak A, Masson J-Y (2005) Fission yeast rad51 and dmc1, two efficient DNA recombinases forming helical nucleoprotein filaments. *Molecular and cellular biology* 25: 4377-4387

Schaetzlein S, Kodandaramireddy NR, Ju Z, Lechel A, Stepczynska A, Lilli DR, Clark AB, Rudolph C, Kuhnel F, Wei K *et al.* (2007) Exonuclease-1 deletion impairs DNA damage signaling and prolongs lifespan of telomere-dysfunctional mice. *Cell* 130: 863-877

Schatz DG, Oettinger MA, Baltimore D (1989) The V(D)J recombination activating gene, RAG-1. *Cell* 59: 1035-1048

Schild D, Lio YC, Collins DW, Tsomondo T, Chen DJ (2000) Evidence for simultaneous protein interactions between human Rad51 paralogs. *The Journal of biological chemistry* 275: 16443-16449

Schipler A, Iliakis G (2013) DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. *Nucleic acids research* 41: 7589-7605

Schuermann D, Fritsch O, Lucht JM, Hohn B (2009) Replication Stress Leads to Genome Instabilities in *Arabidopsis* DNA Polymerase δ Mutants. 21: 2700–2714

Schulte-Uentrop L, El-Awady RA, Schliecker L, Willers H, Dahm-Daphi J (2008) Distinct roles of XRCC4 and Ku80 in non-homologous end-joining of endonuclease- and ionizing radiation-induced DNA double-strand breaks. *Nucleic acids research* 36: 2561-2569

Schwacha A, Kleckner N (1995) Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell* 83: 783-791

Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J, Livingston DM (1997a) Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell* 90: 425-435

Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM (1997b) Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* 88: 265-275

Seeliger K, Dukowic-Schulze S, Wurzel-Wildersinn R, Pacher M, Puchta H (2012) BRCA2 is a mediator of RAD51- and DMC1-facilitated homologous recombination in *Arabidopsis thaliana*. *New Phytol* 193: 364-375

Sehorn MG, Sigurdsson S, Bussen W, Unger VM, Sung P (2004) Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. *Nature* 429: 433-437

Sehorn MG, Sung P (2004) Meiotic recombination: an affair of two recombinases. *Cell cycle (Georgetown, Tex)* 3: 1375-1377

Sekelsky JJ, Brodsky MH, Burtis KC (2000) DNA repair in *Drosophila*: insights from the *Drosophila* genome sequence. *J Cell Biol* 150: F31-36

Serra H, Da Ines O, Degroote F, Gallego ME, White CI (2013) Roles of XRCC2, RAD51B and RAD51D in RAD51-Independent SSA Recombination. *PLoS genetics* 9: e1003971

Shah PP, Zheng X, Epshtein A, Carey JN, Bishop DK, Klein HL (2010) Swi2/Snf2-related translocases prevent accumulation of toxic Rad51 complexes during mitotic growth. *Molecular cell* 39: 862-872

Shaked H, Avivi-Ragolsky N, Levy AA (2006) Involvement of the Arabidopsis SWI2/SNF2 chromatin remodeling gene family in DNA damage response and recombination. *Genetics* 173: 985-994

Sharan SK, Bradley A (1997) Murine Brca2: sequence, map position, and expression pattern. *Genomics* 40: 234-241

Sharan SK, Pyle A, Coppola V, Babus J, Swaminathan S, Benedict J, Swing D, Martin BK, Tessarollo L, Evans JP et al. (2004) BRCA2 deficiency in mice leads to meiotic impairment and infertility. *Development* 131: 131-142

She Z, Gao Z-Q, Liu Y, Wang W-J, Liu G-F, Shtykova EV, Xu J-H, Dong Y-H (2012) Structural and SAXS analysis of the budding yeast SHU-complex proteins. *FEBS letters* 586: 2306-2312

Shen SX, Weaver Z, Xu X, Li C, Weinstein M, Chen L, Guan XY, Ried T, Deng CX (1998) A targeted disruption of the murine Brca1 gene causes gamma-irradiation hypersensitivity and genetic instability. *Oncogene* 17: 3115-3124

Sheridan SD, Yu X, Roth R, Heuser JE, Sehorn MG, Sung P, Egelman EH, Bishop DK (2008) A comparative analysis of Dmc1 and Rad51 nucleoprotein filaments. *Nucleic Acids Research* 12: 4057-4066

Shim EY, Chung W-H, Nicolette ML, Zhang Y, Davis M, Zhu Z, Paull TT, Ira G, Lee SE (2010) *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. *The EMBO journal* 29: 3370-3380

Shim KS, Schmutte C, Tomblin G, Heinen CD, Fishel R (2004) hXRCC2 enhances ADP/ATP processing and strand exchange by hRAD51. *The Journal of biological chemistry* 279: 30385-30394

Shinohara A, Gasior S, Ogawa T, Kleckner N, Bishop DK (1997a) *Saccharomyces cerevisiae* recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. *Genes to cells : devoted to molecular & cellular mechanisms* 2: 615-629

Shinohara A, Ogawa H, Ogawa T (1992) Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* 69: 457-470

Shinohara A, Shinohara M, Ohta T, Matsuda S, Ogawa T (1998) Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. *Genes to cells : devoted to molecular & cellular mechanisms* 3: 145-156

Shinohara M, Gasior SL, Bishop DK, Shinohara A (2000) Tid1/Rdh54 promotes colocalization of rad51 and dmc1 during meiotic recombination. *Proceedings of the National Academy of Sciences of the United States of America* 97: 10814-10819

Shinohara M, Sakai K, Shinohara A, Bishop DK (2003) Crossover interference in *Saccharomyces cerevisiae* requires a TID1/RDH54- and DMC1-dependent pathway. *Genetics* 163: 1273-1286

Shinohara M, Shita-Yamaguchi E, Buerstedde JM, Shinagawa H, Ogawa H, Shinohara A (1997b) Characterization of the roles of the *Saccharomyces cerevisiae* RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. *Genetics* 147: 1545-1556

Shive HR, West RR, Embree LJ, Azuma M, Sood R, Liu P, Hickstein DD (2010) brca2 in zebrafish ovarian development, spermatogenesis, and tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America* 107: 19350-19355

Shor E, Weinstein J, Rothstein R (2005) A genetic screen for top3 suppressors in *Saccharomyces cerevisiae* identifies SHU1, SHU2, PSY3 and CSM2: four genes involved in error-free DNA repair. *Genetics* 169: 1275-1289

Shu Z, Smith S, Wang L, Rice MC, Kmiec EB (1999) Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(-/-) background. *Molecular and cellular biology* 19: 8686-8693

Siaud N, Dray E, Gy I, Gérard E, Takvorian N, Doutriaux M-P (2004) Brca2 is involved in meiosis in *Arabidopsis thaliana* as suggested by its interaction with Dmc1. *The EMBO journal* 23: 1392-1401

Sibanda BL, Critchlow SE, Begun J, Pei XY, Jackson SP, Blundell TL, Pellegrini L (2001) Crystal structure of an Xrcc4-DNA ligase IV complex. *Nature structural biology* 8: 1015-1019

Siebert R, Puchta H (2002) Efficient repair of genomic double-strand breaks by homologous recombination between directly repeated sequences in the plant genome. *The Plant cell* 14: 1121-1131

Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, Sung P (2001) Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. *Genes & development* 15: 3308-3318

Sigurdsson S, Van Komen S, Petukhova G, Sung P (2002) Homologous DNA pairing by human recombination factors Rad51 and Rad54. *The Journal of biological chemistry* 277: 42790-42794

Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis A-K, Van Houten B, Shuman S, McKinnon PJ, Jasin M (2011) Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. *Nature* 471: 245-248

Simsek D, Jasin M (2010) Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. *Nature structural & molecular biology* 17: 410-416

Singh KK, Samson L (1995) Replication protein A binds to regulatory elements in yeast DNA repair and DNA metabolism genes. *Proceedings of the National Academy of Sciences of the United States of America* 92: 4907-4911

Singleton MR, Dillingham MS, Wigley DB (2007) Structure and mechanism of helicases and nucleic acid translocases. *Annual review of biochemistry* 76: 23-50

Singleton MR, Wentzell LM, Liu Y, West SC, Wigley DB (2002) Structure of the single-strand annealing domain of human RAD52 protein. *Proceedings of the National Academy of Sciences of the United States of America* 99: 13492-13497

Smagulova F, Gregoret IV, Brick K, Khil P, Camerini-Otero RD, Petukhova GV (2011) Genome-wide analysis reveals novel molecular features of mouse recombination hotspots. *Nature* 472: 375-378

Smiraldo PG, Gruver AM, Osborn JC, Pittman DL (2005) Extensive chromosomal instability in Rad51d-deficient mouse cells. *Cancer research* 65: 2089-2096

Smith CE, Llorente B, Symington LS (2007) Template switching during break-induced replication. *Nature* 447: 102-105

Solinger JA, Kiianitsa K, Heyer WD (2002) Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. *Mol Cell* 10: 1175-1188

Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H, Takata M, Yamaguchi-Iwai Y, Takeda S (1998) Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *EMBO J* 17: 598-608

Soulas-Sprauel P, Rivera-Munoz P, Malivert L, Le Guyader G, Abramowski V, Revy P, de Villartay J-P (2007) V(D)J and immunoglobulin class switch recombinations: a paradigm to study the regulation of DNA end-joining. *Oncogene* 26: 7780-7791

Staeva-Vieira E, Yoo S, Lehmann R (2003) An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. *The EMBO journal* 22: 5863-5874

Stark JM, Pierce AJ, Oh J, Pastink A, Jasin M (2004) Genetic steps of mammalian homologous repair with distinct mutagenic consequences. *Molecular and cellular biology* 24: 9305-9316

Stasiak AZ, Larquet E, Stasiak A, Muller S, Engel A, Van Dyck E, West SC, Egelman EH (2000) The human Rad52 protein exists as a heptameric ring. *Curr Biol* 10: 337-340

Stauffer ME, Chazin WJ (2004) Physical interaction between replication protein A and Rad51 promotes exchange on single-stranded DNA. *The Journal of biological chemistry* 279: 25638-25645

Story RM, Bishop DK, Kleckner N, Steitz TA (1993) Structural relationship of bacterial RecA proteins to recombination proteins from bacteriophage T4 and yeast. *Science (New York, NY)* 259: 1892-1896

Strande N, Roberts SA, Oh S, Hendrickson EA, Ramsden DA (2012) Specificity of the dRP/AP Lyase of Ku Promotes Nonhomologous End Joining (NHEJ) Fidelity at Damaged Ends. *THE JOURNAL OF BIOLOGICAL CHEMISTRY* 287: 13686–13693

Strathern JN, Klar AJ, Hicks JB, Abraham JA, Ivy JM, Nasmyth KA, McGill C (1982) Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the MAT locus. *Cell* 31: 183-192

Studamire B, Price G, Sugawara N, Haber JE, Alani E (1999) Separation-of-function mutations in *Saccharomyces cerevisiae* MSH2 that confer mismatch repair defects but do not affect nonhomologous-tail removal during recombination. *Molecular and cellular biology* 19: 7558-7567

Sugawara N, Haber JE (1992) Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Molecular and cellular biology* 12: 563-575

Sugawara N, Ira G, Haber JE (2000) DNA length dependence of the single-strand annealing pathway and the role of *Saccharomyces cerevisiae* RAD59 in double-strand break repair. *Molecular and cellular biology* 20: 5300-5309

Sugawara N, Pâques F, Colaiácovo M, Haber JE (1997) Role of *Saccharomyces cerevisiae* Msh2 and Msh3 repair proteins in double-strand break-induced recombination. *Proceedings of the National Academy of Sciences of the United States of America* 94: 9214-9219

Sugawara N, Wang X, Haber JE (2003) In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. *Molecular cell* 12: 209-219

Sugiyama T, Kowalczykowski SC (2002) Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation. *The Journal of biological chemistry* 277: 31663-31672

Sugiyama T, New JH, Kowalczykowski SC (1998) DNA annealing by RAD52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. *Proceedings of the National Academy of Sciences of the United States of America* 95: 6049-6054

- Sugiyama T, Zaitseva EM, Kowalczykowski SC** (1997) A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. *The Journal of biological chemistry* 272: 7940-7945
- Sung P** (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science (New York, NY)* 265: 1241-1243
- Sung P** (1997a) Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *The Journal of biological chemistry* 272: 28194-28197
- Sung P** (1997b) Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes & development* 11: 1111-1121
- Sung P, Robberson DL** (1995) DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell* 82: 453-461
- Surtees JA, Alani E** (2006) Mismatch repair factor MSH2-MSH3 binds and alters the conformation of branched DNA structures predicted to form during genetic recombination. *Journal of molecular biology* 360: 523-536
- Sutherland BM, Bennett PV, Cintron-Torres N, Hada M, Trunk J, Monteleone D, Sutherland JC, Laval J, Stanislaus M, Gewirtz A** (2002) Clustered DNA damages induced in human hematopoietic cells by low doses of ionizing radiation. *Journal of radiation research* 43 Suppl: S149-152
- Sutherland BM, Bennett PV, Sidorkina O, Laval J** (2000) Clustered damages and total lesions induced in DNA by ionizing radiation: oxidized bases and strand breaks. *Biochemistry* 39: 8026-8031
- Suwaki N, Klare K, Tarsounas M** (2011) RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. *Seminars in cell & developmental biology* 22: 898-905
- Svendsen JM, Harper JW** (2010) GEN1/Yen1 and the SLX4 complex: Solutions to the problem of Holliday junction resolution. *Genes & development* 24: 521-536
- Svendsen JM, Smogorzewska A, Sowa ME, O'Connell BC, Gygi SP, Elledge SJ, Harper JW** (2009) Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. *Cell* 138: 63-77
- Swagemakers SM, Essers J, de Wit J, Hoeijmakers JH, Kanaar R** (1998) The human RAD54 recombinational DNA repair protein is a double-stranded DNA-dependent ATPase. *The Journal of biological chemistry* 273: 28292-28297
- Symington LS** (2002a) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiology and molecular biology reviews : MMBR* 66: 630-670

Symington LS (2002b) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiology and molecular biology reviews* 66: 630-670

Symington LS, Gautier J (2011) Double-strand break end resection and repair pathway choice. *Annual review of genetics* 45: 247-271

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983) The double-strand-break repair model for recombination. *Cell* 33: 25-35

Takata M, Sasaki MS, Sonoda E, Fukushima T, Morrison C, Albala JS, Swagemakers SMA, Kanaar R, Thompson LH, Takeda S (2000) The Rad51 Paralog Rad51B Promotes Homologous Recombinational Repair. *MOLECULAR AND CELLULAR BIOLOGY* 20: 6476-6482

Takata M, Sasaki MS, Tachiiri S, Fukushima T, Sonoda E, Schild D, Thompson LH, Takeda S (2001) Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. *Molecular and cellular biology* 21: 2858-2866

Tambini CE, George AM, Rommens JM, Tsui LC, Scherer SW, Thacker J (1997) The XRCC2 DNA repair gene: identification of a positional candidate. *Genomics* 41: 84-92

Tambini CE, Spink KG, Ross CJ, Hill MA, Thacker J (2010) The importance of XRCC2 in RAD51-related DNA damage repair. *DNA Repair (Amst)* 9: 517-525

Tamura K, Adachi Y, Chiba K, Oguchi K, Takahashi H (2002) Identification of Ku70 and Ku80 homologues in Arabidopsis thaliana: evidence for a role in the repair of DNA double-strand breaks. *The Plant journal : for cell and molecular biology* 29: 771-781

Tan TL, Essers J, Citterio E, Swagemakers SM, de Wit J, Benson FE, Hoeijmakers JH, Kanaar R (1999) Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. *Current biology : CB* 9: 325-328

Tanaka K, Hiramoto T, Fukuda T, Miyagawa K (2000) A novel human rad54 homologue, Rad54B, associates with Rad51. *J Biol Chem* 275: 26316-26321

Tanaka K, Kagawa W, Kinebuchi T, Kurumizaka H, Miyagawa K (2002) Human Rad54B is a double-stranded DNA-dependent ATPase and has biochemical properties different from its structural homolog in yeast, Tid1/Rdh54. *Nucleic acids research* 30: 1346-1353

Tang CM, Tomkinson AE, Lane WS, Wold MS, Seto E (1996) Replication protein A is a component of a complex that binds the human metallothionein IIA gene transcription start site. *J Biol Chem* 271: 21637-21644

Tao Y, Li X, Liu Y, Ruan J, Qi S, Niu L, Teng M (2012) Structural analysis of Shu proteins reveals a DNA binding role essential for resisting damage. *The Journal of biological chemistry* 287: 20231-20239

- Tarsounas M, Davies AA, West SC** (2004a) RAD51 localization and activation following DNA damage. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 359: 87-93
- Tarsounas M, Munoz P, Claas A, Smiraldi PG, Pittman DL, Blasco MA, West SC** (2004b) Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* 117: 337-347
- Tebbs RS, Zhao Y, Tucker JD, Scheerer JB, Siciliano MJ, Hwang M, Liu N, Legerski RJ, Thompson LH** (1995) Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene. *Proceedings of the National Academy of Sciences of the United States of America* 92: 6354-6358
- Thacker J** (1999) A surfeit of RAD51-like genes? *Trends in genetics : TIG* 15: 166-168
- Thoma NH, Czyzewski BK, Alexeev AA, Mazin AV, Kowalczykowski SC, Pavletich NP** (2005) Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic Rad54. *Nature structural & molecular biology* 12: 350-356
- Thompson LH, Schild D** (2001) Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutation research* 477: 131-153
- Thorslund T, McIlwraith MJ, Compton SA, Lekomtsev S, Petronczki M, Griffith JD, West SC** (2010) The breast cancer tumor suppressor BRCA2 promotes the specific targeting of RAD51 to single-stranded DNA. *Nature structural & molecular biology* 17: 1263-1265
- Titus TA, Selvig DR, Qin B, Wilson C, Starks AM, Roe BA, Postlethwait JH** (2006) The Fanconi anemia gene network is conserved from zebrafish to human. *Gene* 371: 211-223
- Toh GW-L, Sugawara N, Dong J, Toth R, Lee SE, Haber JE, Rouse J** (2010) Mec1/Tell1-dependent phosphorylation of Slx4 stimulates Rad1-Rad10-dependent cleavage of non-homologous DNA tails. *DNA repair* 9: 718-726
- Trapp O, Seeliger K, Puchta H** (2011) Homologs of breast cancer genes in plants. *Frontiers in plant science* 2: 19
- Trujillo KM, Sung P** (2001) DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50-Mre11 complex. *The Journal of biological chemistry* 276: 35458-35464
- Trujillo KM, Yuan SS, Lee EY, Sung P** (1998) Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. *The Journal of biological chemistry* 273: 21447-21450
- Tsai CJ, Kim SA, Chu G** (2007) Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends. *Proceedings of the National Academy of Sciences of the United States of America* 104: 7851-7856
- Tsodikov OV, Enzlin JH, Schärer OD, Ellenberger T** (2005) Crystal structure and DNA binding functions of ERCC1, a subunit of the DNA structure-specific endonuclease XPF-

ERCC1. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11236-11241

Tsubouchi H, Roeder GS (2003) The importance of genetic recombination for fidelity of chromosome pairing in meiosis. *Developmental cell* 5: 915-925

Tsutsui Y, Morishita T, Iwasaki H, Toh H, Shinagawa H (2000) A recombination repair gene of *Schizosaccharomyces pombe*, *rhp57*, is a functional homolog of the *Saccharomyces cerevisiae* *RAD57* gene and is phylogenetically related to the human *XRCC3* gene. *Genetics* 154: 1451-1461

Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, Morita T (1996) Targeted disruption of the *Rad51* gene leads to lethality in embryonic mice. *Proc Natl Acad Sci U S A* 93: 6236-6240

Turner JM (2007) Meiotic sex chromosome inactivation. *Development* 134: 1823-1831

Uanschou C, Siwiec T, Pedrosa-Harand A, Kerzendorfer C, Sanchez-Moran E, Novatchkova M, Akimcheva S, Woglar A, Klein F, Schlögelhofer P (2007) A novel plant gene essential for meiosis is related to the human CtIP and the yeast COM1/SAE2 gene. *The EMBO journal* 26: 5061-5070

Uchiyama Y, Suzuki Y, Sakaguchi K (2008) Characterization of plant *XRCC1* and its interaction with proliferating cell nuclear antigen. *Planta* 227: 1233-1241

Uematsu N, Weterings E, Yano K-i, Morotomi-Yano K, Jakob B, Taucher-Scholz G, Mari P-O, van Gent DC, Chen BPC, Chen DJ (2007) Autophosphorylation of DNA-PKCS regulates its dynamics at DNA double-strand breaks. *The Journal of cell biology* 177: 219-229

van den Bosch M, Vreeken K, Zonneveld JB, Brandsma JA, Lombaerts M, Murray JM, Lohman PH, Pastink A (2001) Characterization of *RAD52* homologs in the fission yeast *Schizosaccharomyces pombe*. *Mutat Res* 461: 311-323

van den Bosch M, Zonneveld JBM, Vreeken K, de Vries FAT, Lohman PHM, Pastink A (2002) Differential expression and requirements for *Schizosaccharomyces pombe* *RAD52* homologs in DNA repair and recombination. *Nucleic acids research* 30: 1316-1324

van der Heijden T, Seidel R, Modesti M, Kanaar R, Wyman C, Dekker C (2007) Real-time assembly and disassembly of human *RAD51* filaments on individual DNA molecules. *Nucleic acids research* 35: 5646-5657

Van Dyck E, Stasiak AZ, Stasiak A, West SC (2001) Visualization of recombination intermediates produced by *RAD52*-mediated single-strand annealing. *EMBO reports* 2: 905-909

Van Gent DC, McBlane JF, Ramsden DA, Sadofsky MJ, Hesse JE, Gellert M (1996) Initiation of V(D)J recombinations in a cell-free system by *RAG1* and *RAG2* proteins. *Current topics in microbiology and immunology* 217: 1-10

- Van Komen S, Petukhova G, Sigurdsson S, Stratton S, Sung P** (2000) Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54. *Molecular cell* 6: 563-572
- Van Komen S, Petukhova G, Sigurdsson S, Sung P** (2002) Functional cross-talk among Rad51, Rad54, and replication protein A in heteroduplex DNA joint formation. *The Journal of biological chemistry* 277: 43578-43587
- Van Veelen LR, Essers J, van de Rakt MWMM, Odijk H, Pastink A, Zdzienicka MZ, Paulusma CC, Kanaar R** (2005) Ionizing radiation-induced foci formation of mammalian Rad51 and Rad54 depends on the Rad51 paralogs, but not on Rad52. *Mutation research* 574: 34-49
- Vannier J-B, Depeiges A, White C, Gallego ME** (2009) ERCC1/XPF protects short telomeres from homologous recombination in *Arabidopsis thaliana*. *PLoS genetics* 5: e1000380
- Veuger SJ, Curtin NJ, Richardson CJ, Smith GCM, Durkacz BW** (2003) Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. *Cancer research* 63: 6008-6015
- Veuger SJ, Curtin NJ, Smith GCM, Durkacz BW** (2004) Effects of novel inhibitors of poly(ADP-ribose) polymerase-1 and the DNA-dependent protein kinase on enzyme activities and DNA repair. *Oncogene* 23: 7322-7329
- Villeneuve AM, Hillers KJ** (2001) Whence meiosis? *Cell* 106: 647-650
- Voelkel-Meiman K, Roeder GS** (1990) Gene conversion tracts stimulated by HOT1-promoted transcription are long and continuous. *Genetics* 126: 851-867
- Walker JR, Corpina RA, Goldberg J** (2001) Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412: 607-614
- Wang H, Rosidi B, Perrault R, Wang M, Zhang L, Windhofer F, Iliakis G** (2005) DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. *Cancer research* 65: 4020-4030
- Wang H-C, Chou W-C, Shieh S-Y, Shen C-Y** (2006) Ataxia telangiectasia mutated and checkpoint kinase 2 regulate BRCA1 to promote the fidelity of DNA end-joining. *Cancer research* 66: 1391-1400
- Wang S, Durrant WE, Song J, Spivey NW, Dong X** (2010) Arabidopsis BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses. *Proc Natl Acad Sci U S A* 107: 22716-22721
- Wang X, Ira G, Tercero JA, Holmes AM, Diffley JF, Haber JE** (2004) Role of DNA replication proteins in double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Mol Cell Biol* 24: 6891-6899

Wang Y, Xiao R, Wang H, Cheng Z, Li W, Zhu G, Wang Y, Ma H (2014) The Arabidopsis RAD51 paralogs RAD51B, RAD51D and XRCC2 play partially redundant roles in somatic DNA repair and gene regulation. *New Phytol* 201: 292-304

Ward JD, Barber LJ, Petalcorin MI, Yanowitz J, Boulton SJ (2007) Replication blocking lesions present a unique substrate for homologous recombination. *The EMBO journal* 26: 3384-3396

Ward JF (1988) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. *Progress in nucleic acid research and molecular biology* 35: 95-125

Warren M, Lord CJ, Masabanda J, Griffin D, Ashworth A (2003) Phenotypic effects of heterozygosity for a BRCA2 mutation. *Human molecular genetics* 12: 2645-2656

Waterworth WM, Altun C, Armstrong SJ, Roberts N, Dean PJ, Young K, Weil CF, Bray CM, West CE (2007) NBS1 is involved in DNA repair and plays a synergistic role with ATM in mediating meiotic homologous recombination in plants. *The Plant journal : for cell and molecular biology* 52: 41-52

Waterworth WM, Drury GE, Bray CM, West CE (2011) Repairing breaks in the plant genome: the importance of keeping it together. *New Phytol* 192: 805-822

Weinstock DM, Brunet E, Jasin M (2007) Formation of NHEJ-derived reciprocal chromosomal translocations does not require Ku70. *Nature cell biology* 9: 978-981

Welsh PL, King MC (2001) BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Human molecular genetics* 10: 705-713

Wesoly J, Agarwal S, Sigurdsson S, Bussen W, Van Komen S, Qin J, van Steeg H, van Benthem J, Wassenaar E, Baarends WM *et al.* (2006) Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. *Molecular and cellular biology* 26: 976-989

West CE, Waterworth WM, Jiang Q, Bray CM (2000) Arabidopsis DNA ligase IV is induced by gamma-irradiation and interacts with an Arabidopsis homologue of the double strand break repair protein XRCC4. *The Plant journal : for cell and molecular biology* 24: 67-78

Westermarck UK, Reyngold M, Olshen AB, Baer R, Jasin M, Moynahan ME (2003) BARD1 participates with BRCA1 in homology-directed repair of chromosome breaks. *Mol Cell Biol* 23: 7926-7936

Whitaker SJ (1992) DNA damage by drugs and radiation: what is important and how is it measured? *European journal of cancer* 28: 273-276

Wiese C, Collins DW, Albala JS, Thompson LH, Kronenberg A, Schild D (2002) Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells. *Nucleic acids research* 30: 1001-1008

- Wilson TE, Lieber MR** (1999) Efficient processing of DNA ends during yeast nonhomologous end joining. Evidence for a DNA polymerase beta (Pol4)-dependent pathway. *The Journal of biological chemistry* 274: 23599-23609
- Wold MS, Kelly T** (1988) Purification and characterization of replication protein A, a cellular protein required for in vitro replication of simian virus 40 DNA. *Proceedings of the National Academy of Sciences of the United States of America* 85: 2523-2527
- Wolner B, Van Komen S, Sung P, Peterson CL** (2003) Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. *Molecular cell* 12: 221-232
- Wong AK, Pero R, Ormonde PA, Tavtigian SV, Bartel PL** (1997) RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*. *The Journal of biological chemistry* 272: 31941-31944
- Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G** (1995) Identification of the breast cancer susceptibility gene *BRCA2*. *Nature* 378: 789-792
- Wu HI, Brown JA, Dorie MJ, Lazzeroni L, Brown JM** (2004) Genome-wide identification of genes conferring resistance to the anticancer agents cisplatin, oxaliplatin, and mitomycin C. *Cancer research* 64: 3940-3948
- Wu L, Hickson ID** (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426: 870-874
- Wu P-Y, Frit P, Meesala S, Dauvillier S, Modesti M, Andres SN, Huang Y, Sekiguchi J, Calsou P, Salles B *et al.*** (2009) Structural and functional interaction between the human DNA repair proteins DNA ligase IV and XRCC4. *Molecular and cellular biology* 29: 3163-3172
- Wu Y, Kantake N, Sugiyama T, Kowalczykowski SC** (2008) Rad51 protein controls Rad52-mediated DNA annealing. *The Journal of biological chemistry* 283: 14883-14892
- Wu Y, Sugiyama T, Kowalczykowski SC** (2006) DNA annealing mediated by Rad52 and Rad59 proteins. *The Journal of biological chemistry* 281: 15441-15449
- Wyatt HDM, Sarbajna S, Matos J, West SC** (2013) Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. *Molecular cell* 52: 234-247
- Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM** (2006) Control of *BRCA2* cellular and clinical functions by a nuclear partner, *PALB2*. *Mol Cell* 22: 719-729
- Xu X, Ball L, Chen W, Tian X, Lambrecht A, Hanna M, Xiao W** (2013) The Yeast Shu Complex Utilizes Homologous Recombination Machinery for Error-free Lesion Bypass via Physical Interaction with a Rad51 Parologue. *PloS one* 8: e81371

- Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX** (1999) Conditional mutation of *Brca1* in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nature genetics* 22: 37-43
- Yamada T, Ohta K** (2013) Initiation of meiotic recombination in chromatin structure. *Journal of biochemistry* 154: 107-114
- Yamaguchi-Iwai Y, Sonoda E, Buerstedde JM, Bezzubova O, Morrison C, Takata M, Shinohara A, Takeda S** (1998) Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in RAD52. *Molecular and cellular biology* 18: 6430-6435
- Yan CT, Boboila C, Souza EK, Franco S, Hickernell TR, Murphy M, Gumaste S, Geyer M, Zarrin AA, Manis JP *et al.*** (2007) IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature* 449: 478-482
- Yang H, Lu P, Wang Y, Ma H** (2011) The transcriptome landscape of Arabidopsis male meiocytes from high-throughput sequencing: the complexity and evolution of the meiotic process. *Plant J* 65: 503-516
- Yano K-i, Morotomi-Yano K, Wang S-Y, Uematsu N, Lee K-J, Asaithamby A, Weterings E, Chen DJ** (2008) Ku recruits XLF to DNA double-strand breaks. *EMBO reports* 9: 91-96
- Yanowitz JL** (2008) Genome integrity is regulated by the *Caenorhabditis elegans* Rad51D homolog rfs-1. *Genetics* 179: 249-262
- Yokoyama H, Sarai N, Kagawa W, Enomoto R, Shibata T, Kurumizaka H, Yokoyama S** (2004) Preferential binding to branched DNA strands and strand-annealing activity of the human Rad51B, Rad51C, Rad51D and Xrcc2 protein complex. *Nucleic acids research* 32: 2556-2565
- Yonetani Y, Hochegger H, Sonoda E, Shinya S, Yoshikawa H, Takeda S, Yamazoe M** (2005) Differential and collaborative actions of Rad51 paralog proteins in cellular response to DNA damage. *Nucleic Acids Res* 33: 4544-4552
- Yoo S-D, Cho Y-H, Sheen J** (2007) Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nature protocols* 2: 1565-1572
- Yu X, Chen J** (2004) DNA damage-induced cell cycle checkpoint control requires CtIP, a phosphorylation-dependent binding partner of BRCA1 C-terminal domains. *Molecular and cellular biology* 24: 9478-9486
- Yu X, Jacobs SA, West SC, Ogawa T, Egelman EH** (2001) Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. *Proceedings of the National Academy of Sciences of the United States of America* 98: 8419-8424
- Yun MH, Hiom K** (2009) CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature* 459: 460-463

Zhang Z, Fan H-Y, Goldman JA, Kingston RE (2007) Homology-driven chromatin remodeling by human RAD54. *Nature structural & molecular biology* 14: 397-405

Zhong Q, Chen C-F, Chen P-L, Lee W-H (2002) BRCA1 facilitates microhomology-mediated end joining of DNA double strand breaks. *The Journal of biological chemistry* 277: 28641-28647

Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD, Lee WH (1999) Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science (New York, NY)* 285: 747-750

Zhu X-D, Niedernhofer L, Kuster B, Mann M, Hoeijmakers JHJ, de Lange T (2003) ERCC1/XPF removes the 3' overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. *Molecular cell* 12: 1489-1498

Zhu Z, Chung W-H, Shim EY, Lee SE, Ira G (2008) Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134: 981-994

Zhuang J, Zhang J, Willers H, Wang H, Chung JH, van Gent DC, Hallahan DE, Powell SN, Xia F (2006) Checkpoint kinase 2-mediated phosphorylation of BRCA1 regulates the fidelity of nonhomologous end-joining. *Cancer Res* 66: 1401-1408

