



Role of Poly-(ADP-ribose)-ylation signaling pathway in the chromatin remodeling after DNA damage

Hafida Sellou

► To cite this version:

Hafida Sellou. Role of Poly-(ADP-ribose)-ylation signaling pathway in the chromatin remodeling after DNA damage. Molecular biology. Université de Rennes, 2016. English. NNT : 2016REN1B029 . tel-01691649

HAL Id: tel-01691649

<https://theses.hal.science/tel-01691649>

Submitted on 24 Jan 2018

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.



THÈSE / UNIVERSITÉ DE RENNES 1

sous le sceau de l'Université Bretagne Loire

pour le grade de

DOCTEUR DE L'UNIVERSITÉ DE RENNES 1

Mention : Biologie

Ecole doctorale Vie-Agro-Santé

présentée par

Hafida SELLOU

Préparée à l'unité de recherche UMR6290, IGDR
Institut de génétique et développement de Rennes
(Université Rennes 1)

**The role of Poly-
(ADP-ribose)-ylation
in chromatin
remodeling after DNA
damage**

**Thèse soutenue à Rennes
le 30 septembre 2016**

devant le jury composé de :

Dr. Gaëlle LEGUBE

Directrice de recherche, Université de Toulouse/
rapporteur

Dr. Emmanuelle FABRE

Directrice de recherche, Université Paris-Diderot /
rapporteur

Dr. Christian JAULIN

Directeur de recherche, Université Rennes 1 /
examineur/ Président du jury

Dr. Gyula TIMINSZKY

Group leader, Ludwig-Maximilians-Universität /
examineur

Dr. Sébastien HUET

Maitre de conférence, Université Rennes 1/
directeur de thèse

Résumé

Chaque jour, nos cellules sont constamment soumises à des stress endogènes, provenant de la production de métabolites par les cellules elles-mêmes et exogènes, comme l'exposition aux rayons Ultra-Violets, agents chimiques, etc. Ces agressions conduisent à des dommages dans l'ADN. Si ces dommages ne sont pas réparés correctement, ils peuvent induire un dérèglement des fonctions de base de la cellule qui peut alors devenir cancéreuse. En réponse à ces lésions au niveau de l'ADN et afin de préserver l'intégrité de leur génome, les cellules activent différents mécanismes de réparation et établissent une signalisation au niveau des sites endommagés.

Dans les cellules eucaryotes, le matériel génétique est organisé en une structure complexe constituée d'ADN et de protéines appelées histones. Cette structure est connue sous le nom de chromatine. La chromatine se caractérise par différents niveaux d'organisation, aboutissant à la formation d'une structure très compacte. La chromatine a pour unité fondamentale le nucléosome. Il constitue le premier niveau de compaction de l'ADN dans le noyau. Cette structure est ensuite régulièrement répétée pour former la fibre de chromatine qui présente, elle-même, des niveaux d'organisation d'ordre supérieurs : boucles, domaines topologiques et enfin territoires chromosomiques. Cependant, ces différents niveaux de structuration permettant d'atteindre un haut niveau de compaction de la chromatine, peuvent représenter une barrière pour la machinerie de réparation. En effet, cette dernière a besoin d'avoir accès à l'ADN endommagé pour le réparer.

Les cellules ont développé des mécanismes permettant d'accéder à l'ADN endommagé. Les étapes précoces du processus de réparation se caractérisent ainsi par des mécanismes de remodelage actifs de la chromatine qui pourraient faciliter l'accès de la machinerie de réparation à l'ADN endommagé. La voie de signalisation par poly-ADP-ribosylation (PAR) est une modification post-traductionnelle composée d'une répétition de petites molécules appelées Poly-ADP-Riboses, qui s'accrochent notamment sur les histones pour signaler la présence de cassures dans l'ADN. Cette voie joue un rôle régulateur essentiel au cours de ces premières étapes de la réponse cellulaire aux dommages dans l'ADN notamment via le recrutement de facteurs de remodelage de la chromatine au niveau de ces dommages. Des données *in vitro* ont démontré que PARP1, protéine majeure de la voie PAR, se liait à l'ADN au niveau de la fibre de chromatine et induisait la compaction de la chromatine. Lorsque PARP1 est activé, il synthétise des chaînes de PAR qui sont reconnues par des protéines de la voie de réparation via un domaine de liaison à ce type de chaînes. *In vitro*, la présence de chaînes de PAR le long de la fibre de chromatine semble aussi induire son désassemblage. Parmi les protéines recrutées via les chaînes de PAR, plusieurs remodeleurs de la chromatine ont été décrits. Cependant, le rôle exact de la signalisation via la PARylation durant les étapes précoces de la réponse aux dommages à l'ADN et plus particulièrement lors du remodelage de la chromatine reste encore mal caractérisé.

Durant ma thèse, j'ai utilisé des techniques avancées en microscopie de fluorescence permettant de caractériser quantitativement et avec une résolution spatio-temporelle optimale les événements de remodelage de la chromatine accompagnant la réponse

aux dommages dans l'ADN. La visualisation directe de ce processus en cellules vivantes a permis de préciser le rôle de la PARylation dans les processus de remodelage de la chromatine au niveau des sites de dommages à l'ADN. Pour cela, nous avons utilisé des lignées cellulaires exprimant des histones marquées à l'aide de protéines fluorescentes photo-activables comme la protéine PATagRFP ou la PA-GFP, qui après irradiation avec un laser à 405 nm deviennent fluorescentes respectivement dans le rouge et le vert, permettant ainsi de visualiser la zone illuminée par le laser à 405 nm. L'irradiation avec le laser à 405 nm permet dans le même temps d'induire des cassures dans l'ADN de cellules pre-sensibilisés avec du Hoechst (intercalant de l'ADN). Afin de mesurer les changements dans la structure de la chromatine après induction de dommages à l'ADN, nous avons développé une routine d'analyse qui permet de mesurer automatiquement la largeur de la zone irradiée photo-activée. Nous avons observé une augmentation rapide de la largeur de la zone irradiée en fonction du temps que nous avons interprété comme une décondensation de la chromatine. Lorsque la voie de PARylation est inhibée, la décondensation de la chromatine n'est plus observée ce qui suggère que cette décondensation est dépendante de la voie de PARylation. Nos travaux suggèrent que le remodeleur de la chromatine Alc1, dont l'activité est contrôlée par la PARylation joue un rôle prépondérant dans le remodelage de la chromatine associé aux dommages dans l'ADN. Lors d'induction de dommages, Alc1 est activé par la voie de signalisation PAR et s'accumule rapidement au niveau des sites de dommages. Le recrutement de Alc1 au niveau des cassures de l'ADN se fait via son domaine macro (domaine de reconnaissance des chaînes de PAR). La suppression d'Alc1 a montré une diminution significative de la décondensation de la chromatine. De plus, la surexpression de Alc1 a montré une sur-décondensation de la

chromatine. Nos résultats démontrent donc que le recrutement de Alc1 permet une décondensation rapide de la chromatine qui pourrait faciliter l'accès d'autres protéines de réparation.

De façon similaire, nos travaux préliminaires suggèrent qu'un autre remodeleur de la chromatine appelé CHD4, dont l'activité est aussi contrôlée par la PARylation jouerait un rôle dans le mécanisme de restructuration de la chromatine associé aux dommages dans l'ADN. Lors d'induction de dommages, CHD4 est recruté au niveau des cassures de l'ADN. L'inhibition de l'expression de CHD4 à l'aide d'ARN interférant semble montrer une diminution significative de la décondensation de la chromatine. Par conséquent, de manière similaire au remodeleur Alc1, le remodeleur de la chromatine CHD4 semble participer au processus de décondensation rapide de la chromatine et permettrait de faciliter l'accès d'autres protéines de réparation.

En plus de la voie de signalisation par PARylation, d'autres voies peuvent être activées par les cellules suite aux dommages à l'ADN comme les voies ATM ou DNA-PK. Ces deux voies impliquent la phosphorylation de différents facteurs dans le but de permettre le recrutement de protéines de réparation au niveau des cassures de l'ADN. Nous avons recherché l'implication de ces voies de réparation dans le processus de décondensation de la chromatine suite aux dommages à l'ADN. Nos résultats préliminaires montrent que l'inhibition de la voie DNA-PK, mais pas celle de la voie ATM conduisent à une diminution significative de la décondensation de la chromatine. Ces résultats suggèrent que la voie DNA-PK pourrait aussi jouer un rôle dans le processus de décondensation de la chromatine suite aux dommages à l'ADN.

En résumé, nos travaux ont démontré que l'induction de dommages à l'ADN par irradiation avec un laser à 405 nm induisait une décondensation de la chromatine. Ce processus de décondensation est dépendant de la voie de PARylation et implique le recrutement et l'action de remodeleurs de la chromatine comme les protéines Alc1 et CHD4. De plus, d'autres voies de signalisation dont la voie DNA-PK sembleraient jouer un rôle dans ce processus. Une étude plus approfondie est encore nécessaire afin de déterminer le mécanisme de coordination entre ces différents acteurs moléculaires au cours du processus de remodelage de la chromatine.

To my parents, sister and
brothers, your love and support make
impossible become possible...

Acknowledgment

“Knowledge is a treasure, but practice is the key to it.”

— Lao Tzu.

A recipe for a good thesis always begins with good mentors...

Because having one mentor was so easy... we thought two would be better!

One from France,

I would like to thank Dr. Sébastien Huet for welcoming me in his team, for his continual support and constant encouragement throughout my thesis. I learned so much during these three years in your team. You showed me the magic of fluorescence and microscopy world and taught me how to be part of it. Your insights and advice have been key to my success as a graduate student. You gave me the freedom to explore and pursue my project. I went in your lab as an apprentice and you allowed me to grow as a researcher.

One from Germany,

I would like to thank Dr. Gyula Timinszky for his support and encouragement during my stay in Munich. With you, I learned how much science can be fun. Our discussion, your advices and insights have been precious to my success during my thesis. I spent very good time in your lab and learned how to appreciate coffee! Thanks you and Zsuzsa for helping me every time I came to Munich.

Each one has a team, which acts as a second family (because a thesis is not a job it's a life)!

I would also like to thank all past and present members of the Huet's team. Especially, Catherine for her helps her smile and her good mood. It was a pleasure to discuss, work and spend time with you. I would also like to thank Théo who was so kind, helping me when computers, software and particularly Image J conspired against me! I spent good time working with you. Good luck with your thesis! Even if

we did not have enough time to know each other, I would like to thank Benjamin D. for his smile and kindness. Wish you all the best for the future.

In Gyula's team, I met a special person coming from a far far away country where there are more sheep than humans and where movies are very good! A special Thanks to my favorite kiwi Rebecca! Watching Deadpool at 10 am, having the best brunch ever, walking under the rain to find a book store, loving cheese, being angry against the microscope, asking you thousands times for help. We spent such good time together! I also had the chance to meet Barbara, a nice Italian girl from Naples where you can find the best pizza by the way! Thanks you Barbara for your time, help, smile and making me discovering Munich. Thanks you girls, I spent so much good time with you and thanks for being there for me! Hope to see you again!

I also would like to thanks Anna, Julia and Giuliana for their smile, help, time, and discussion. Wish you all the best for you girls!

Being in a lab means to have a lot of neighbors...

Third floor and microscopy platform:

During my thesis, I was surrounded by Marc Tramier and his team, let say they were the closest neighbors which means spending a lot of time with them. Thanks you Marc for your advices and insights during group meeting and your good mood! I would also like to thank Claire a.k.a Sponge Bob for all the great time we spent together during our thesis and wish her the best for the future. I would like to thank Otmane for his smile and good mood. Even if we did not have enough time to know each other, I would like to thank Florian for good time and wish him good luck for his thesis. And a special thanks to Giulia for all the help and advices she gave me during my thesis. Every time I felt bad, or I wanted to give up, you were there as a big sister listening to me and giving me courage to continue! Wish you all the best for your future and can't wait to listen to your band on live!

I also would like to thank Jacques Pécreaux and his team for all the good time we spent together. For his advices and insights during group meeting and help with

microscopy image processing! Thanks to Benjamin M. for his jokes and good mood, I wish you good luck for your defense and future career. Thanks to Hélène for advices and her good mood. Thanks to Xavier for fun and jokes, I wish you all the best for the future. And I would like to thank Yann for his smile and jokes, for help, advices and all the great time I got the pleasure to share with him and his wife Domi. Wish you all the best for both of you!

Doing microscopy means spending time with a microscope, I could thanks the spinning disk for being a good partner during all the microscopy sessions I did, but I would rather thank Stéphanie, Clément and Marine (past member) from the MRic photonics facility for their help and kindness. Wish you all the best!

I also would like to thanks Guillaume for all the great time we spent together, wish you all the best for your future!

Second floor:

Thesis is not only working on the bench, but it is also coffee breaks and partying. I would like to thanks the canine genetics team for all the good time during coffee breaks (Mélanie, Morgane, Doudou, Pascale, Ronan, Christophe, Valentin, Clotilde, Anne-So, Benoit, Thomas, Laetitia, Nadine, Aline, Maud, Solenne...). I also would like to thanks Stéphane for his good mood, jokes and all the funny time as well as his kindness!

I would also like to thank Ghislain for his forever good mood, smile, great time and long discussion about life in the corridor, thank you for showing me what a worm looks like under a microscope! Wish you all the best for your defense and your career. Another good guy from the second floor is Renaud, thank you for discussion about rugby, your smile and showing me what a fly looks like under a microscope! Wish all the best for your defense and future life with Elise in UK!

Ground floor:

I would like to thank Feriel and Blanca for their smile and the good time I spent with you girls! Thanks for your advices and your good mood. Wish you all the best and hope to see you in the future! Thanks to Miguel, Javier, Anthony (and Laura) for their good mood and good discussion.

I do not forget past members of the institute I spent good time with: Manu, Olivia, Nicolas, Pauline and Jocelyn. Thanks for the good moments we spent together!

In Munich, there is only one floor but full of great people! I would like to thanks all the Ladurner Department and the head of the department Andreas. I also would like to thanks Anton for all the help with the housing and other many things he did to make my stay in Munich better. I would like to thank two other kiwis Ava and Corey for their kindness and smile; I spent very good time with you. Wish you all the best for your future! Muchas gracias to Ramon and Marta for their kindness, smile and time, Thanks again Marta for the Jazz session we went to see together with Corey and Ava! I also would like to thank Thomas, Rupa, Umut, and Lisa for good time we spent together. I also would like to thank past member of this department, Andrew and Marek. Wish you all the best guys!

Because a thesis is not just making science and experiments...

I would like to thanks all the management office of the institute, the administration office and the washing and sterilization service for their great job! A special thanks to Géraldine and Nadine G., you are treasures both of you! Thanks for the help, all the great time, and your smile. It was a pleasure to spend my thesis surrounded by you!

A thesis is practice, failure, and sometimes good results...

When it comes to the end of the thesis, you should be ready to be evaluated and recognized by your peers. Also, I would like to thanks my dissertation committee, Drs. Gaëlle Legube, Emmanuelle Fabre, Christian Jaulin and Pr. Kerstin Bystricky for accepting to evaluate my thesis works. I hope that this dissertation matches your expectations.

Because thesis is a part of life where family and friends are the best supporters...

I would like to thank all my friends: Marion, Camille, Amélie, Christelle and former past and present colleagues from the Bystricky's lab Virginie, Fatima, Sylvia, Mathieu, Luca, Isabelle, Anne-Claire, Sylvain and Thomas from my hometown Toulouse for their support and for being there for me. Special thanks to Kerstin for her support, advices and insights. Coming back home and spending time with you were a breath of air!

I also would like to thanks Sarah A. who accepted to be my English proofreader, thanks for your help and please do not look at my mistakes in these acknowledgment!

A special thanks to my best friends Nora and Gaet. You were my sunshine during my thesis especially during this last year. You were so supportive; pushing me to reach my goals, listening to me complaining about how hard is the thesis and life. You can't picture how much you are precious for me. You taught me to follow my dreams and success. Hope to see you soon! Love you so much!

I also would like to thanks my auntie Karima and her husband Djamel for their help when I began my new life in Rennes. Merci Karima pour tes paroles remplies de sagesse, parler avec toi fut une thérapie !

Mais le plus important reste la famille. Je voudrais remercier chaleureusement mes parents, mes deux frères et ma sœur pour leur présence et leur soutien sans faille. Merci papa et maman pour toutes les heures passées au téléphone à m'écouter, me réconforter, à m'apprendre la vie. Vous avez vécu ma thèse avec moi, avec ses hauts et ses bas. Vous m'avez appris la patience, donné le goût du travail et appris la valeur de l'humilité. Vous êtes à mes yeux les racines solides d'un arbre sur lequel je grandis, l'échelle qui me conduit à la réussite, ainsi que la sagesse qui me fait grandir chaque jour. Merci à Adel, Ounissa et Rayan, vous avoir comme frères et sœur m'a appris à être généreuse, à partager et aider mon prochain. J'espère que vous serez toujours autant fiers de moi que je le serais de vous.

Table of Contents

| | |
|--|-----|
| INTRODUCTION | 20 |
| I. Chromatin: structural organization and function | 21 |
| A. The Nucleosome: Structural Unit of the Chromatin | 21 |
| B. The Chromatin Fiber: from 11nm to 30nm | 25 |
| C. The Chromatin Loops | 28 |
| D. TADs: topologically associating domains | 31 |
| E. The Chromosomes Territories | 33 |
| II. Chromatin Dynamics | 36 |
| A. Chromatin Dynamics at the Nucleosome Scale | 36 |
| B. Chromatin Dynamics at the Genomic-Locus Scale | 37 |
| C. Chromatin Dynamics at the Chromosome Territory Scale | 41 |
| D. Processes that Influence Chromatin Dynamics | 41 |
| III. DNA Repair | 43 |
| A. The Different Types of DNA Damages | 44 |
| B. The Canonical Repair Mechanism | 46 |
| C. PARP Signalling Pathway | 50 |
| IV. Chromatin Remodeling at DNA Damage Sites | 57 |
| A. The Multiscale Changes in Chromatin Architecture and Dynamics at DNA Breaks | 58 |
| B. The Molecular Players | 60 |
| C. Functional Role of Chromatin Remodeling and Dynamics at DNA damage | 76 |
| RESULTS | 83 |
| I. Establishment of a Methodology to Study Chromatin Dynamics upon DNA Damage | 84 |
| II. PARP1 Activation Controls the Transient Chromatin Relaxation at DNA Damage Sites | 91 |
| III. PARylation Levels | 95 |
| IV. Contribution of ATP-dependent Processes in Chromatin Relaxation at DNA Lesions | 98 |
| V. The ATP-Dependent Chromatin Remodeler Alc1 Contributes to Chromatin Relaxation | 101 |
| VI. The ATP-Dependent Chromatin Remodeler CHD4 Contributes to Chromatin Relaxation | 112 |

| | |
|--|-----|
| VII. Histone Variants..... | 116 |
| VIII. Cross Talk Between PARP1, ATM and DNA PK Signalling Pathways | 118 |
| DISCUSSION | 120 |
| I. The Assay to Induce and Follow Chromatin Dynamics upon DNA Damage: Advantages and Inconvenient | 121 |
| II. Why Do Cells Relax their Chromatin upon DNA Damage? | 123 |
| III. Which Factors are implicated in the Chromatin Relaxation? | 128 |
| IV. How Do Structural Changes Affecting the Chromatin Fiber Lead to Global Chromatin Relaxation?..... | 131 |
| EXPERIMENTAL PROCEDURES | 136 |
| BIBLIOGRAPHY | 146 |
| ANNEXES | 165 |

Summary

In each human cell, many thousands of DNA lesions arise every day, challenging continuously the genome integrity. The majority of these lesions results from byproducts of normal cell metabolism or DNA replication, but they are also induced by exposure to radiations and genotoxic chemicals. The integrity of the genome is preserved by a plethora of different DNA damage signalling and repair machinery arranged by the cells.

In the cell nucleus, DNA associates with scaffolding proteins to form the chromatin. The chromatin is tightly packed in the nucleus through several levels of organization. Such high-packing state poses a significant challenge for the repair machinery. Indeed, the damaged DNA needs to be accessible to repair proteins, and for that, cells have developed several mechanisms to allow the access to the damaged chromatin. The early steps of the DNA damage response involve the activation of proteins that are part of signalling pathways. One of the proteins activated upon DNA damage is PARP1, which synthesizes long and branched chains of ADP-ribose (poly-ADP-ribose or PAR) on itself and other chromatin factors, including histones. The activation of PARP1 leads to the recruitment of several effectors involved in DNA repair and chromatin remodeling. However the exact function of the PAR-signalling during early DNA damage response and in particular during chromatin remodeling at DNA breaks remains unclear.

During my PhD, I used advanced fluorescent imaging tools to study in living cells the dynamics of chromatin in the nucleus at a local scale upon DNA damage. I used

these tools to study PAR-dependent chromatin relaxation after DNA damage and to screen factors that selectively alter the dynamic behaviour of the damaged chromatin. This methodology allowed us to identify PAR-dependent factors involved in the local chromatin remodeling upon DNA damage.

Résumé

Chaque cellule humaine est constamment soumise à des agressions extérieures comme l'exposition aux rayons Ultra-Violets, agents chimiques, etc. ou endogènes provenant de la production de métabolites par la cellule elle-même. Ces agressions induisent des dommages dans l'ADN. Ces dommages, s'ils ne sont pas réparés correctement, peuvent induire un dérèglement des fonctions de base de la cellule qui peut alors devenir cancéreuse. Pour réparer leur ADN, les cellules activent divers mécanismes de réparation et établissent une signalisation au niveau des sites endommagés.

Dans le noyau, l'ADN est associé à des protéines appelées histones pour former la chromatine. La chromatine se caractérise par différents niveaux d'organisation, aboutissant à la formation d'une structure très compacte. Cette compaction élevée de la chromatine peut représenter une barrière pour la machinerie de réparation. En effet, pour être réparé, l'ADN endommagé doit être accessible à la machinerie de réparation. Pour cela, les cellules ont développé des mécanismes permettant d'accéder à l'ADN endommagé. Ces mécanismes de réponse aux dommages à l'ADN impliquent l'activation de voies de signalisation. L'un des signaux précurseurs activés après dommage à l'ADN est la Poly-ADP-Ribosylation (PARylation). La PARylation est une modification post-traductionnelle composée d'une répétition de petites molécules appelées Poly-ADP-Riboses, qui s'accrochent notamment sur les histones pour signaler la présence de cassures dans l'ADN et permettent ainsi de recruter les protéines impliquées dans la réparation des dommages. Lorsque l'ADN est endommagé, l'activation de processus de réparation induit de manière précoce le

recrutement de facteurs de remodelage de la chromatine. Le rôle exact de la signalisation via la PARylation durant les étapes précoces de la réponse aux dommages à l'ADN et plus particulièrement lors du remodelage de la chromatine reste encore mal caractérisé.

Durant ma thèse, j'ai utilisé des techniques avancées en microscopie pour étudier la dynamique de la chromatine après induction de dommages à l'ADN. J'ai ainsi tenté d'élucider le rôle de la PARylation dans le mécanisme de remodelage de la chromatine au niveau des dommages dans l'ADN, en recherchant des facteurs permettant d'altérer de manière spécifique la dynamique de la chromatine. Cette méthodologie nous a permis d'identifier différents facteurs impliqués dans le remodelage de la chromatine après dommage à l'ADN.

Table of figures:

| | |
|---|-----|
| Figure 1. The nucleosome particle..... | 22 |
| Figure 2 The nucleosome particle..... | 24 |
| Figure 3. 2 Models for chromatin fiber folding..... | 27 |
| Figure 4. Overview of 3C-based methods..... | 30 |
| Figure 5. Hierarchical organization from chromatin loops to chromosome territories. | 32 |
| Figure 6. Chromosome territories visualization..... | 35 |
| Figure 7. Example of Chromosome labelling with dUTP of U2OS nucleus and corresponding MSD analysis. | 40 |
| Figure 8. The source and type of DNA damage and their main associated repair pathways | 45 |
| Figure 9. The Canonical repair mechanism..... | 47 |
| Figure 10. The “access-repair-restore” model. | 49 |
| Figure 11. The Poly(ADP-ribose)-ylation as a post-translational modification..... | 52 |
| Figure 12. PARP1 in SSBs and DSBs repair pathways..... | 56 |
| Figure 13. PARP1 involvement in DNA repair. | 63 |
| Figure 14. The histone chaperones: regulators of histone exchanges. | 67 |
| Figure 15. Chromatin Remodeler Families..... | 69 |
| Figure 16. Chromatin remodeling mechanism..... | 75 |
| Figure 17. Changes in chromatin motion and compaction state at DNA damage sites for yeast and mammalian cells..... | 81 |
| Figure 18. Using photo-activable dyes to observe chromatin sub regions..... | 85 |
| Figure 19. 405 nm laser irradiation of Hoechst treated cells induces DNA damage..... | 85 |
| Figure 20. Chromatin relaxes after induction of DNA damage..... | 86 |
| Figure 21. Alternative approach to label the chromatin using the incorporation of fluorescent nucleotides in the DNA..... | 88 |
| Figure 22. The amplitude of chromatin relaxation scales with the intensity of the laser power..... | 89 |
| Figure 23. chromatin re-condenses after the initial phase of fast relaxation..... | 90 |
| Figure 24. PARP1 activation regulates the transient chromatin relaxation at DNA damage sites..... | 92 |
| Figure 25. PARP1 knockout using CRISPR-Cas9 method..... | 93 |
| Figure 26. PARP1 binding leads to chromatin over-compaction..... | 94 |
| Figure 27. The effect of PARylation levels on the transient chromatin relaxation at DNA damage sites..... | 95 |
| Figure 28. PARylation levels in cells over-expressing PARP1 WT and PARP1 delta CD mutant..... | 97 |
| Figure 29. Chromatin relaxation at DNA damage sites partially depends on ATP (1)..... | 99 |
| Figure 30. Chromatin relaxation at DNA damage sites partially depends on ATP (2)..... | 100 |
| Figure 31. The chromatin remodeler Alc1 is quickly recruited at the DNA lesion sites..... | 102 |
| Figure 32. The chromatin remodeler Alc1 is recruited in a PAR-dependent manner at the DNA damage sites..... | 103 |

| | |
|---|-----|
| Figure 33. Alc1 knockout using CRISPR-Cas9..... | 104 |
| Figure 34. The knockout of Alc1 has no effect on chromatin architecture in the absence of DNA damage | 105 |
| Figure 35. The chromatin remodeler Alc1 contributes to the chromatin relaxation upon DNA damage (1) | 106 |
| Figure 36. The chromatin remodeler Alc1 contributes to the chromatin relaxation upon DNA damage (2) | 107 |
| Figure 37. Alc1 effect on chromatin relaxation is dependent on the laser intensity used to induce DNA damage | 108 |
| Figure 38. The over-expression of Alc1 leads to an over-relaxation of the chromatin | 109 |
| Figure 39. The chromatin remodeler Alc1 does not seem to contribute to the chromatin recondensation upon DNA damage..... | 110 |
| Figure 40. Cells over-expressing Alc1 WT fail to recover their initial condensation state..... | 111 |
| Figure 41. The chromatin remodeler CHD4 is recruited at DNA lesion sites in a PAR-dependent manner | 113 |
| Figure 42.siRNA-mediated knock-down of CHD4 | 113 |
| Figure 43. The chromatin remodeler CHD4 contributes to the chromatin remodeling upon DNA damage..... | 114 |
| Figure 44. The over-expression of chromatin remodeler CHD4 seems to have no effect on chromatin relaxation DNA damage | 115 |
| Figure 45. The H2A histone variants contribution to the chromatin remodeling upon DNA damage | 117 |
| Figure 46. DNA-PK signalling pathway seems to be involved in chromatin relaxation upon DNA damage..... | 119 |
| Figure 48. Speculative model for chromatin re-organization upon DNA damage. | 134 |

Abbreviation:

4C: Chromatin Conformation Capture on Chip

53BP1: p53-binding protein 1

5C: Chromatin Conformation Capture Carbon copy

ADP: Adenosine diphosphate

APLF: Aprataxin and PNK-like factor

ARH3: ADP-ribosylhydrolase 3

ATM: Ataxia Telangiectasia Mutated

ATP: Adenosine triphosphate

ATR: ATM- and RAD3-related

Bp: base pair

BRCA1: BReast Cancer 1

BRCT domain: BRCA1 C Terminus domain

CAF1: Chromatin assembly factor

CHD1L: Chromodomain helicase DNA binding protein 1 like

CHD2/4: Chromodomain helicase DNA binding protein 2/4

Chk1/2: Checkpoint kinase 1/2

CRISPR/Cas9: Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein-9 nuclease

CFTR: cystic fibrosis transmembrane conductance regulator

DNA: deoxyribonucleic acid

DNA-PK: DNA-dependent protein kinase

EGFP: Enhanced Green Fluorescent Protein

FACS: Fluorescence-activated cell sorting

FHA domain: forkhead-associated domain

H2A bbd: H2A Barr body-deficient

hSPT16: human Facilitates chromatin transcription complex subunit SPT16

INO80: inositol requiring mutant 80

IR: infrared

ISWI: Imitation SWItch

kDa: kilo Dalton

Mb: mega base

Mdc1: Mediator of DNA damage checkpoint protein 1

MRE11: meiotic recombination 11

MRN complex: Mre11-Rad50-Nbs1

mw: milli watts

NAD: Nicotinamide adenine dinucleotide

NBS1: Nijmegen Breakage Syndrome 1

NTP/dNTP: Nucleotide triphosphate/ deoxy Nucleotide triphosphate

PATagRFP/PAGFP: Photo-activable Tag red fluorescent protein/ photo-activable green fluorescent protein

PCNA: proliferating cell nuclear antigen

PHD: plant homeodomain

RNF168: Ring finger protein 168

RSC complex: Chromatin structure remodeling

SCM3: Structural maintenance of chromosomes protein 3

sgRNA: single-guide RNA

SNF2H: Sucrose Non-fermenting Protein 2 Homolog

SSRP1: Structure Specific Recognition Protein 1

SWR1: SWI/SNF-related

TARG1: terminal ADP-ribose protein glycohydrolase

Trrap: Transformation/Transcription Domain-Associated Protein

U2OS: human osteosarcoma

UBD: Ubiquitin-binding domain

WWE domain: W (Tryptophan) W (Tryptophan) E (Glutamic acid)

XRCC1: X-ray repair cross-complementing protein 1

YFP: Yellow Fluorescent Protein

INTRODUCTION

I. Chromatin: structural organization and function

The chromatin is a complex structure characterized by a multiscale architecture. In fact, eukaryotic DNA is split into many chromosomes confined within a nucleus with a diameter of about 10^{-5} m. If we stretch all the chromosomes from a single human cell, the resulting length of DNA can reach about two meters long. Therefore, the DNA is packed into several condensation levels to accommodate the requirement of storing, organizing and protecting the genetic information while ensuring its access when needed. In this first part I will give an overview of the chromatin multiscale architecture from the nucleosome particle to the chromosome territories in the absence of DNA damage.

A. The Nucleosome: Structural Unit of the Chromatin

Early observations of chromatin using electron microscopy have shown the existence of a “beads-on-string” structure, which was described as a succession of particles linked to each other by a DNA filament ¹. These particles establish the first level of DNA condensation and are called “nucleosomes” ².

The nucleosome is the elementary unit of the chromatin, formed by 146 base pairs (bp) of DNA wrapped around an octamer of two copies each of the four core histone proteins (H2A, H2B, H3, and H4) ³ (Figure 1). The core histone octamer is sealed with a single molecule of linker histone H1 that clamps the DNA entry/exit point of the nucleosome to compact the chromatin fiber ⁴⁻⁶.

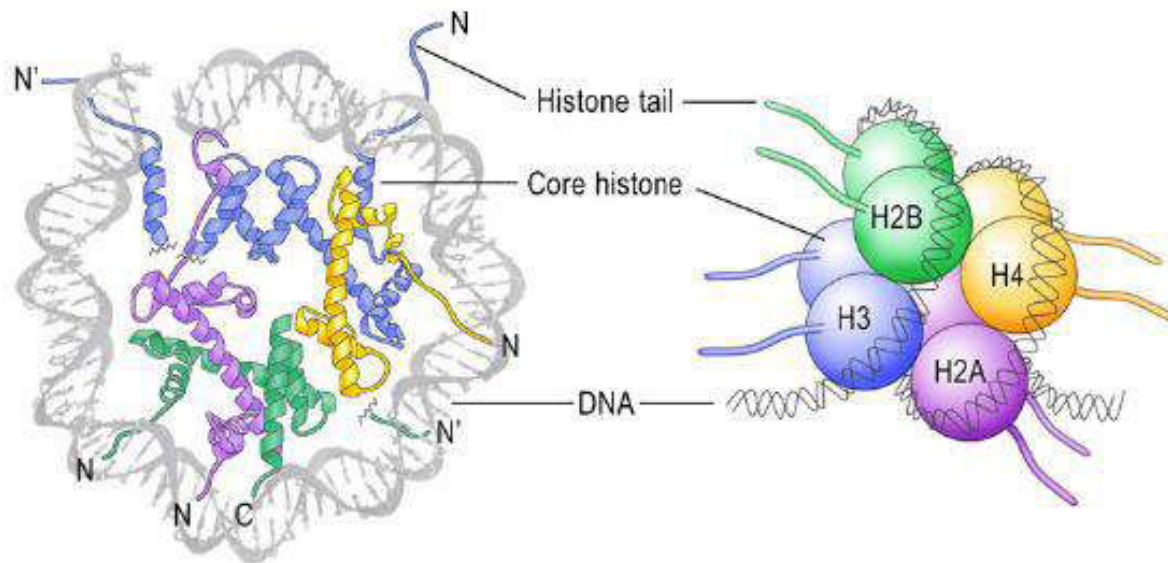


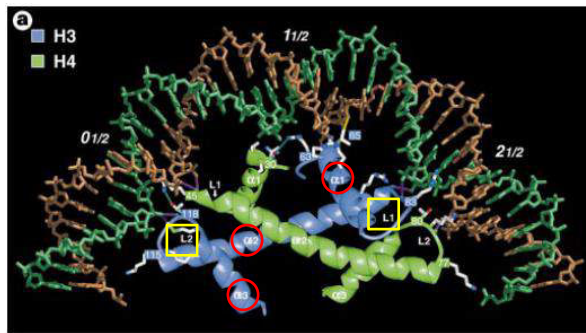
Figure 1. The nucleosome particle

The nucleosome is formed of DNA (146bp) wrapped around an octamer of two copies of each of the four core histone proteins (H2A, H2B, H3 and H4). The eight histone proteins form two types of similarly organized heterodimers, H2A-H2B and H3-H4. The N-terminal tails are flexible and can be modified by post-translational modifications. (Adapted from ⁷)

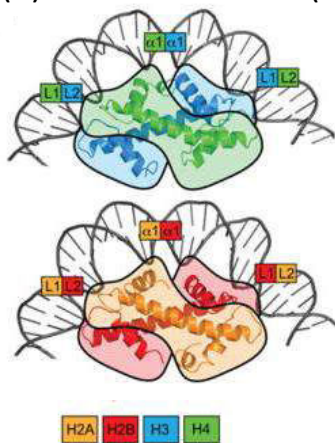
The core histones are positively charged proteins that contain a relatively large amount of lysine and arginine. They carry a C-terminal domain with a “histone fold” motif composed of three successive alpha-helices separated by flexible linkers ⁸ (Figure 2) which is involved in histone-histone and DNA-histone interactions. The DNA-histone interaction occurs every one turn of the DNA helix at the minor groove facing the histone proteins. To describe positions around the nucleosome, the “Super Helical Locations” (SHLs) nomenclature is used ³ (Figure 2), which reference the successive contacts between the DNA minor groove and the core histones as the DNA wraps around the histone octamer. It has been shown that the histone-DNA contacts vary greatly in strength between the central dyad (dyad is defined as SHL 0) and entry/exit sites ^{9,10}. Three strong regions of histone-DNA contacts exist: a strong contact around the dyad and two lesser but energetically significant contacts about 50pb away on either side of the dyad ¹¹.

The core histones also carry N-terminal tails which point outside the nucleosome core particle (Figure 1 and 2) and have been shown to be mobile and flexible using high resolution nuclear magnetic resonance (NMR) ¹². These core histone's tails are highly conserved but frequently modified by post-translational modifications which are involved in the modulation of the chromatin fiber conformation and participate in specific interactions with many chromatin-associated regulatory proteins ¹³.

(a)



(b)



(c)

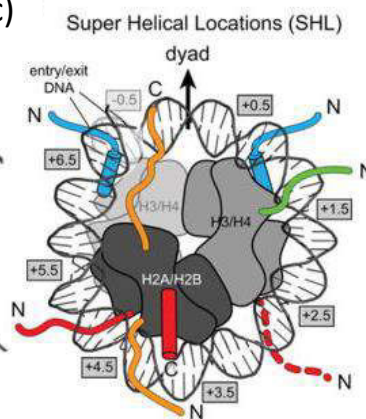


Figure 2 The nucleosome particle

(a) Histone H3-H4 histone fold pair is shown. The three helices (from H3) $\alpha 1$, $\alpha 2$, $\alpha 3$ are shown in blue and red circled, the loops L1 and L2 (from H3) are shown in blue and yellow squares. (Adapted from³). (b) Schematic view of histone-DNA interaction. Each histone dimer grips three consecutive minor grooves of DNA in a similar fashion, with the central contact made by the N-terminal part and backbone of the $\alpha 1$ helix for each histone of the dimer, and two outer contact made by the loops preceding the second helix of one histone and the third helix of the other (L1-L2). (c) Schematic diagram showing positions around the nucleosome given by SHL. The dyad is referred as SHL0. (b and c were adapted from¹¹)

B. The Chromatin Fiber: from 11 nm to 30 nm

When considering the chromatin packing, it seems to follow a hierarchical organization model, where nucleosome arrays first establish a “beads-on-string” fiber of 10-11 nm in diameter, which in turn is thought to be folded into higher ordered fibers of 30 nm, which then are compacted into larger fibers of 100-200 nm¹⁴⁻¹⁶.

30 nm Fiber: Higher Packing of the Chromatin Fiber

In vitro, linear nucleosome arrays fold into a 30 nm fiber upon increasing ionic strength¹⁷ in a process that relies on the length of the linker DNA (i.e DNA segment between two nucleosomes)¹⁸ and the presence of the linker histone H1^{5,14}. Based on *in vitro* data, two models were proposed to represent the structure of the 30 nm fiber. In the solenoid model (Figure 3), the nucleosomes follow each other along the same helical path^{14,19}. Alternatively, in the zigzag model (Figure 3), the chromatin fiber is a two-start helix in which nucleosomes are arranged in a zigzag manner such that a nucleosome in the fiber binds to the second neighbor nucleosome^{20,21}. The existence of the 30 nm fiber *in vivo* have been debated several times in the past decade, and recent studies using cryo-electron microscopy, small-angle X-ray scattering (SAXS), and electron spectroscopy imaging experiments failed to detect the 30 nm fiber in fixed interphase or mitotic cells²²⁻²⁵. These works rather suggested that, in the nucleus, chromatin is mainly composed by irregularly folded nucleosome fibers. Recent work using super-resolution nanoscopy to visualize chromatin fiber with a resolution of approximately 20 nm has shown that nucleosomes tend to cluster in heterogeneous groups (number of nucleosomes per group is cell-specific

and ranges from 2 to 8) interspersed with nucleosome free regions to form chromatin fibers ²⁶. These results can be consistent with the model proposed by Hsieh and colleagues ²⁷. Using a nucleosome-resolution chromosome folding method called Micro-C (Hi-C based method, described in the “chromatin loop” section) ²⁷, they showed that nucleosomes are clustered into a tri- or tetra nucleosome clusters along the chromatin fiber ²⁷.

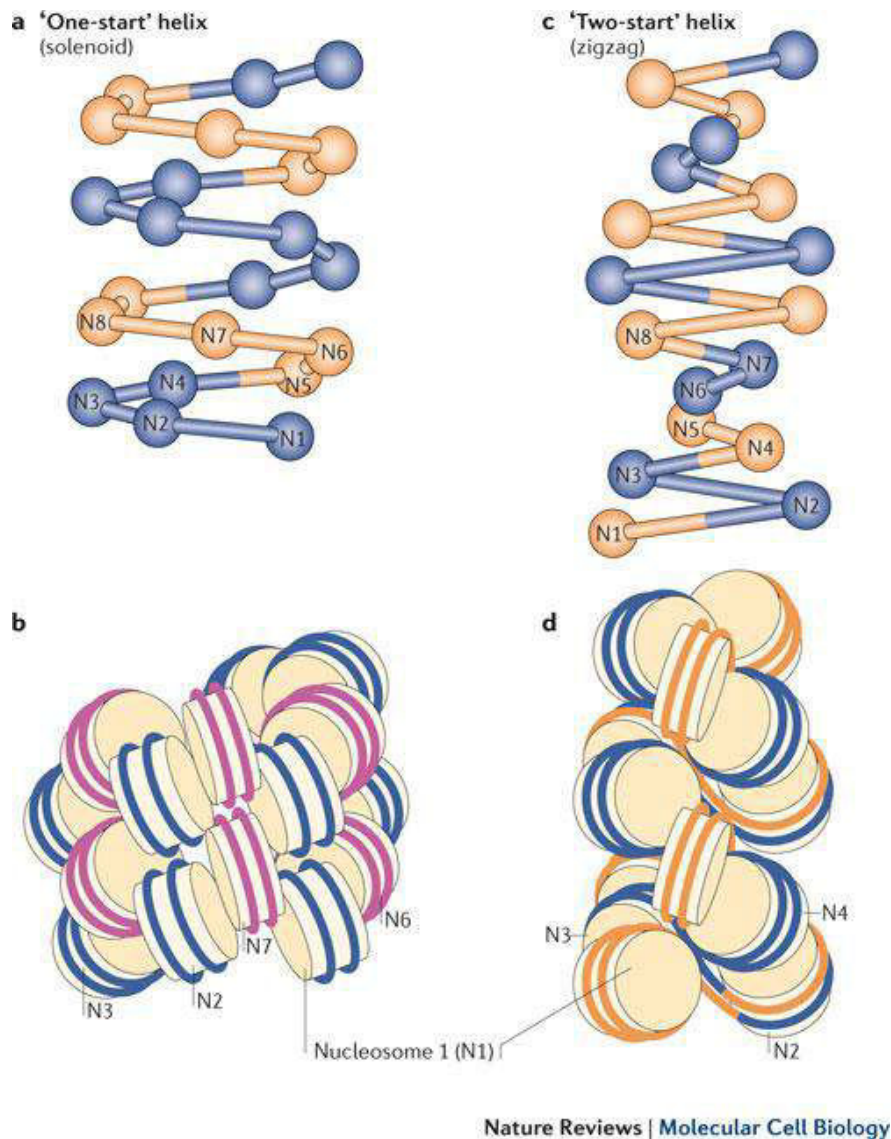


Figure 3.2 Models for chromatin fiber folding

The solenoid model is based on the interactions between consecutive nucleosomes ($n, n + 1$; a,b). The alternative nucleosomes are numbered from N1 to N8. In this model, the 30 nm chromatin fiber is a one-start helix in which a nucleosome interacts with its fifth and sixth neighbor nucleosomes. Alternative helical gyres are colored blue and magenta (b). The zigzag model implies interactions between alternate nucleosomes ($n, n + 2$; c,d). Here, the chromatin fiber is a two-start helix in which nucleosomes are arranged in a zigzag manner. Alternative nucleosome pairs are colored blue and orange (d). (adapted from ¹⁸).

C. The Chromatin Loops

Beyond the internal organization of the chromatin fiber, multiple additional levels of compaction have been described, including the chromatin loops. The looping of the chromatin fiber has been often studied in the transcription context and is thought to be a mechanism that brings genes and distal regulatory elements in close physical proximity ²⁸. Studies of chromatin loops and their organization in domains use either fluorescence in situ hybridization (FISH) ^{29,30} or chromosome conformation capture (3C) methods ³¹. The FISH technique uses fluorescent probes that bind specifically to a genomic DNA sequence allowing a direct visualization of the spatial localization of different loci as well as their spatial proximity ^{29,30}. However, this method is usually applied to few loci at time. In comparison, the 3C based methods including 4C, 5C, Hi-C (Figure 4) are based on cross-linking with formaldehyde to link covalently chromatin segments that have close spatial proximity ³¹. After chromatin fragmentation by restriction enzymes digestion or sonication, crosslinked fragments are then ligated to form a hybrid DNA molecule, each corresponding to an interaction event of a pair of genomic loci ³¹. Detection and quantification of interactions between two loci are dependent on the technique (3C/4C by PCR or sequencing, 5C by sequencing or microarray, Hi-C by sequencing). These approaches allow the measurement of sequence interactions frequencies genome-wide, as well as the mapping of all the possible interactions in a genomic region of interest ^{32,33}.

Pioneering studies in the β -globin locus (a cluster of five genes) showed that the loops formed between the genes and the locus control region (LCR) were specific to

erythroid cells where the genes are active, suggesting that enhancer-promoter interaction may be needed for regulation of transcription ^{34,35}. Since then, a large number of 3C studies have confirmed loop mediated interactions between regulatory elements and their target genes, including the α -globin genes ³⁶, CFTR gene ³⁷, yeast silent mating type loci ³⁸. Loops can be formed in cis (interaction between two sequences located on the same chromosome) or in trans (interaction between two sequences located on different chromosomes) ³¹. A recent work using Hi-C ³⁹ has identified ~10,000 loops all over the genome. The authors identified these loops (in their densest Hi-C map) by looking for pairs of loci that have significantly more contact with one another than they do with other nearby loci. Most observed loops were short (<2 Mb) (Figure 5) and strongly conserved across cell types and between human and mouse. Promoter-enhancer loops were the most commonly observed and usually associated with gene activation. Loop anchors typically occur at specific chromatin domains (detailed in the next section) and bind chromatin non-histone architectural protein CTCF (CCCTC-binding factor) ⁴⁰.

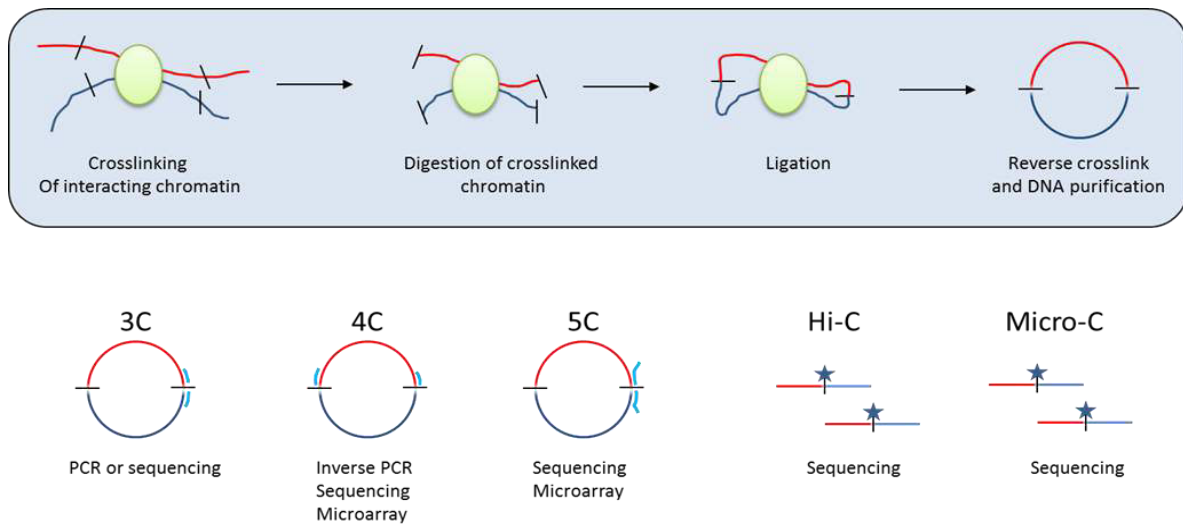


Figure 4. Overview of 3C-based methods

Chromosome conformation capture (3C) is a powerful technique for identifying and mapping long-range interactions, by converting spatial proximity into specific ligation products. The upper panel shows the cross-linking, digestion, and ligation steps common to all of the “C” methods. The resulting sticky ends are filled in with nucleotides. In the case of Hi-C or micro-C one of the nucleotides is biotinylated (blue star). Biotinylated junctions are isolated with streptavidin beads and identified by paired-end sequencing.

The lower panel indicates the steps that are specific to separate methods. In the case of Micro-C, micrococcal nuclease (MNase) is used instead of restriction enzymes to digest chromatin. This enables nucleosome resolution chromosome folding maps, thus, produces maps with higher resolution than Hi-C (restriction enzyme: ~1kb, MNase: ~0.1kb⁴¹).

D. TADs: topologically associating domains

Studies on several organisms including *Drosophila melanogaster*, mouse and human genome using the approaches cited previously (3C/5C, 4C, Hi-C) have identified structural features named topologically associating domains (TADs) which can reach hundreds of kilobases and more in size (Figure 5) ^{42,43}. TADs are defined as chromatin modules which favor internal, rather than external, chromatin interactions and are delimited by boundary elements such as insulators sites, which prevent the interaction between two TADs ⁴⁴. TADs divide chromatin into distinct and autonomous regions which display distinct features in terms of histones modifications, gene expression, association with lamina, or replication timing ⁴⁵⁻⁴⁸. Groups of TADs sharing similar features are reminiscent of euchromatin/heterochromatin organization of the genome, where euchromatin appears as a loosely packed and actively transcribed chromatin whereas heterochromatin occurs as densely packed and silent repressed chromatin. The mechanisms that establish TADs remain unclear. Recent work has established a correlation between the binding of specific factors and the boundaries that defines the TADs ⁴⁹, and the deletion of a boundary between two TADs in the case of the X-chromosome led to partial fusion of flanking TADs ⁵⁰ suggesting a dynamic organization of the TADs rather than a rigid compartmentalization.

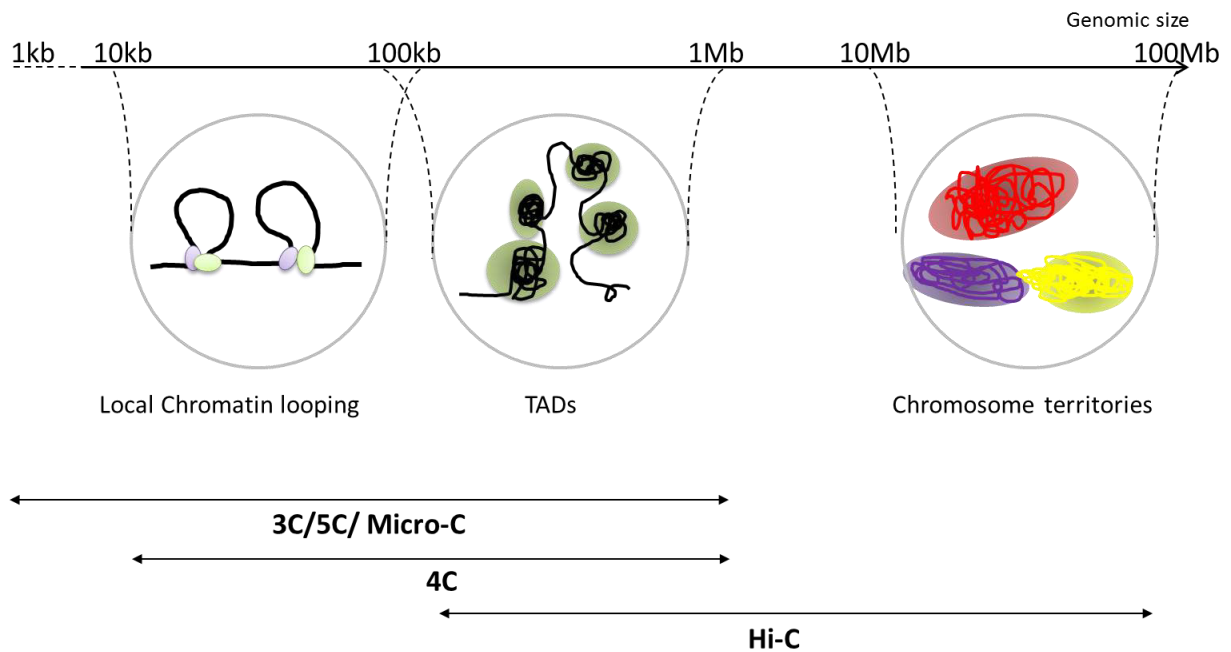


Figure 5. Hierarchical organization from chromatin loops to chromosome territories. Rough-size of chromatin loops, TADs and chromosome territories. These sizes have been estimated using 3C-based methods.

E. The Chromosomes Territories

At a large scale, the highest chromatin organizational level is described as a territorial organization. The term “territory” refers to a discrete region in an interphase nucleus where each chromosome is confined. This model has been proposed more than a century ago by Rabl and Boveri and validated in the 80s by Cremer and colleagues using UV-laser micro-irradiation experiments ⁵¹.

The chromosome territories can be visualized by chromosome painting using FISH technique in the whole nucleus where fluorescently labeled probes bind to single chromosomes ^{52,53} (Figure 6). This approach allows the assessment of the volume occupied by each chromosome into the interphase nucleus ⁵⁴. It is also possible to simultaneously visualize several chromosome territories using different fluorescent probes ^{52,53} (Figure 6) which allow analyzing their positions relative to each other.

Chromosome territories are not organized randomly into the nucleus. Gene-rich chromosomes tend to be located in the nuclear interior while gene-poor chromosomes are typically found at the nuclear periphery ^{55,56}. Historically, the first proposed model suggested that the nucleus is compartmentalized into chromosome territories (CTs) and interchromatin compartments (ICs) which contain proteins and complexes required for several cellular processes including replication, transcription, splicing and repair ⁵². In this model, each chromosome is confined in its own territory and interactions with its neighbors are very rare to prevent extensive intermingling between two CTs. Nevertheless, it was shown by high resolution in situ hybridization approach that preserves chromatin structure that chromosomes intermingle in an

interphase nucleus in human cells ⁵⁷. The degree of overlapping between specific chromosome pairs correlates with the frequency of chromosome translocations and is influenced by transcription-dependent interactions between chromosomes ⁵⁷.

In summary, the chromatin undergoes multiple compaction steps to organize the genetic information. However, we still do not clearly know how much the higher folding levels depend on the lower ones.

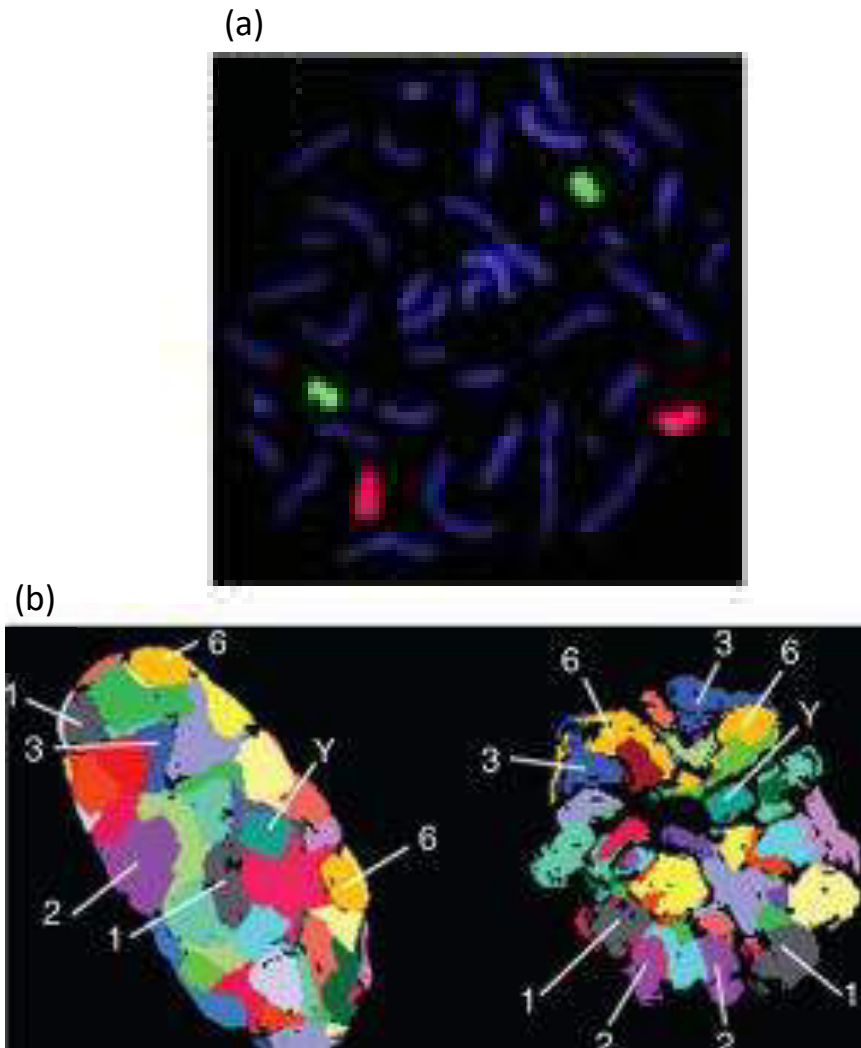


Figure 6. Chromosome territories visualization

(a) Visualization of individual chromosomes in a human metaphase plate (chr.18 in red, chr.19 in green) after fluorescence in situ hybridization (FISH) using labeled chromosome painting probes. (b) Simultaneous labelling of all chromosomes in a human fibroblast nucleus (left) and a prometaphase rosette (right) by multi-color FISH. (Adapted from ⁵⁸)

II. Chromatin Dynamics

At a global chromosome scale, the chromatin is usually described as a stable entity throughout interphase ⁵⁹. However, cells undergo dynamic biological processes, including replication, transcription and repair that require dynamic changes in the local chromatin architecture. Studies based on imaging approaches have begun to explore the biological implications of chromatin dynamics within the nucleus. In both prokaryotes and eukaryotes ^{60,61}, micron-scale movements have been reported. Clearly, the chromatin dynamics is a very complex phenomenon operating at different scales in time and space. In this part, I will describe the current status about chromatin dynamics in the absence of DNA damage, from a nucleosome scale to a global chromosome scale, obtained through the development of biochemical and imaging techniques.

A. Chromatin Dynamics at the Nucleosome Scale

Constant changes in nucleosomes composition and shifts in nucleosome positioning ensure that chromatin is dynamic ^{62,63}. Chromosomal core histones have a constant turn-over ⁶⁴. The canonical core histones are mainly incorporated during replication-coupled nucleosome assembly ^{65,66}. The histone variants are synthesized throughout the cell cycle and can be incorporated into chromatin in a replication-independent manner during G1 and G2 ^{65,67}. The chromatin composition in terms of histone variant correlates with its functional properties and affects chromatin dynamics locally or in a global manner ⁶⁸. For example, the H3 variant H3.3 and the H2A variant H2A.Bbd co-localize predominantly with transcriptionally active

chromatin ^{69,70}, CenH3 or CENPA is only found at centromeres, where it generates a chromatin structure suitable to the formation of the kinetochore ⁷¹, and the variant histone macroH2A is enriched at the transcriptionally silent X-chromosome of female mammals ⁶⁸. If, at the nucleosome-scale, we now have a relatively precise characterization of the histone turnover, our description of the nucleosome motions within the interphase nucleus remains quite poor. Using fluorescence microscopy approach, Nozaki and colleagues ⁷² have imaged single tagged nucleosomes in living cells. They observed local movements of nucleosomes (50nm displacements per 30ms) ⁷². These movements appeared to be similar in interphase and mitotic chromatin ⁷². Due to resolution limitation of current live cell imaging tool, the study of chromatin dynamics at this scale remains a difficult challenge.

B. Chromatin Dynamics at the Genomic-Locus Scale

The development of fluorescent live cell imaging microscopy and fluorescent labelling of genomic loci makes possible the visualization and tracking of genomic locus dynamics. In the 90s, Fluorescent Repressor Operator System (FROS) approaches (Table 1) opened the door to the real-time study of chromosomal loci positions in living cells ⁷³. These techniques are based on the insertion in the genome of numerous repeats of bacterial sequences called “operator” (Lac/ Tet/ Lambda operator) to which fluorescently tagged repressor proteins bind. More recently, new systems arose based on the same principle but without the drawbacks (fragile sites, interfering with transcription) linked to the repetitive nature of operator sequences ⁷⁴⁻⁷⁶. Other techniques have been developed to track chromatin at a genomic scale in living cells and rely on the incorporation of fluorescently labelled deoxy-or-ribo NTP

analogues (Table 1 and figure 7) ⁷⁷. With this approach, genomic loci can be visualized thanks to the incorporation of fluorescent analogues into replicating genomic DNA during S phase.

| Technic | Applications/Advantages and drawbacks | DNA sequence integration |
|--------------------------|---|---|
| FROS sequence | chromosomal loci labelling, fragile sites, interfering with transcription | insertion repetitive DNA (arrays of 5-10kb) |
| Fluorescent dNTPs | whole genome labelling | No |
| Photo-activable Histones | whole genome, stable fluorescence activation of individual chromosomes | No |

Table 1. Major chromatin labelling tools used for monitoring chromatin dynamics in living cells.

Using these approaches to track chromosomal loci motion, it has been shown that depending on the nuclear localization, tagged loci do not follow the same dynamics. Loci located at nucleoli or at the nuclear periphery are less mobile than more nucleoplasmic loci. Disruption of the nucleoli or loss of Lamina A (protein of the nuclear envelope) increases the mobility of associated loci which mean that physical

attachment of the chromatin to a sub-nuclear compartment constrains its mobility^{78,79}.

To characterize quantitatively the mobility of the chromatin motion, the classical approach is to calculate the Mean Square Displacement (MSD) (Figure 7). Using the MSD, it is possible to estimate quantitative movements parameters such as the diffusion coefficient and radius of constraint (volume within which the particle moves). Using this method, experiments have shown that the diffusion coefficient of chromatin movement ranges from 10^{-4} to $10^{-3} \mu\text{m}^2/\text{s}$ ^{80,81} (by comparison, the diffusion coefficient of a 30 kD globular protein in mammalian nuclei is several order of magnitudes higher, $10\text{--}40 \mu\text{m}^2/\text{s}$)⁸².

It also interesting to mention that mobility is usually higher in yeast cells than in mammalian cells, which may be explained by the chromosomes size which is longer in mammals than the yeast ones and thus more difficult to move⁸³.

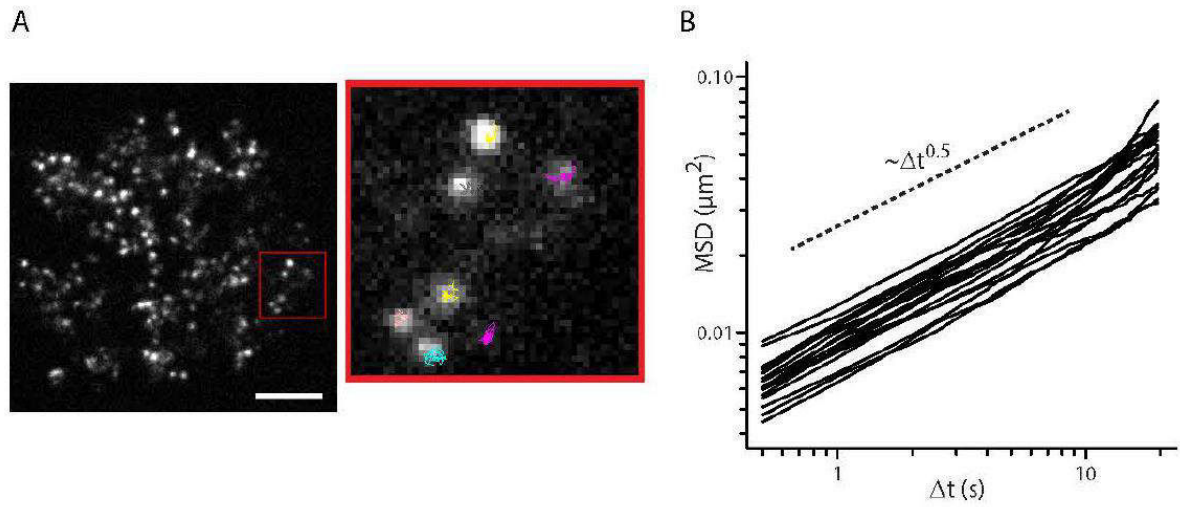


Figure 7. Example of Chromosome labelling with dUTP of U2OS nucleus and corresponding MSD analysis.

(A) Nucleus of a U2OS cell with its DNA labeled using fluorescent nucleotides. The magnification shows examples of trajectories displayed by the labeled chromatin foci. Bar = 5 μ m. (B) Curves of the mean square displacement (MSD) calculated from the trajectories of the labeled chromatin foci. (Adapted from ⁸²).

C. Chromatin Dynamics at the Chromosome Territory Scale

A chromosome territory (CT) can be considered as an entity which can be morphologically deformed or mobile inside the nucleus. To assess the deformation of CTs, photo-bleaching patterns of GFP-tagged H2B have been used ^{59,84}, showing globally no deformation of CTs during interphase ⁸⁴⁻⁸⁶ except after the first hour of G1 ⁸⁶. In addition, using, photo-activable fluorescent proteins fused to histones ^{87,88} or incorporation of fluorescent analogues which after multiple cell divisions form groups of labeled replication foci identifying individual chromosomes ⁸⁴, it has been shown that the position of CTs regarding to each other's did not change inside the nucleus during interphase ^{84,88}, and become more mobile during mitosis ⁸⁴.

D. Processes that Influence Chromatin Dynamics

The chromatin mobility seems to be dependent on the nuclear environment and mainly due to ATP-dependent processes rather than thermal fluctuations ^{60,61}. Temperature modulation leads to molecular agitation but could not explain the increased magnitude of locus motion suggesting the involvement of an additional factor ⁸⁹.

Several active processes could be responsible for chromatin dynamics such as transcription or differentiation. In the context of transcription, changes in chromatin dynamics have been reported to correlate with modulation of transcription levels ⁹⁰ as well as with ATP-dependent chromatin remodeling events ⁹¹.

In this way, chromatin dynamics could reflect changes in nuclear position inside the nucleus after transcription activation, for example IgH and Igk genes change their

position inside the nucleus by moving from the nuclear periphery in hematopoietic precursors toward the interior as they are activated in B-cells ⁹². In addition, it has been shown that co-regulated genes located on the same or different chromosomes could be clustered into nuclear “transcription center” ⁹³. Another modality of chromatin motion is the re-localization of a gene locus outside of its chromosome territory after transcription activation. Such re-localization has been linked to high expression of larger chromatin domains such as the Hox gene cluster ⁹⁴.

And more recently, some studies have begun to examine the chromatin mobility in contexts other than transcription, including DNA damage context and this aspect will be discussed later (see section “chromatin remodeling at DNA damage sites”, part A).

III. DNA Repair

Every day, many thousands of DNA lesions arise in each human cell ⁹⁵. The majority of these lesions occur through endogenous processes such as byproducts of normal cell metabolism (DNA replication, respiration...), as well as through exogenous sources such as radiations and toxic environmental chemicals ^{96,97}. We can distinguish DNA lesions with a physiological role that occur during developmentally regulated genome rearrangements in lymphocytes and germ cells ^{96,98,99} from the ones arising from internal or external assaults to the DNA. Both types of lesions can lead to deleterious effects which result in mutations and/or chromosomal aberrations ^{100,101}. To avoid these deleterious effects, cells have developed several mechanisms to correct DNA damages. Thereby, genome integrity is preserved through DNA damage signalling and repair machinery. Depending on the type of damage that occurs, the cells activate specific signalling pathways that allow the repair machinery to correct the damage, re-establish the integrity of the genome and prevent their transmission to daughter cells ^{97,102}. Defects in DNA damage signalling or repair machinery contribute to various disorders including the Ataxia-Telangiectasia (A-T) hereditary disorder, Fanconi Anemia, Cockayne's syndrome, Xeroderma pigmentosum, breast cancer and many others ^{103,104} highlighting the critical importance of an efficient DNA damage response and repair for cell viability. In this section, I will give an overview of the DNA damage response and repair, with a focus on the Poly (ADP-ribose)-ylation pathway.

A. The Different Types of DNA Damages

There are many types of DNA lesions from a structural point of view (figure 8), including base modifications (deamination), base losses, base-pair mismatches, covalent modifications (pyrimidine dimers), bulky adduct, single strand breaks (SSBs) and double strand breaks (DSBs) ¹⁰⁵. The majority of these damages are resolved by specialized mechanisms developed by cells. For example, base incorporation errors or base damages are repaired by the mismatch repair (MMR) ¹⁰² (Figure 8) and the nucleotide excision repair (NER) removes bulky DNA adducts ¹⁰² (Figure 8). The SSBs arise from several sources of damage: oxidative attack by endogenous reactive oxygen species (ROS), during the base excision repair (BER), from errors of DNA topoisomerase 1 (TOP1) activity or from DNA replication ¹⁰⁶. They are repaired by the SSB and BER repair pathways ¹⁰² (Figure 8). The DSBs are induced by ionizing radiations or by enzymes that cleave DNA such as topoisomerase 2 (TOP2) ¹⁰⁷. The DSBs are repaired by two main repair pathways (Figure 8), homologous recombination (HR) and non-homologous end joining (NHEJ) ¹⁰⁷.

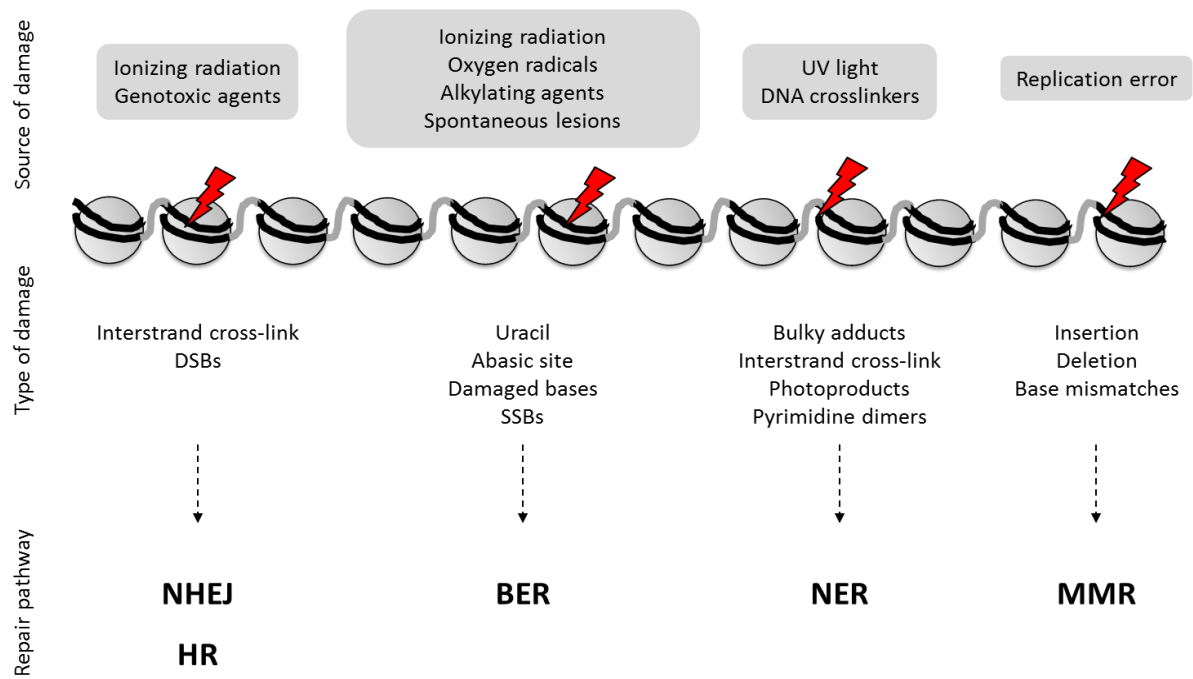


Figure 8. The source and type of DNA damage and their main associated repair pathways

The cells are constantly exposed to exogenous as well as endogenous sources of damage. Depending on the type of damage, cells will activate particular repair pathways to repair the damage.

B. The Canonical Repair Mechanism

Given the heterogeneity of DNA lesions, eukaryotic cells use distinct, albeit partly overlapping repair pathways. In this way, according to the type of lesion, each repair pathway activates specific signalling pathways leading to a sequential recruitment of sensors, transducers, mediators and effectors.

The canonical repair mechanism follows a relay race-like pattern ⁹⁶ (Figure 9). First, the damage is recognized by the sensor proteins (Figure 9). For example in the case of DSBs and SSBs damage, ATM, ATR, DNA-PK or PARP1, are recruited in order to sense and signal the damage by modifying histones by phosphorylation (ATM, ATR and DNA-PK are kinases) or Poly(ADP-ribose)-ylation (PARP1 is an ADP-ribose transferase) ^{106,108}. Then, the mediator proteins are recruited (Figure 9). For instance in the context of DSBs, Mdc1, 53BP1, MRN complex or BRCA1 act directly downstream of sensor proteins ¹⁰⁹. They act both as recruiters of additional substrates of sensor proteins and as scaffolds upon which proteins complexes are assembled ¹⁰⁹. And finally, effector proteins (Figure 9) such as checkpoint kinases Chk1 and Chk2 elicit the appropriate response to the damage by, for instance, delaying transiently the cell cycle allowing the correct repair of damaged chromatin by repair machinery ¹¹⁰. The repair itself of the damage involves several factors such as DNA polymerases and DNA ligases, which have the ability to re-establish the integrity of DNA double helix ¹⁰⁹.

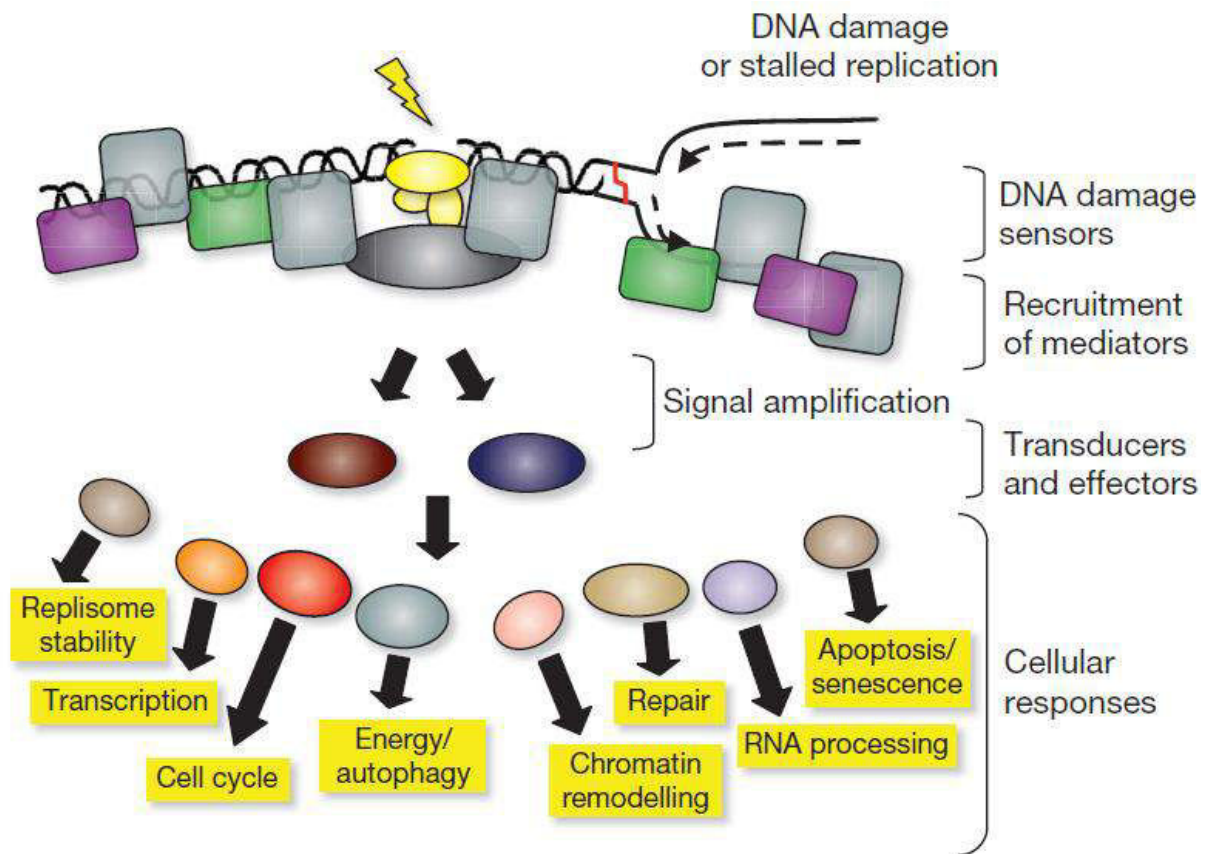


Figure 9. The Canonical repair mechanism

Successively, sensors factors will sense the damage and activate the signalization of the damage, and then mediators will serve as a platform of recruitment of repair factors, transducing the signal and finally, effectors will modulate the cell response by controlling the cell cycle and allowing the repair. (Adapted from ⁹⁶).

Concomitant to these signalling steps, the chromatin undergoes structural changes to allow the correct repair of the damage ¹¹¹. The current model describing these structural changes is known as the “access-repair-restore” model (Figure 10). In this model, the access to DNA break lesions by repair machinery is ensured by a destabilization of the damaged chromatin, which is subsequently reorganized after completion of repair ¹¹². This specific step of the repair mechanism will be discussed later (see the section “chromatin remodeling at DNA damage sites”).



Figure 10. The “access-repair-restore” model.

This model illustrates the chromatin re-organization that occurs in response to damage induction. The repair of the damage requires the access to the damaged area, thus several factors involved in the chromatin remodeling process are recruited to the damage in order to regulate access to the damaged chromatin. Then the repair machinery is recruited to the damage to complete the repair and finally the chromatin architecture is restored to allow the continuity of cell activities (Transcription, growth, division, cell cycle...).

C. PARP Signalling Pathway

The poly(ADP-ribose)ylation or PARylation is a covalent post-translational modification (PTM) of proteins by ADP-ribose polymers ¹¹³. PARylation is an abundant and dynamic modification which is involved in several biological processes such as transcription ¹¹⁴, replication ¹¹⁴, cell cycle regulation ¹¹⁵ and apoptosis ¹¹⁶. However, the most extensively studied role of PARylation is its implication in the DNA damage response ¹¹⁷, where it seems to be a key regulator of chromatin architecture and as well as DNA repair machinery.

a. The PARylation: A Dynamic Post-Translational Modification

The PARylation modification is catalyzed by PAR polymerases (PARP) (Figure 11) whose founding member is PARP1 in humans. This family comprises now 17 members identified from homology to PARP1 ^{118,119}. Not all PARP enzymes share the same PARylation activity with PARP1. PARP2 and Tankyrases are able to synthesize and transfer **poly**(ADP-ribose) onto proteins, whereas many other PARPs are only **mono**(ADP-ribose) transferases and can only make very short ADP-ribose oligomers ¹²⁰. PARP1 is the main poly (ADP-ribose) producer and also the major acceptor of poly (ADP-ribose) ¹²¹. The PARPs enzymes transfer the first ADP-ribose unit from co-enzyme NAD⁺ onto a protein acceptor (Figure 11), on aspartate or glutamate residues ¹²²⁻¹²⁶. Some PARPs such as PARP1/2 and Tankyrases are able to repeat the transfer of additional ADP-ribose group following the establishment of the first ester bond to produce long chains of poly-ADP-ribose (PAR) ¹²⁷⁻¹²⁹. The PAR chains can reach up to 200 ADP-ribose units in length and some reports suggest that

PAR chains can contain branching^{130,131}. Protein-free PAR also exists in the cell, but their exact role remains unknown. When they are in excess, they can trigger cell death via parthanatos^{121,132}.

Like other PTMs, the cellular level of PAR is dynamic and tightly controlled. The PAR half-life (i.e the time it takes to remove half of the PAR chains that are attached to proteins) ranges from 1 to 6 min¹³³. The PAR chains are removed by the PAR Glycohydrolase (PARG) enzyme, which is the major enzyme for the removal of cytoplasmic and nuclear PAR (Figure 11)¹³⁴⁻¹³⁶. To efficiently reverse PARylation, PARG enzyme cleaves the O-glycosidic ribose-ribose bonds within the PAR chains, releasing free ADP-ribose molecule^{137,138}. More recently, it has been reported that a second hydrolase ARH3 exhibits a similar activity^{139,140}. However, PARG and ARH3 enzymes are not able to hydrolyze the proximal ADP-ribose group that is directly attached to the acceptor proteins^{137,139}. Recently, three enzymes have been reported to possess an enzymatic activity to cleave mono-(ADP-ribose)-ylated protein substrate, TARG1/C6orf130¹²⁵, MacroD1 and MacroD2^{121,141,142}.

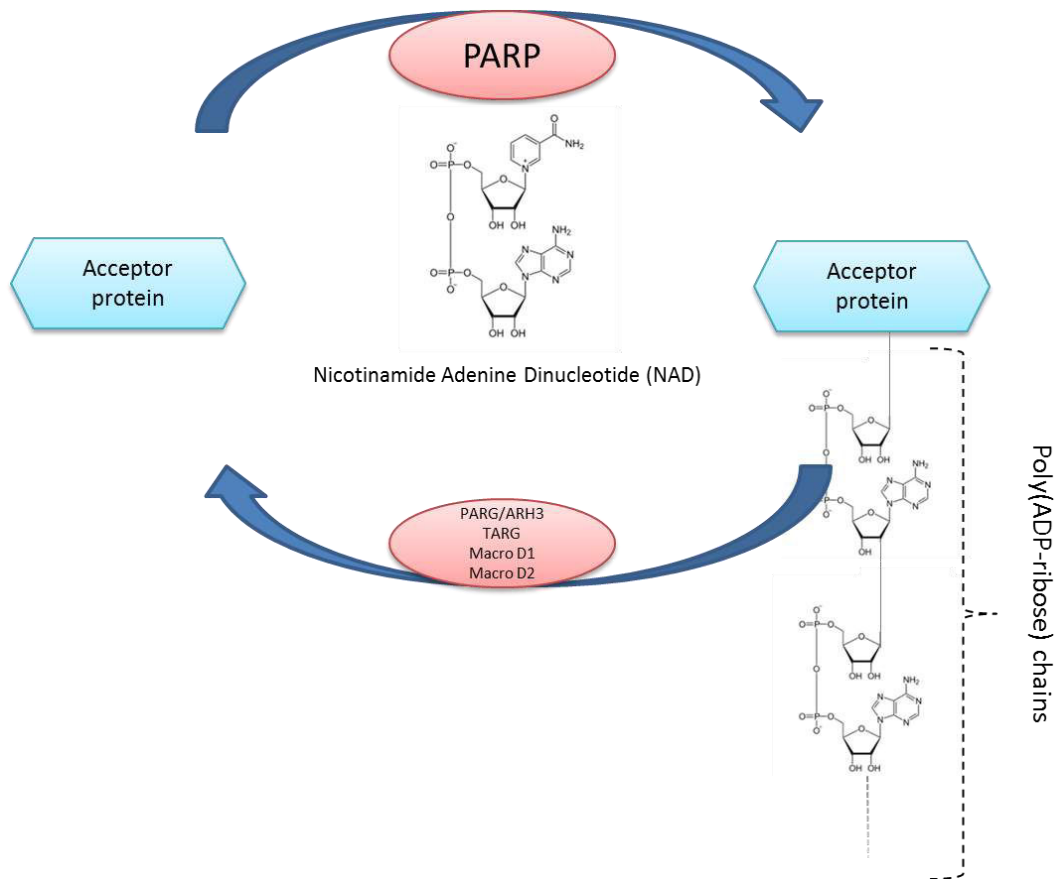


Figure 11. The Poly(ADP-ribose)-ylation as a post-translational modification.

To PARylate proteins, PARP polymerases transfer ADP ribose units from NAD⁺ to acceptor proteins. This reaction is reversible and PAR chains can be removed by specific enzymes (PARG, ARH3, TARG, MacroD1/D2).

a. Classical Functions of PARP1 in SSB Repair

Historically, the role of PARP1 in BER/SSB repair pathway has been identified in PARP1^{-/-} mice which exhibit increased sensitivity to ionizing radiation and oxidative damages. As result, cells derived from these mice are hypersensitive to alkylating reagent ¹⁴³. The BER/SSB repair pathways serve to correct lesions induced by a variety of sources including reactive oxygen species damages and ionizing irradiations. In order to repair oxidized bases, the cells transform the lesion into a SSB intermediate damage site leading to the recruitment of PARP1 and its activation ¹⁰⁶. In the BER process, damaged bases are cleaved by DNA glycosylases, producing apurinic/ apyrimidinic sites (AP sites) also known as abasic sites, which next are processed by AP endonuclease (APE) to create a SSB ⁹⁵. Then, this SSB is repaired through two distinct pathways: short-patch repair and long-patch repair pathways (Figure 12). These two pathways are different in terms of patch sizes and DNA repair factors involved ¹⁰⁶(Figure 12).

PARP1 rapidly binds to SSB and subsequently modifies itself and other substrates to further recruit repair proteins at the breakage site (Figure 12). PARP1 has been shown to interact with the SSB repair factor XRCC1 ¹⁴⁴, which plays a role in the SSB repair pathway ¹⁰⁶. In addition, PARP1 is able to interact with other factors of the BER/ SSB repair pathway including the DNA glycosylase 8-oxoguanine glycosylase 1 (OGG1), DNA polymerase β , DNA ligase III, PCNA and aprataxin ^{145,146}.

The binding and activity of PARP1 at DNA lesion sites is transient, because poly ADP-ribosylated PARP1 rapidly dissociates from DNA and PAR chains are rapidly

degraded by PARG¹²¹. It has been shown that PARP1 might accelerate SSB repair by promoting the focal accumulation of SSB repair factors bearing dedicated PAR recognition modules at DNA lesion sites^{109,120}. For instance, FHA (ForkHead Associated) domains of APTX (Aprataxin) and the BRCT (BRCA1 C Terminus) domains of XRCC1 recognize Poly-ADP-ribose^{120,147}. However, PARP1 is not only involved in SSBs repair but also in DSBs repair pathways including HR and NHEJ.

b. PARP1 Role in DSB Repair

To repair DSBs, cells can activate two major DNA repair mechanisms, Homologous recombination (HR) and classical non-homologous end joining (C-NHEJ). HR requires a sister chromatid to be used as homologous template for the accurate repair during S and G2 phases¹⁰⁷. NHEJ does not need a template DNA and can operate throughout the cell cycle¹⁰⁷. Several secondary pathways arise in more specific situations: Single Strand Annealing (SSA) if the DSBs are in repeated regions¹⁴⁸, alternative non-homologous end joining (alt-NHEJ) when C-NHEJ is defective¹⁴⁹...

PARP1 Role in HR

In PARP-depleted cells, HR occurs normally, suggesting that PARylation has little impact on HR¹⁵⁰. Nevertheless, PARP1 has been shown to be involved in HR-mediated repair (figure 12) and reactivation of stalled replication forks^{151,152} by recruiting Mre11^{152,153} and NBS1¹⁵³ proteins which are part of the MRN (Mre11/Rad50/Nbs1) complex (implicated in the DNA end resection).

PARP1 Role in C-NHEJ

In the C-NHEJ pathway, the Ku70/Ku80 heterodimer is recruited to DNA lesion sites, which in turn leads to the recruitment of DNA-PK catalytic subunit ¹⁵⁴. PARP1 has been shown to form a complex with DNA-PKcs ¹⁵⁵ to elicit an architectural rearrangement of the DNA-PK-mediated synapsis ¹⁵⁵ and to interact with several C-NHEJ factors including APLF and DNA ligase IV ^{156,157} (Figure 12).

PARP1 Role in Alt-NHEJ

Alt-NHEJ is a new DSB repair pathways that occurs when C-NHEJ is deficient ¹⁵⁸. Alt-NHEJ is initiated by the resection of damaged DNA ends and uses complementary microhomologies sequences of 1-10 nucleotides to guide the DNA repair ¹⁵⁸. In this pathway, PARP1 is able to recognize the broken ends and to create a scaffold for the recruitment of additional alt-NHEJ factors (Figure 12) including DNA ligase III /XRCC1 complex and polynucleotide kinase/phosphatase (PNKP) ¹⁵⁷.

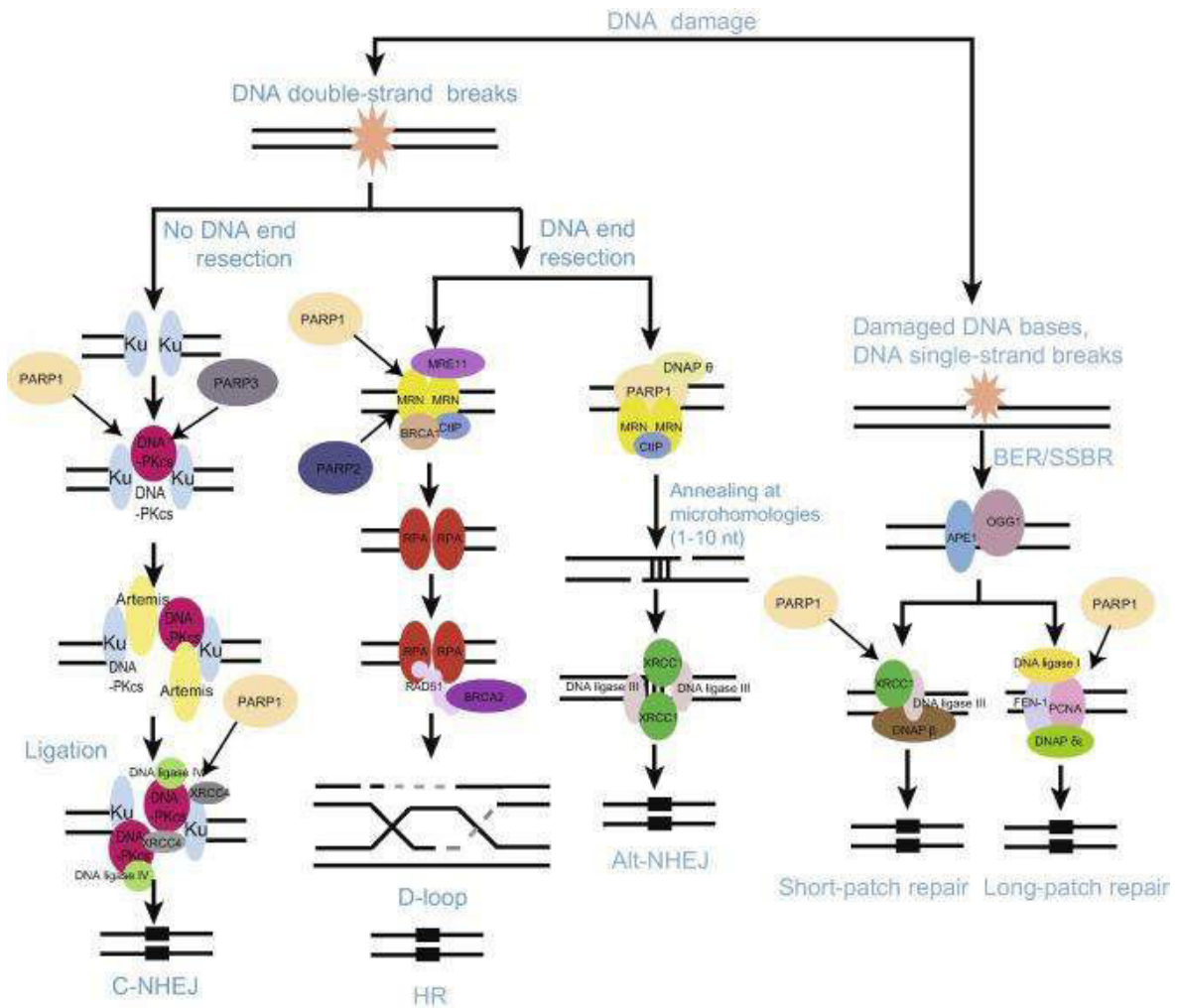


Figure 12. PARP1 in SSBs and DSBs repair pathways

Schema showing DNA repair pathway regulated by PARP1. PARP1 interact with several factors involved in these different repair pathways in order to promote their recruitment to the site of DNA lesion. (adapted from ¹⁵⁷)

IV. Chromatin Remodeling at DNA Damage Sites

The packing of DNA into nucleosomes as well as higher order chromatin folding levels are thought to act as a barrier for the binding of proteins involved in DNA-dependent processes such as DNA repair proteins which need to bind DNA to allow the repair of the breaks ¹⁵⁹. To regulate DNA accessibility, cells have developed mechanisms to remodel chromatin thus allowing the timely binding of specific DNA repair proteins. Thereby, cells have at their disposal several interdependent mechanisms to remodel the architecture of the chromatin fiber. First, post-translational modifications (PTMs) that are present on the tails of the core histones may, *per se*, impact on the chromatin structure. This is true in particular for PAR chains ¹⁶⁰. Second, these PTMs are also able to recruit factors involved in the regulation of chromatin structure ¹⁶¹. Among those, chromatin remodeling enzymes can displace the nucleosomes along and from the DNA to expose or protect underlying DNA to regulatory factors that are involved in DNA repair ¹⁶². In addition, histone chaperones and histone variants are also part of these nucleosomes remodeling mechanisms ^{163,164}. In this part, I will describe the local and large scale changes in chromatin architecture and dynamics occurring at the DNA breaks, explain the molecular players involved in such processes and discuss the functional roles of chromatin remodeling at DNA damage.

A. The Multiscale Changes in Chromatin Architecture and Dynamics at DNA Breaks

The changes in chromatin architecture at DNA breaks upon DNA damage have been mainly studied for the most deleterious form of DNA damage namely DSBs. As mentioned above (Chapter III, DNA repair), eukaryotic organisms activate two main mechanisms for the repair of DSBs: HR and NHEJ. The activation of these repair pathways might be associated with changes in chromatin architecture. I will start by describing the situation in yeast and then I will talk about mammalian cells.

In yeast, the induction of DSBs by restriction enzymes or genotoxic treatment is associated with an expansion of the nuclear zone explored by the moving damaged locus. The extent of this expansion is variable and relies on the locus of interest and the ploidy of the cell ^{165,166}. Indeed, DNA damage induction in diploids cells increases the dynamics of the damaged chromatin as well as the global chromatin mobility ¹⁶⁷. In similar DNA damaging conditions, this increase of the overall mobility is not observed in haploid cells ¹⁶⁵. Moreover, the modulation of chromatin motion at DNA break sites seems to rely on the type of DNA damage since spontaneous lesions occurring during DNA replication display decreased mobility compared to non-damaged DNA ¹⁶⁸. When DSBs persist and no homology is found, homology pairing-associated protein Rad 51 remains on the broken chromatin ¹⁶⁹. This indicates a persistent homology search which at the end leads to the relocation of the DSBs to nuclear periphery ^{169,170}.

The situation in mammalian cells appears much more complex compared to yeast where we have nowadays a good characterization of the modulation of chromatin dynamics after DSBs induction. Similar to yeast, damaged chromatin in mammalian cells could gain mobility and in some cases was found to relocate to repair clusters^{171,172}. It has shown that DSBs produced by the topoisomerase II inhibitor etoposide are substantially more mobile than DSBs induced by ionizing radiation¹⁷¹ suggesting that chromatin mobility upon DNA damage might depend on the type of DNA damaging method. In addition the mobility of damaged chromatin might be dependent on chromatin compaction state. Indeed, it has been shown that DNA breaks in euchromatin region were more mobile than those in condensed heterochromatin¹⁷¹. Despite all these data, numerous reports failed to observe pronounced changes in chromatin mobility upon damages regardless to the source of damage^{87,173-176}. Kruhlak and colleagues⁸⁷ have shown in their study that the mobility of chromatin pre-sensitized by Hoechst and damaged by laser UV light was similar to that of intact chromatin when measured over a 20-minute period.

Alongside the characterization of chromatin motion, many works also investigated the changes in compaction state at DNA breaks. Early investigations have shown that UV-induced DNA damage leads to nucleosome rearrangements and increased sensitivity to nucleases¹⁷⁷. This sensitivity to nucleases reflects a higher accessibility at the nucleosomal level upon DNA damage induction, which is correlated with chromatin relaxation at the micron-scale visualized by fluorescence microscopy^{87,178}. This chromatin relaxation at the damaged area is followed by a

slow recondensation of the chromatin ¹⁷⁹ which, ultimately, can lead to chromatin compaction states higher than before DNA damage ¹⁸⁰.

Finally, particular chromatin remodeling processes seem to be observed for DNA lesions induced in heterochromatin. In these areas, characterized by highly repetitive sequences, it has been shown that DSBs tend to relocate into euchromatin area where γ -H2AX foci are formed ^{181,182}. Similar mechanism has been observed in *Drosophila melanogaster* ¹⁸³ which can be interpreted as a limitation of the risk of deleterious chromosomal rearrangement within highly repetitive heterochromatin. In addition, it has been shown that breaks in heterochromatin move to the nuclear periphery to continue repair ¹⁸⁴. While our description of the chromatin remodeling mechanisms at DNA breaks remains unclear, different molecular players that may directly act on the chromatin architecture are recruited at the DNA breaks.

B. The Molecular Players

Multiple proteins recruited to the damaged chromatin are involved in chromatin remodeling. We can distinguish four classes of players that are thought to be involved in the chromatin remodeling process that occurs at DNA breaks: (a) histone variants, (b) Post-translational modifications, (c) histones chaperones, and (d) chromatin remodelers.

a. Histone Variants

The incorporation of histone variants into the nucleosome is one of the strategies that the cell uses to modulate chromatin structure. Several histones variant have been

described to modulate the chromatin architecture including H2A Bbd¹⁸⁵, H2AZ^{186,187}, macroH2A¹⁸⁸, H3.3¹⁸⁹ and CENP-A¹⁹⁰.

In the context of DNA damage, the histone variant H2AZ has been shown to play a role in the control of chromatin remodeling during DSB repair¹⁹¹. H2A.Z is exchanged onto nucleosomes at DSBs by the p400 remodeling ATPase at DSBs and shifts the chromatin to an open conformation¹⁹¹. In addition, the histone chaperone APLF binding to histones in the response to DNA breaks has been suggested to promote histone removal and recruitment of the MacroH2A1 histone variant¹⁹² which was shown to re-organize chromatin structure in response to PARylation^{193,194}.

b. Post-Translational Modifications (PTMs)

Post-translational protein modifications may be involved in chromatin remodeling in two ways: directly acting on the chromatin structure or recruiting proteins. First, PTMs can modulate histone-DNA interactions. For instance, Histone H3K56 acetylation has been shown to facilitate chromatin accessibility during the DNA damage response by weakening histone–DNA interactions at the entry and exit points in the nucleosome¹⁹⁵. Second, PTMs can also regulate nucleosome-nucleosome interactions. For example, histone H3K79 methylation promotes local changes of the nucleosomal surface in order to alter nucleosome-nucleosome interaction¹⁹⁶. The poly-(ADP-ribose)-ylation has also been described to act directly on the chromatin structure. In the absence of damage, PARP1 binds to chromatin and promote chromatin condensation (Figure 13). PARP1 is activated by its binding to damaged chromatin¹⁹⁷. Through its activity, PARP1 is able to PARylate itself and the surrounding

histones¹⁹⁸ leading to a modification of the local chromatin structure. In vitro data have shown a more relaxed chromatin structure due to the negatively charged PAR chains¹⁹⁸.

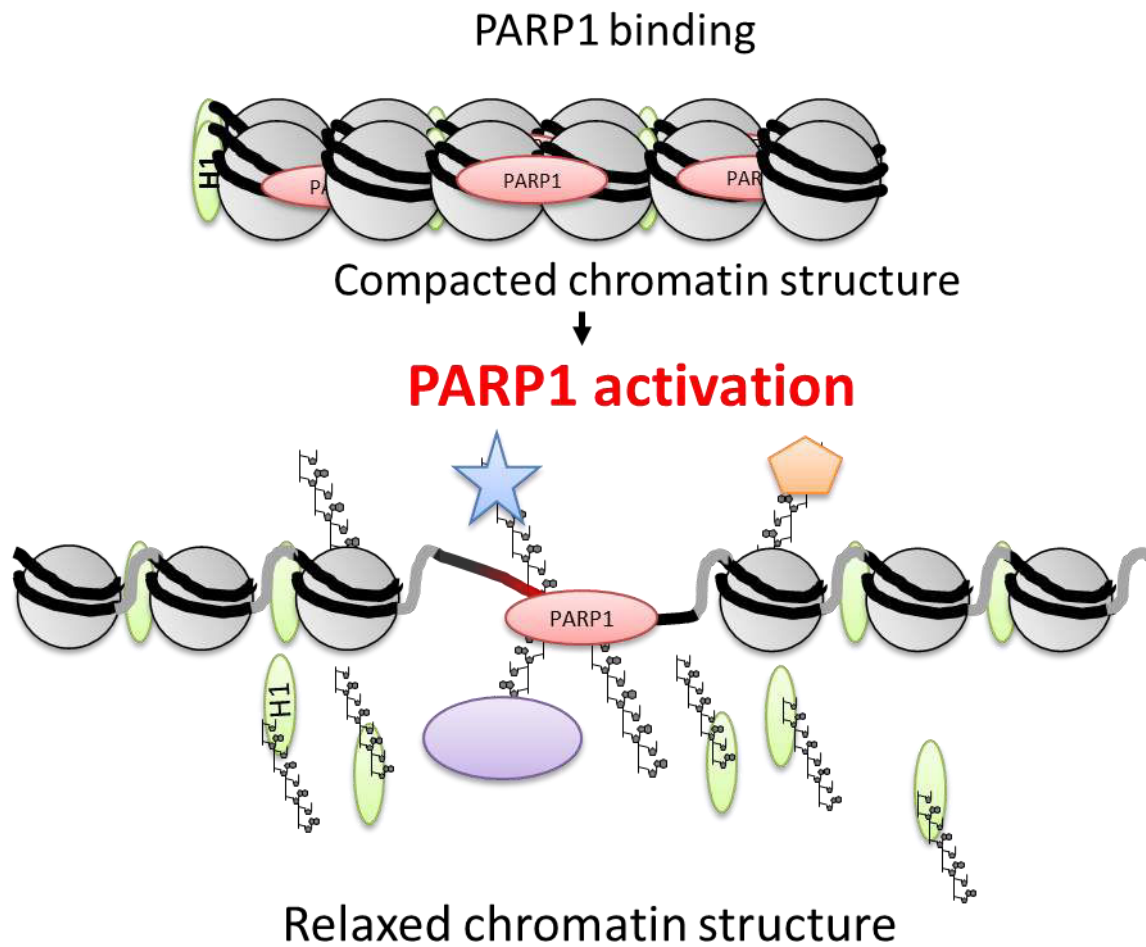


Figure 13. PARP1 involvement in DNA repair.

After DNA damage induction, PARP1 induces structural changes at the chromatin fiber level. By catalyzing auto-PARylation, PARP1 promotes local chromatin remodeling as well as the recruitment of chromatin modifying enzymes.

PTMs also promote the recruitment and/or the dissociation of repair factors at DNA breaks sites ¹⁰⁹. For many years, the so-called phosphorylation has been considered as the main PTM involved in such mechanisms ¹⁹⁹. The prime example is the phosphorylation of histone variant H2AX which has been described to participate to chromatin remodeling by interacting and facilitating the recruitment of Arp4 (a subunit of the Ino80 and Swr1 chromatin remodeling complexes) at DSBs site ²⁰⁰ (see below for more details about the chromatin remodelers). Additionally, PARP1 through its PAR chains allow the recruitment of chromatin modifying enzymes including chromatin remodelers Alc1 ²⁰¹, SMARCA5/SNF2H ²⁰², CHD4²⁰³ and histone chaperone APLF²⁰⁴ (Figure 13). Several studies have shown that the recruitment and/or the dissociation of repair factors at DNA breaks sites are also regulated by other PTMs, including ubiquitylation²⁰², sumoylation, methylation, and acetylation¹⁹⁸. Finally, PTMs could also modulate the binding of histones chaperones. For example, H3K122 acetylation is involved in the interaction between the histone H3 and chaperone ASF1 (Anti-Silencing Function 1), and mutation of this residue leads to defects in the DNA damage response ²⁰⁵.

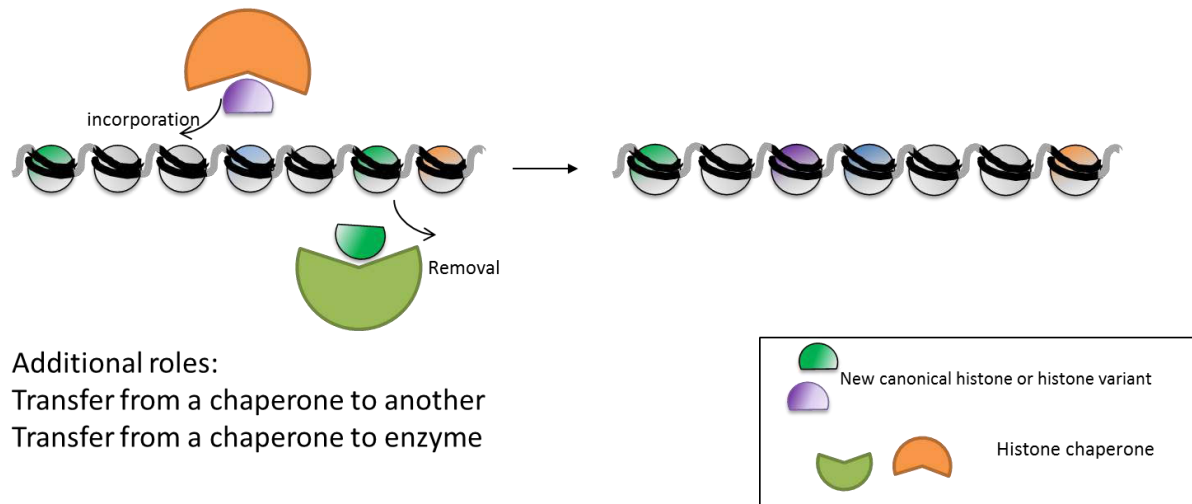
c. Histone Chaperones

In a cellular context, free core histones basically do not exist in the nucleus. They are either incorporated into a nucleosome or bound to histone chaperones^{206,207}. Histone chaperones are proteins that protect either the hydrophobic histone-histone interface or the charged histone-DNA interface to prevent unintended interactions of histones with other factors and tightly control the transfer of histones onto the DNA^{208,209}. In this way, histone chaperones play a crucial role in fundamental processes including DNA repair²⁰⁶. The major role of histone chaperones comprises the association with histones and histone transfer either onto or off DNA, however additional roles also comprises transfer from one chaperone to another, or to enzymes using histones as substrate (Figure 14)²⁰⁷. The histone chaperones can act as single chaperone, in multi-chaperone complexes or as part of enzymatic complexes²⁰⁷. In humans, three multi-subunits chaperone complexes have been shown to be involved in the DNA damage response: CAF-1²¹⁰⁻²¹², FACT^{213,214} and HIRA²¹⁵. The CAF-1 (chromatin assembly factor-1) complex is composed by p150, p60 and p48 subunits and is the associated chaperone of H3 and H4^{112,207}. In particular, CAF-1 mediate H3.1 variant incorporation after UV-induced damages²¹¹. The FACT (facilitates chromatin transcription) complex comprises a heterodimer of hSpt16 and SSRP1 and interacts with H2A-H2B dimer as well as H3-H4 to modulate histone-DNA interaction²¹⁶. FACT is also known as major regulator involved in H2AX exchange²¹³. Its binding to nucleosomes is regulated by PARP1 through the PARylation of hSpt16 which alters its binding property to nucleosomes and leads to the release of FACT from chromatin during damage response²¹⁴. The HIRA (histone regulator A) complex has been

shown to be recruited very early to UV-C damage regions in order to promote H3.3 deposition at damage sites ²¹⁵. This H3.3 deposition, acts as a marking system, licensing transcription restart following DNA damage repair ²¹⁵. In addition, the histone chaperone APLF is also recruited at DNA damage sites through the interaction with PAR chains ^{192,204,217}.

Major role:

Association with histones and transfer onto or off DNA



Additional roles:

Transfer from a chaperone to another

Transfer from a chaperone to enzyme

Figure 14. The histone chaperones: regulators of histone exchanges.

The histones chaperones associate with histones to promote histone transfer, without being part of the final product. The chaperones allow the transfer onto DNA (deposition), off DNA (eviction) as well as the transfer from one chaperone to another or to enzymes that use histones as a substrate.

d. Chromatin Remodelers

In addition to histone variants, PTMs and histone chaperones, chromatin remodelers are also recruited to damaged chromatin in order to mediate DNA accessibility to repair machinery. ATP-dependent chromatin remodelers are translocase enzymes which use the ATP energy to create enough force that is necessary to reposition, evict and exchange nucleosome core particles facilitating the different step of the repair process ^{11,162}. In human, several chromatin remodeling enzyme families are present suggesting specialized functions of these proteins and their associated complexes ²¹⁸. They display nucleosomes repositioning ²¹⁹⁻²²¹ and eviction ²²² activities. Studies on chromatin remodeling provide plenty of data on the remodeling mechanism *in vitro*, however, not much is known about their remodeling mechanism and their regulation *in vivo*.

Chromatin Remodeling Enzymes

In eukaryotes, at least four families of chromatin remodelers have been described: SWI/SNF, ISWI, CHD and INO80 ²²³ (Figure 15). In this part, I will only focus on chromatin remodelers involved in DNA repair by giving examples for each of these families.

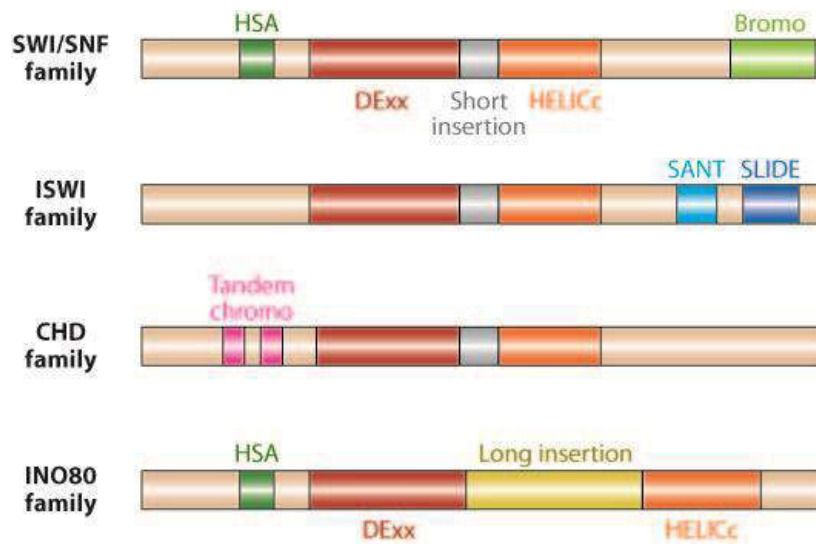


Figure 15. Chromatin Remodeler Families.

All remodeler families contain a SWI2/SNF2-family ATPase subunit. The ATPase domain that is split in two parts: DExx (*red*) and HELICc (*orange*). Remodelers of the SWI/SNF, ISWI, and CHD families each have a distinctive short insertion (*gray*) within the ATPase domain. The remodelers of INO80 family contain a long insertion (*yellow*). Each family is further defined by distinct combinations of flanking domains: Bromodomain (*light green*) and HSA (helicase-SANT) domain (*dark green*) for SWI/SNF family, SANT-SLIDE module (*blue*) for ISWI family, tandem chromodomains (*magenta*) for the CHD family, and HSA domain (*dark green*) for the INO80 family. Adapted from ¹⁶².

SWI/ SNF Family

The SWI/SNF (switching defective/sucrose nonfermenting) family remodelers were initially purified from *Saccharomyces cerevisiae* ²²⁴. The RSC (Remodels the Structure of Chromatin) complex belong to this family and is the most abundant ATP-dependent chromatin-remodeling complex in yeast (~ 1000–2000 molecules per cell) ²²⁵. RSC is very rapidly recruited to DNA breaks via its subunits Sth1, Rsc8 and Rsc1 and mobilizes nucleosomes to promote phosphorylation of H2A S129 and resection²²⁶.

ISWI Family

The ISWI (imitation switch) family includes NURF, CHRAC, and ACF complexes which were initially purified from *Drosophila melanogaster* ²²⁷. The ACF (ATP-utilizing Chromatin assembly and remodeling Factor) remodeling complex has been shown to be involved in DNA damage ²²⁸. Its subunit ACF1 (ATP-utilizing Chromatin assembly and remodeling Factor 1, also known as BAZ1A) has been shown to be recruited at DNA breaks sites²²⁹ and is thought to enhance the efficiency of nucleosome sliding ^{228,230}. Similarly, SMARCA5/SNF2H (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5), the catalytic subunit of ISWI chromatin remodeling complexes in humans, is also recruited to damaged DNA sites through the interaction with RNF168 (an E3 ubiquitin ligase) in a PARP1-dependent manner ²⁰². Then, SMARCA5/SNF2H promotes γ H2AX ubiquitylation and BRCA1 accumulation at damaged sites²⁰².

CHD Family

The CHD (chromodomain, helicase, DNA binding) family includes the NuRD ATPase catalytic subunit CHD4 which is recruited to DNA breaks sites in a PAR-dependent manner^{203,231} and is phosphorylated by ATM in response to DNA damage. It has been also established that CHD4 controls cell-cycle progression by regulating checkpoints signalling, and promotes damage repair and cell survival after DNA-damage induction^{203,232,233}. More recently, CHD2 has also been shown to promote chromatin expansion and the deposition of histone variant H3.3 at sites of DNA damage by being recruited to DNA break sites in a PAR-dependent manner²³⁴.

The CHD1L (CHD1-like) chromatin remodeler also known as Alc1 (Amplified in Liver Cancer 1) is a macro-domain containing SNF2-like ATPase remodeler that is rapidly recruited to damaged chromatin in a PARP1-dependent manner^{201,235}. Its ATP-dependent nucleosome remodeling activity is stimulated by NAD⁺ and PARylation^{201,235}. It has been shown that the activation of Alc1 relies on the formation of a stable Alc1-PARP1-nucleosome intermediate where Alc1 binds to PARylated PARP1 through its macro-domain^{201,236} suggesting a role for Alc1 in chromatin remodeling at the DNA damage sites.

INO 80 Family

The INO80 (inositol requiring 80) family includes the INO80 chromatin remodeling complex which is known to be involved in the DNA repair in both yeast and mammals^{237,238}. The yeast INO80 complex subunits Nhp 10 and Arp4 allow the recruitment of the complex to the DNA breaks through a direct interaction with phosphorylated

H2AX, participating to DNA end processing in HR repair^{200,239,240}. In contrast, the mammalian INO80 complex is recruited to DNA break sites through Arp8 subunit and in a phosphorylated H2AX-independent manner²⁴¹. Moreover, the INO80 nucleosome remodeling complex has been shown to modulate chromatin mobility at DNA breaks in yeast¹⁶⁶, linking the increase of chromatin mobility upon DNA damage to chromatin remodeling events occurring at the chromatin fiber scale.

The Remodeling Mechanism

The canonical chromatin remodeling mechanism can be divided into 3 steps¹¹: (1) initiation step, which requires the specific recognition and binding to the chromatin, (2) translocation step, where multiple translocations of nucleosome core particle occur depending on the particular remodeling enzyme and on the properties of the DNA sequence to be remodeled, (3) release step. Three models¹¹ have been proposed to illustrate such mechanism (Figure 16). The “twist diffusion model” proposes to that energy fluctuations would be sufficient to twist the DNA helix at the edge of the nucleosome¹¹. In this way, the twist of the DNA helix could lead to the nucleosome sliding and the replacement of canonical histone-DNA interactions. The distortion is continuously propagated through the nucleosome and would change the position of the nucleosome. The DNA rotates around its axis over the histone octamer surface with a step length of one base pair (Figure 16). However, several studies argue against a mechanism based solely on DNA twisting. Indeed, experiment using nicked and gapped DNA still allowed SWI/SNF and ISWI dependent nucleosome remodeling^{242,243}.

A first alternative model has been suggested, the “loop recapture” model ¹¹ (Figure 15). This model proposes a detachment of a DNA segment from the histone octamer surface at the entry/exit site of the nucleosome instead of a thermal energy that twists DNA at the nucleosomal entry ^{244,245}. The histone octamer surface that is exposed would interact with more distant regions of the DNA molecule leading to the formation of a DNA loop on the surface of the octamer. The resulting DNA loop would then translocate over the nucleosome surface by releasing and rebinding adjacent sequences on the core histones surface. The propagation of the DNA loop would change the translational position of the octamer, according to the size of the DNA loop ²⁴⁵. This model is supported by biochemical and single molecule studies of the ACF remodeling complex. ACF was shown to un-wrap the DNA, roughly 20 and 40bp, from the border of the nucleosome ²⁴⁶. The remodeling by SWI/SNF and RSC on nucleosomal template also promotes DNA loops, and in the case of translocation by RCS complex produces a remodeled intermediate with internal DNA loops ²⁴⁷.

A third model known as the “octamer swiveling” model ¹¹ (Figure 16), proposed that remodelers can disrupt major contacts between the DNA and the core histones allowing a united swiveling of the DNA fiber relative to the histone octamer ¹¹.

However, there are still contradictions between the models and the experimental data. Indeed, a recent work, using single-molecule fluorescence resonance energy transfer technique providing unprecedented resolution concerning nucleosome sliding catalyzed by yeast ISWI complexes ²⁴⁸ suggests that base pairs exit the nucleosome one by one preceding multi-pb steps of DNA movement on the entry site.

All together these results suggest that ATP-dependent chromatin remodeling events are an important process for chromatin structure modulation. More investigations are needed to provide new insights into the *in vivo* mechanisms by which these chromatin remodeling enzymes act in the DNA repair process.

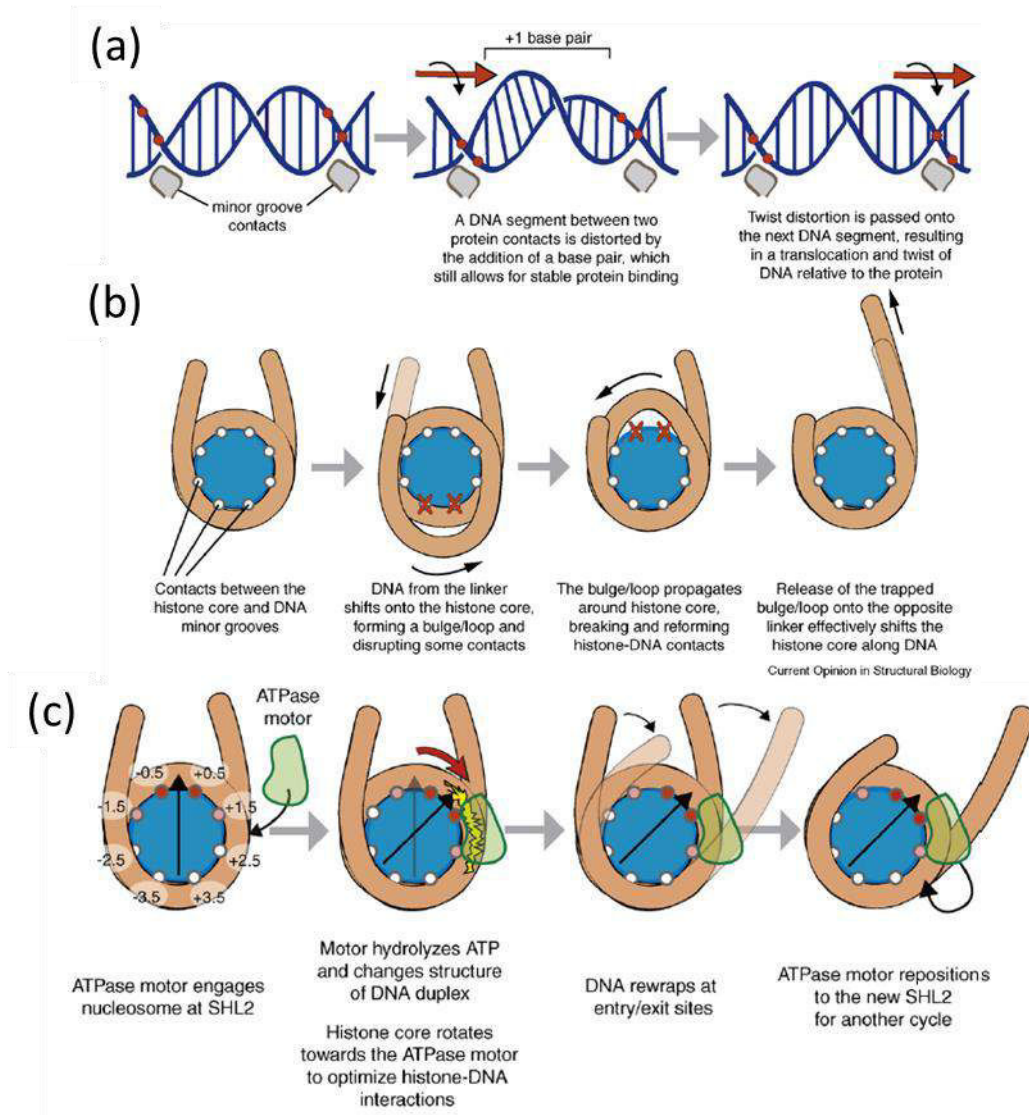


Figure 16. Chromatin remodeling mechanism

The canonical mechanism follows 3 steps: initiation, translocation and release. (a) The “twist diffusion” model proposes that energy fluctuations would be sufficient to twist the DNA helix at the edge of the nucleosome. The distortion is continuously propagated through the nucleosome 1pb at time.

(b) The “loop recapture” model proposes a detachment of DNA segment from the octamer surface at the entry/exit site of the nucleosome.

(c) Another model, “octamer swiveling” model, proposes a disruption of major contacts between DNA and core histones allowing a united swiveling of the DNA fiber relative to histone octamer. Adapted from ¹¹.

C. Functional Role of Chromatin Remodeling and Dynamics at DNA damage

a. A Facilitated Access to the Damage

The activation of the DNA repair machinery is usually associated with changes in chromatin compaction state. New findings suggest that chromatin compaction impedes the access of DNA repair factors to sites of DNA breaks^{249,250}. Murr and colleagues showed that Tip60 Histone Acetyl-Transferase and cofactor Trrap bind to the chromatin surrounding sites of DSBs in vivo. The depletion of Trrap impaired both DNA-damage-induced histone H4 hyperacetylation, which is thought to promote chromatin accessibility²⁴⁹, and accumulation of repair molecules at sites of DSBs, resulting in defective repair by HR. Importantly, Murr and colleagues were able to counteract by chromatin relaxation the impaired loading of repair proteins and the defect in DNA repair in Trrap-deficient cells. This suggests that chromatin relaxation upon DNA damage induction is a necessary step for repair process since it promotes the accessibility to the damaged chromatin. However, such results should be carefully considered since several works have reported that diffusing tracers with sizes up to few hundred kDa can easily diffuse through the nucleus and access even the densely compacted heterochromatin^{251,252}. Complementary, it has been proposed that the chromatin compaction state may influence the way proteins scan for binding sites (DNA breaks in the case of repair proteins), along the chromatin fiber²⁵². And more recently, Burgess and colleagues suggested that the recruitment of some DNA damage response factors may be triggered by chromatin over-compaction at DNA break sites rather than its relaxation¹⁸⁰. In addition, it has been described a transient formation of repressive chromatin at DNA break sites through

the recruitment of heterochromatin protein complex containing kap-1, HP1, and the H3K9 methyltransferase suv39h1. Subsequent cycles of H3K9 methylation and loading of kap-1/HP1/suv39h1 complexes on chromatin result in the spreading of heterochromatin along the chromatin domains flanking the DSBs, leading to the rapid formation of repressive chromatin at the DSBs²⁵³. This transient formation of repressive chromatin has been suggested to be necessary for stabilizing the damaged chromatin and for remodeling the chromatin to create an efficient template for the DNA repair machinery²⁵³.

b. Spatial Organization of the Repair Machinery

A Spatial Organization to Form Repair Foci

The direct implication of chromatin remodeling in the spatial organization of the repair machinery has not been yet well elucidated. However, it appears that the distinct repair components could be assembled in “foci”^{254,255}. Studies in yeast have shown that two DSBs induced by restriction enzyme localized in two separated loci are able to co-localize in the same Rad 52 focus²⁵⁴. These foci could be assimilated to “repair factories”, and by analogy to “transcription factories”²⁵⁶, repair factories could be described as “compartments” in the nucleus where multiples DSB are clustered in order to be repaired (Figure 16). These repair centers may facilitate the repair of multiple DNA breaks at once clustering several factors involved in the DNA damage signalling and repair. For instance, it has been described the co-localization of proteins such as Rad9 (implicated in DNA damage checkpoint signalling), Rad22 (implicated in DNA repair by homologous recombination), and PCNA (implicated in

DNA synthesis) in nuclear foci after induction of DSBs by gamma irradiation of living fission yeast cells ²⁵⁷.

In mammals, the existence of repair factories has remained controversial. However, there are experimental evidences for the induction of DSBs clusters under different experimental settings and conditions ^{255,258}. DSBs clustering could be considered in terms of co-localization of DSBs and in terms of radiation induced foci (RIF) which correspond to the local recruitment of DNA sensing proteins. In this way, Neumaier and colleagues have shown that multiple DSBs spatially distant can rapidly cluster into repair centers ²⁵⁸. They also showed that the yield of RIF was much smaller at higher doses of irradiation ²⁵⁸ which might suggest that DSBs tend to cluster leading to lower RIF counts. Another work has shown DSBs clustering by looking at changes in gamma-H2AX track morphology within minutes after DSB induction, indicating movement of the gamma-H2AX domains ²⁵⁹. In this study, Aten et al. have shown extensive migration and subsequent clustering of different DSBs induced by α -particle source irradiation ²⁵⁹. Juxtaposition of the DSB-containing chromosomes was more apparent in G1 phase cells and depended on the repair protein Mre11, one of several proteins implicated in interconnecting damaged chromatin ²⁵⁹.

It is also interesting to note that the study of translocations in mammalian cells brought evidence of DSBs mobility and their tendency to cluster. Two models for how broken chromosomes may undergo translocations have been suggested ²⁶⁰. The “breakage-first” model proposes that DNA breaks occurring at distant locations are able to roam the nucleus for potential partners and are brought together to produce translocation ^{261,259}. In contrast, a “contact-first” model postulates that joining of

broken chromosomes ends can only occur when the DNA breaks are formed in chromatid fibers that co-localize at the time of DNA damage ²⁶¹.

A Spatial Organization to Re-localize DNA Breaks to Favorable Repair Environment

In addition to the functional roles previously cited, the chromatin remodeling might facilitate the mobility of DSBs in order to relocate them to favorable repair environment when they cannot be repair in their environment of origin. It has been reported the implication of SWR1 and INO80 chromatin remodelers in the choice of DSBs perinuclear anchorage site when they relocate to nuclear periphery in yeast ²⁶². And in the context of heterochromatin, opposing ISWI-and CHD- chromatin remodeling activities have been implicated in the spatial organization of heterochromatic DNA repair ²⁶³.

DSBs located in the nuclear interior and at nuclear pores can be repaired both by NHEJ and HR, which is not the case for compact heterochromatin associated with lamina at the inner nuclear membrane where DNA damage signalling is delayed and HR impaired ²⁶⁴. In addition, DSBs within lamina-associated domains are not re-localized to HR permissive compartment (nuclear interior) but are repaired in situ by C-NHEJ or alt-NHEJ ²⁶⁵. Contrastly, in *Drosophila*, DNA breaks in heterochromatin move to the nuclear periphery to be repaired by HR ¹⁸⁴. The dynamic re-localization of repair foci to the periphery of heterochromatin regions, observed both in *Drosophila* and mammalians cells ^{181,183}, is thought to play a role in the prevention of ectopic recombination between heterochromatic repetitive sequences.

In addition of heterochromatin environment, the nucleolus is thought to be also a compartment where DSBs repair could be deleterious ²⁶⁶. The nucleolus shelters highly repetitive and transcribed ribosomal DNA ²⁶⁷. In yeast, it has been shown that a break induced by restriction enzyme in the nucleolus was re-localized outside of the nucleolus to be repaired ²⁶⁸. In mammalian cells, a similar mechanism might exist. Indeed, it has been reported that inducing DSBs in ribosomal DNA with restriction enzyme lead to a re-organization of the nucleolar compartment. The DSBs are then relocated into “nucleolar caps” in order to be repaired ^{269,270}, however the underlying mechanism remains unclear.

Finally, the re-localization of breaks that occur in heterochromatin as well as in nucleolar compartment or persistent breaks (section III-A) involves the cohesion-associated SMC5/6 (Structural maintenance of chromosomes 5/6) complex as well as post-translational modification SUMOylation ^{271,272}.

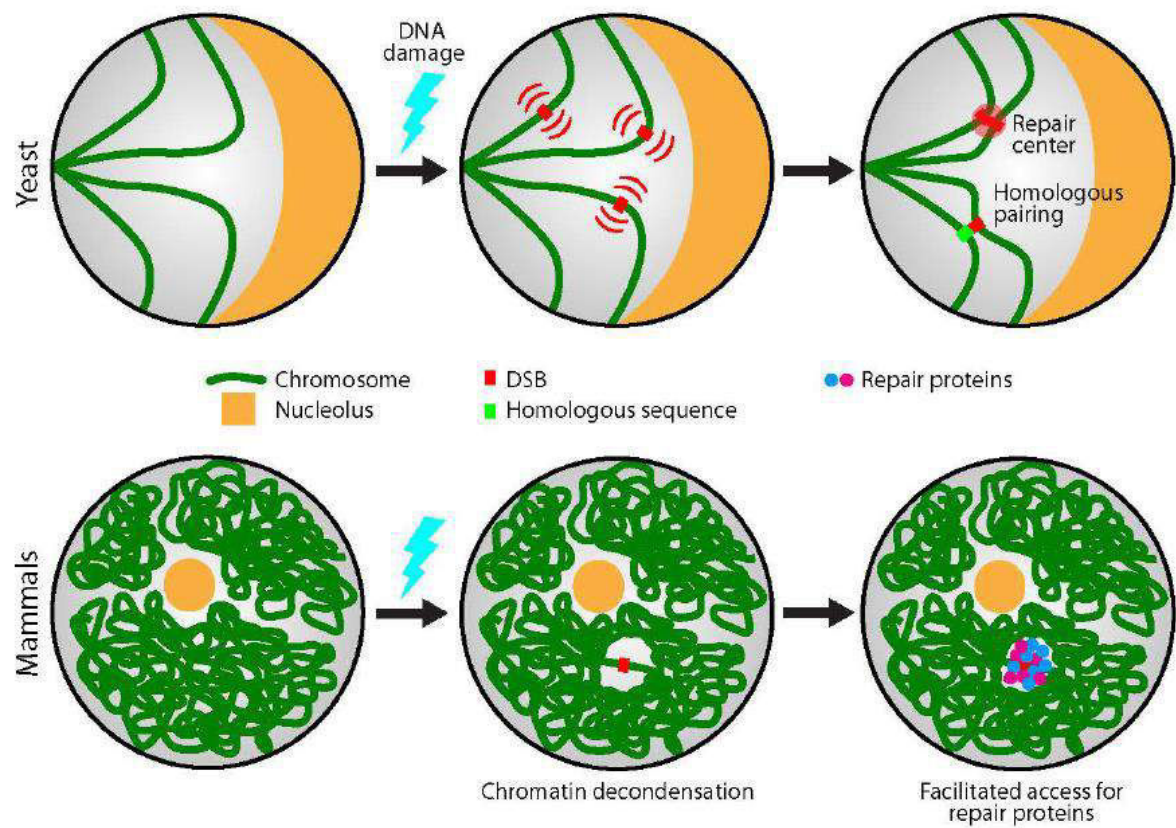


Figure 17. Changes in chromatin motion and compaction state at DNA damage sites for yeast and mammalian cells.

Adapted from⁸².

In summary, DNA repair is an essential and fundamental biological process involved in the maintenance of genome stability. This process occurs in the context of the highly organized multiscale architecture displayed by chromatin within the interphase nucleus. The assembly of the repair machineries is thought to occur through the accumulation of repair factors at sites of DNA damage to form repair foci. The assembly of these repair foci might require complex spatial and temporal coordination of repair factors as well as the involvement of chromatin-modifying enzymes. In addition the progress of the repair process relies on the structure of the chromatin where the DNA lesions occur. The chromatin remodeling events may modulate chromatin mobility at different organization scales and are emerging as key determinant in repair efficiency and in maintenance of genome stability.

RESULTS

During my PhD, I focused on the early steps of the cellular response to DNA damage. I studied the chromatin relaxation upon UV-induced damage in order to understand the role of PARP1 and PARylation in the chromatin decondensation. My results reveal that the local chromatin relaxation at the DNA lesion sites is regulated by PARP1 enzymatic activity and identify the chromatin remodeler Alc 1 as an important player in the PARP1-dependent chromatin relaxation upon DNA damage *in vivo*.

I. Establishment of a Methodology to Study Chromatin Dynamics upon DNA Damage

In order to assess large-scale chromatin re-organization at DNA damage sites in living cells, we established an assay based on human U2OS (human osteosarcoma) cells expressing either stably or transiently core histone H2B protein tagged with photo-activable dyes PAGFP or PATagRFP to label the chromatin. A predefined area is irradiated with a 405 nm laser to photo-convert the labeled histone which in turn fluoresces in red (PATagRFP) or in green (PAGFP), highlighting the region of interest (Figure 18).

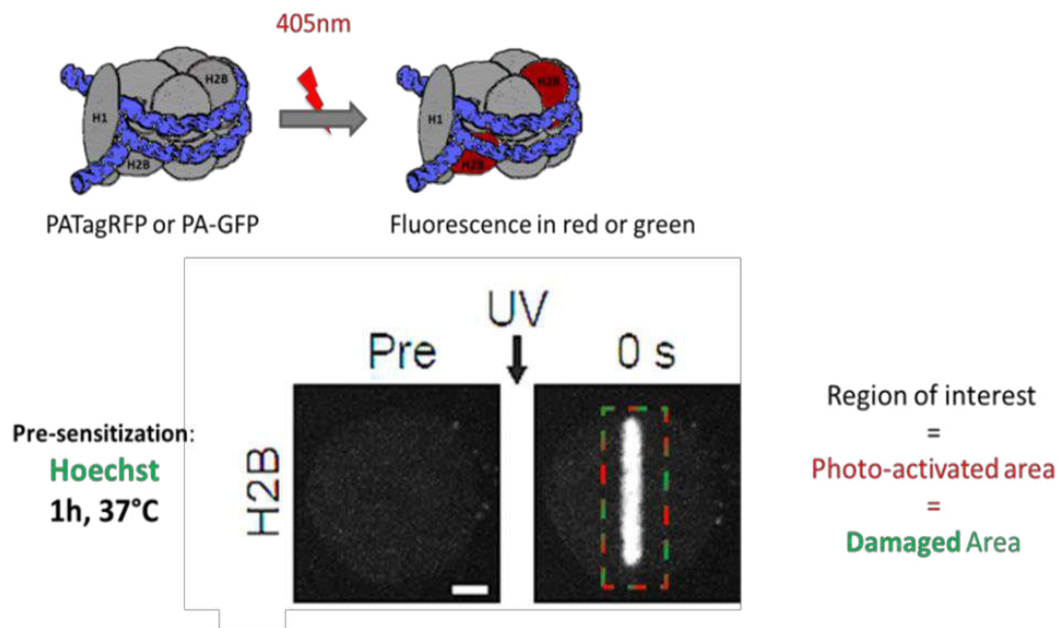


Figure 18. Using photo-activable dyes to observe chromatin sub regions

Schematic representation of the photo-activation of the core histone H2B fused to PA-TagRFP or PA-GFP (upper panel), Visualization of photo-activated histones after laser micro-irradiation in cells pre-sensitized with Hoechst (1h).

The 405 nm laser irradiation of Hoechst pre-sensitized cells induces in the same time photo-activation and DNA damage (Figure 18 and 19).

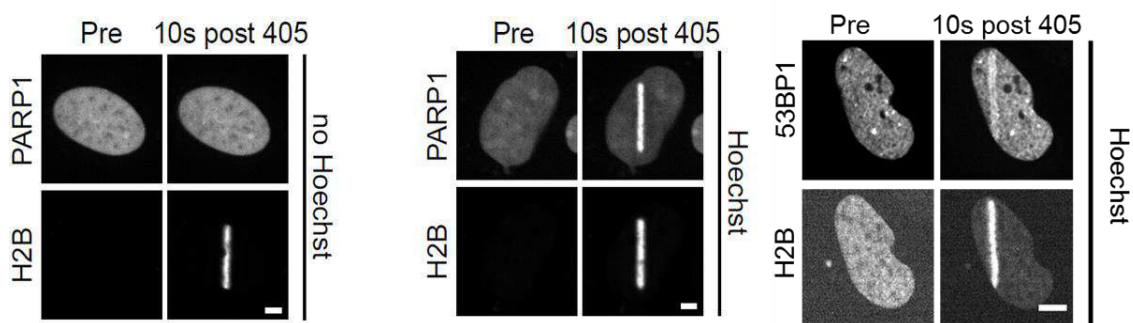


Figure 19. 405 nm laser irradiation of Hoechst treated cells induces DNA damage

Recruitment of PARP1 and at the micro-irradiated area in cells co-expressing PARP1-mCherry and H2B-PAGFP. Bar = 4 μ m. In cells non-pre-sensitized with Hoechst, the 405 nm irradiation induces local photo-activation of the H2B-PAGFP but no recruitment of PARP1-mCherry. In contrast, in case of Hoechst pre-sensitization, the 405 nm irradiation induces both photo-activation of the H2B-PAGFP and a marked recruitment of PARP1-mCherry, indicating the presence of DNA lesions. Similarly, recruitment of 53BP1 and at the micro-irradiated area in cells co-expressing 53BP1-GFP and H2B-PATagRFP, we observed the recruitment of 53BP1 only in cells pre-sensitized with Hoechst.

After the induction of DNA damage with the 405 nm laser, we observed an expansion of the laser irradiated region (Figure 20 A). To analyze the changes in the chromatin compaction level that occur after laser micro-irradiation, we used a custom-made routine written in MatLab (MathWorks). Briefly, the chromatin area micro-irradiated at 405 nm and tagged with the photo-activatable H2B is segmented (Figure 20 A) and then the thickness of the photo-activated line is measured for each frame. The size of the photo-converted chromatin region rapidly increases in the Hoechst-pre-sensitized cells (Figure 20 B), reflecting chromatin relaxation at DNA damage sites, as previously reported^{87,179}.

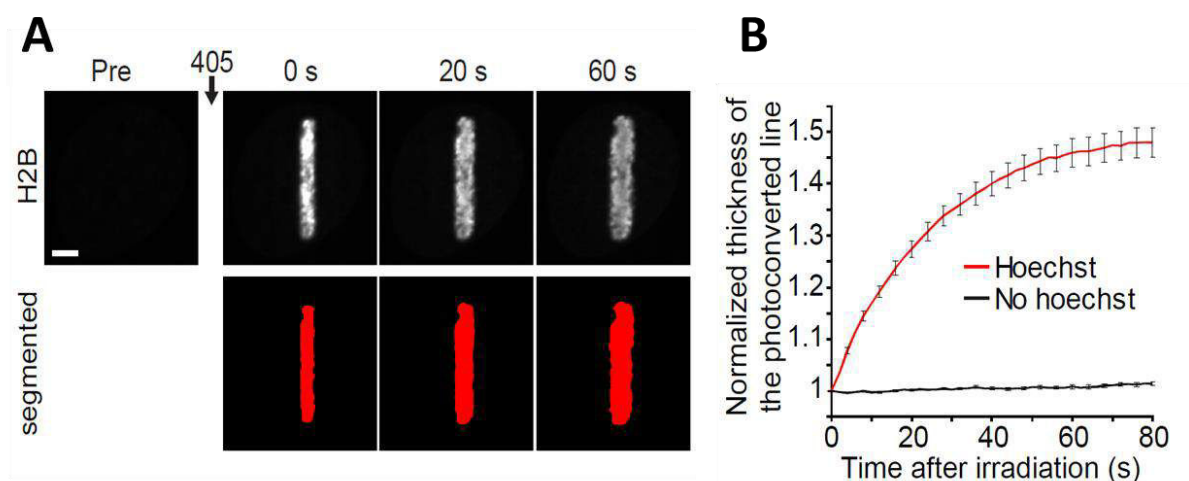


Figure 20. Chromatin relaxes after induction of DNA damage

(A) Confocal image sequence of a human U2OS nucleus expressing H2B-PAGFP. Bar = 4 μ m. The automatic segmentation of the histone H2B channel is shown in red below the raw images. The average thickness of the segmented line can be plotted as a function of time after irradiation, as shown in (B) for cells pre-sensitized or not with Hoechst (mean \pm SEM). Based on this analysis, the ratio between the thicknesses of the photo-converted line at time = 60s and time = 0s can be calculated to estimate the relative relaxation of the irradiated region.

This relaxation has also been observed using alternative laser micro-irradiation methods (without a need for Hoechst sensitization) such as Ionizing radiations (IR,

800nm laser) (data from Sébastien Huet), pulsed UV (355nm laser) (data from Gyula Timinszky). To verify that this observed chromatin decondensation is not a local release of the photo-converted H2B, since the induction of DNA damage leads to massive remodeling at the nucleosome level ²⁷³, we used an alternative approach to label the chromatin using the incorporation of fluorescent nucleotides in the DNA in living cells ^{77,84}. After laser irradiation, we observed that the fluorescent spots move in a directional manner away from the irradiated area (Figure 21 A). This movement occurs at speeds similar to the expanding speed of the H2B photo-converted region (Figure 21 B). These results validate that the changes we observe in the size of the photo-converted H2B region observed after DNA damage induction reflect the relaxation of the chromatin, rather than the local release of photo-converted H2B, validating that H2B photo-labelling is a suitable tool to assess chromatin dynamics upon DNA damage.

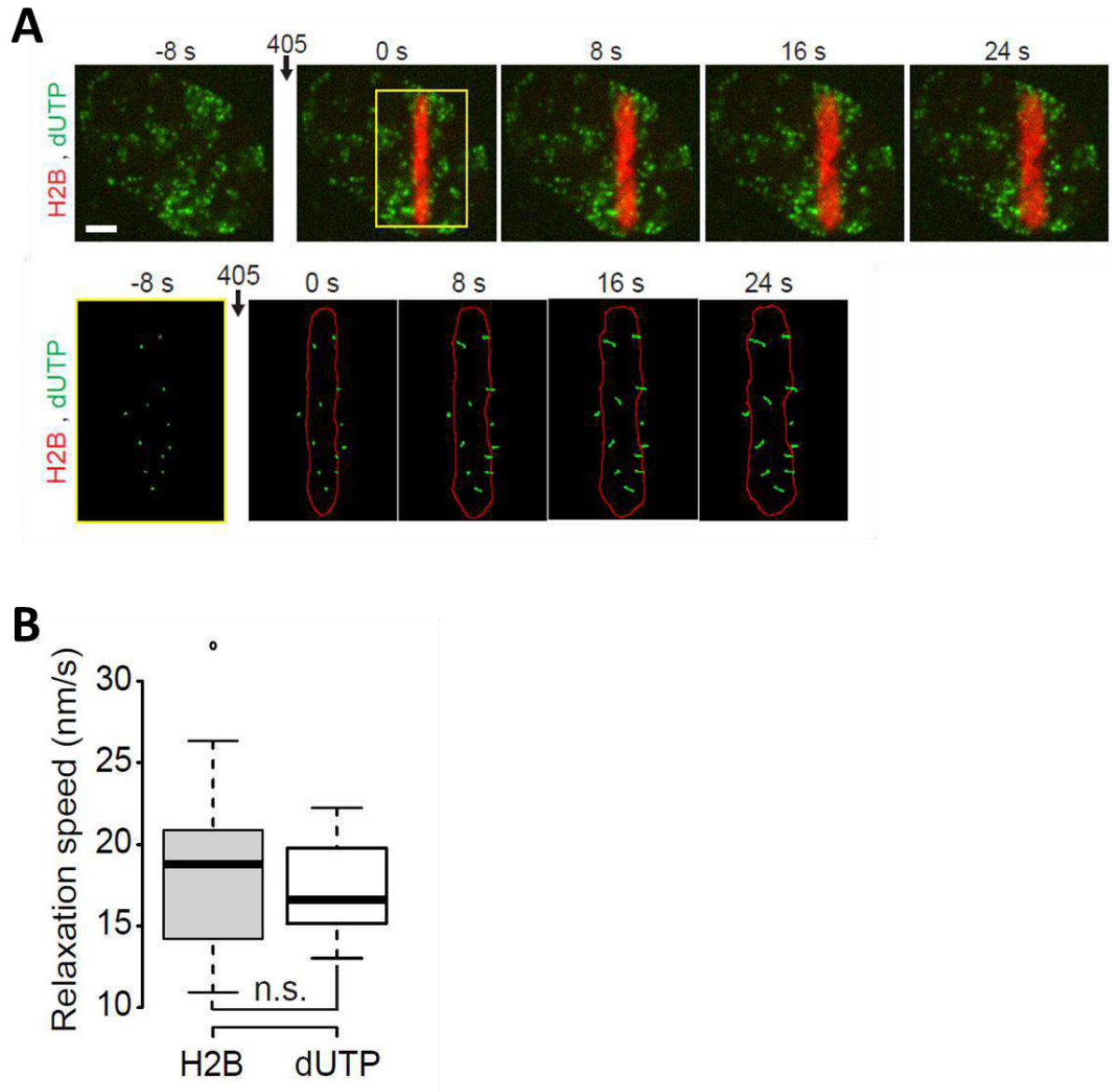


Figure 21. Alternative approach to label the chromatin using the incorporation of fluorescent nucleotides in the DNA

(A) Upper panel: Confocal image sequence of a U2OS cell expressing H2B-PATagRFP (red) and labeled with fluorescent nucleotides dUTP-ATTO633 (green). Bar = 4 μ m. Lower panel: Enlarged view of the region overlaid in yellow on the previous panel. Next to the images: segmentation of the photo-converted chromatin area (red outline) and trajectories of individual foci labeled with fluorescent nucleotides (green). For this experiment, the power of the 405 nm laser used for simultaneous photo-activation and micro-irradiation was set to 250 μ W at the sample level, instead of 125 μ W, to induce an enhanced chromatin relaxation allowing an easier identification of the phase of directed motion for the dUTP-labeled foci. (B) Comparison between the speed at which the width of the H2B labeled region is growing and the speed of the dUTP-labeled foci perpendicular to the irradiation line. We show the average speed for the 30s subsequent to laser micro-irradiation. 12 cells were analyzed. p values were calculated by paired t-test.

For the laser micro-irradiation, we used two laser power intensities for the 405nm laser, low intensity (0.125mw), and high intensity (0.250mw) by measuring the intensity of the laser at the objective. We observed that the chromatin relaxation increases when we increase the intensity of the laser power suggesting that the amplitude of chromatin relaxation scales with the amount of DNA breaks induced by laser irradiation (Figure 22).

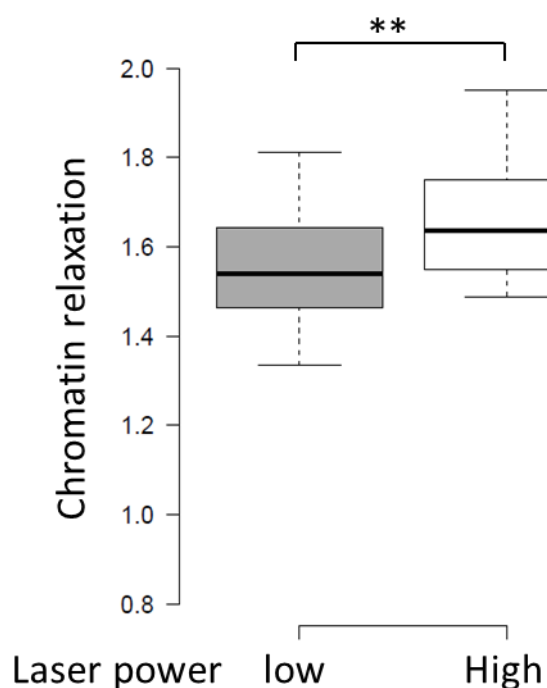


Figure 22. The amplitude of chromatin relaxation scales with the intensity of the laser power
Comparison of relative chromatin relaxation at 60s after laser micro-irradiation in wild-type U2OS cells expressing H2B-PATagRFP laser micro-irradiated at Low and High laser power. The laser micro-irradiation was set to 0.250 mW for High laser power and 0.125mW for low laser power at the sample level.

Following the initial phase of fast decondensation upon DNA damage, we observed that chromatin slowly re-condenses and recovers its initial state i.e pre-damage compaction state in about 15 min (Figure 23), in agreement with previous studies

179,274

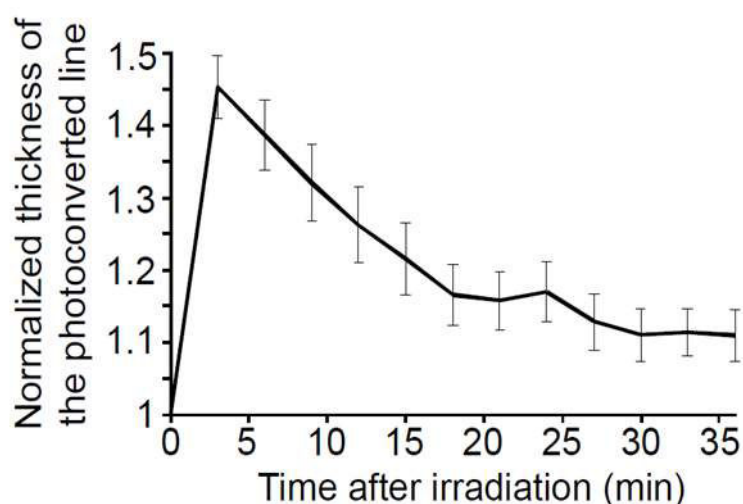


Figure 23. chromatin re-condenses after the initial phase of fast relaxation
Dynamics of the chromatin compaction state at DNA damage sites over long time scales measured in wild type U2OS cell expressing H2B-PATagRFP (mean ± SEM).

In conclusion, the methodology that we established is suitable to follow chromatin dynamics upon DNA damage. I used this methodology to study the role of PARylation in the chromatin relaxation after DNA damage.

II. PARP1 Activation Controls the Transient Chromatin Relaxation at DNA Damage Sites

In vitro data suggests that PARP1 and PARylation can modulate the chromatin compaction state, either by PARP1 binding to chromatin or by the activation of its catalytic activity. On one hand, in its inactive form, PARP1 is associated with nucleosomes and forms a compact chromatin *in vitro*²⁷⁵. On the other hand, PARylation has been shown to promote major loosening of the chromatin fiber *in vitro*¹⁶⁰. Considering these previous works, we investigated the role of PARP1 and PARylation in the decondensation of chromatin upon laser micro-irradiation in human living cells. First, we inhibited PARylation through PARP inhibitors treatment (Figure 24) and we observed that the DNA damage-induced chromatin decondensation was fully abolished (Figure 24). In fact, we even observed chromatin over-compaction upon laser micro-irradiation (Figure 24). This result implicates PARylation as the determining factor of chromatin relaxation upon DNA damage.

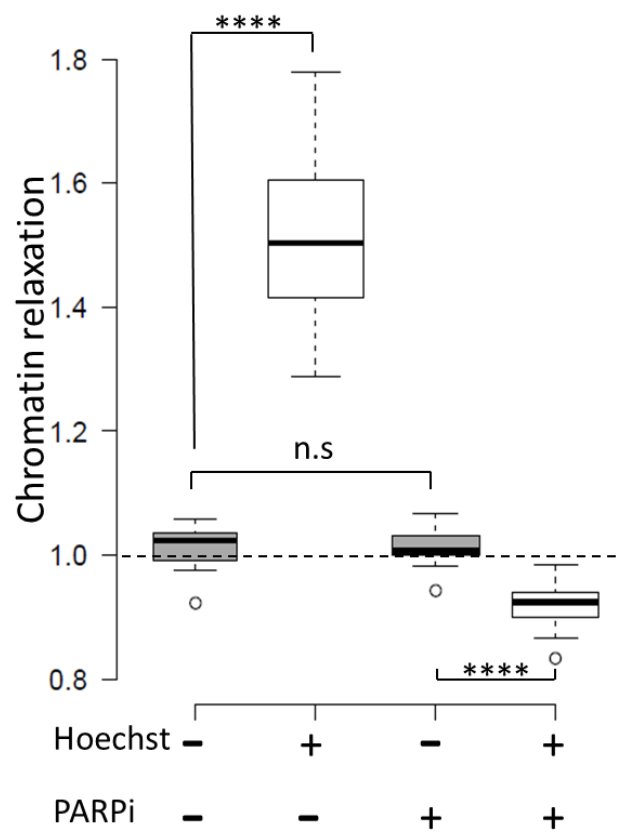


Figure 24. PARP1 activation regulates the transient chromatin relaxation at DNA damage sites

Relative chromatin relaxation at 60s after laser micro-irradiation in wild-type U2OS cells expressing H2B-PATagRFP and treated or not with the PARP1 inhibitor AG14361 (30μM, 1h).

Several PARPs are recruited at the DNA breaks including PARP1, PARP2 and PARP3 (data from Théo Lebeaupin). These PARPs are all inhibited by the PARP inhibitors so to distinguish between them we need to do specific Knockout. To assess the role of PARP1 in the chromatin re-organization after DNA damage, we used the CRISPR-Cas9 approach (see experimental procedure for more details) to delete PARP1. We designed CRISPR-Cas9 GFP plasmids containing sgRNA that target potential splicing site in the PARP1 gene. We obtained two PARP1 Knock-out cell lines (Figure 25).

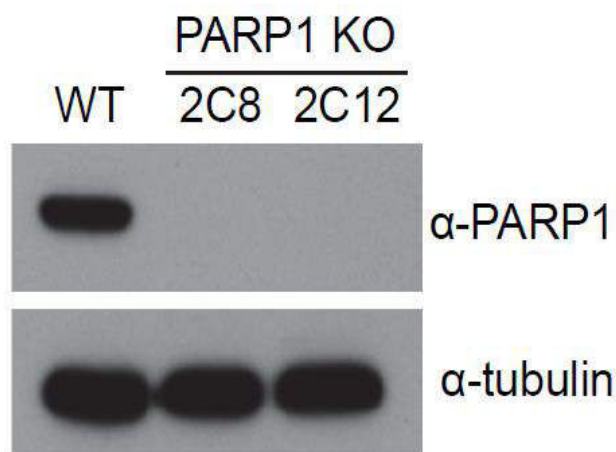


Figure 25. PARP1 knockout using CRISPR-Cas9 method
 Western-blot of wild-type U2OS cells and the PARP1 knockout cell lines.

Using these PARP1 KO cells, we observed that the chromatin relaxation at DNA lesions was dramatically reduced compared to wild-type cells, highlighting the predominant role of PARP1 in the PARylation associated to DNA damage induction (Figure 26). Interestingly, we did not observe an over-compaction of the chromatin in these KO cells, even after inhibition of PARylation with PARP inhibitors (Figure 26).

PARP inhibitors do not block the binding of PARP1 to DNA damage ¹⁹³, which suggest that the over-compaction of chromatin that we observe upon PARylation inhibition in wild-type cells is due to PARP1 binding to DNA breaks, whereas its product, PAR chains, is directly and/or indirectly responsible for chromatin relaxation.

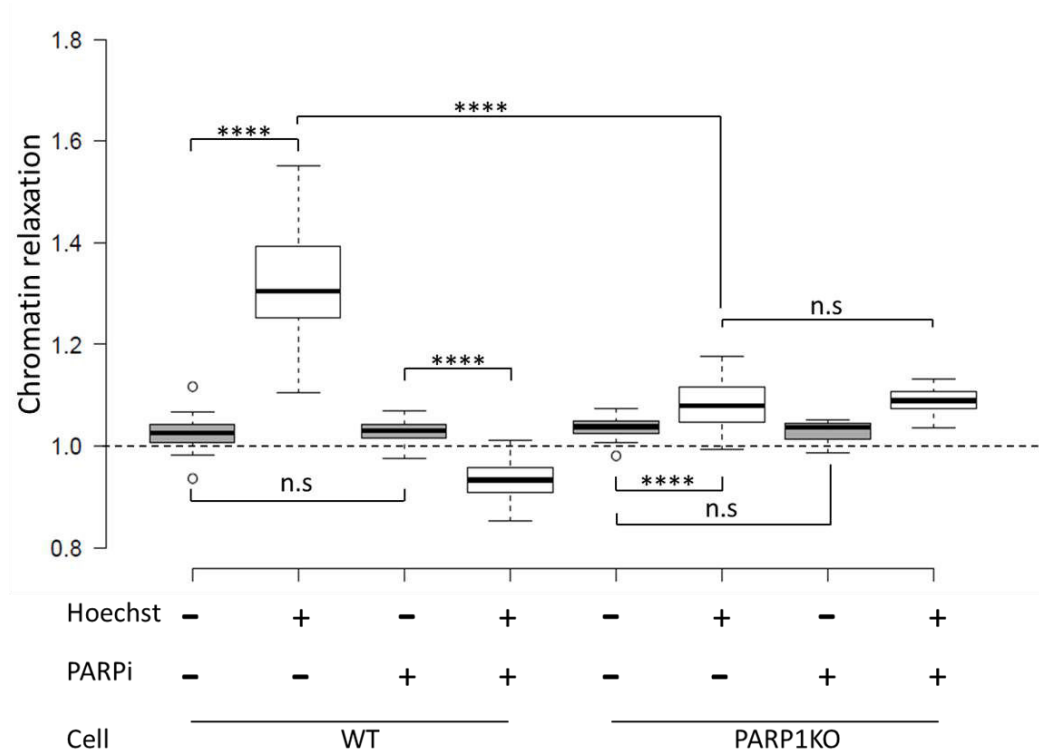


Figure 26. PARP1 binding leads to chromatin over-compaction

Relative chromatin relaxation at 60s after laser micro-irradiation in wild type and PARP1 knockout cells (clone 2C8) transfected with H2B-PAGFP and treated or not with the PARP1 inhibitor AG14361 (30 μ M, 1h). Similar results were obtained with a second PARP1 knockout cell clone (clone 2C12, data not shown).

In conclusion, we showed that the activation of PARP1 regulates the chromatin compaction state at the DNA lesion sites, either by its binding to DNA lesions or by its PAR chains.

III. PARylation Levels

To better understand the effect of PARP1 on chromatin relaxation, we looked at the PARylation levels in cells over-expressing PARP1 WT as well as cells over-expressing a mutant of PARP1 that lacks its catalytic domain (PARP1 delta CD). The chromatin relaxation in U2OS cells over-expressing PARP1 WT is significantly decreased compared to control cells (Figure 27 A). While, over-expressing PARP1 deltaCD, not only abolished chromatin relaxation but also induced a significant chromatin over-compaction upon laser micro-irradiation (Figure 27 B).

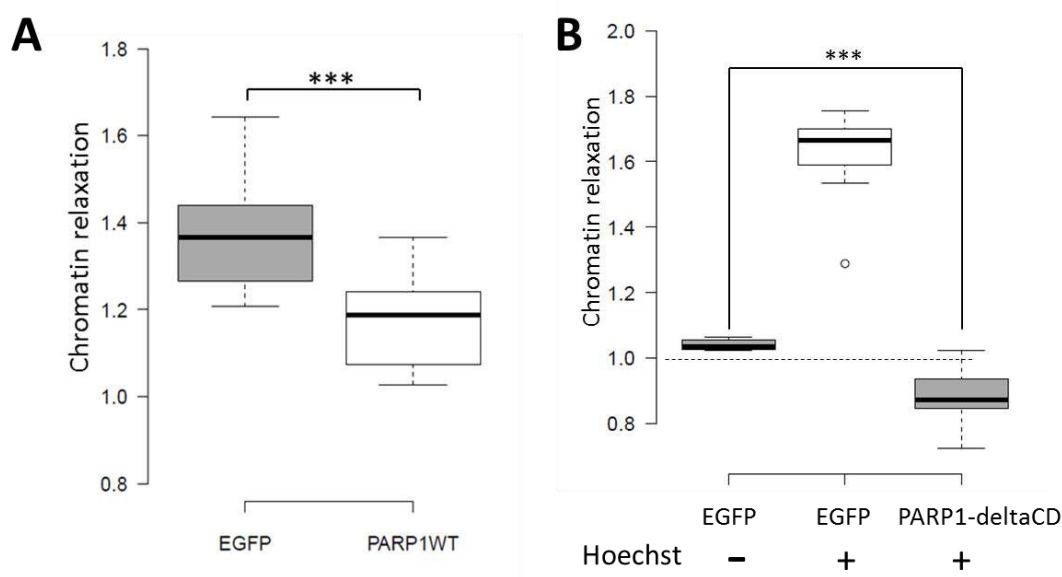


Figure 27. The effect of PARylation levels on the transient chromatin relaxation at DNA damage sites

(A) Relative chromatin relaxation at 60s after laser micro-irradiation in U2OS expressing H2B-PATagRFP and transfected with uncoupled EGFP and PARP1WT fused to GFP and micro-irradiation was set to 0.250 mW at the sample level, instead of 0.125 mW. (B) Relative chromatin relaxation at 60s after laser micro-irradiation in U2OS stably expressing H2B-PATagRFP and transfected with uncoupled EGFP and PARP1WT fused to GFP and micro-irradiation was set to 0.250 mW at the sample level, instead of 0.125 mW.

To assess the influence of PARylation levels on the chromatin relaxation, we measured the level of PARylation in cells over-expressing PARP1 WT or PARP1 deltaCD (lacking the catalytic domain), and treated or not with DNA damaging agent H₂O₂ (Figure 28). The level of PARylation increases after H₂O₂ treatment and seems to be non-significantly different in cells over-expressing PARP1 WT compared to GFP control cells (Figure 28), while the cells over-expressing PARP1 deltaCD show an abolition of the PARylation (Figure 28). PARP1 deltaCD seems to act as dominant-negative. In addition, these results suggest that even we have a decrease in chromatin relaxation in cells over-expressing PARP1WT, the level of PARylation in the latter stay unchanged compared to control cells.

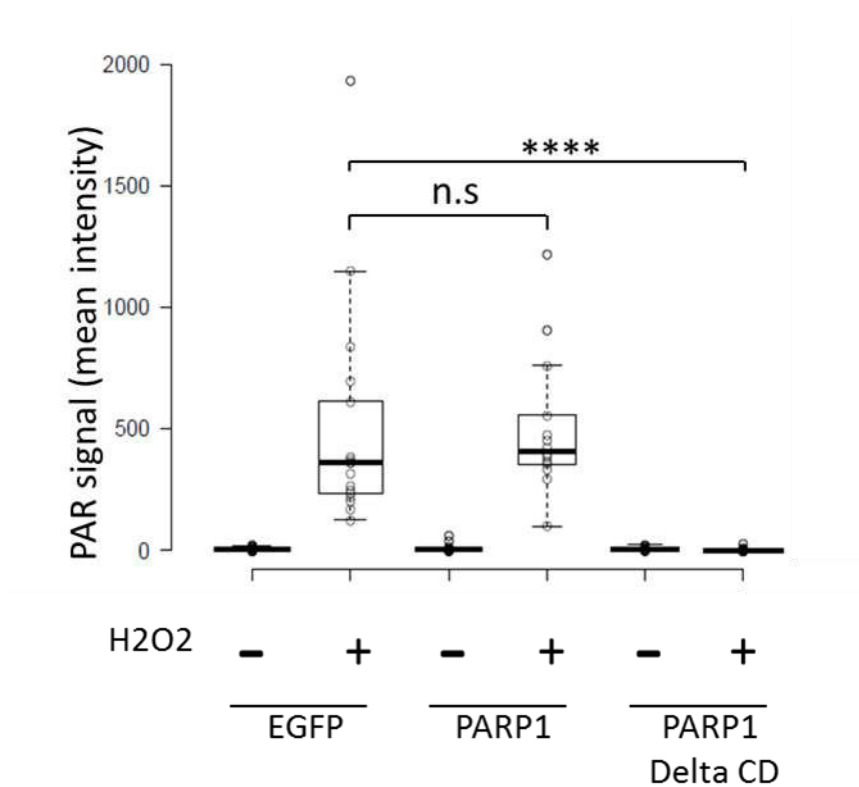


Figure 28. PARylation levels in cells over-expressing PARP1 WT and PARP1 delta CD mutant

Mean intensity of PAR signal for wild-type U2OS cells stably expressing H2B-PATagRFP and transfected with uncoupled EGFP or PARP1, PARP1 deltaCD fused to GFP, treated or not with H2O2 (1mM, 10 min).

In conclusion, we showed that the effect of PARP1 on chromatin structure after DNA damage might be a balance between its catalytic activity, PARylation which leads to chromatin relaxation, and its binding to chromatin which leads to chromatin compaction.

IV. Contribution of ATP-dependent Processes in Chromatin Relaxation at DNA Lesions

While, *in vitro*, chromatin loosening due to PARP1 activation does not require ATP¹⁶⁰, it has been shown that depleting ATP in living cells has been shown to lead to the suppression of chromatin relaxation at the DNA break sites⁸⁷. One could imagine that depleting ATP in living cells blocks the activation of PARP1. However, it has been shown that the presence of ATP, rather than its absence inhibits PARP1 activity²⁷⁶. In order to assess the role of ATP in our assays, we quantified the levels of PARylation as well as the chromatin relaxation upon laser micro-irradiation in cells where ATP was depleted. We observed that the depletion of ATP significantly impaired chromatin relaxation after DNA damage induction (Figure 29 A), while the level of PARylation was not affected at the DNA break sites (Figure 29 B). The level of PARylation is reflected through the recruitment of the WWE domain of RNF146 that recognize PAR chains²⁷⁷.

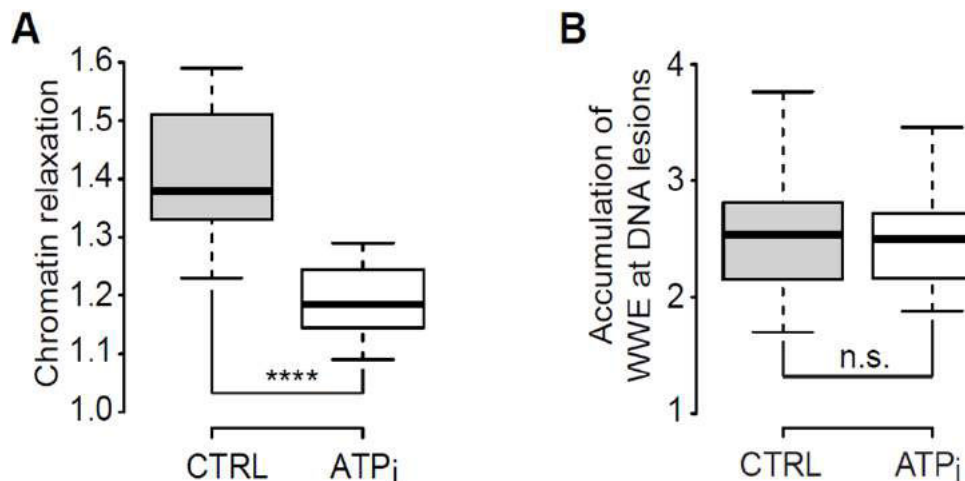


Figure 29. Chromatin relaxation at DNA damage sites partially depends on ATP (1)

(A) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type cells expressing H2B-PAGFP and depleted or not for ATP. (B) Accumulation of the WWE domain of RNF146 at the DNA lesions estimated 60s after laser micro-irradiation in wild type cells expressing an EGFP tagged version of WWE and depleted or not for ATP.

Nevertheless, the depletion of ATP did not fully abolished the chromatin relaxation, indeed the amplitude of the chromatin relaxation corresponds approximately half of the control situation, suggesting that PARylation has an effect on chromatin in both ATP-dependent and -independent manner. A cofounding effect of the inhibition of ATP is chromatin over-condensation. This effect could affect chromatin decondensation at DNA break sites. Using hypertonic treatment²⁷⁸ we induced chromatin hyper-compaction similar to that of ATP depletion (Figure 30 A). In cells treated with hypertonic medium, decondensation was slightly increased as compared to control cell treated with isotonic medium (Figure 30 B), while the level of PARylation at DNA lesions was unchanged (Figure 30 C). Therefore, tighter chromatin packing increases loosening, rather than reducing it, which is observed upon ATP depletion.

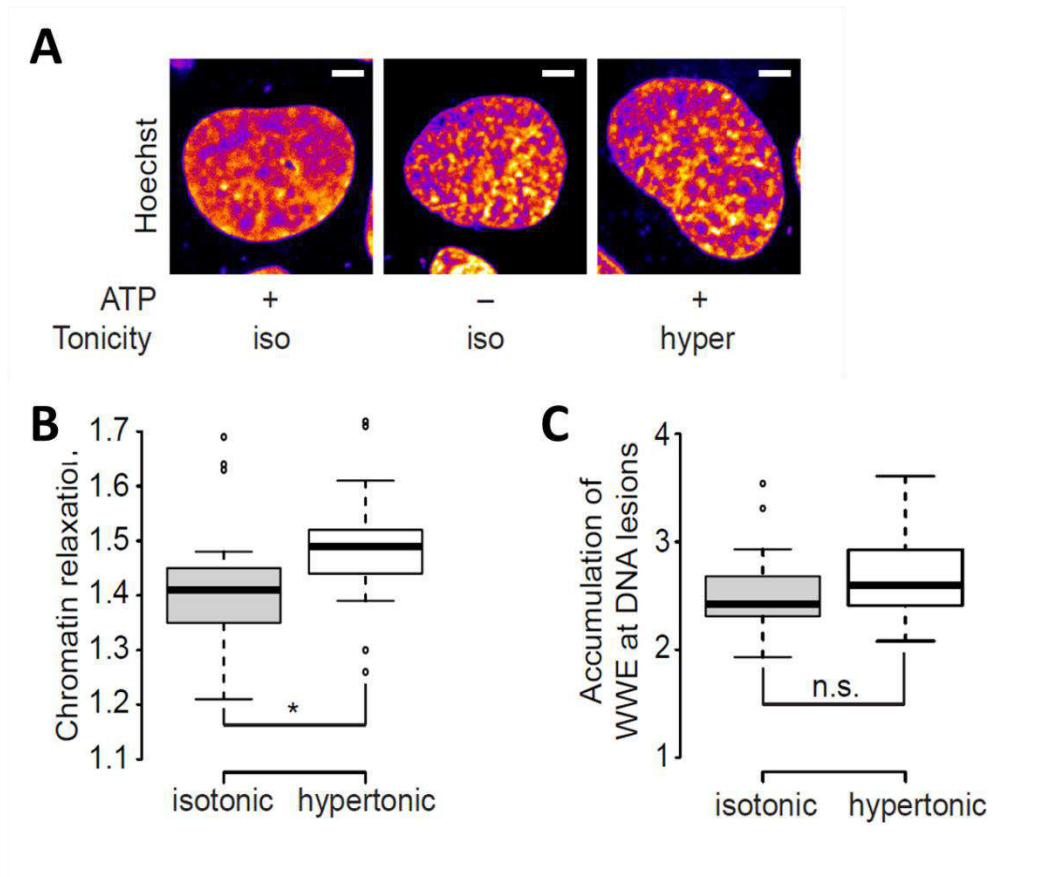


Figure 30. Chromatin relaxation at DNA damage sites partially depends on ATP (2)
(A) Confocal image of U2OS cell nuclei stained with Hoechst and left untreated, depleted for ATP or bathed with hypertonic medium. Bar = 4 μ m. **(B)** Relative chromatin relaxation at 60s after laser micro-irradiation in wild type cells expressing H2B-PAGFP and bathed in isotonic or hypertonic media. **(C)** Accumulation of the WWE domain of RNF146 at the DNA lesions estimated 60s after laser micro-irradiation in wild type cells bathed in isotonic or hypertonic media.

In conclusion, we showed that chromatin relaxation at DNA lesion sites not only depends on PARylation but also partially on ATP.

V. The ATP-Dependent Chromatin Remodeler Alc1 Contributes to Chromatin Relaxation

Trying to identify factors involved in the early step of the DNA Damage Response and particularly in the chromatin relaxation, we focused on ATP-dependent chromatin-remodeling enzymes. Several chromatin remodelers have been shown to be regulated by PARP activation ²⁷⁹, including Alc1 ^{201,235}, CHD4 ^{203,231}, CHD2 ²³⁴, SMARCA5/SNF2H ²⁰². We performed a mini-screen of siRNA knock-down using siRNA against several PAR-dependent chromatin remodelers including Alc1, CHD1, CHD4, SMARCA1 and SMARCA5. We used 3 different siRNA per targeted protein. We observed a significant decrease in chromatin relaxation when Alc1, CHD1, CHD4 and SMARCA1 were impaired (data from Gyula Timinszky).

Alc1, also known as CHD1L is a recently identified oncogene located at genomic region 1q21, a frequently amplified region in hepatocellular carcinoma (HCC) ²⁸⁰. Alc1 has been shown to play important roles in transcriptional regulation, maintenance of chromosome integrity and DNA repair ²⁸¹. Alc1 is rapidly recruited at DNA damage sites, with a maximum recruitment a few second after laser micro-irradiation ^{193,235} (Figure 31 A and B). This rapid recruitment is compatible with a role for Alc1 in chromatin relaxation at DNA lesion sites, a process that lasts approximately 60s (Figure 31 B).

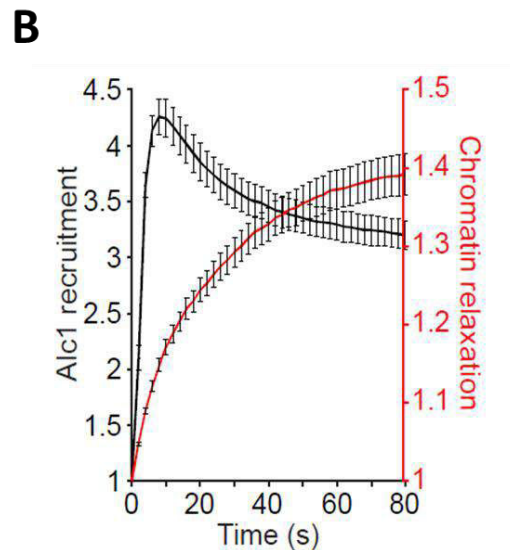
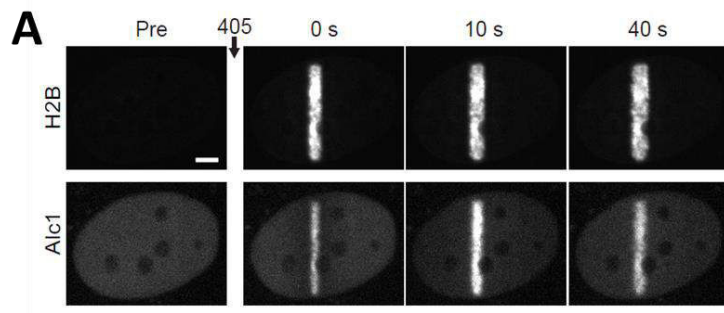


Figure 31. The chromatin remodeler Alc1 is quickly recruited at the DNA lesion sites
(A) Confocal image sequence of a U2OS nucleus co-expressing H2B-PAGFP and Alc1-mCherry. Bar = 4 μm . **(B)** Kinetics of Alc1 recruitment and chromatin relaxation at the DNA lesions measured in Alc1 knockout cells co-expressing H2B-PAGFP and Alc1-mCherry (mean \pm SEM).

In addition, Alc1 is the only chromatin remodeler to carry a macro domain which is able to bind PAR chains and allows its recruitment to the DNA lesion sites (Figure 32).

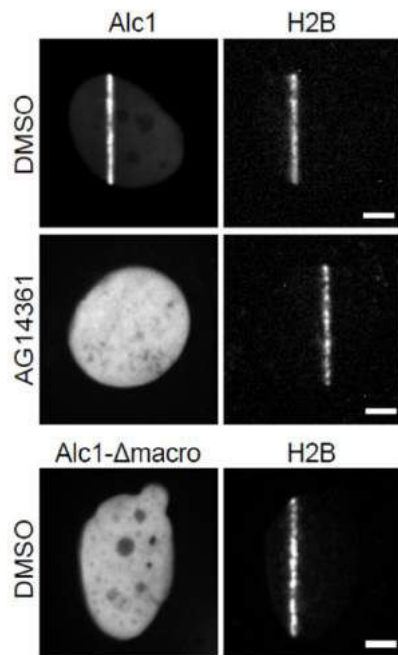


Figure 32. The chromatin remodeler Alc1 is recruited in a PAR-dependent manner at the DNA damage sites

Recruitment of Alc1 at the DNA damage sites in cells co-expressing the wild-type version of Alc1 fused to EGFP or a mutant lacking the macro domain (Alc1- Δ macro) fused to YFP, and H2B-PATagRFP. Cells were pre-sensitized with Hoechst and treated or not with the PARP inhibitors AG14361 (30 μ M, 1h). Images were acquired 4 s after laser micro-irradiation Bar = 4 μ m. The recruitment of Alc1 at DNA lesions was fully abolished upon treatment with AG14361 or for an Alc1 mutant lacking the macro domain.

Alc1 has not been shown to be part of a complex like CHD4 or other chromatin remodelers but rather seems to function as an individual protein so we decided to focus on it first.

To address the role of Alc1 in chromatin relaxation, we generated an Alc1 knockout U2OS cell line using CRISPR/Cas 9 approach (Figure 33).

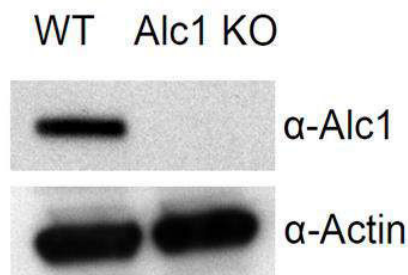


Figure 33. Alc1 knockout using CRISPR-Cas9
 Western-blot of wild type U2OS cells and the Alc1 knockout cell line.

First, we verified that the loss of Alc1 has no detectable effect on chromatin architecture in the absence of DNA damage (Figure 34 A). To address this question we analyzed the chromatin “texture” using an Image J plugging named “GLCM” (Gray Level Co-occurrence Matrices) written by Julio E. Cabrera. The GLCM method is a classical tool used to analyze the statistical distribution of pixel intensities at specified positions relative to each other in the image. A number of texture features (also known as Haralick features) may be extracted from the GLCM (Haralick et al. 1979) including the homogeneity, contrast, correlation and many other features.

We choose the contrast and correlation parameters to characterize chromatin texture using a pixel-to-pixel distance of 7 pixels, which allowed maximizing the differences measured between control cells and those subjected to osmotic stress inducing chromatin over-compaction or decompaction. When chromatin is condensed as it occurs when we treat with hypertonic medium, the correlation is decreased (Figure 34 B) and the contrast is increased (Figure 34 C). Instead, when we treat with hypotonic medium, the correlation increased (Figure 34 B), and the contrast decreased (Figure 34 C) reflecting a more relaxed chromatin state. We observe that

the absence of Alc1 had no detectable effect on chromatin architecture in the absence of DNA lesions (Figure 34 A, B and C).

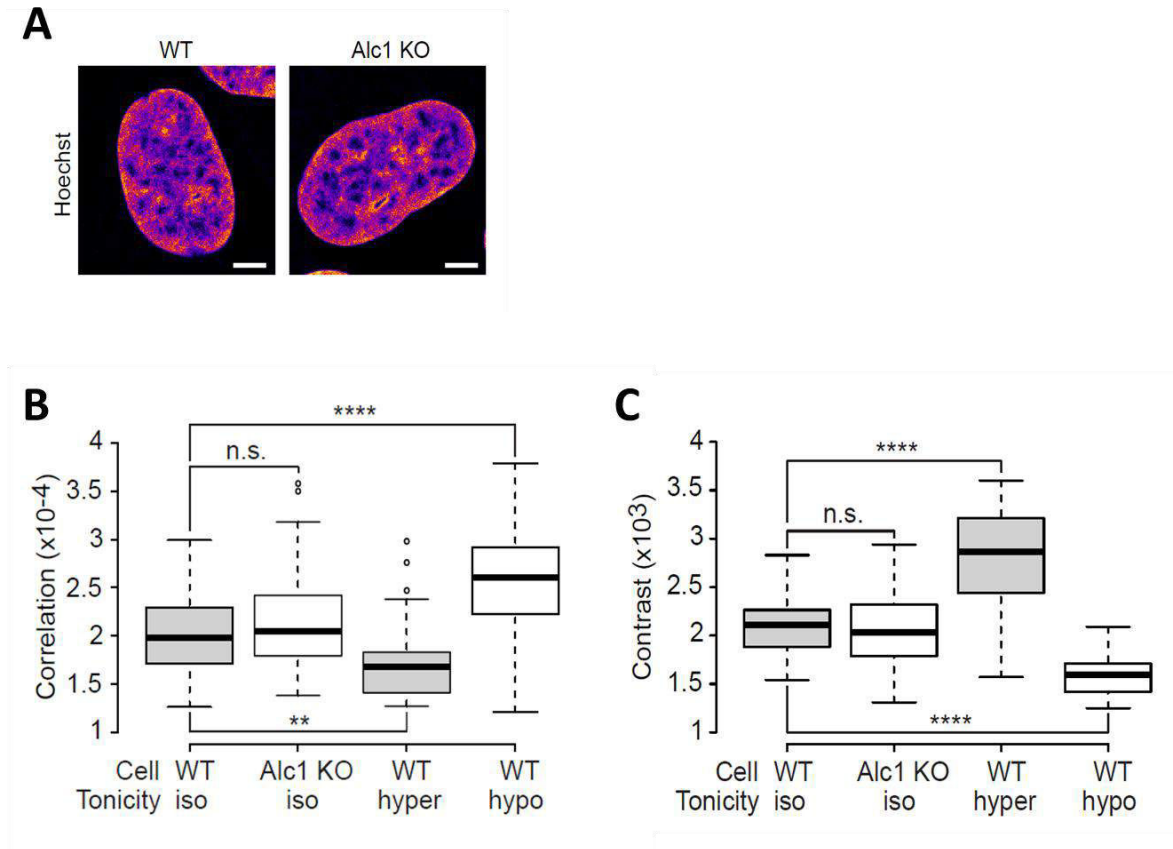


Figure 34. The knockout of Alc1 has no effect on chromatin architecture in the absence of DNA damage

(A) Confocal images of wild-type and Alc knockout U2OS cells labeled with Hoechst. Bar = 4 μ m. (B-C) Quantitative analysis of the Hoechst patterns in wild-type and Alc knockout U2OS cells. Two parameters were assessed to characterize the chromatin compaction state: the pixel-to-pixel correlation (B) and the contrast (C). As positive controls, we analyzed the chromatin patterns in cells bathed with hypertonic or hypotonic medium to induce chromatin hyper-compaction or decompaction, respectively. More than 70 cells conditions for the wild-type and Alc1 knockout cells. More than 25 cells conditions for the wild-type cells subjected to osmotic stresses.

Nevertheless loss of Alc1 leads to impaired chromatin relaxation upon laser irradiation (Figure 35). Re-expressing wild-type Alc1 in Alc1 deleted cells fully restored chromatin relaxation, whereas the ATP-ase-dead mutant (Alc1-E175Q) did not (Figure 35).

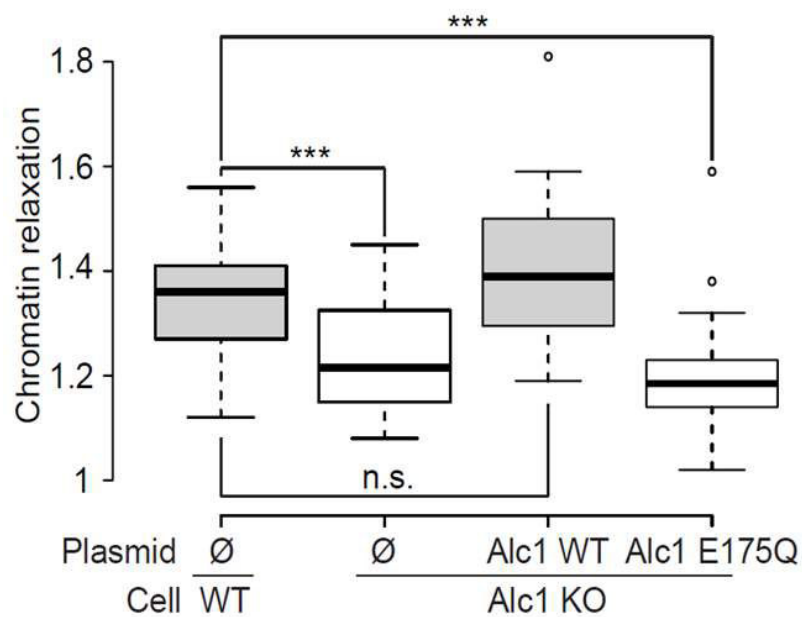


Figure 35. The chromatin remodeler Alc1 contributes to the chromatin relaxation upon DNA damage (1)

Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells versus Alc1 knockout cells co-transfected with H2B-PAGFP and an empty plasmid (\emptyset), wild type Alc1 or the catalytic-dead mutant Alc1 E175Q, both fused to mCherry.

We also used Alc1 RNAi treatment, and we observed a similar behaviour compared to knock-out cells (Figure 36).

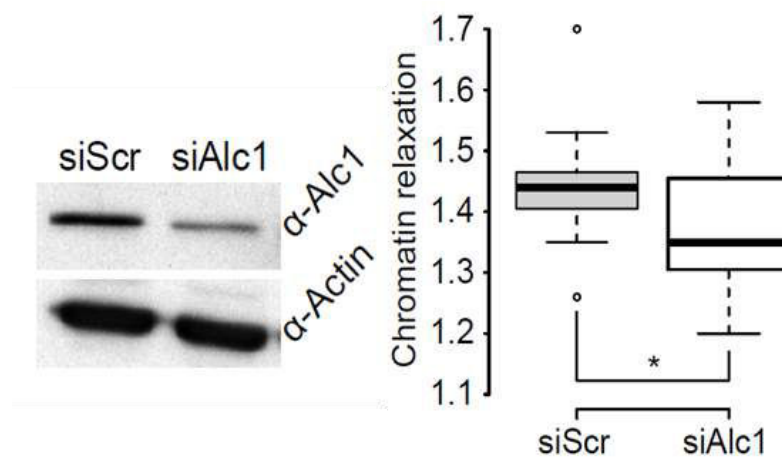


Figure 36. The chromatin remodeler Alc1 contributes to the chromatin relaxation upon DNA damage (2)

Western-blot of U2OS cells treated with a scrambled siRNA or with a siRNA directed against Alc1 (on the left). Relative chromatin relaxation at 60s after laser micro-irradiation for wild type U2OS cells stably expressing H2B-PATagRFP and transfected with a scrambled siRNA or a siRNA directed against Alc1. More than 15 cells per condition were imaged (on the right).

Interestingly, we observed that Alc1 effect was dependent on the intensity of the laser power. Increasing the laser power at a higher intensity (0.250mw instead of 0.125mw) showed no effect of Alc1 impairment on the chromatin relaxation (Figure 37).

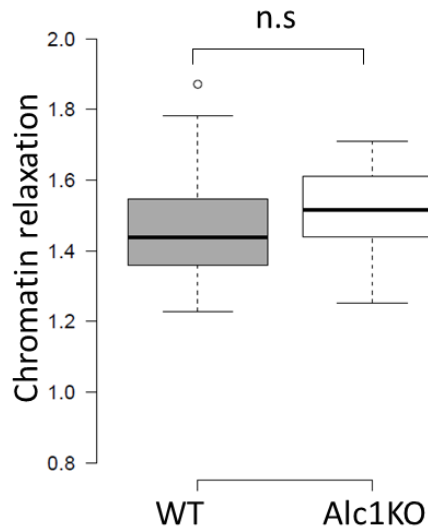


Figure 37. Alc1 effect on chromatin relaxation is dependent on the laser intensity used to induce DNA damage

Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells versus Alc1 knockout cells co-transfected with H2B-PAGFP and an empty plasmid (Ø) and micro-irradiation was set to 0.250 mW at the sample level, instead of 0.125 mW.

In addition, we observed a strongly increased chromatin relaxation at DNA lesions while we over-expressed Alc1 wild-type but not the ATP-ase dead mutant (Alc1-E175Q) (Figure 38 A). The over-expression of Alc1 wild-type seems to have no effect on the PARylation level (Figure 38 B). Altogether these results identify Alc1 as a mediator of PAR-dependent chromatin relaxation through its ATP dependent remodeling activity.

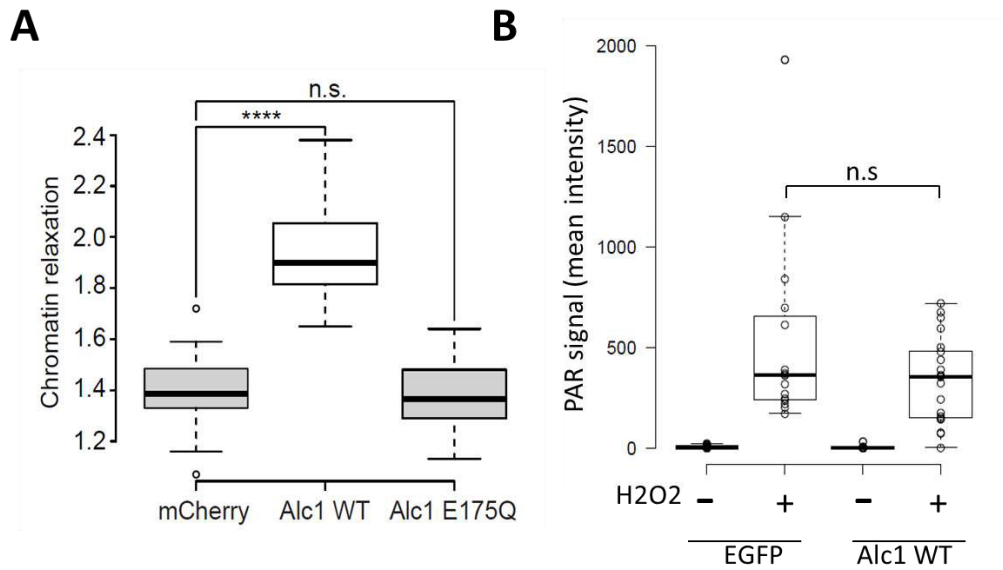


Figure 38. The over-expression of Alc1 leads to an over-relaxation of the chromatin (A) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells expressing H2B-PAGFP and transfected with uncoupled mCherry, wild type Alc1 fused to mCherry or the catalytic-dead mutant Alc1 E175Q fused to mCherry. (B) Mean intensity of PAR signal for wild-type U2OS cells stably expressing H2B-PATagRFP and transfected with uncoupled EGFP or Alc1 WT fused to GFP, treated or not with H2O2 (1mM, 10 min).

We next examined whether Alc1 could also be involved in the slow chromatin recondensation observed after the initial relaxation. We followed the chromatin recondensation in the Alc1 knock-out cells. We observed that chromatin slowly recondenses and seems to recover its initial state i.e pre-damage compaction similar to what is observed in wild-type cells (Figure 39 A). Interestingly, we observed no significant difference in the recondensation time between the Alc1KO cells and wild-type cells (figure 39 B). These results were obtained from one experiment and we need further investigations to figure out the Alc1 involvement in the chromatin recondensation.

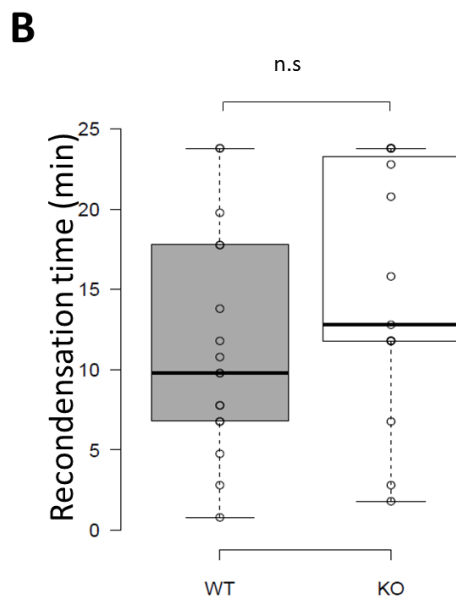
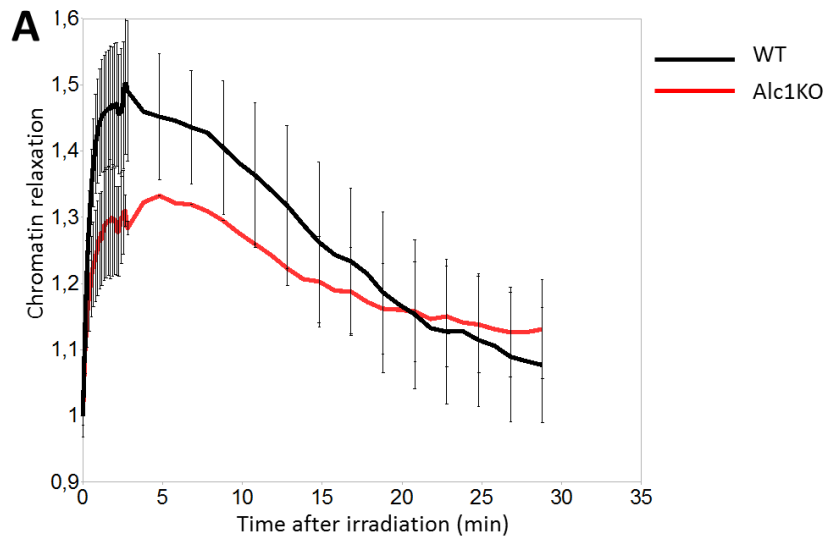


Figure 39. The chromatin remodeler Alc1 does not seem to contribute to the chromatin recondensation upon DNA damage

(A) Dynamics of the chromatin compaction state at DNA damage sites over long time scales in wild-type U2OS cells and the Alc1 knockout cell line transfected with H2B-PAGFP. Mean \pm SEM. 15 cells per condition were imaged. (B) Chromatin recondensation time in wild-type U2OS cells and the Alc1 knockout cell line transfected with H2B-PAGFP.

Similarly, we followed the chromatin re-condensation in cells over-expressing Alc1. We observed that the chromatin in the cells over-expressing Alc1 WT failed to recover its initial state and stayed opened during the 30 min time lapse (Figure 40).

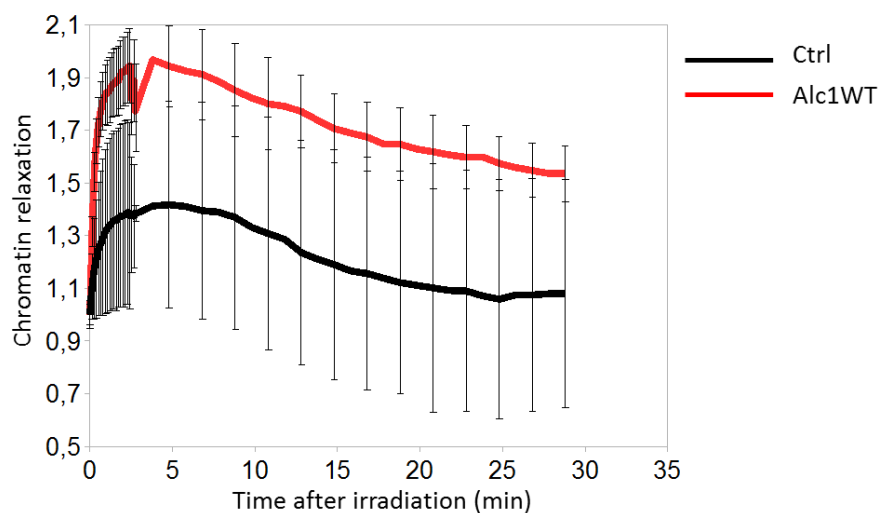


Figure 40. Cells over-expressing Alc1 WT fail to recover their initial condensation state
Dynamics of the chromatin compaction state at DNA damage sites over long time scales in wild-type U2OS cells co-transfected with H2B-PAGFP and uncoupled mCherry, Alc1 wild-type fused to mCherry. Mean \pm SEM. 10 and 6 cells per condition were imaged, respectively.

In conclusion, we showed that Alc1 induces local chromatin relaxation upon DNA damage through its catalytic activity, but do not seems to contribute to the chromatin recondensation upon DNA damage.

VI. The ATP-Dependent Chromatin Remodeler CHD4 Contributes to Chromatin Relaxation

CHD4 chromo-domain helicase DNA-binding protein 4 (CHD4; also known as Mi-2b) is a major subunit of the repressive nucleosome remodeling and deacetylase complex (NuRD) that is unique in combining chromatin remodeling activity with histone deacetylase and demethylase functions involved in transcriptional repression²⁸²⁻²⁸⁵. Loss of function of CHD4 or other NuRD components causes accumulation of DNA damage and features of accelerated ageing²⁸⁶.

The NuRD complex contains a helicase/ATPase domain that facilitates histone deacetylation in controlling chromatin reorganization and transcriptional regulation²⁸⁷. CHD4 is involved in the DNA Damage Response and cell-cycle control²⁰³. CHD4 have been shown to mediate the recruitment of the NuRD complex to DNA break sites in a PAR-dependent manner²⁰³. In our experimental conditions, CHD4 is recruited to DNA lesions sites in a PAR-dependent manner (Figure 41).

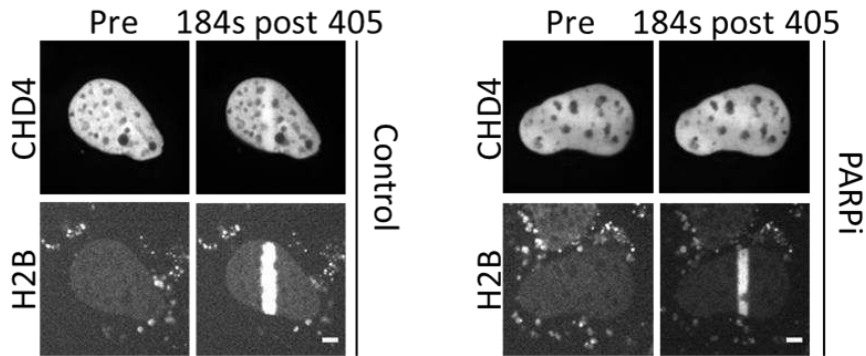


Figure 41. The chromatin remodeler CHD4 is recruited at DNA lesion sites in a PAR-dependent manner

Recruitment of CHD4 at the micro-irradiated area in cells co-expressing H2B-PATagRFP and transfected with uncoupled EGFP or CHD4 wild-type fused to GFP. Bar = 4 μ m. In cells non-treated with PARP inhibitor, the 405 nm irradiation induces both local photo-activation of the H2B-PATagRFP and a marked recruitment of CHD4-GFP. In contrast, in case of cells treated with PARP inhibitor, the 405 nm irradiation induces local photo-activation of the H2B-PAGFP but no recruitment of CHD4-GFP.

So, it was interesting to investigate its potential involvement in the chromatin relaxation that occurs after DNA damage induction. To assess the role of CHD4 in the chromatin decondensation process, we knocked-down CHD4 with siRNA treatment (Figure 23 B).

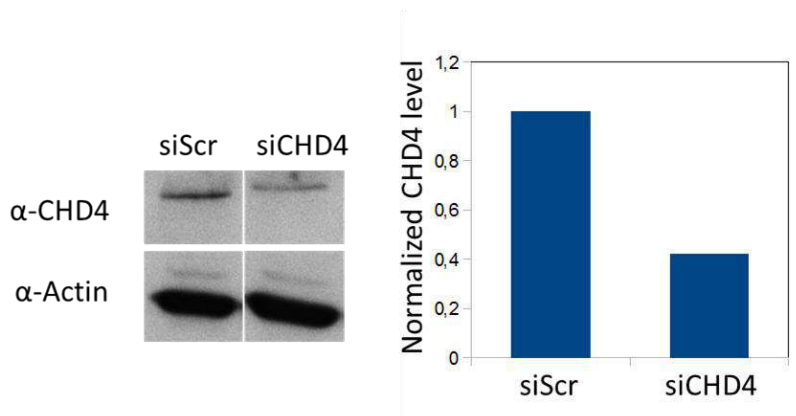


Figure 42. siRNA-mediated knock-down of CHD4

Western-blot of U2OS cells treated with a scrambled siRNA or with a siRNA directed against CHD4 (on the left), and normalized quantification of CHD4 protein level (on the right).

We found that CHD4 siRNA-mediated depletion significantly impaired the chromatin relaxation upon damage (Figure 43). The effect of CHD4 depletion is observed at low as well as at high laser power intensity and thus seems to be independent on the laser power intensity (Figure 43).

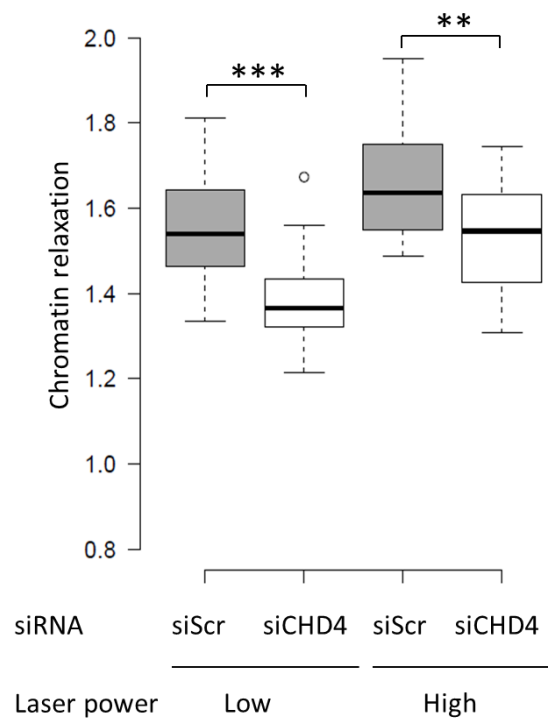


Figure 43. The chromatin remodeler CHD4 contributes to the chromatin remodeling upon DNA damage

Relative chromatin relaxation at 60s after laser micro-irradiation for wild-type U2OS cells stably expressing H2B-PATagRFP and transfected with scrambled siRNA directed against CHD4. The laser micro-irradiation was set to 0.250 mW for High laser power and 0.125mW for low laser power at the sample level. More than 15 cells per condition were imaged.

Over-expressing CHD4 does not lead to a particular phenotype in contrast to what was observed with Alc1 over-expression (Figure 44).

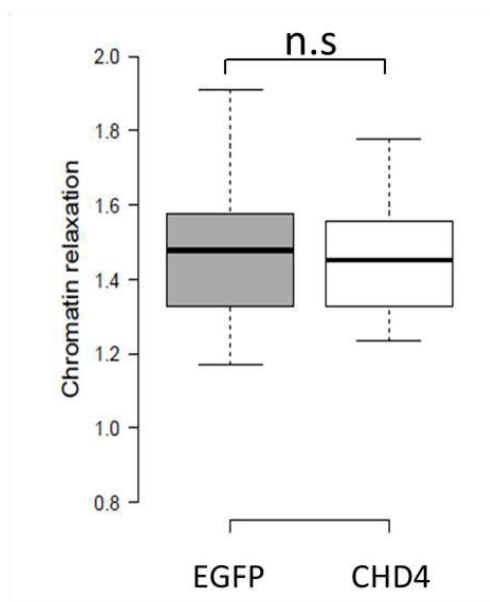


Figure 44. The over-expression of chromatin remodeler CHD4 seems to have no effect on chromatin relaxation DNA damage

Relative chromatin relaxation at 60s after laser micro-irradiation in U2OS stably expressing H2B-PATagRFP and transfected with uncoupled EGFP and CHD4 fused to GFP and micro-irradiation was set to 0.250 mW at the sample level, instead of 0.125 mW. More than 25 cells per condition were imaged.

VII. Histone Variants

Besides chromatin remodelers that directly act on chromatin, the composition of the nucleosome in terms of histone variant may also modulate the chromatin relaxation at the DNA breaks. Several histone variants have been shown to be involved in the DNA damage response including H2AX²⁸⁸, H2AZ¹⁹¹, H2A Bbd²⁸⁹.

In order to assess the involvement of these histone variants in the chromatin remodeling after DNA damage, we over-expressed these different histone variants in U2OS stably expressing H2B-PATagRFP and we followed the chromatin relaxation. We observed no significant difference between control cells expressing EGFP and the cells expressing, H2AX-GFP and H2A.Z-GFP cells (Figure 45). The over-expression of H2ABbd-GFP seems to lead to a small decrease of the chromatin relaxation (Figure 45). Since the results presented here are preliminary and represent only one experiment, further investigations are needed to better understand the role of these histone variants in the chromatin relaxation upon DNA damage.

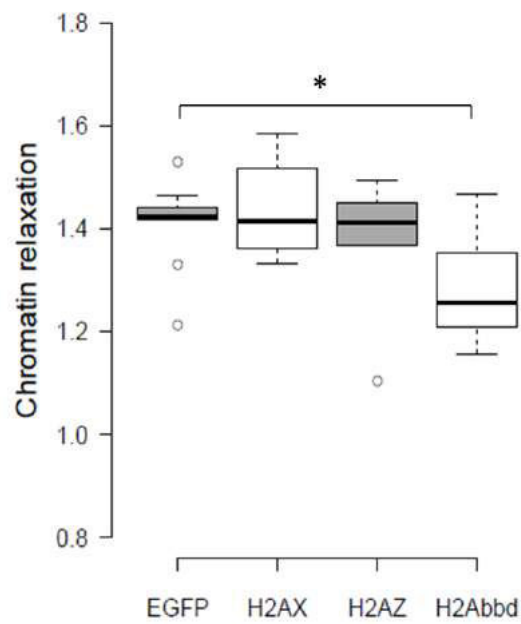


Figure 45. The H2A histone variants contribution to the chromatin remodeling upon DNA damage

Relative chromatin relaxation at 60s after laser micro-irradiation for wild-type U2OS cells stably expressing H2B-PATagRFP and transfected with uncoupled EGFP or H2AX, H2AZ and H2A Bbd fused to GFP. Few cells per condition were imaged (9; 11; 6; 10 cells respectively). The micro-irradiation was set to 0.250 mW at the sample level, instead of 0.125 mW.

VIII. Cross Talk Between PARP1, ATM and DNA PK Signalling Pathways

ATM and DNA-PK signalling pathways are also involved in the DNA damage response and cross talks exist between these pathways. To assess such possibility in the context of chromatin relaxation at DNA damage, we inhibited ATM and DNA-PK signalling pathway using inhibitors. Inhibiting DNA-PK seems to impair chromatin relaxation, while inhibiting ATM showed no significant difference compared to control cells (Figure 46). This preliminary data suggest an involvement of DNA-PK in the chromatin relaxation after DNA damage. As described previously, in the absence of PARP1 (PARP1 KO cells), a mild chromatin relaxation remains even after PARylation inhibition. We thought that this residual chromatin relaxation could involve an alternative signalling pathway which can be DNA-PK pathway. It will be interesting to see if we can abolish totally the chromatin relaxation after DNA damage by inhibiting DNA-PK in PARP1 KO cells.

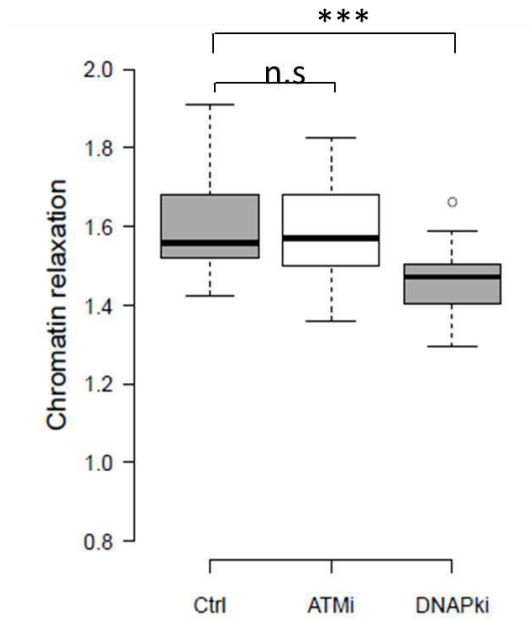


Figure 46. DNA-PK signalling pathway seems to be involved in chromatin relaxation upon DNA damage

Relative chromatin relaxation at 60s after laser micro-irradiation for wild-type U2OS cells stably expressing H2B-PATagRFP and treated or not with ATM inhibitor (20 μ M, 6h) and DNA-PK inhibitor (2 μ M, 6h), respectively. More than 15 cells per condition were imaged. The laser micro-irradiation was set to 0.250 mW at the sample level, instead of 0.125 mW.

DISCUSSION

I. The Assay to Induce and Follow Chromatin Dynamics upon DNA Damage: Advantages and Inconvenient

Methods to Induce DNA damages

Nowadays, several methods are used to induce DNA damage, including drug treatment such as etoposide (inhibitor of topoisomerase II)¹⁷¹, restriction enzymes which induce multiple DSBs in the genome^{255,290-292} and laser micro-irradiation^{87,179,293}.

To assess the chromatin re-organization upon DNA damage, we used photo-activables dyes and a 405 nm laser micro-irradiation to induce simultaneously the photo-activation and DNA damage in living cells. In this way we can easily follow the changes in chromatin compaction state at the site of DNA damage depending on different experimental conditions (protein over-expression, siRNA treatment, inhibitor treatments). A major advantage of the laser track as compared to other method to induce DNA damage such as restriction enzymes is its temporal resolution. With the laser micro-irradiation, we have a precise “time 0” (time after laser irradiation), which is crucial for analyzing fast repair processes. Nevertheless, some inconvenient are associated with such assay.

Type of Damages

The use of restriction enzymes or drugs to induce DNA damage provides a good tool to study at a molecular level, multiple DSBs, while laser micro-irradiation induce different types of DNA damage^{293,294}. In our assay, we observed the recruitment of PARP1 (recruited at SSBs as well as at DSBs), and 53BP1 (DSBs

marker) at the DNA lesion sites (Figure 18 B) suggesting that the 405 nm laser induces several types of DNA damages including SSBs and DSBs. We also observed an increase of the chromatin relaxation when we increase the laser power (Figure 18 H) which can reflect an overall increase of the amount of damage. Similarly, Alc1 knockout does not have the same effect depending on the 405nm laser power (Figure 22 D and 22 L). At low laser power, we observed a significant decrease of the chromatin relaxation between wild-type cells and Alc1 KO cells, whereas at high laser power, we did not observed difference in chromatin relaxation between wild-type cells and Alc1 KO cells. These results might suggest that when we have a small amount of DNA breaks (at low laser power), Alc1 might be the major mediator of chromatin relaxation. However, when the amount of DNA breaks is higher (at high laser power), other chromatin remodelers might be activated, making Alc1 dispensable for the relaxation process. Another possibility is that different types of DNA damages are induced depending on the laser irradiation condition. Alc1 would be involved specifically in the chromatin remodeling at DNA lesions induced at low laser power. This latter possibility is in line with the fact that PARylation signaling, which controls Alc1 recruitment^{201,235}, seems mostly involved in SSB repair rather than DSB repair¹⁴⁵. To test this hypothesis, it will be interesting to assess the relative contribution to chromatin relaxation of other chromatin remodelers depending on the power used for laser irradiation.

Localization and Number of Damages

The restriction enzymes are able to induce multiple DSBs located within various chromatin states ²⁹⁰ depending on the chosen restriction enzyme and the area where the restriction sites are integrated in the genome, while laser micro-irradiation induce DNA damage along a line or a spot in the nucleus in a region of interest regardless of the genomic position. In addition, the number of damages induced by restriction enzymes is theoretically known since we know the number of restriction sites. However, due to the efficiency of cutting, the number of DSBs induced effectively could differ. It is possible to quantify the real number of damages using Chromatin immunoprecipitation at the restriction sites and mapping the recruitment of DNA damage factors before and after DNA damage induction ²⁹⁰. In the case for damages induced by laser micro-irradiation where no genomic landmark exist, it is possible to assess the number of DNA damages using techniques such as TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling) assay where break are detected by terminal deoxynucleotidyl transferase mediated dUTP-biotin end labeling and followed by incubation with avidin coupled to fluorochrome ²⁹⁵.

II. Why Do Cells Relax their Chromatin upon DNA Damage?

Open the Access to Damaged Chromatin

Chromatin is a dynamic structure that is able to adopt different conformations. For instance, chromatin display contrasting levels of compaction depending on the cell cycle phase: highly compacted during mitosis, more relaxed during interphase

²⁹⁶. In the case of transcription, it is now well established that chromatin undergoes different conformation changes in order to modulate transcriptional activity ²⁹⁷. A “compacted” chromatin structure is generally considered as inhibitory for the binding of transcriptional regulators ²⁹⁸. As a solution to counteract this inhibitory effect of chromatin compaction, cells activate molecular machinery that uses the energy of ATP to remodel the nucleosomal structure ²⁹⁸.

Until recently, the functional interplay between chromatin conformation and the DNA repair process has remained poorly understood. Recently, several studies have implicated chromatin-remodeling complexes and histone-modifying enzymes in DNA lesion repair ^{198,201,203,204,239,299-302}. Consistent with other studies, we observed that after the induction of DNA damage, chromatin relaxes at the site of DNA lesion ^{87,179,274}. In addition we showed that chromatin remodelers Alc1 and CHD4 are involved in this chromatin relaxation, linking chromatin remodeling events at the fiber level to global chromatin relaxation. But why cells need to relax their chromatin after DNA damage?

A straightforward reason would be that chromatin relaxation facilitates DNA access to repair proteins (similarly to a transcription context) as illustrated by the hypersensitivity of the chromatin to nucleases at the DNA breaks and in line with the “access-repair-restore” model proposed by Smerdon in 1991 ³⁰³. Recently, it has been shown that the compaction of chromatin can alter the DNA damage response ²⁴⁹. The histone variant macroH2A1 has been shown to promote chromatin condensation at DSBs sites¹⁷⁹. By measuring the effect of compaction on the recruitment of Ku70, which forms a heterodimer with Ku80 that binds to

double-strand breaks, it has been described that the recruitment of endogenous Ku70 was reduced in cells expressing macroH2A1.1¹⁹³. This suggests that chromatin compaction by macroH2A1.1 suppresses Ku70 recruitment. In addition, Murr and colleagues²⁴⁹, provided evidence to support the hypothesis that chromatin structure might be a barrier to the recruitment of DNA repair machinery at sites of DNA lesions. The authors were able to counteract impaired recruitment of repair molecules at sites of damages by inducing chromatin relaxation using chloroquine, sodium butyrate or hypotonic conditions. Consequently, these studies highlight the importance to decondense the chromatin in order to facilitate the repair of DNA lesions and ensure the integrity of the genome. Interestingly, it has been proposed that the localization of proteins such as transcription factors to damaged chromatin may not only rely on the recognition of PTMs but also the ability to bind DNA through DNA-binding domain³⁰⁴ which suggest that chromatin relaxation may facilitate such DNA-binding. More recently, a new biophysical mechanism named liquid demixing has been proposed to modulate access of repair proteins to the DNA damage sites³⁰⁵. The liquid demixing is defined as a process that generates membrane-less compartments within the subcellular space in which certain components are enriched while others are excluded³⁰⁵. The liquid demixing might create a phase separation in order to provide a platform of proteins to initiate and or /select DNA damage response pathways. Altogether, these results might suggest that the DNA relaxation is not necessary for accessing the DNA breaks since compact chromatin structure still allow penetration of fluorescent tracers²⁵² but may

contribute to a potentially more efficient scanning of the chromatin fiber while searching for the breaks. This would be consistent with the model proposed by Bancaud and colleagues when comparing protein binding to chromatin in euchromatin versus heterochromatin ²⁵².

Facilitate the Repair of Damaged Chromatin

The relaxation of chromatin may also facilitate the research of homology in the context of homologous recombination. The DNA break ends may have more probabilities to access to numerous DNA sequences in order to find homologous template for repair. In diploid yeast, the induction of DSBs by restriction enzymes or drug treatment was also associated with an expansion of the nuclear area explored by the damaged locus ^{165,166} suggesting a need for the damaged chromatin to explore the nucleus in order to find and pair with its homologous sequence. In line with the results obtained in yeast, our data in mammalian cells (from other lab members) show that chromatin relaxation is associated with an increased local chromatin movement.

The chromatin over-condensation or re-condensation following chromatin relaxation upon DNA damage has been shown associated to the recruitment of heterochromatin proteins ^{179,180,253}. This may originate from the necessity to both inhibit transcription ³⁰⁶ of the damaged DNA and keep the loose broken DNA ends in close proximity to facilitate repair. However keeping in close proximity broken DNA ends from two spatially close chromosomes may lead to chromosome translocations ³⁰⁷.

Chromatin Sensitivity to DNA Damage

The chromatin relaxation process also asks the question of the influence of the chromatin architecture prior to micro-irradiation. As yet, it has been shown that condensed chromatin is much less susceptible to DSBs induced by γ -rays than decondensed chromatin^{308,309}. We can speculate that the lower sensitivity of DNA to the damage induced by ionizing radiation in heterochromatin is not only due to the chromatin compacted state but also caused by the presence of a large amount of proteins bound in these regions which can provide protection against DNA damage. For example, in bacteria, it has been shown that Dps, an inducible nonspecific DNA-binding protein was able to protect DNA from DNA damage induced by oxidative stress³¹⁰. One could imagine that similar mechanisms might exist in eukaryotes. Further work is needed to better characterize the factors and conditions that drive chromatin sensitivity regarding DNA damage by testing sensitivity of different chromatin template (condensed, relaxed, with +/- bound proteins) to DNA damage.

III. Which Factors are implicated in the Chromatin Relaxation?

PARP1 and Its Alternatives

In our study, consistent with other reports, we showed that the activation of PARP1 regulates the chromatin structure and the transient chromatin relaxation at DNA lesion sites. In addition, we showed that if the knock-out of PARP1 dramatically decreases chromatin relaxation, a mild chromatin relaxation is still observed in the absence of PARP1 in these cells (Figure 19 C). So in the case where PARP1 is not present, which factor could modulate chromatin structure? One first possibility could be the involvement of PARP2 or PARP3 proteins. PARP2 and PARP3 are also involved in DNA damage response^{128,311} and are recruited at the DNA lesion sites (data from Théo Lepeaubin). Although PARP2 has been shown able to “rescue” PARP1 deficiency^{312,313}, PARP2 and PARP3 seem to be involved in different pathway than PARP1 in the DNA damage response^{313,314}.

A second possibility could be the involvement of other DNA damage signalling pathways including ATM pathway or DNA-PK pathway. Our preliminary results have shown that the inhibition of DNA-PK and not the inhibition of ATM leads to a decrease of chromatin relaxation after DNA damage. On one hand, DNA-PK has been reported by several studies to interact with PARP1 enzyme¹⁵⁵. On the other hand, other reports imply that they have separate roles within the NHEJ DNA repair pathway by competing for DNA break sites³¹⁵. In addition, no direct effect on chromatin structure have been described in contrast to what is as

observed for PARP1, leading to a non-elucidated question. More experiments need to be performed for a better understanding of DNA-PK role in the chromatin relaxation after DNA damage.

Involvement of Histone Variants

Our preliminary results have shown that the different histone variants we tested γ H2AX and H2AZ seem to have no significant effect on the chromatin relaxation after DNA damage. Histone variant H2ABbd could have a small effect but need to be confirmed with more experiments. The macroH2A1 variant has been shown to mediate a shift from accessible to condensed chromatin in a DSBs context ¹⁷⁹. In addition, following chromatin relaxation after DNA damage, using our assay, work performed by Marek Kozlowski (manuscript in preparation) in our lab showed an impairment of chromatin relaxation at the DNA lesions sites in cells over-expressing macroH2A variants. This impaired chromatin relaxation associated to the over-expression of macroH2A variants promotes PARP1 retention at DNA lesion sites. This suggests that macroH2A variants may act as a regulator of chromatin relaxation in order to limit the level of chromatin relaxation to avoid deleterious effects.

Coordination Between The Chromatin Remodelers

The direct recruitment of chromatin remodelers relies on PTMs such as PARylation which probably act in a sequential and interdependent fashion ^{109,201-203,235}. The binding of chromatin remodeling enzymes to DNA lesion further alters chromatin structure by inducing nucleosome sliding and/or histone exchange

^{228,230,240}. We identified chromatin-remodeling enzymes Alc1 and CHD4 that are recruited to DNA damage sites as involved in the chromatin relaxation after DNA damage. Alc1 is recruited at the DNA lesion sites without being part of a complex ^{201,235}, whereas CHD4 is known to be recruited as part of the NuRD complex ²⁰³. CHD4 has been described to promote heterochromatin formation ³¹⁶, which contrasts with its role in chromatin relaxation. Alternatively, CHD4 may act on chromatin relaxation being part of another complex, in association with acetyltransferase p300 ³¹⁷. More recently, chromatin remodeler CHD2 has also been reported to play a role in chromatin remodeling after DNA damage in a PAR-dependent manner ²³⁴. CHD2 appears to be recruited slightly later than Alc1 and CHD4 to DNA damage site, so we can speculate that the three remodelers may act sequentially to allow chromatin loosening. Further work is needed to understand how the activities of these three chromatin remodelers are coordinated. Do we have a chromatin remodeler that may act as a priming factor to open the chromatin and then allow the recruitment of additional chromatin remodelers to open efficiently the chromatin for a facilitated access to DNA lesion sites? Do these remodelers possess specific activities that target specific type of damage or remodel specific chromatin domains? To answer these numerous questions, we need to better understand the interplay between these different chromatin remodelers, comparing their kinetics, knocking them down and see if they compensate each other to rescue the phenotype by expressing CHD2 or CHD4 in Alc1 KO cells.

IV. How Do Structural Changes Affecting the Chromatin Fiber Lead to Global Chromatin Relaxation?

Effect of PARylation Itself on the Chromatin Architecture

In the absence of damage, PARP1 binding to chromatin leads to chromatin compaction. Instead, the activation of PARP1 promotes local chromatin relaxation¹⁶⁰. This local chromatin relaxation could be due to electrostatic repulsion between the negatively charged PAR chains, or between the PAR chains and the DNA helix¹⁹⁸. Steric hindrance induced by the bulky PAR chains attached to the chromatin fiber may also contribute to chromatin fiber loosening¹¹⁴. *In vitro* studies described the linker histone H1 as instrumental in the transition of the loose, bead-on-a-string chromatin fiber to compacted chromatin⁵. H1 is a substrate of PARP1 and its PARylation has been shown to trigger its dissociation from chromatin in the context of transcription³¹⁸. One could imagine that the PARylation of H1 might lead to its dissociation from chromatin and promote chromatin relaxation. However, we found that the mobilization of linker histone H1 at DNA lesions does not trigger directly the PARP1-dependent chromatin relaxation (data from Théo Lepeaubin).

Nucleosome Repositioning by Chromatin Remodelers

The chromatin relaxation involves chromatin remodeling events as well as post-translational modifications. Depending on the chromatin remodeling enzymes and its associated subunits, the remodeling reaction can include nucleosome sliding (i.e lateral movement to a new position without histone loss), partial or complete

nucleosome eviction, and changes in histone octamer composition such as histone dimer exchange^{162,248,319,320}. Several data on the remodeling mechanism *in vitro* have been generated, however, not much is known about the targeting and regulation of the remodelers *in vivo*¹⁶². The chromatin remodelers could regulate nucleosome positioning after DNA damage by disrupting the genomic landscape of nucleosome positions³¹⁹. In yeast for example, Isw2 and Isw1a complex shift nucleosomes at the 5' ends of genes from 3' to 5', toward the nucleosome-depleted region (NDR). Loss of one or the other complex leads to +1 and +2 nucleosomes shifting downstream into target genes³¹⁹. In the context of DNA lesions, chromatin remodelers might change the chromatin positioning pattern in order to modulate chromatin packing state to influence the scanning and binding of repair factors at the DNA lesion sites²⁵².

The remaining question is what could be the impact of nucleosome sliding at the chromatin fiber level on the global chromatin compaction state? So when chromatin relaxes after DNA damage one could imagine that chromatin remodelers could release or slide the nucleosomes and allow the removal or displacement of non-histone architectural proteins (Figure 26). In our case, we do not get release of H2B proteins at DNA lesion sites (data from Théo Lebeaupin) and Alc1 has been shown to slide nucleosomes *in vitro*²³⁵, so we can speculate that sliding by Alc1 at the DNA breaks sites might allow the removal or displacement of non-histone architectural proteins such as CTCF proteins.

The zinc finger factor, CCCTC-binding factor (CTCF)⁴⁰ was initially described as a transcription factor³²¹, and has been gradually characterized as an organizer of

higher-order chromatin that facilitates the establishment of TADs ⁴⁰. CTCF is associated with cohesin to form or stabilize long-range chromatin loops from CTCF binding sites ^{322,323}. To validate this model, it would be interesting to check whether CTCF and cohesion dynamics at the chromatin is changed upon DNA damage induction. In addition, the analysis of the impact on the chromatin relaxation of the knocking down of these proteins would be informative.

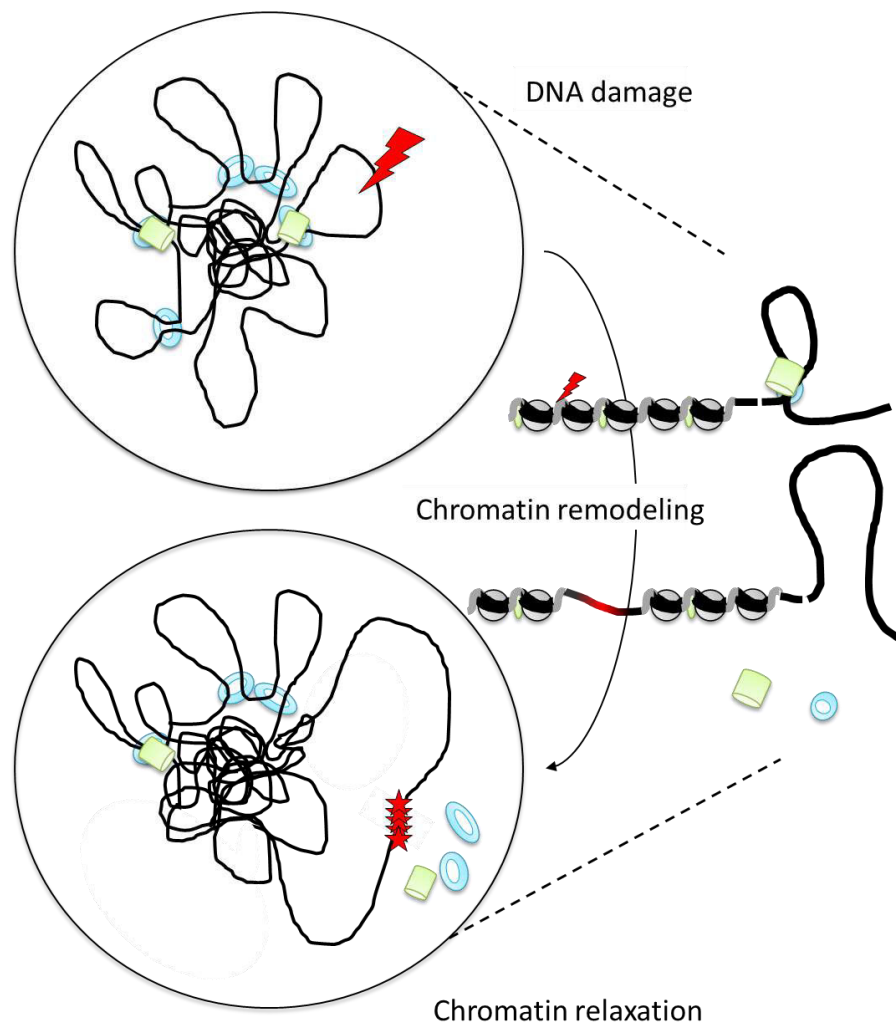


Figure 47. Speculative model for chromatin re-organization upon DNA damage. CTCF proteins are represented by green barrels and cohesins by blue rings. After DNA damage, CTCF proteins and cohesins might be removed or displaced to allow chromatin remodeling and chromatin relaxation

So, we still do not know exactly why cells need to relax their chromatin after DNA damage. Nevertheless, we have a beginning of responses that draw possible mechanisms. But due to the diversity of the different type of induced damages, we still lack essential information to puzzle the complex changes that occur in chromatin structure after DNA damage. Comparison with transcription-dependent chromatin re-organization could help us to reach a better understanding for such events.

EXPERIMENTAL PROCEDURES

Plasmids

The core histone H2B, subcloned from the pH2B-mCherry vector (gift from J. Ellenberg³²⁴, Euroscarf, accession number P30632), was cloned into pPATagRFP-N1 using NdeI and BamHI restriction sites. pPATagRFP-N1 was a gift from V. Verkhusha³²⁵, Addgene plasmid # 31941). The histone H2B-PAGFP was gift from J. Ellenberg³²⁶, Euroscarf accession number P30499 and P30503, respectively. Wild type Alc1 and E175Q Alc1 mutant fused to the C-terminus of EGFP or mCherry were obtained by exchanging YFP for the respective fluorescent protein in the constructs described previously²³⁵. The Alc1- Δ macro mutant fused to YFP was described previously²³⁵. The WWE domain of RNF146 (amino acids 99-183) was cloned into pmEGFP-C1 using BglII and EcoRI by PCR amplifying it from a cDNA library. For PARP1-EGFP, we exchanged mCherry with EGFP from a PARP1-mCherry construct published previously¹⁹³. PARP1- Δ CD-EGFP and 53BP1-EGFP constructs were gift from Gyula Timinszky. Mammalian expression was under the control of CMV promoter. The CHD4-GFP construct was gift from Sophie Polo²⁰³. The histone variant H2AX, H2A Bbd²⁸⁹ and H2AZ were obtained from Sandra B. Hake. All constructs were sequence verified.

Cell Culture, Inhibitor Treatments, and Osmotic Shocks

Cells used for this work were wild-type U2OS cells or knockout cells made from parental U2OS cells. Cells were routinely cultured in Dulbecco's modified Eagle's medium (with 4.5 g/L glucose) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μ g/mL penicillin, 100 U/mL streptomycin in 5% CO₂ at 37 °C. For

microscopy, cells were plated on Lab-Tek II chambered coverglass (Thermo Scientific). Pre-sensitization was achieved by bathing cells for 1h in culture medium containing 0.3 $\mu\text{g/mL}$ Hoechst 33342 (Life Technologies). Immediately before imaging, the growth medium was replaced by Leibovitz's L-15 medium (Life Technologies) supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 $\mu\text{g/mL}$ penicillin and 100 U/mL streptomycin. The PARP1 inhibitors AG14361 and Olaparib (Euromedex) were used at 30 μM and 50 μM , respectively. ATP depletion was achieved as described in ³²⁷. The osmotic shock procedure was previously described in ²⁷⁸.

Live cell DNA Labelling with Fluorescent Nucleotides

U2OS cells expressing H2B-PATagRFP were synchronized at the G1/S phase transition by treating the cells with aphidicolin (Sigma) at 5 $\mu\text{g/mL}$ for 18h. After aphidicolin release, the cell layer, bathed with growing medium containing 10 μM of dUTP-ATTO633 (Jena-Bioscience), was scraped using a silicon stick to allow nucleotide loading and integration to the DNA during replication.

Transfections and Generation of Stable and Knockout Cell Lines

Transient transfections were performed 12-24h after plating cells using XtremeGENE HP (Roche) or JetPRIME (Polyplus Transfection) according to manufacturer's instructions. Cells were imaged 48 to 72h after transfection.

To establish U2OS cell lines stably expressing H2B-PATagRFP, wild-type cells were transfected with the H2B-PATagRFP construct using XtremeGENE HP (Roche) and grown in culture medium containing Geneticin (PAA) for selection. Clones with stably integrated H2B-PATagRFP were picked after two weeks of Geneticin selection. Once selected, these cells were cultured in normal medium supplemented with 500 µg/mL Geneticin (PAA).

The knockout cell lines were made according to the protocol described by the Zhang lab³²⁸. The target sequence for *ALC1* (5'-GACTTCCCTCAAGTACGTTAG-3') and PARP1 (5'-GTCCAACAGAAGTACGTGCAA-3') was chosen according to the web-based CRISPR design tool from Zhang lab (<http://www.genome-engineering.org>). The sgRNA oligos were introduced into pX458 expressing Cas9 nuclease fused to GFP (Addgene #48138). pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang (Addgene plasmid # 48138). We transfected the plasmids using the transfection reagent XtremeGENE HP (Roche) according to manufacturer's protocol. Single GFP positive cells were sorted into 96-well plates using FACS. The knockout cell lines grown up from the single cells were identified by western blot using specific antibodies against PARP1 or Alc1.

siRNA knock down

For RNAi-mediated knockdown, we used Silencer® Select Negative Control No. 2 (ref 4390846), siRNA against Alc1 (CHD1L) (ref s18358), and siRNA against CHD4 (ref s2984) from Ambion (Thermo Fisher Scientific). Cells grown in normal culture

medium were transfected with 500 nM siRNA using Oligofectamine (Life Technologies) according to the manufacturer's instructions. After 48h, cells were used for imaging or harvested for protein analysis.

Western Blot

Cell lysates were separated using SDS-PAGE, and transferred to nitrocellulose membranes (GE Healthcare) and blocked in 5% (w/v) milk powder in 0.05% (v/v) PBS-Tween 20 at room temperature. The primary antibodies were diluted in 5% (w/v) milk powder in 0.05% (v/v) PBS-Tween 20 and used at the following concentrations: affinity purified anti-Alc1 rabbit polyclonal, 1:2500; anti-Actin (Sigma-Aldrich, A5060), 1:1000; anti-PARP1 rabbit polyclonal, 1:10000; anti-CHD4 mouse monoclonal (Abcam ab54603), 1:1000; the mouse monoclonal (DM1A) anti-tubulin (Sigma-Aldrich T9026), 1:20000. To detect primary antibodies, HRP-conjugated secondary antibodies were used. The HRP-conjugated anti-rabbit IgG and anti-mouse IgG antibodies (Bio-Rad) were used at 1:10000 and the blot was developed using the ELC reagent (Merck Millipore).

Immunofluorescence

Measure of PARylation level in cells overexpressing PARP1, PARP1 deltaCD and Alc1: Cells were transfected with the different construct 48h hours before being fixed following the Immunofluorescence procedure described in ¹⁹³. Images of fixed cells were taken on an inverted confocal spinning disk (imaging scan head CSU-X1 from Yokogawa and microscope body Ti-E from Nikon) and the images were assembled

using ImageJ. To quantify PAR signal intensities into the nucleus, images were background subtracted, the nuclei were segmented using Hoechst staining and average signal intensities were measured using ImageJ, and subsequently analyzed and plotted in Excel.

Live Cell Imaging and Laser Micro-Irradiation

Live cell imaging was performed on an inverted confocal spinning disk (imaging scan head CSU-X1 from Yokogawa and microscope body Ti-E from Nikon) equipped with a single-point scanning head to allow laser micro-irradiation and local photo-activation using a 405 nm laser. We used a Plan APO 63x oil immersion objective lens (O.N. 1.4) and a sCMOS ORCA Flash 4.0 camera (Hamamatsu) for imaging the cells. The pixel resolution at the object plane was 108 nm. The fluorescence of EGFP and the activated form of PAGFP was excited with a laser at 488 nm and the one of mCherry and the activated form of PATagRFP was excited with a laser at 561 nm. For fluorescence detection, we used band pass filters adapted to the fluorophores. Laser powers were adjusted to minimize bleaching during the time-lapse acquisitions. Photo-activation and DNA damage were induced simultaneously using a 405 nm laser. The power of the 405 nm laser used for photo-activation and, for cells pre-sensitized with Hoechst, induction of DNA lesions, was set to 0,125 mW at the sample level, unless stated otherwise. Cells were irradiated along a 16 μm -long line crossing the nucleus. The microscope is equipped with a heating chamber to maintain cells at 37 °C during the imaging experiments.

For the images shown on Figure 18 B, 20 A, 22 B and 22 G, a different setup was used. The images were taken on an inverted AxioObserver Z1 confocal spinning-disk microscope (Zeiss) equipped with a single-point scanning head for laser micro-irradiation and local photoactivation using a 405 nm laser (Rapp OptoElectronic). We used a C-Apo 63× water immersion objective lens (O.N. 1.2) and the images were acquired on a AxioCam HRm CCD camera (Zeiss). The pixel resolution at the object plane was 171 nm. The fluorescence of EGFP and YFP was excited with a laser at 488 nm and the one of the activated form of PTagRFP was excited with a laser at 561 nm. For fluorescence detection, we used band pass filters adapted to the fluorophores. The micro-irradiation conditions at 405 nm were adjusted to obtain amplitudes of the chromatin relaxation at DNA lesions which were similar to those obtained with the system described above. The cells were maintained at 37°C using a heating chamber.

Image Analysis

The time-lapse sequences were analyzed automatically using custom-made routines written in MatLab (MathWorks) to quantify chromatin relaxation at DNA lesions. The chromatin areas micro-irradiated at 405 nm and tagged with the photoactivatable H2B were segmented by k-means segmentation. An ellipsoid was fitted to the segmented area and its minor axis length was used to estimate the width of the micro-irradiated chromatin area and thus assess changes in the chromatin compaction level.

To characterize Alc1 recruitment kinetics, the Alc1 integrated intensity was measured inside the segmented micro-irradiated chromatin area in cells co-expressing Alc1 and H2B tagged with two different fluorophores. This intensity was divided by the Alc1 intensity integrated over the whole nucleus to correct for bleaching and small focus drifts. For this step, the whole nuclei were segmented using the low fluorescence signal coming from the non-activated tagged H2B proteins.

When necessary, nuclei movements occurring during the time-lapse experiments were corrected using the ImageJ plugin StackReg ³²⁹.

The accumulation of the fluorescently tagged WWE domain of RNF146 at the DNA lesions was quantified as follows. By manual segmentation, the mean fluorescence intensity in three areas was estimated: at the site of DNA damage (I_d), in a region of the nucleus not subjected to laser irradiation (I_{nd}) and outside the cells (I_{bg}). The accumulation of the WWE domain at the DNA lesions A_{WWE} was then calculated as:

$$A_{WWE} = \frac{I_d - I_{bg}}{I_{nd} - I_{bg}}$$

For chromatin texture analysis, wild-type and Alc1 knockout U2OS cells were plated on Lab-TekII-chambered coverglass, fixed with 4% paraformaldehyde for 10 min at room temperature and stained with Hoechst 33342 (1 μ g/mL) for 1h. Confocal

images were captured on a Leica SP8 confocal microscope using a Plan APO 63x oil immersion objective lens (O.N. 1.4). Hoechst staining was excited with a 405-nm laser and the emission band was chosen to optimize fluorescence collection. The pinhole was set to one Airy unit and we used a pixel size of 60 nm. To analyze chromatin texture, the GLCM ImageJ texture plugin written by Julio E. Cabrera was used. The correlation and contrast parameters were chosen to characterize chromatin texture using a pixel-to-pixel distance of 7 pixels, which allowed maximizing the differences measured between the cells bathed with the isotonic medium and those subjected to osmotic stress.

In cells labeled with fluorescent nucleotides, the chromatin dynamics was assessed by tracking the fluorescently labeled DNA replication foci using the plugin Particle Tracker from Image J³³⁰.

Statistics

Unless stated otherwise, for all curves and boxplots shown on the figures, more than 15 cells per condition were analyzed. Boxplots were generated using a web-tool developed by the Tyers and Rappsilber labs (<http://boxplot.tyerslab.com/>). The box limits correspond to the 25th and 75th percentiles and the bold line indicates the median value. The whiskers extend 1.5 times the interquartile range and outliers are shown by dots. Unless stated otherwise, p values were calculated using unpaired Student's t-test assuming unequal variances. On the boxplots, * refers to $p < 0.05$, ** to $p < 0.01$, *** to $p < 0.001$, **** to $p < 0.0001$ and n.s. to non-significant.

BIBLIOGRAPHY

- 1 Olins, A. L. & Olins, D. E. Spheroid chromatin units (v bodies). *Science (New York, N.Y.)* **183**, 330-332 (1974).
- 2 Oudet, P., Gross-Bellard, M. & Chambon, P. Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* **4**, 281-300 (1975).
- 3 Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251-260, doi:10.1038/38444 (1997).
- 4 Woodcock, C. L., Skoultchi, A. I. & Fan, Y. Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length. *Chromosome Res.* **14**, 17-25, doi:10.1007/s10577-005-1024-3 (2006).
- 5 Thoma, F., Koller, T. & Klug, A. Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J Cell Biol* **83**, 403-427 (1979).
- 6 Horn, P. J. & Peterson, C. L. Molecular biology. Chromatin higher order folding--wrapping up transcription. *Science (New York, N.Y.)* **297**, 1824-1827, doi:10.1126/science.1074200 (2002).
- 7 Graff, J. & Mansuy, I. M. Epigenetic codes in cognition and behaviour. *Behav Brain Res* **192**, 70-87, doi:10.1016/j.bbr.2008.01.021 (2008).
- 8 Arents, G., Burlingame, R. W., Wang, B. C., Love, W. E. & Moudrianakis, E. N. The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10148-10152 (1991).
- 9 Brower-Toland, B. D. *et al.* Mechanical disruption of individual nucleosomes reveals a reversible multistage release of DNA. *Proc Natl Acad Sci U S A* **99**, 1960-1965, doi:10.1073/pnas.022638399 (2002).
- 10 Mihardja, S., Spakowitz, A. J., Zhang, Y. & Bustamante, C. Effect of force on mononucleosomal dynamics. *Proc Natl Acad Sci U S A* **103**, 15871-15876, doi:10.1073/pnas.0607526103 (2006).
- 11 Bowman, G. D. Mechanisms of ATP-dependent nucleosome sliding. *Curr. Opin. Struct. Biol.* **20**, 73-81, doi:10.1016/j.sbi.2009.12.002 (2010).
- 12 Schroth, G. P., Yau, P., Imai, B. S., Gatewood, J. M. & Bradbury, E. M. A NMR study of mobility in the histone octamer. *FEBS Lett.* **268**, 117-120 (1990).
- 13 Hansen, J. C., Tse, C. & Wolffe, A. P. Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry* **37**, 17637-17641 (1998).
- 14 Finch, J. T. & Klug, A. Solenoidal model for superstructure in chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1897-1901 (1976).
- 15 Song, F. *et al.* Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units. *Science (New York, N.Y.)* **344**, 376-380, doi:10.1126/science.1251413 (2014).
- 16 Widom, J. A relationship between the helical twist of DNA and the ordered positioning of nucleosomes in all eukaryotic cells. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1095-1099 (1992).
- 17 Huynh, V. A., Robinson, P. J. & Rhodes, D. A method for the in vitro reconstitution of a defined "30 nm" chromatin fibre containing stoichiometric amounts of the linker histone. *J Mol Biol* **345**, 957-968, doi:10.1016/j.jmb.2004.10.075 (2005).
- 18 Luger, K., Dechassa, M. L. & Tremethick, D. J. New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nat Rev Mol Cell Biol* **13**, 436-447, doi:10.1038/nrm3382 (2012).
- 19 Widom, J. & Klug, A. Structure of the 300Å chromatin filament: X-ray diffraction from oriented samples. *Cell* **43**, 207-213 (1985).

- 20 Woodcock, C. L., Frado, L. L. & Rattner, J. B. The higher-order structure of chromatin: evidence for a helical ribbon arrangement. *J Cell Biol* **99**, 42-52 (1984).
- 21 Dorigo, B. *et al.* Nucleosome arrays reveal the two-start organization of the chromatin fiber. *Science (New York, N.Y.)* **306**, 1571-1573, doi:10.1126/science.1103124 (2004).
- 22 Fussner, E. *et al.* Constitutive heterochromatin reorganization during somatic cell reprogramming. *EMBO J* **30**, 1778-1789, doi:10.1038/emboj.2011.96 (2011).
- 23 Joti, Y. *et al.* Chromosomes without a 30-nm chromatin fiber. *Nucleus* **3**, 404-410, doi:10.4161/nucl.21222 (2012).
- 24 Nishino, Y. *et al.* Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure. *EMBO J* **31**, 1644-1653, doi:10.1038/emboj.2012.35 (2012).
- 25 Maeshima, K. *et al.* Nucleosomal arrays self-assemble into supramolecular globular structures lacking 30-nm fibers. *EMBO J* **35**, 1115-1132, doi:10.15252/embj.201592660 (2016).
- 26 Ricci, Maria A., Manzo, C., García-Parajo, M. F., Lakadamyali, M. & Cosma, Maria P. Chromatin Fibers Are Formed by Heterogeneous Groups of Nucleosomes In Vivo. *Cell* **160**, 1145-1158, doi:10.1016/j.cell.2015.01.054 (2015).
- 27 Hsieh, T.-H. S. *et al.* Mapping Nucleosome Resolution Chromosome Folding in Yeast by Micro-C. *Cell* **162**, 108-119, doi:10.1016/j.cell.2015.05.048 (2015).
- 28 Tom Sexton, F. B. Genomic interactions: Chromatin loops and gene meeting points in transcriptional regulation. *Seminars in cell & developmental biology* **20**, 849-855, doi:10.1016/j.semcdb.2009.06.004 (2009).
- 29 Price, C. M. Fluorescence in situ hybridization. *Blood Rev* **7**, 127-134 (1993).
- 30 Simonis, M. & de Laat, W. FISH-eyed and genome-wide views on the spatial organisation of gene expression. *Biochim Biophys Acta* **1783**, 2052-2060, doi:10.1016/j.bbamcr.2008.07.020 (2008).
- 31 Miele, A. & Dekker, J. Mapping cis- and trans- chromatin interaction networks using chromosome conformation capture (3C). *Methods Mol Biol* **464**, 105-121, doi:10.1007/978-1-60327-461-6_7 (2009).
- 32 Dekker, J., Rippe, K., Dekker, M. & Kleckner, N. Capturing chromosome conformation. *Science (New York, N.Y.)* **295**, 1306-1311, doi:10.1126/science.1067799 (2002).
- 33 Dekker, J., Marti-Renom, M. A. & Mirny, L. A. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. *Nat. Rev. Genet.* **14**, 390-403, doi:10.1038/nrg3454 (2013).
- 34 Tolhuis, B., Palstra, R. J., Splinter, E., Grosveld, F. & de Laat, W. Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol. Cell* **10**, 1453-1465 (2002).
- 35 Carter, D., Chakalova, L., Osborne, C. S., Dai, Y.-f. & Fraser, P. Long-range chromatin regulatory interactions in vivo. *Nat Genet* **32**, 623-626, doi:10.1038/ng1051 (2002).
- 36 Vernimmen, D., De Gobbi, M., Sloane-Stanley, J. A., Wood, W. G. & Higgs, D. R. Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J* **26**, 2041-2051, doi:10.1038/sj.emboj.7601654 (2007).
- 37 Ott, C. J. *et al.* Intronic enhancers coordinate epithelial-specific looping of the active CFTR locus. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 19934-19939, doi:10.1073/pnas.0900946106 (2009).
- 38 Miele, A., Bystrycky, K. & Dekker, J. Yeast silent mating type loci form heterochromatic clusters through silencer protein-dependent long-range interactions. *PLoS Genet.* **5**, e1000478, doi:10.1371/journal.pgen.1000478 (2009).
- 39 Rao, S. S. *et al.* A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665-1680, doi:10.1016/j.cell.2014.11.021 (2014).

- 40 Ong, C. T. & Corces, V. G. CTCF: an architectural protein bridging genome topology and function. *Nat Rev Genet* **15**, 234-246, doi:10.1038/nrg3663 (2014).
- 41 Mozziconacci, J. & Koszul, R. Filling the gap: Micro-C accesses the nucleosomal fiber at 100-1000 bp resolution. *Genome Biol* **16**, 169, doi:10.1186/s13059-015-0744-8 (2015).
- 42 Sexton, T. *et al.* Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell* **148**, 458-472, doi:10.1016/j.cell.2012.01.010 (2012).
- 43 Hou, C., Li, L., Qin, Z. S. & Corces, V. G. Gene Density, Transcription, and Insulators Contribute to the Partition of the Drosophila Genome into Physical Domains. *Mol. Cell* **48**, 471-484, doi:10.1016/j.molcel.2012.08.031 (2012).
- 44 Ciabrelli, F. & Cavalli, G. Chromatin-Driven Behavior of Topologically Associating Domains. *J. Mol. Biol.* **427**, 608-625, doi:10.1016/j.jmb.2014.09.013 (2015).
- 45 Dixon, J. R. *et al.* Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376-380, doi:10.1038/nature11082 (2012).
- 46 Le Dily, F. *et al.* Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. *Genes Dev.* **28**, 2151-2162, doi:10.1101/gad.241422.114 (2014).
- 47 Le Dily, F. & Beato, M. TADs as modular and dynamic units for gene regulation by hormones. *FEBS Lett.* **589**, 2885-2892, doi:10.1016/j.febslet.2015.05.026 (2015).
- 48 Pope, B. D. *et al.* Topologically associating domains are stable units of replication-timing regulation. *Nature* **515**, 402-405, doi:10.1038/nature13986 (2014).
- 49 Phillips-Cremins, J. E. *et al.* Architectural protein subclasses shape 3D organization of genomes during lineage commitment. *Cell* **153**, 1281-1295, doi:10.1016/j.cell.2013.04.053 (2013).
- 50 Nora, E. P. *et al.* Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* **485**, 381-385, doi:10.1038/nature11049 (2012).
- 51 Cremer, T. *et al.* Rabl's model of the interphase chromosome arrangement tested in Chinese hamster cells by premature chromosome condensation and laser-UV-microbeam experiments. *Hum Genet* **60**, 46-56 (1982).
- 52 Cremer, T. & Cremer, C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat. Rev. Genet.* **2**, 292-301, doi:10.1038/35066075 (2001).
- 53 Habermann, F. A. *et al.* Arrangements of macro- and microchromosomes in chicken cells. *Chromosome Res.* **9**, 569-584 (2001).
- 54 Schardin, M., Cremer, T., Hager, H. D. & Lang, M. Specific staining of human chromosomes in Chinese hamster x man hybrid cell lines demonstrates interphase chromosome territories. *Hum. Genet.* **71**, 281-287 (1985).
- 55 Kurz, A. *et al.* Active and inactive genes localize preferentially in the periphery of chromosome territories. *J Cell Biol* **135**, 1195-1205 (1996).
- 56 Croft, J. A. *et al.* Differences in the localization and morphology of chromosomes in the human nucleus. *J Cell Biol* **145**, 1119-1131 (1999).
- 57 Branco, M. R. & Pombo, A. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLoS Biol.* **4**, e138, doi:10.1371/journal.pbio.0040138 (2006).
- 58 Cremer, T. & Cremer, M. Chromosome territories. *Cold Spring Harb Perspect Biol* **2**, a003889, doi:10.1101/cshperspect.a003889 (2010).
- 59 Gerlich, D. *et al.* Global Chromosome Positions Are Transmitted through Mitosis in Mammalian Cells. *Cell* **112**, 751-764, doi:10.1016/S0092-8674(03)00189-2 (2003).

- 60 Zidovska, A., Weitz, D. A. & Mitchison, T. J. Micron-scale coherence in interphase chromatin
dynamics. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 15555-15560, doi:10.1073/pnas.1220313110
(2013).
- 61 Weber, S. C., Spakowitz, A. J. & Theriot, J. A. Nonthermal ATP-dependent fluctuations
contribute to the in vivo motion of chromosomal loci. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7338-
7343, doi:10.1073/pnas.1119505109 (2012).
- 62 Campos, E. I. & Reinberg, D. Histones: Annotating Chromatin. *Annual Review of Genetics* **43**,
559-599, doi:10.1146/annurev.genet.032608.103928 (2009).
- 63 Segal, E. & Widom, J. From DNA sequence to transcriptional behaviour: a quantitative
approach. *Nat. Rev. Genet.* **10**, 443-456, doi:10.1038/nrg2591 (2009).
- 64 Kimura, H. & Cook, P. R. Kinetics of core histones in living human cells: little exchange of H3
and H4 and some rapid exchange of H2B. *J Cell Biol* **153**, 1341-1353 (2001).
- 65 Annunziato, A. T. Split decision: what happens to nucleosomes during DNA replication? *J Biol
Chem* **280**, 12065-12068, doi:10.1074/jbc.R400039200 (2005).
- 66 Thiriet, C. & Hayes, J. J. A novel labeling technique reveals a function for histone H2A/H2B
dimer tail domains in chromatin assembly in vivo. *Genes Dev* **15**, 2048-2053,
doi:10.1101/gad.910201 (2001).
- 67 Henikoff, S. & Ahmad, K. Assembly of variant histones into chromatin. *Annu Rev Cell Dev Biol*
21, 133-153, doi:10.1146/annurev.cellbio.21.012704.133518 (2005).
- 68 Talbert, P. B. & Henikoff, S. Histone variants--ancient wrap artists of the epigenome. *Nat Rev
Mol Cell Biol* **11**, 264-275, doi:10.1038/nrm2861 (2010).
- 69 Tolstorukov, M. Y. *et al.* Histone variant H2A.Bbd is associated with active transcription and
mRNA processing in human cells. *Mol Cell* **47**, 596-607, doi:10.1016/j.molcel.2012.06.011
(2012).
- 70 McKittrick, E., Gafken, P. R., Ahmad, K. & Henikoff, S. Histone H3.3 is enriched in covalent
modifications associated with active chromatin. *Proc Natl Acad Sci U S A* **101**, 1525-1530,
doi:10.1073/pnas.0308092100 (2004).
- 71 Van Hooser, A. A. *et al.* Specification of kinetochore-forming chromatin by the histone H3
variant CENP-A. *J Cell Sci* **114**, 3529-3542 (2001).
- 72 Nozaki, T. *et al.* Flexible and dynamic nucleosome fiber in living mammalian cells. *Nucleus* **4**,
349-356, doi:10.4161/nucl.26053 (2013).
- 73 Straight, A. F., Belmont, A. S., Robinett, C. C. & Murray, A. W. GFP tagging of budding yeast
chromosomes reveals that protein-protein interactions can mediate sister chromatid
cohesion. *Curr. Biol.* **6**, 1599-1608 (1996).
- 74 Saad, H. *et al.* DNA dynamics during early double-strand break processing revealed by non-
intrusive imaging of living cells. *PLoS Genet.* **10**, e1004187,
doi:10.1371/journal.pgen.1004187 (2014).
- 75 Chen, B. *et al.* Dynamic imaging of genomic loci in living human cells by an optimized
CRISPR/Cas system. *Cell* **155**, 1479-1491, doi:10.1016/j.cell.2013.12.001 (2013).
- 76 Bystricky, K. Chromosome dynamics and folding in eukaryotes: Insights from live cell
microscopy. *FEBS Lett* **589**, 3014-3022, doi:10.1016/j.febslet.2015.07.012 (2015).
- 77 Zink, D. *et al.* Structure and dynamics of human interphase chromosome territories in vivo.
Hum. Genet. **102**, 241-251 (1998).
- 78 Chubb, J. R., Boyle, S., Perry, P. & Bickmore, W. A. Chromatin motion is constrained by
association with nuclear compartments in human cells. *Curr. Biol.* **12**, 439-445 (2002).
- 79 Bronshtein, I. *et al.* Loss of lamin A function increases chromatin dynamics in the nuclear
interior. *Nat Commun* **6**, 8044, doi:10.1038/ncomms9044 (2015).

- 80 Marshall, W. F. *et al.* Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr. Biol.* **7**, 930-939 (1997).
- 81 Bornfleth, H., Edelmann, P., Zink, D., Cremer, T. & Cremer, C. Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy. *Biophys J* **77**, 2871-2886, doi:10.1016/S0006-3495(99)77119-5 (1999).
- 82 Lebeaupin, T., Sellou, H., Timinszky, G. & Huet, S. Chromatin dynamics at DNA breaks: what, how and why? *AIMS Biophysics* **2**, 458-475, doi:10.3934/biophy.2015.4.458 (2015).
- 83 Rosa, A. & Everaers, R. Structure and dynamics of interphase chromosomes. *PLoS Comput. Biol.* **4**, e1000153, doi:10.1371/journal.pcbi.1000153 (2008).
- 84 Walter, J., Schermelleh, L., Cremer, M., Tashiro, S. & Cremer, T. Chromosome order in HeLa cells changes during mitosis and early G1, but is stably maintained during subsequent interphase stages. *J Cell Biol* **160**, 685-697, doi:10.1083/jcb.200211103 (2003).
- 85 Gerlich, D. *et al.* Global chromosome positions are transmitted through mitosis in mammalian cells. *Cell* **112**, 751-764 (2003).
- 86 Muller, I., Boyle, S., Singer, R. H., Bickmore, W. A. & Chubb, J. R. Stable morphology, but dynamic internal reorganisation, of interphase human chromosomes in living cells. *PLoS One* **5**, e11560, doi:10.1371/journal.pone.0011560 (2010).
- 87 Kruhlak, M. J. *et al.* Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J Cell Biol* **172**, 823-834, doi:10.1083/jcb.200510015 (2006).
- 88 Wiesmeijer, K., Krouwels, I. M., Tanke, H. J. & Dirks, R. W. Chromatin movement visualized with photoactivable GFP-labeled histone H4. *Differentiation* **76**, 83-90, doi:10.1111/j.1432-0436.2007.00234.x (2008).
- 89 Weber, S. C., Spakowitz, A. J. & Theriot, J. A. Nonthermal ATP-dependent fluctuations contribute to the in vivo motion of chromosomal loci. *PNAS* **109**, 7338-7343, doi:10.1073/pnas.1119505109 (2012).
- 90 Hu, Y., Kireev, I., Plutz, M., Ashourian, N. & Belmont, A. S. Large-scale chromatin structure of inducible genes: transcription on a condensed, linear template. *J Cell Biol* **185**, 87-100, doi:10.1083/jcb.200809196 (2009).
- 91 Neumann, F. R. *et al.* Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. *Genes Dev.* **26**, 369-383, doi:10.1101/gad.176156.111 (2012).
- 92 Kosak, S. T. *et al.* Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* **296**, 158-162, doi:10.1126/science.1068768 (2002).
- 93 Osborne, C. S. *et al.* Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat Genet* **36**, 1065-1071, doi:10.1038/ng1423 (2004).
- 94 Chambeyron, S. & Bickmore, W. A. Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. *Genes Dev.* **18**, 1119-1130, doi:10.1101/gad.292104 (2004).
- 95 Lindahl, T. & Barnes, D. E. Repair of endogenous DNA damage. *Cold Spring Harb Symp Quant Biol* **65**, 127-133 (2000).
- 96 Jackson, S. P. & Bartek, J. The DNA-damage response in human biology and disease. *Nature* **461**, 1071-1078, doi:10.1038/nature08467 (2009).
- 97 Ciccia, A. & Elledge, S. J. The DNA damage response: making it safe to play with knives. *Mol. Cell* **40**, 179-204, doi:10.1016/j.molcel.2010.09.019 (2010).
- 98 Longhese, M. P., Bonetti, D., Manfrini, N. & Clerici, M. Mechanisms and regulation of DNA end resection. *EMBO J* **29**, 2864-2874, doi:10.1038/emboj.2010.165 (2010).
- 99 Tsai, A. G. & Lieber, M. R. Mechanisms of chromosomal rearrangement in the human genome. *BMC Genomics* **11**, 1-9, doi:10.1186/1471-2164-11-S1-S1 (2010).

- 100 Durante, M. *et al.* From DNA damage to chromosome aberrations: joining the break. *Mutat Res* **756**, 5-13, doi:10.1016/j.mrgentox.2013.05.014 (2013).
- 101 Larmonie, N. S. *et al.* Breakpoint sites disclose the role of the V(D)J recombination machinery in the formation of T-cell receptor (TCR) and non-TCR associated aberrations in T-cell acute lymphoblastic leukemia. *Haematologica* **98**, 1173-1184, doi:10.3324/haematol.2012.082156 (2013).
- 102 Hoeijmakers, J. H. Genome maintenance mechanisms for preventing cancer. *Nature* **411**, 366-374, doi:10.1038/35077232 (2001).
- 103 Bernstein, C., Bernstein, H., Payne, C. M. & Garewal, H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat. Res.* **511**, 145-178 (2002).
- 104 Thompson, L. H. & Schild, D. Recombinational DNA repair and human disease. *Mutat. Res.* **509**, 49-78 (2002).
- 105 Helleday, T., Eshtad, S. & Nik-Zainal, S. Mechanisms underlying mutational signatures in human cancers. *Nat Rev Genet* **15**, 585-598, doi:10.1038/nrg3729 (2014).
- 106 Caldecott, K. W. Single-strand break repair and genetic disease. *Nat. Rev. Genet.* **9**, 619-631, doi:10.1038/nrg2380 (2008).
- 107 Shrivastav, M., De Haro, L. P. & Nickoloff, J. A. Regulation of DNA double-strand break repair pathway choice. *Cell Res* **18**, 134-147, doi:10.1038/cr.2007.111 (2008).
- 108 Harper, J. W. & Elledge, S. J. The DNA damage response: ten years after. *Mol. Cell* **28**, 739-745, doi:10.1016/j.molcel.2007.11.015 (2007).
- 109 Polo, S. E. & Jackson, S. P. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes Dev.* **25**, 409-433, doi:10.1101/gad.2021311 (2011).
- 110 Stracker, T. H., Usui, T. & Petrini, J. H. Taking the time to make important decisions: the checkpoint effector kinases Chk1 and Chk2 and the DNA damage response. *DNA Repair (Amst)* **8**, 1047-1054, doi:10.1016/j.dnarep.2009.04.012 (2009).
- 111 Lans, H., Marteiijn, J. A. & Vermeulen, W. ATP-dependent chromatin remodeling in the DNA-damage response. *Epigenetics Chromatin* **5**, 4, doi:10.1186/1756-8935-5-4 (2012).
- 112 Adam, S., Polo, S. E. & Almouzni, G. How to restore chromatin structure and function in response to DNA damage--let the chaperones play: delivered on 9 July 2013 at the 38th FEBS Congress in St Petersburg, Russia. *FEBS J.* **281**, 2315-2323, doi:10.1111/febs.12793 (2014).
- 113 Hakmé, A., Wong, H.-K., Dantzer, F. & Schreiber, V. The expanding field of poly(ADP-ribosyl)ation reactions. 'Protein Modifications: Beyond the Usual Suspects' Review Series. *EMBO Rep.* **9**, 1094-1100, doi:10.1038/embor.2008.191 (2008).
- 114 Beneke, S. Regulation of chromatin structure by poly(ADP-ribosyl)ation. *Front Genet* **3**, 169, doi:10.3389/fgene.2012.00169 (2012).
- 115 Yang, L. *et al.* Identification of poly(ADP-ribose) polymerase-1 as a cell cycle regulator through modulating Sp1 mediated transcription in human hepatoma cells. *PLoS One* **8**, e82872, doi:10.1371/journal.pone.0082872 (2013).
- 116 D'Amours, D., Sallmann, F. R., Dixit, V. M. & Poirier, G. G. Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. *J Cell Sci* **114**, 3771-3778 (2001).
- 117 Dantzer, F. *et al.* Poly(ADP-ribose) polymerase-1 activation during DNA damage and repair. *Methods Enzymol* **409**, 493-510, doi:10.1016/S0076-6879(05)09029-4 (2006).
- 118 Amé, J.-C., Spenlehauer, C. & de Murcia, G. The PARP superfamily. *Bioessays* **26**, 882-893, doi:10.1002/bies.20085 (2004).
- 119 Otto, H. *et al.* In silico characterization of the family of PARP-like poly(ADP-ribosyl)transferases (pARTs). *BMC Genomics* **6**, 139, doi:10.1186/1471-2164-6-139 (2005).

- 120 Li, N. & Chen, J. ADP-ribosylation: activation, recognition, and removal. *Mol. Cells* **37**, 9-16, doi:10.14348/molcells.2014.2245 (2014).
- 121 Barkauskaite, E., Jankevicius, G., Ladurner, A. G., Ahel, I. & Timinszky, G. The recognition and removal of cellular poly(ADP-ribose) signals. *FEBS J.* **280**, 3491-3507, doi:10.1111/febs.12358 (2013).
- 122 Chapman, J. D., Gagné, J.-P., Poirier, G. G. & Goodlett, D. R. Mapping PARP-1 auto-ADP-ribosylation sites by liquid chromatography-tandem mass spectrometry. *J. Proteome Res.* **12**, 1868-1880, doi:10.1021/pr301219h (2013).
- 123 Matic, I., Ahel, I. & Hay, R. T. Reanalysis of phosphoproteomics data uncovers ADP-ribosylation sites. *Nat. Methods* **9**, 771-772, doi:10.1038/nmeth.2106 (2012).
- 124 Riquelme, P. T., Burzio, L. O. & Koide, S. S. ADP ribosylation of rat liver lysine-rich histone in vitro. *J. Biol. Chem.* **254**, 3018-3028 (1979).
- 125 Sharifi, R. *et al.* Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. *EMBO J* **32**, 1225-1237, doi:10.1038/emboj.2013.51 (2013).
- 126 Tao, Z., Gao, P. & Liu, H.-W. Studies of the expression of human poly(ADP-ribose) polymerase-1 in *Saccharomyces cerevisiae* and identification of PARP-1 substrates by yeast proteome microarray screening. *Biochemistry* **48**, 11745-11754, doi:10.1021/bi901387k (2009).
- 127 Alvarez-Gonzalez, R. & Jacobson, M. K. Characterization of polymers of adenosine diphosphate ribose generated in vitro and in vivo. *Biochemistry* **26**, 3218-3224 (1987).
- 128 Gibson, B. A. & Kraus, W. L. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat. Rev. Mol. Cell Biol.* **13**, 411-424, doi:10.1038/nrm3376 (2012).
- 129 Ruf, A., Rolli, V., de Murcia, G. & Schulz, G. E. The mechanism of the elongation and branching reaction of poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis. *J. Mol. Biol.* **278**, 57-65, doi:10.1006/jmbi.1998.1673 (1998).
- 130 Juarez-Salinas, H., Levi, V., Jacobson, E. L. & Jacobson, M. K. Poly(ADP-ribose) has a branched structure in vivo. *J. Biol. Chem.* **257**, 607-609 (1982).
- 131 Miwa, M., Saikawa, N., Yamaizumi, Z., Nishimura, S. & Sugimura, T. Structure of poly(adenosine diphosphate ribose): identification of 2'-[1''-ribosyl-2''-(or 3''-)(1'''-ribosyl)]adenosine-5',5'',5'''-tris(phosphate) as a branch linkage. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 595-599 (1979).
- 132 Wang, Y. *et al.* Poly(ADP-ribose) (PAR) binding to apoptosis-inducing factor is critical for PAR polymerase-1-dependent cell death (parthanatos). *Sci Signal* **4**, ra20, doi:10.1126/scisignal.2000902 (2011).
- 133 Alvarez-Gonzalez, R. & Althaus, F. R. Poly(ADP-ribose) catabolism in mammalian cells exposed to DNA-damaging agents. *Mutat. Res.* **218**, 67-74 (1989).
- 134 Dunstan, M. S. *et al.* Structure and mechanism of a canonical poly(ADP-ribose) glycohydrolase. *Nat Commun* **3**, 878, doi:10.1038/ncomms1889 (2012).
- 135 Miwa, M. & Sugimura, T. Splitting of the ribose-ribose linkage of poly(adenosine diphosphate-ribose) by a calf thymus extract. *J. Biol. Chem.* **246**, 6362-6364 (1971).
- 136 Kim, I.-K. *et al.* Structure of mammalian poly(ADP-ribose) glycohydrolase reveals a flexible tyrosine clasp as a substrate-binding element. *Nat. Struct. Mol. Biol.* **19**, 653-656, doi:10.1038/nsmb.2305 (2012).
- 137 Slade, D. *et al.* The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature* **477**, 616-620, doi:10.1038/nature10404 (2011).

- 138 Ueda, K., Oka, J., Naruniya, S., Miyakawa, N. & Hayaishi, O. Poly ADP-ribose glycohydrolase from rat liver nuclei, a novel enzyme degrading the polymer. *Biochem. Biophys. Res. Commun.* **46**, 516-523 (1972).
- 139 Mueller-Dieckmann, C. *et al.* The structure of human ADP-ribosylhydrolase 3 (ARH3) provides insights into the reversibility of protein ADP-ribosylation. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15026-15031, doi:10.1073/pnas.0606762103 (2006).
- 140 Niere, M. *et al.* ADP-ribosylhydrolase 3 (ARH3), not poly(ADP-ribose) glycohydrolase (PARG) isoforms, is responsible for degradation of mitochondrial matrix-associated poly(ADP-ribose). *J. Biol. Chem.* **287**, 16088-16102, doi:10.1074/jbc.M112.349183 (2012).
- 141 Jankevicius, G. *et al.* A family of macrodomain proteins reverses cellular mono-ADP-ribosylation. *Nat. Struct. Mol. Biol.* **20**, 508-514, doi:10.1038/nsmb.2523 (2013).
- 142 Rosenthal, F. *et al.* Macrodomain-containing proteins are new mono-ADP-ribosylhydrolases. *Nat. Struct. Mol. Biol.* **20**, 502-507, doi:10.1038/nsmb.2521 (2013).
- 143 de Murcia, J. M. *et al.* Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7303-7307 (1997).
- 144 El-Khamisy, S. F., Masutani, M., Suzuki, H. & Caldecott, K. W. A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. *Nucleic Acids Res* **31**, 5526-5533 (2003).
- 145 Dantzer, F. *et al.* Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. *Biochemistry* **39**, 7559-7569 (2000).
- 146 Harris, J. L. *et al.* Aprataxin, poly-ADP ribose polymerase 1 (PARP-1) and apurinic endonuclease 1 (APE1) function together to protect the genome against oxidative damage. *Hum Mol Genet* **18**, 4102-4117, doi:10.1093/hmg/ddp359 (2009).
- 147 Li, M., Lu, L.-Y., Yang, C.-Y., Wang, S. & Yu, X. The FHA and BRCT domains recognize ADP-ribosylation during DNA damage response. *Genes Dev.* **27**, 1752-1768, doi:10.1101/gad.226357.113 (2013).
- 148 Liang, F., Han, M., Romanienko, P. J. & Jasin, M. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proc Natl Acad Sci U S A* **95**, 5172-5177 (1998).
- 149 Chiruvella, K. K., Liang, Z. & Wilson, T. E. Repair of double-strand breaks by end joining. *Cold Spring Harb Perspect Biol* **5**, a012757, doi:10.1101/cshperspect.a012757 (2013).
- 150 Schultz, N., Lopez, E., Saleh-Gohari, N. & Helleday, T. Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. *Nucleic Acids Res* **31**, 4959-4964 (2003).
- 151 Ying, S., Hamdy, F. C. & Helleday, T. Mre11-dependent degradation of stalled DNA replication forks is prevented by BRCA2 and PARP1. *Cancer Res* **72**, 2814-2821, doi:10.1158/0008-5472.CAN-11-3417 (2012).
- 152 Bryant, H. E. *et al.* PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. *EMBO J* **28**, 2601-2615, doi:10.1038/emboj.2009.206 (2009).
- 153 Haince, J.-F. *et al.* PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *J. Biol. Chem.* **283**, 1197-1208, doi:10.1074/jbc.M706734200 (2008).
- 154 Davis, A. J. & Chen, D. J. DNA double strand break repair via non-homologous end-joining. *Transl Cancer Res* **2**, 130-143, doi:10.3978/j.issn.2218-676X.2013.04.02 (2013).
- 155 Spagnolo, L., Barbeau, J., Curtin, N. J., Morris, E. P. & Pearl, L. H. Visualization of a DNA-PK/PARP1 complex. *Nucleic Acids Res* **40**, 4168-4177, doi:10.1093/nar/gkr1231 (2012).
- 156 Eustermann, S. *et al.* Solution structures of the two PBZ domains from human APLF and their interaction with poly(ADP-ribose). *Nat Struct Mol Biol* **17**, 241-243, doi:10.1038/nsmb.1747 (2010).

- 157 Wei, H. & Yu, X. Functions of PARylation in DNA Damage Repair Pathways. *Genomics Proteomics Bioinformatics*, doi:10.1016/j.gpb.2016.05.001 (2016).
- 158 Deriano, L. & Roth, D. B. Modernizing the nonhomologous end-joining repertoire: alternative and classical NHEJ share the stage. *Annu Rev Genet* **47**, 433-455, doi:10.1146/annurev-genet-110711-155540 (2013).
- 159 Goodarzi, A. A. & Jeggo, P. A. The heterochromatic barrier to DNA double strand break repair: how to get the entry visa. *Int J Mol Sci* **13**, 11844-11860, doi:10.3390/ijms130911844 (2012).
- 160 Poirier, G. G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C. & Mandel, P. Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. *Proc Natl Acad Sci U S A* **79**, 3423-3427 (1982).
- 161 Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41-45, doi:10.1038/47412 (2000).
- 162 Clapier, C. R. & Cairns, B. R. The biology of chromatin remodeling complexes. *Annual Review of Biochemistry* **78**, 273-304, doi:10.1146/annurev.biochem.77.062706.153223 (2009).
- 163 Polo, S. E. & Almouzni, G. Chromatin dynamics after DNA damage: The legacy of the access-repair-restore model. *DNA Repair (Amst.)* **36**, 114-121, doi:10.1016/j.dnarep.2015.09.014 (2015).
- 164 Altaf, M., Auger, A., Covic, M. & Cote, J. Connection between histone H2A variants and chromatin remodeling complexes. *Biochem Cell Biol* **87**, 35-50, doi:10.1139/O08-140 (2009).
- 165 Dion, V., Kalck, V., Horigome, C., Towbin, B. D. & Gasser, S. M. Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat. Cell Biol.* **14**, 502-509, doi:10.1038/ncb2465 (2012).
- 166 Seeber, A., Dion, V. & Gasser, S. M. Checkpoint kinases and the INO80 nucleosome remodeling complex enhance global chromatin mobility in response to DNA damage. *Genes Dev.* **27**, 1999-2008, doi:10.1101/gad.222992.113 (2013).
- 167 Miné-Hattab, J. & Rothstein, R. Increased chromosome mobility facilitates homology search during recombination. *Nat. Cell Biol.* **14**, 510-517, doi:10.1038/ncb2472 (2012).
- 168 Dion, V., Kalck, V., Seeber, A., Schleker, T. & Gasser, S. M. Cohesin and the nucleolus constrain the mobility of spontaneous repair foci. *EMBO Rep.* **14**, 984-991, doi:10.1038/embor.2013.142 (2013).
- 169 Kalocsay, M., Hiller, N. J. & Jentsch, S. Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol. Cell* **33**, 335-343, doi:10.1016/j.molcel.2009.01.016 (2009).
- 170 Nagai, S. *et al.* Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science (New York, N.Y.)* **322**, 597-602, doi:10.1126/science.1162790 (2008).
- 171 Krawczyk, P. M. *et al.* Chromatin mobility is increased at sites of DNA double-strand breaks. *J Cell Sci* **125**, 2127-2133, doi:10.1242/jcs.089847 (2012).
- 172 Aten, J. A. *et al.* Dynamics of DNA Double-Strand Breaks Revealed by Clustering of Damaged Chromosome Domains. *Science* **303**, 92-95, doi:10.1126/science.1088845 (2004).
- 173 Nelms, B. E., Maser, R. S., MacKay, J. F., Lagally, M. G. & Petrini, J. H. In situ visualization of DNA double-strand break repair in human fibroblasts. *Science (New York, N.Y.)* **280**, 590-592 (1998).
- 174 Jakob, B., Splinter, J., Durante, M. & Taucher-Scholz, G. Live cell microscopy analysis of radiation-induced DNA double-strand break motion. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 3172-3177, doi:10.1073/pnas.0810987106 (2009).

- 175 Soutoglou, E. *et al.* Positional stability of single double-strand breaks in mammalian cells. *Nat. Cell Biol.* **9**, 675-682, doi:10.1038/ncb1591 (2007).
- 176 Roukos, V. *et al.* Spatial dynamics of chromosome translocations in living cells. *Science (New York, N.Y.)* **341**, 660-664, doi:10.1126/science.1237150 (2013).
- 177 Smerdon, M. J. & Lieberman, M. W. Nucleosome rearrangement in human chromatin during UV-induced DNA- reapiir synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4238-4241 (1978).
- 178 Ziv, Y. *et al.* Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat. Cell Biol.* **8**, 870-876, doi:10.1038/ncb1446 (2006).
- 179 Khurana, S. *et al.* A macrohistone variant links dynamic chromatin compaction to BRCA1-dependent genome maintenance. *Cell Rep* **8**, 1049-1062, doi:10.1016/j.celrep.2014.07.024 (2014).
- 180 Burgess, R. C., Burman, B., Kruhlak, M. J. & Misteli, T. Activation of DNA damage response signaling by condensed chromatin. *Cell Rep* **9**, 1703-1717, doi:10.1016/j.celrep.2014.10.060 (2014).
- 181 Jakob, B. *et al.* DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. *Nucleic Acids Res* **39**, 6489-6499, doi:10.1093/nar/gkr230 (2011).
- 182 Ježková, L. *et al.* Function of chromatin structure and dynamics in DNA damage, repair and misrepair: γ-rays and protons in action. *Appl Radiat Isot* **83 Pt B**, 128-136, doi:10.1016/j.apradiso.2013.01.022 (2014).
- 183 Chiolo, I. *et al.* Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* **144**, 732-744, doi:10.1016/j.cell.2011.02.012 (2011).
- 184 Ryu, T. *et al.* Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. *Nat. Cell Biol.* **17**, 1401-1411, doi:10.1038/ncb3258 (2015).
- 185 Gautier, T. *et al.* Histone variant H2ABbd confers lower stability to the nucleosome. *EMBO Rep* **5**, 715-720, doi:10.1038/sj.embor.7400182 (2004).
- 186 Suto, R. K., Clarkson, M. J., Tremethick, D. J. & Luger, K. Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat Struct Biol* **7**, 1121-1124, doi:10.1038/81971 (2000).
- 187 Park, Y. J., Dyer, P. N., Tremethick, D. J. & Luger, K. A new fluorescence resonance energy transfer approach demonstrates that the histone variant H2AZ stabilizes the histone octamer within the nucleosome. *J Biol Chem* **279**, 24274-24282, doi:10.1074/jbc.M313152200 (2004).
- 188 Abbott, D. W. *et al.* Structural characterization of macroH2A containing chromatin. *Biochemistry* **43**, 1352-1359, doi:10.1021/bi035859i (2004).
- 189 Santenard, A. *et al.* Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. *Nat Cell Biol* **12**, 853-862, doi:10.1038/ncb2089 (2010).
- 190 Tachiwana, H. & Kurumizaka, H. Structure of the CENP-A nucleosome and its implications for centromeric chromatin architecture. *Genes Genet Syst* **86**, 357-364 (2011).
- 191 Xu, Y. *et al.* Histone H2A.Z controls a critical chromatin remodeling step required for DNA double-strand break repair. *Mol Cell* **48**, 723-733, doi:10.1016/j.molcel.2012.09.026 (2012).
- 192 Mehrotra, P. V. *et al.* DNA repair factor APLF is a histone chaperone. *Mol. Cell* **41**, 46-55, doi:10.1016/j.molcel.2010.12.008 (2011).
- 193 Timinszky, G. *et al.* A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. *Nat. Struct. Mol. Biol.* **16**, 923-929, doi:10.1038/nsmb.1664 (2009).

- 194 Xu, C., Xu, Y., Gursoy-Yuzugullu, O. & Price, B. D. The histone variant macroH2A1.1 is
recruited to DSBs through a mechanism involving PARP1. *FEBS Lett.* **586**, 3920-3925,
doi:10.1016/j.febslet.2012.09.030 (2012).
- 195 Masumoto, H., Hawke, D., Kobayashi, R. & Verreault, A. A role for cell-cycle-regulated
histone H3 lysine 56 acetylation in the DNA damage response. *Nature* **436**, 294-298,
doi:10.1038/nature03714 (2005).
- 196 Lu, X. *et al.* The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and
chromatin structure. *Nat. Struct. Mol. Biol.* **15**, 1122-1124, doi:10.1038/nsmb.1489 (2008).
- 197 Tallis, M., Morra, R., Barkauskaite, E. & Ahel, I. Poly(ADP-ribosyl)ation in regulation of
chromatin structure and the DNA damage response. *Chromosoma* **123**, 79-90,
doi:10.1007/s00412-013-0442-9 (2014).
- 198 Bowman, G. D. & Poirier, M. G. Post-translational modifications of histones that influence
nucleosome dynamics. *Chem Rev* **115**, 2274-2295, doi:10.1021/cr500350x (2015).
- 199 Paull, T. T. *et al.* A critical role for histone H2AX in recruitment of repair factors to nuclear
foci after DNA damage. *Curr Biol* **10**, 886-895 (2000).
- 200 Downs, J. A. *et al.* Binding of chromatin-modifying activities to phosphorylated histone H2A
at DNA damage sites. *Mol. Cell* **16**, 979-990, doi:10.1016/j.molcel.2004.12.003 (2004).
- 201 Ahel, D. *et al.* Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin
remodeling enzyme ALC1. *Science (New York, N.Y.)* **325**, 1240-1243,
doi:10.1126/science.1177321 (2009).
- 202 Smeenk, G. *et al.* Poly(ADP-ribosyl)ation links the chromatin remodeler SMARCA5/SNF2H to
RNF168-dependent DNA damage signaling. *Journal of Cell Science* **126**, 889-903,
doi:10.1242/jcs.109413 (2013).
- 203 Polo, S. E., Kaidi, A., Baskcomb, L., Galanty, Y. & Jackson, S. P. Regulation of DNA-damage
responses and cell-cycle progression by the chromatin remodelling factor CHD4. *EMBO J* **29**,
3130-3139, doi:10.1038/emboj.2010.188 (2010).
- 204 Ahel, I. *et al.* Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins.
Nature **451**, 81-85, doi:10.1038/nature06420 (2008).
- 205 English, C. M., Adkins, M. W., Carson, J. J., Churchill, M. E. A. & Tyler, J. K. Structural basis for
the histone chaperone activity of Asf1. *Cell* **127**, 495-508, doi:10.1016/j.cell.2006.08.047
(2006).
- 206 Gurard-Levin, Z. A., Quivy, J. P. & Almouzni, G. Histone chaperones: assisting histone traffic
and nucleosome dynamics. *Annu Rev Biochem* **83**, 487-517, doi:10.1146/annurev-biochem-
060713-035536 (2014).
- 207 De Koning, L., Corpet, A., Haber, J. E. & Almouzni, G. Histone chaperones: an escort network
regulating histone traffic. *Nat. Struct. Mol. Biol.* **14**, 997-1007, doi:10.1038/nsmb1318 (2007).
- 208 Natsume, R. *et al.* Structure and function of the histone chaperone CIA/ASF1 complexed with
histones H3 and H4. *Nature* **446**, 338-341, doi:10.1038/nature05613 (2007).
- 209 Andrews, A. J., Chen, X., Zevin, A., Stargell, L. A. & Luger, K. The histone chaperone Nap1
promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions.
Mol. Cell **37**, 834-842, doi:10.1016/j.molcel.2010.01.037 (2010).
- 210 Moggs, J. G. *et al.* A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing
DNA damage. *Mol. Cell. Biol.* **20**, 1206-1218 (2000).
- 211 Polo, S. E., Roche, D. & Almouzni, G. New Histone Incorporation Marks Sites of UV Repair in
Human Cells. *Cell* **127**, 481-493, doi:10.1016/j.cell.2006.08.049 (2006).
- 212 Okano, S., Lan, L., Caldecott, K. W., Mori, T. & Yasui, A. Spatial and Temporal Cellular
Responses to Single-Strand Breaks in Human Cells. *Mol. Cell. Biol.* **23**, 3974-3981,
doi:10.1128/MCB.23.11.3974-3981.2003 (2003).

- 213 Heo, K. *et al.* FACT-mediated exchange of histone variant H2AX regulated by phosphorylation
of H2AX and ADP-ribosylation of Spt16. *Mol. Cell* **30**, 86-97,
doi:10.1016/j.molcel.2008.02.029 (2008).
- 214 Huang, J.-Y. *et al.* Modulation of nucleosome-binding activity of FACT by poly(ADP-
ribosylation). *Nucleic Acids Res* **34**, 2398-2407, doi:10.1093/nar/gkl241 (2006).
- 215 Adam, S., Polo, S. E. & Almouzni, G. Transcription recovery after DNA damage requires
chromatin priming by the H3.3 histone chaperone HIRA. *Cell* **155**, 94-106,
doi:10.1016/j.cell.2013.08.029 (2013).
- 216 Hondele, M. *et al.* Structural basis of histone H2A-H2B recognition by the essential
chaperone FACT. *Nature* **499**, 111-114, doi:10.1038/nature12242 (2013).
- 217 Iles, N., Rulten, S., El-Khamisy, S. F. & Caldecott, K. W. APLF (C2orf13) is a novel human
protein involved in the cellular response to chromosomal DNA strand breaks. *Mol. Cell. Biol.*
27, 3793-3803, doi:10.1128/MCB.02269-06 (2007).
- 218 Saha, A., Wittmeyer, J. & Cairns, B. R. Chromatin remodelling: the industrial revolution of
DNA around histones. *Nat Rev Mol Cell Biol* **7**, 437-447, doi:10.1038/nrm1945 (2006).
- 219 Udugama, M., Sabri, A. & Bartholomew, B. The INO80 ATP-dependent chromatin remodeling
complex is a nucleosome spacing factor. *Mol Cell Biol* **31**, 662-673, doi:10.1128/MCB.01035-
10 (2011).
- 220 Gkikopoulos, T. *et al.* A role for Snf2-related nucleosome-spacing enzymes in genome-wide
nucleosome organization. *Science* **333**, 1758-1760, doi:10.1126/science.1206097 (2011).
- 221 Sala, A. *et al.* Genome-wide characterization of chromatin binding and nucleosome spacing
activity of the nucleosome remodelling ATPase ISWI. *EMBO J* **30**, 1766-1777,
doi:10.1038/emboj.2011.98 (2011).
- 222 Hogan, C. J. *et al.* Fission yeast lec1-ino80-mediated nucleosome eviction regulates
nucleotide and phosphate metabolism. *Mol Cell Biol* **30**, 657-674, doi:10.1128/MCB.01117-
09 (2010).
- 223 Flaus, A., Martin, D. M., Barton, G. J. & Owen-Hughes, T. Identification of multiple distinct
Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Res* **34**, 2887-2905,
doi:10.1093/nar/gkl295 (2006).
- 224 Mohrmann, L. & Verrijzer, C. P. Composition and functional specificity of SWI2/SNF2 class
chromatin remodeling complexes. *Biochim Biophys Acta* **1681**, 59-73,
doi:10.1016/j.bbaexp.2004.10.005 (2005).
- 225 Cairns, B. R. *et al.* RSC, an essential, abundant chromatin-remodeling complex. *Cell* **87**, 1249-
1260 (1996).
- 226 Chambers, A. L. & Downs, J. A. The RSC and INO80 chromatin-remodeling complexes in DNA
double-strand break repair. *Prog Mol Biol Transl Sci* **110**, 229-261, doi:10.1016/B978-0-12-
387665-2.00009-2 (2012).
- 227 Corona, D. F. & Tamkun, J. W. Multiple roles for ISWI in transcription, chromosome
organization and DNA replication. *Biochim Biophys Acta* **1677**, 113-119,
doi:10.1016/j.bbaexp.2003.09.018 (2004).
- 228 Aydin, O. Z., Vermeulen, W. & Lans, H. ISWI chromatin remodeling complexes in the DNA
damage response. *Cell Cycle* **13**, 3016-3025, doi:10.4161/15384101.2014.956551 (2014).
- 229 Erdel, F. & Rippe, K. Binding kinetics of human ISWI chromatin-remodelers to DNA repair
sites elucidate their target location mechanism. *Nucleus* **2**, 105-112,
doi:10.4161/nucl.2.2.15209 (2011).
- 230 He, X., Fan, H. Y., Garlick, J. D. & Kingston, R. E. Diverse regulation of SNF2h chromatin
remodeling by noncatalytic subunits. *Biochemistry* **47**, 7025-7033, doi:10.1021/bi702304p
(2008).

- 231 Chou, D. M. *et al.* A chromatin localization screen reveals poly (ADP ribose)-regulated
recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc.*
Natl. Acad. Sci. U.S.A. **107**, 18475-18480, doi:10.1073/pnas.1012946107 (2010).
- 232 Larsen, D. H. *et al.* The chromatin-remodeling factor CHD4 coordinates signaling and repair
after DNA damage. *J Cell Biol* **190**, 731-740, doi:10.1083/jcb.200912135 (2010).
- 233 Smeenk, G. *et al.* The NuRD chromatin-remodeling complex regulates signaling and repair of
DNA damage. *J Cell Biol* **190**, 741-749, doi:10.1083/jcb.201001048 (2010).
- 234 Luijsterburg, M. S. *et al.* PARP1 Links CHD2-Mediated Chromatin Expansion and H3.3
Deposition to DNA Repair by Non-homologous End-Joining. *Mol Cell* **61**, 547-562,
doi:10.1016/j.molcel.2016.01.019 (2016).
- 235 Gottschalk, A. J. *et al.* Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-
dependent chromatin remodeler. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13770-13774,
doi:10.1073/pnas.0906920106 (2009).
- 236 Gottschalk, A. J., Trivedi, R. D., Conaway, J. W. & Conaway, R. C. Activation of the SNF2 family
ATPase ALC1 by poly(ADP-ribose) in a stable ALC1-PARP1-nucleosome intermediate. *J. Biol.*
Chem. **287**, 43527-43532, doi:10.1074/jbc.M112.401141 (2012).
- 237 Shen, X., Mizuguchi, G., Hamiche, A. & Wu, C. A chromatin remodelling complex involved in
transcription and DNA processing. *Nature* **406**, 541-544, doi:10.1038/35020123 (2000).
- 238 Wu, S. *et al.* A YY1-INO80 complex regulates genomic stability through homologous
recombination-based repair. *Nat. Struct. Mol. Biol.* **14**, 1165-1172, doi:10.1038/nsmb1332
(2007).
- 239 Morrison, A. J. *et al.* INO80 and gamma-H2AX interaction links ATP-dependent chromatin
remodeling to DNA damage repair. *Cell* **119**, 767-775, doi:10.1016/j.cell.2004.11.037 (2004).
- 240 van Attikum, H., Fritsch, O., Hohn, B. & Gasser, S. M. Recruitment of the INO80 complex by
H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand
break repair. *Cell* **119**, 777-788, doi:10.1016/j.cell.2004.11.033 (2004).
- 241 Kashiwaba, S.-i. *et al.* The mammalian INO80 complex is recruited to DNA damage sites in an
ARP8 dependent manner. *Biochem. Biophys. Res. Commun.* **402**, 619-625,
doi:10.1016/j.bbrc.2010.10.066 (2010).
- 242 Aoyagi, S., Wade, P. A. & Hayes, J. J. Nucleosome sliding induced by the xMi-2 complex does
not occur exclusively via a simple twist-diffusion mechanism. *J. Biol. Chem.* **278**, 30562-
30568, doi:10.1074/jbc.M304148200 (2003).
- 243 Längst, G. & Becker, P. B. ISWI induces nucleosome sliding on nicked DNA. *Mol. Cell* **8**, 1085-
1092 (2001).
- 244 Widom, J. Structure, dynamics, and function of chromatin in vitro. *Annu Rev Biophys Biomol*
Struct **27**, 285-327, doi:10.1146/annurev.biophys.27.1.285 (1998).
- 245 Schiessel, H., Widom, J., Bruinsma, R. F. & Gelbart, W. M. Polymer reptation and nucleosome
repositioning. *Phys. Rev. Lett.* **86**, 4414-4417, doi:10.1103/PhysRevLett.86.4414 (2001).
- 246 Strohner, R. *et al.* A 'loop recapture' mechanism for ACF-dependent nucleosome remodeling.
Nat. Struct. Mol. Biol. **12**, 683-690, doi:10.1038/nsmb966 (2005).
- 247 Liu, N., Peterson, C. L. & Hayes, J. J. SWI/SNF- and RSC-catalyzed nucleosome mobilization
requires internal DNA loop translocation within nucleosomes. *Mol. Cell. Biol.* **31**, 4165-4175,
doi:10.1128/MCB.05605-11 (2011).
- 248 Deindl, S. *et al.* ISWI remodelers slide nucleosomes with coordinated multi-base-pair entry
steps and single-base-pair exit steps. *Cell* **152**, 442-452, doi:10.1016/j.cell.2012.12.040
(2013).
- 249 Murr, R. *et al.* Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and
repair of DNA double-strand breaks. *Nat. Cell Biol.* **8**, 91-99, doi:10.1038/ncb1343 (2006).

- 250 Downey, M. & Durocher, D. Chromatin and DNA repair: the benefits of relaxation. *Nat. Cell Biol.* **8**, 9-10, doi:10.1038/ncb0106-9 (2006).
- 251 Verschure, P. J. *et al.* Condensed chromatin domains in the mammalian nucleus are accessible to large macromolecules. *EMBO Rep.* **4**, 861-866, doi:10.1038/sj.embor.embor922 (2003).
- 252 Bancaud, A. *et al.* Molecular crowding affects diffusion and binding of nuclear proteins in heterochromatin and reveals the fractal organization of chromatin. *EMBO J* **28**, 3785-3798, doi:10.1038/emboj.2009.340 (2009).
- 253 Ayrapetov, M. K., Gursoy-Yuzugullu, O., Xu, C., Xu, Y. & Price, B. D. DNA double-strand breaks promote methylation of histone H3 on lysine 9 and transient formation of repressive chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 9169-9174, doi:10.1073/pnas.1403565111 (2014).
- 254 Lisby, M., Mortensen, U. H. & Rothstein, R. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nature Cell Biology* **5**, 572-577, doi:10.1038/ncb997 (2003).
- 255 Caron, P. *et al.* Non-redundant Functions of ATM and DNA-PKcs in Response to DNA Double-Strand Breaks. *Cell Rep* **13**, 1598-1609, doi:10.1016/j.celrep.2015.10.024 (2015).
- 256 Sutherland, H. & Bickmore, W. A. Transcription factories: gene expression in unions? *Nature reviews. Genetics* **10**, 457-466, doi:10.1038/nrg2592 (2009).
- 257 Meister, P. *et al.* Nuclear factories for signalling and repairing DNA double strand breaks in living fission yeast. *Nucleic Acids Res* **31**, 5064-5073 (2003).
- 258 Neumaier, T. *et al.* Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 443-448, doi:10.1073/pnas.1117849108 (2012).
- 259 Aten, J. A. *et al.* Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* **303**, 92-95, doi:10.1126/science.1088845 (2004).
- 260 Meaburn, K. J., Misteli, T. & Soutoglou, E. Spatial genome organization in the formation of chromosomal translocations. *Seminars in cancer biology* **17**, 80-90, doi:10.1016/j.semcancer.2006.10.008 (2007).
- 261 Savage, J. R. Cancer. Proximity matters. *Science* **290**, 62-63 (2000).
- 262 Horigome, C. *et al.* SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. *Mol. Cell* **55**, 626-639, doi:10.1016/j.molcel.2014.06.027 (2014).
- 263 Klement, K. *et al.* Opposing ISWI- and CHD-class chromatin remodeling activities orchestrate heterochromatic DNA repair. *J Cell Biol* **207**, 717-733, doi:10.1083/jcb.201405077 (2014).
- 264 Lemaitre, C. *et al.* Nuclear position dictates DNA repair pathway choice. *Genes Dev* **28**, 2450-2463, doi:10.1101/gad.248369.114 (2014).
- 265 Dabin, J., Fortuny, A. & Polo, S. E. Epigenome Maintenance in Response to DNA Damage. *Mol Cell* **62**, 712-727, doi:10.1016/j.molcel.2016.04.006 (2016).
- 266 (!!! INVALID CITATION !!!).
- 267 Boisvert, F. M., van Koningsbruggen, S., Navascues, J. & Lamond, A. I. The multifunctional nucleolus. *Nature reviews. Molecular cell biology* **8**, 574-585, doi:10.1038/nrm2184 (2007).
- 268 Torres-Rosell, J. *et al.* The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nature cell biology* **9**, 923-931, doi:10.1038/ncb1619 (2007).
- 269 Harding, S. M., Boiarsky, J. A. & Greenberg, R. A. ATM Dependent Silencing Links Nucleolar Chromatin Reorganization to DNA Damage Recognition. *Cell reports* **13**, 251-259, doi:10.1016/j.celrep.2015.08.085 (2015).

- 270 van Sluis, M. & McStay, B. A localized nucleolar DNA damage response facilitates recruitment
of the homology-directed repair machinery independent of cell cycle stage. *Genes &
development* **29**, 1151-1163, doi:10.1101/gad.260703.115 (2015).
- 271 Kalocsay, M., Hiller, N. J. & Jentsch, S. Chromosome-wide Rad51 spreading and SUMO-H2A.Z-
dependent chromosome fixation in response to a persistent DNA double-strand break. *Molecular cell* **33**, 335-343, doi:10.1016/j.molcel.2009.01.016 (2009).
- 272 Ryu, T. *et al.* Heterochromatic breaks move to the nuclear periphery to continue
recombinational repair. *Nature cell biology* **17**, 1401-1411, doi:10.1038/ncb3258 (2015).
- 273 Polo, S. E. Reshaping chromatin after DNA damage: the choreography of histone proteins. *J
Mol Biol* **427**, 626-636, doi:10.1016/j.jmb.2014.05.025 (2015).
- 274 Strickfaden, H. *et al.* Poly(ADP-ribosyl)ation-dependent Transient Chromatin Decondensation
and Histone Displacement following Laser Microirradiation. *J Biol Chem* **291**, 1789-1802,
doi:10.1074/jbc.M115.694992 (2016).
- 275 Ji, Y. & Tulin, A. V. The roles of PARP1 in gene control and cell differentiation. *Curr Opin
Genet Dev* **20**, 512-518, doi:10.1016/j.gde.2010.06.001 (2010).
- 276 Kim, M. Y., Mauro, S., Gevry, N., Lis, J. T. & Kraus, W. L. NAD⁺-dependent modulation of
chromatin structure and transcription by nucleosome binding properties of PARP-1. *Cell* **119**,
803-814, doi:10.1016/j.cell.2004.11.002 (2004).
- 277 Wang, Z. *et al.* Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WWE
domains suggests a general mechanism for poly(ADP-ribosyl)ation-dependent ubiquitination.
Genes Dev **26**, 235-240, doi:10.1101/gad.182618.111 (2012).
- 278 Walter, A., Chapuis, C., Huet, S. & Ellenberg, J. Crowded chromatin is not sufficient for
heterochromatin formation and not required for its maintenance. *J Struct Biol* **184**, 445-453,
doi:10.1016/j.jsb.2013.10.004 (2013).
- 279 Smeenk, G. & van Attikum, H. The chromatin response to DNA breaks: leaving a mark on
genome integrity. *Annu Rev Biochem* **82**, 55-80, doi:10.1146/annurev-biochem-061809-
174504 (2013).
- 280 Ma, N. F. *et al.* Isolation and characterization of a novel oncogene, amplified in liver cancer 1,
within a commonly amplified region at 1q21 in hepatocellular carcinoma. *Hepatology* **47**,
503-510, doi:10.1002/hep.22072 (2008).
- 281 Cheng, W., Su, Y. & Xu, F. CHD1L: a novel oncogene. *Mol Cancer* **12**, 170, doi:10.1186/1476-
4598-12-170 (2013).
- 282 Wade, P. A., Jones, P. L., Vermaak, D. & Wolffe, A. P. A multiple subunit Mi-2 histone
deacetylase from *Xenopus laevis* cofractionates with an associated Snf2 superfamily ATPase.
Curr Biol **8**, 843-846 (1998).
- 283 Zhang, Y. *et al.* Analysis of the NuRD subunits reveals a histone deacetylase core complex and
a connection with DNA methylation. *Genes Dev* **13**, 1924-1935 (1999).
- 284 Denslow, S. A. & Wade, P. A. The human Mi-2/NuRD complex and gene regulation. *Oncogene*
26, 5433-5438, doi:10.1038/sj.onc.1210611 (2007).
- 285 Ramirez, J. & Hagman, J. The Mi-2/NuRD complex: a critical epigenetic regulator of
hematopoietic development, differentiation and cancer. *Epigenetics* **4**, 532-536 (2009).
- 286 Pegoraro, G. *et al.* Ageing-related chromatin defects through loss of the NURD complex. *Nat
Cell Biol* **11**, 1261-1267, doi:10.1038/ncb1971 (2009).
- 287 Torchy, M. P., Hamiche, A. & Klaholz, B. P. Structure and function insights into the NuRD
chromatin remodeling complex. *Cell. Mol. Life Sci.* **72**, 2491-2507, doi:10.1007/s00018-015-
1880-8 (2015).

- 288 Turinetto, V. & Giachino, C. Multiple facets of histone variant H2AX: a DNA double-strand-
break marker with several biological functions. *Nucleic Acids Res* **43**, 2489-2498,
doi:10.1093/nar/gkv061 (2015).
- 289 Sansoni, V. *et al.* The histone variant H2A.Bbd is enriched at sites of DNA synthesis. *Nucleic
Acids Res* **42**, 6405-6420, doi:10.1093/nar/gku303 (2014).
- 290 Iacovoni, J. S. *et al.* High-resolution profiling of gammaH2AX around DNA double strand
breaks in the mammalian genome. *EMBO J* **29**, 1446-1457, doi:10.1038/emboj.2010.38
(2010).
- 291 Aymard, F. *et al.* Transcriptionally active chromatin recruits homologous recombination at
DNA double-strand breaks. *Nat Struct Mol Biol* **21**, 366-374, doi:10.1038/nsmb.2796 (2014).
- 292 Caron, P. *et al.* Cohesin protects genes against gammaH2AX Induced by DNA double-strand
breaks. *PLOS Genet* **8**, e1002460, doi:10.1371/journal.pgen.1002460 (2012).
- 293 Kong, X. *et al.* Comparative analysis of different laser systems to study cellular responses to
DNA damage in mammalian cells. *Nucleic Acids Res* **37**, e68, doi:10.1093/nar/gkp221 (2009).
- 294 Dinant, C. *et al.* Activation of multiple DNA repair pathways by sub-nuclear damage induction
methods. *Journal of Cell Science* **120**, 2731-2740, doi:10.1242/jcs.004523 (2007).
- 295 Karmakar, P. *et al.* BLM is an early responder to DNA double-strand breaks. *Biochem Biophys
Res Commun* **348**, 62-69, doi:10.1016/j.bbrc.2006.07.037 (2006).
- 296 Li, G., Sudlow, G. & Belmont, A. S. Interphase cell cycle dynamics of a late-replicating,
heterochromatic homogeneously staining region: precise choreography of
condensation/decondensation and nuclear positioning. *J Cell Biol* **140**, 975-989 (1998).
- 297 Li, B., Carey, M. & Workman, J. L. The role of chromatin during transcription. *Cell* **128**, 707-
719, doi:10.1016/j.cell.2007.01.015 (2007).
- 298 Voss, T. C. & Hager, G. L. Dynamic regulation of transcriptional states by chromatin and
transcription factors. *Nat Rev Genet* **15**, 69-81, doi:10.1038/nrg3623 (2014).
- 299 Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. DNA double-stranded
breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* **273**, 5858-5868
(1998).
- 300 Stiff, T. *et al.* ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure
to ionizing radiation. *Cancer Res* **64**, 2390-2396 (2004).
- 301 Morrison, A. J. & Shen, X. Chromatin remodelling beyond transcription: the INO80 and SWR1
complexes. *Nat Rev Mol Cell Biol* **10**, 373-384, doi:10.1038/nrm2693 (2009).
- 302 Smeenk, G. *et al.* Poly(ADP-ribosyl)ation links the chromatin remodeler SMARCA5/SNF2H to
RNF168-dependent DNA damage signaling. *J Cell Sci* **126**, 889-903, doi:10.1242/jcs.109413
(2013).
- 303 Smerdon, M. J. DNA repair and the role of chromatin structure. *Curr Opin Cell Biol* **3**, 422-428
(1991).
- 304 Izhar, L. *et al.* A Systematic Analysis of Factors Localized to Damaged Chromatin Reveals
PARP-Dependent Recruitment of Transcription Factors. *Cell Rep* **11**, 1486-1500,
doi:10.1016/j.celrep.2015.04.053 (2015).
- 305 Altmeyer, M. *et al.* Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-
ribose). *Nat Commun* **6**, 8088, doi:10.1038/ncomms9088 (2015).
- 306 Heine, G. F., Horwitz, A. A. & Parvin, J. D. Multiple mechanisms contribute to inhibit
transcription in response to DNA damage. *J Biol Chem* **283**, 9555-9561,
doi:10.1074/jbc.M707700200 (2008).
- 307 Roukos, V., Burman, B. & Misteli, T. The cellular etiology of chromosome translocations. *Curr
Opin Cell Biol* **25**, 357-364, doi:10.1016/j.ceb.2013.02.015 (2013).

- 308 Falk, M., Lukasova, E. & Kozubek, S. Chromatin structure influences the sensitivity of DNA to
gamma-radiation. *Biochim Biophys Acta* **1783**, 2398-2414, doi:10.1016/j.bbamcr.2008.07.010
(2008).
- 309 Takata, H. *et al.* Chromatin compaction protects genomic DNA from radiation damage. *PLoS*
One **8**, e75622, doi:10.1371/journal.pone.0075622 (2013).
- 310 Martinez, A. & Kolter, R. Protection of DNA during oxidative stress by the nonspecific DNA-
binding protein Dps. *J Bacteriol* **179**, 5188-5194 (1997).
- 311 De Vos, M., Schreiber, V. & Dantzer, F. The diverse roles and clinical relevance of PARPs in
DNA damage repair: current state of the art. *Biochem Pharmacol* **84**, 137-146,
doi:10.1016/j.bcp.2012.03.018 (2012).
- 312 Ame, J. C. *et al.* PARP-2, A novel mammalian DNA damage-dependent poly(ADP-ribose)
polymerase. *J Biol Chem* **274**, 17860-17868 (1999).
- 313 Menissier de Murcia, J. *et al.* Functional interaction between PARP-1 and PARP-2 in
chromosome stability and embryonic development in mouse. *EMBO J* **22**, 2255-2263,
doi:10.1093/emboj/cdg206 (2003).
- 314 Beck, C., Robert, I., Reina-San-Martin, B., Schreiber, V. & Dantzer, F. Poly(ADP-ribose)
polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. *Exp Cell Res*
329, 18-25, doi:10.1016/j.yexcr.2014.07.003 (2014).
- 315 Wang, H. *et al.* Biochemical evidence for Ku-independent backup pathways of NHEJ. *Nucleic*
Acids Res **31**, 5377-5388 (2003).
- 316 Sims, J. K. & Wade, P. A. Mi-2/NuRD complex function is required for normal S phase
progression and assembly of pericentric heterochromatin. *Mol Biol Cell* **22**, 3094-3102,
doi:10.1091/mbc.E11-03-0258 (2011).
- 317 Qi, W. *et al.* Acetyltransferase p300 collaborates with chromodomain helicase DNA-binding
protein 4 (CHD4) to facilitate DNA double-strand break repair. *Mutagenesis* **31**, 193-203,
doi:10.1093/mutage/gev075 (2016).
- 318 Ju, B. G. *et al.* A topoisomerase II β -mediated dsDNA break required for regulated
transcription. *Science* **312**, 1798-1802, doi:10.1126/science.1127196 (2006).
- 319 Hughes, A. L. & Rando, O. J. Mechanisms underlying nucleosome positioning in vivo. *Annu*
Rev Biophys **43**, 41-63, doi:10.1146/annurev-biophys-051013-023114 (2014).
- 320 Mueller-Planitz, F., Klinker, H. & Becker, P. B. Nucleosome sliding mechanisms: new twists in
a looped history. *Nat Struct Mol Biol* **20**, 1026-1032, doi:10.1038/nsmb.2648 (2013).
- 321 Lobanenko, V. V. *et al.* A novel sequence-specific DNA binding protein which interacts with
three regularly spaced direct repeats of the CCCTC-motif in the 5'-flanking sequence of the
chicken c-myc gene. *Oncogene* **5**, 1743-1753 (1990).
- 322 Hadjur, S. *et al.* Cohesins form chromosomal cis-interactions at the developmentally
regulated IFNG locus. *Nature* **460**, 410-413, doi:10.1038/nature08079 (2009).
- 323 Zuin, J. *et al.* Cohesin and CTCF differentially affect chromatin architecture and gene
expression in human cells. *Proc Natl Acad Sci U S A* **111**, 996-1001,
doi:10.1073/pnas.1317788111 (2014).
- 324 Neumann, B. *et al.* Phenotypic profiling of the human genome by time-lapse microscopy
reveals cell division genes. *Nature* **464**, 721-727, doi:10.1038/nature08869 (2010).
- 325 Subach, F. V., Patterson, G. H., Renz, M., Lippincott-Schwartz, J. & Verkhusha, V. V. Bright
monomeric photoactivatable red fluorescent protein for two-color super-resolution sptPALM
of live cells. *J Am Chem Soc* **132**, 6481-6491, doi:10.1021/ja100906g (2010).
- 326 Beaudouin, J., Mora-Bermudez, F., Klee, T., Daigle, N. & Ellenberg, J. Dissecting the
contribution of diffusion and interactions to the mobility of nuclear proteins. *Biophys J* **90**,
1878-1894, doi:10.1529/biophysj.105.071241 (2006).

- 327 Platani, M., Goldberg, I., Lamond, A. I. & Swedlow, J. R. Cajal body dynamics and association
with chromatin are ATP-dependent. *Nat Cell Biol* **4**, 502-508, doi:10.1038/ncb809 (2002).
- 328 Ran, F. A. *et al.* Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* **8**, 2281-2308,
doi:10.1038/nprot.2013.143 (2013).
- 329 Thevenaz, P., Ruttimann, U. E. & Unser, M. A pyramid approach to subpixel registration
based on intensity. *IEEE Trans Image Process* **7**, 27-41, doi:10.1109/83.650848 (1998).
- 330 Sbalzarini, I. F. & Koumoutsakos, P. Feature point tracking and trajectory analysis for video
imaging in cell biology. *J Struct Biol* **151**, 182-195, doi:10.1016/j.jsb.2005.06.002 (2005).

ANNEXES

*Review***Chromatin dynamics at DNA breaks: what, how and why?****Théo Lebeaupin^{1,2,3}, Hafida Sellou^{1,2,3}, Gyula Timinszky³, and Sébastien Huet^{1,2,*}**¹ CNRS, UMR 6290, Institut Génétique et Développement de Rennes, Rennes, France² Université de Rennes 1, Structure fédérative de recherche Biosit, Rennes, France³ Department of Physiological Chemistry, Adolf Butenandt Institute, Ludwig-Maximilians-Universität München, Munich, Germany*** Correspondence:** Email: sebastien.huet@univ-rennes1.fr; Tel: +33-223234557.

Abstract: Chromatin has a complex, dynamic architecture in the interphase nucleus, which regulates the accessibility of the underlying DNA and plays a key regulatory role in all the cellular functions using DNA as a template, such as replication, transcription or DNA damage repair. Here, we review the recent progresses in the understanding of the interplay between chromatin architecture and DNA repair mechanisms. Several reports based on live cell fluorescence imaging show that the activation of the DNA repair machinery is associated with major changes in the compaction state and the mobility of chromatin. We discuss the functional consequences of these changes in yeast and mammals in the light of the different repair pathways utilized by these organisms. In the final section of this review, we show how future developments in high-resolution light microscopy and chromatin modelling by polymer physics should contribute to a better understanding of the relationship between the structural changes in chromatin and the activity of the repair processes.

Keywords: chromatin; nucleus; DNA repair; double strand break; homologous recombination; non-homologous end joining; fluorescence microscopy; single particle tracking; anomalous diffusion; polymer physics

1. Introduction

Chromatin, one of the most complex supramolecular structures in the cell, displays several organizational levels spanning over four orders of magnitudes in size from the 2-nm diameter of the DNA double helix to the few tens of micrometers of chromosome territories in the nucleus [1]. This

packing state of chromatin is thought to influence all cellular functions acting on DNA. For example, even though the causal link between these two processes remains unclear, the modulation of transcription is associated with major changes in the chromatin organization [2]. While we have a relatively good understanding of nucleosome structure and function and that of the chromosome territories, the multiple organizational levels between these two extreme structures remain poorly understood and are the subject of intense research.

In the present review, we will focus on the interplay between chromatin and DNA repair, which has been receiving growing attention over the last years. Recent studies have shown that major chromatin remodeling events occur in the vicinity of DNA lesions [3,4]. However, it is still largely unknown whether these remodeling events are a mere consequence of the repair processes or play an active role in the resolution of DNA breaks. We will first review our current knowledge about chromatin structure and dynamics in the absence of DNA damage and in response to the induction of such damage. Second, we will examine the potential functional roles of chromatin dynamics during the DNA repair processes. Finally, we will speculate on how recent chromatin polymer models combined with high-resolution spatio-temporal data could help to bridge the gap between the modifications of the internal organization of the chromatin fiber induced by the DNA repair machinery and the changes in chromatin dynamics assessed by light microscopy.

2. The Organizational Levels of Chromatin: from the Nucleosome to Chromosome Territories

Similar to proteins, chromatin displays a hierarchical organization [2]. The primary structure encompasses the nucleosome architecture and the internal packing of the chromatin fiber, meaning the spatial distribution of the nucleosomes along this fiber. For many years, the classical view has been that the beads-on-a-string fiber composed of nucleosomes alternating with linker DNA spontaneously folds into a thicker 30-nm fiber [5,6]. However, the existence of this folding level was recently questioned by several studies that failed to identify the 30-nm fiber in the interphase nucleus using different high resolution imaging methods [7,8]. More recently, data obtained in yeast with a new chromosome conformation capture approach leading to mono-nucleosome resolution [9] suggested the existence of small compact tetranucleosome structures similar to those previously observed in-vitro [6], but did not demonstrate the presence of longer regular 30-nm fibers.

The secondary structuring level of the chromatin fiber relies on the formation of loops due to long-distance interactions along this fiber. Although the existence of chromatin loops of kilobase-to-megabase sizes has been widely documented [9,10], their distribution along the fiber and their stability remain debated [11]. These loops may be the elementary component of a recently identified structural unit: the topologically associated domains (TADs) [12,13,14], which correspond to compact structures encompassing ~1Mb of DNA and characterized by a high probability of contacts along the chromatin fiber.

Finally, the ternary structure of the chromatin corresponds to the spatial distributions of the TADs and, at larger scales, of the whole chromosomes, within the nucleus. The TADs associate to form larger compartments sharing similar features, such as an opened chromatin state or a defined gene density [15], reminiscent of the original definition of euchromatin and heterochromatin areas. Studies analyzing the spatial distributions of whole chromosomes showed that they were not widespread over the nuclear volume but occupy compact and largely mutually exclusive areas called

chromosome territories [16,17]. The positioning of these territories in the nucleus is not random and is probably partially defined by interactions with the inner nuclear membranes [18].

So far, we only described a snapshot of chromatin architecture. However, several studies have reported rapid chromatin motions at scales up to $\sim 1\ \mu\text{m}$ [19–22], which would suggest that chromatin architecture is highly dynamic at all the organizational levels below chromosome territories [23]. These local chromatin motions probably originate both from passive thermal fluctuations and active remodeling mechanisms but the relative contributions of each component is still a subject of investigations [24,25].

3. Current Methodologies Available to Analyze Chromatin Dynamics

Chromatin dynamics in the living interphase nucleus can be directly analyzed at multiple scales in space and time using different fluorescence-based imaging methods. The main difference between these approaches resides in the size of the assessed chromatin area. The movements of chromosome territories within the nucleus can be followed by confocal microscopy using fluorescently tagged histones [26,27]. Single chromosomes or sub-chromosomal areas can be identified by local photobleaching or photoactivation of the fluorescent proteins [28]. This approach can also be used to characterize chromatin compaction, in the context of the DNA damage response [29]. The minimal chromatin area that can be studied with this approach is defined by the size of the laser spot used to photobleach or photoconvert the tagged histones, which probably encompasses several Mb of DNA wrapped around thousands of nucleosomes.

To study the dynamics of smaller chromatin areas, DNA can be directly labeled by the incorporation of fluorescent nucleotides during replication [30]. The labeled areas thus correspond to replication foci that contain $\sim 0.8\text{Mb}$ of DNA [31]. Another common labeling approach uses repeated bacterial sequences, such as the Lac or the Tet operator, integrated into the genome. The binding of the associated repressor proteins tagged by fluorescent dyes to this DNA stretch, whose size is approximately 100 kb, generates a fluorescent spot whose trajectory can be followed under the microscope [32]. Although this strategy has demonstrated its usefulness in analyzing chromatin motion (see below), it is known to suffer from several pitfalls. For instance, the integration of these DNA arrays containing a large number of repeated sequences tightly bound to repressor proteins induce the formation of fragile sites and the transcriptional silencing of the surrounding genes [33,34]. Interestingly, it was recently reported that shorter DNA recognition sequences of only one kilobase can be used to assess chromatin motions [35]. Moreover, the newly developed tools for genome editing such as the TALEs or CRISPR/Cas systems can also be applied to fluorescently tag short target DNA sequences in living cells [36,37]. These new approaches would allow not only to solve the issues related to the repetitive nature of the Lac or Tet arrays but also to follow the dynamics of smaller chromatin regions. The different methods mentioned so far to assess chromatin dynamics were based on the local labeling of predefined chromatin regions. An alternative is to label uniformly the chromatin, using for example fluorescently tagged core histones, and to use image correlation methods to characterize the local chromatin movements [38,39].

4. Chromatin Dynamics in the Absence of DNA Damage

Although the global architecture of chromatin is stable during interphase [26,27], local movements with amplitudes of 0.3 to 1 μm have been reported in multiple organisms: bacteria [22], yeast [19] and higher eukaryotes [40,41]. Most of the reports studying chromatin motion are based on the analysis of the mean squared displacement (MSD) curves calculated from the tracks of fluorescently labeled chromatin foci [42]. Diffusion coefficients derived from these MSDs range between 10^{-5} and 10^{-3} $\mu\text{m}^2/\text{s}$ [40,41]. By comparison, the diffusion coefficient of a 30 kD globular protein in mammalian nuclei is several magnitudes higher, 10–40 $\mu\text{m}^2/\text{s}$. Interestingly, chromatin mobility is usually higher in yeast than in mammals, maybe due to the fact that mammalian chromosomes are longer than the yeast ones and thus more difficult to move [43]. The analysis of the MSD curves also indicates that chromatin dynamics do not correspond to pure diffusion but rather to anomalous diffusion or subdiffusion [44] (Figure 1). Such diffusion patterns arise either when molecules diffuse in complex heterogeneous media [45] or when studying thermal fluctuations within a polymer [46], both of which could explain the observed chromatin dynamics. Interestingly, the subdiffusive motion of the chromatin seems homogeneous within a large range of timescale from 10^{-2} to 10^2 s [44,47], suggesting that the components responsible for these chromatin motions act at multiple timescales. In rare cases, transient directional chromatin movements have been also reported [20].

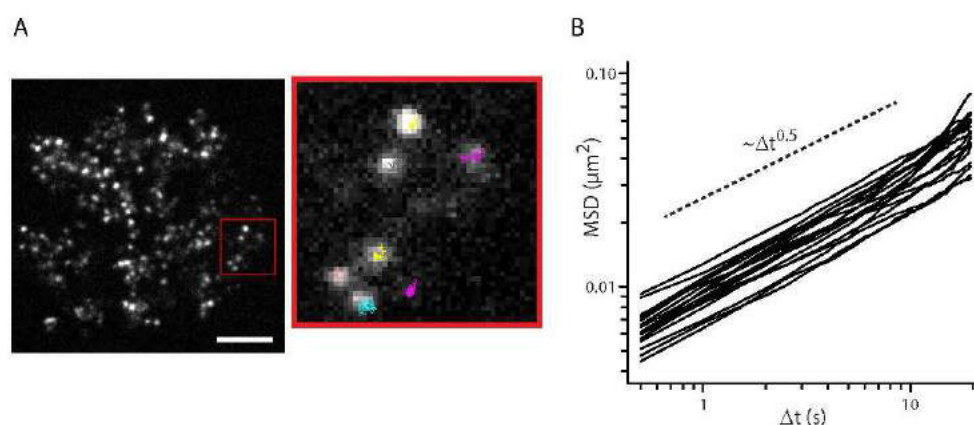


Figure 1. (A) Nucleus of a U2OS cell with its DNA labeled using fluorescent nucleotides. Bar = 5 μm . The inset shows examples of trajectories displayed by the labeled chromatin foci. The trajectories were recorded for 30s at 2 frames per second. (B) Curves of the mean square displacement (MSD) calculated from the trajectories of the labeled foci. Each curve corresponds to the averaged chromatin dynamics within one nucleus (21 nuclei, 40 to 180 track per nucleus). The fact that the curves show a slope of ~ 0.5 in the log-log representation indicates that the chromatin dynamics is subdiffusive at the studied timescales.

Although contradictory results have been reported [25,40], several studies indicate that local chromatin motions are principally due to ATP-dependent processes rather than thermal fluctuations [19,24,38]. Multiple active processes are probably responsible for chromatin dynamics.

While the influence of the DNA replication status is unclear [19,25,48], modulations of transcription levels correlate with changes in chromatin motions [49,50]. In this context, the ATP-dependent chromatin remodeler INO80 is an important regulator of chromatin dynamics [51]. In the case of directed motion related to transcription activation, the involvement of actin dependent transport has been reported [52,53]. Besides these active processes directly acting on chromatin, the nuclear environment surrounding chromatin also influences its movements. The tethering of chromatin to stable nuclear structures such as the lamina or the nucleoli reduces chromatin motions [54]. Moreover, a recent report revealed that the viscoelastic properties of the complex and heterogeneous nuclear environment also modulate chromatin dynamics [55].

5. Chromatin Dynamics upon DNA Damage

Chromatin dynamics in the context of DNA repair mechanisms has been mainly analyzed for the most deleterious form of DNA damage: double strand breaks (DSBs). Eukaryotic organisms activate two main mechanisms to repair DSBs (Figure 2): homologous recombination (HR) and non-homologous end joining (NHEJ). HR requires the pairing between the broken DNA and an intact homologous sequence, which is used as a template for the faithful repair [56]. Instead, NHEJ directly religates the broken ends without the need for an intact template, making this type of repair more error-prone [57]. The changes in chromatin architecture associated with the activation of these DSB repair pathways have been studied mostly in yeast and mammalian nuclei. While chromatin dynamics is in the same range in yeast and mammals in the absence of DNA damage, the induction of DSBs is associated with a very different response of the chromatin architecture in the two model systems. This observation may be related to the fact that HR is the major DSB repair pathway in yeast while NHEJ dominates in differentiated mammalian cell lines [58].

5.1. The yeast paradigm

In yeast, chromatin dynamics was assessed by tracking fluorescently labeled chromosomal loci during two different steps of the DSB repair by HR: the early resection process and the later homologous pairing phase. During resection, a strong inhibition of the chromatin motions was observed [35]. Chromatin dynamics associated with homologous pairing was characterized mainly in terms of confinement radius, which corresponds to the size of the region explored by the tracked locus. The induction of DSBs by restriction enzymes or pharmacological treatment was associated with an expansion of the nuclear area explored by the mobile damaged locus, even if the amplitude of this expansion varies depending on the locus of interest and the ploidy of the cell [59,60]. Surprisingly, the induction of DNA damage not only affects the dynamics of the damaged site but also induces an overall increase of chromatin mobility in diploid cells [3]. The fact that this global effect was not observed in haploid cells under similar conditions [59] suggests that it only occurs when a damaged chromosome needs to explore the nucleus to find and pair with its homologue. It is also important to note that the modulation of chromatin movements at DNA breaks depends on the type of DNA damage since spontaneous breaks occurring during DNA replication display decreased mobility compared to undamaged DNA [48]. Several members of the DNA repair machinery are implicated in the modulation of the chromatin dynamics in relation to DNA damage: the recombinase protein Rad51, the ATR mediator Mec1 and the INO80 nucleosome remodeling

complex [59,60], but the exact mechanism by which these repair proteins regulate chromatin motions remains unknown.

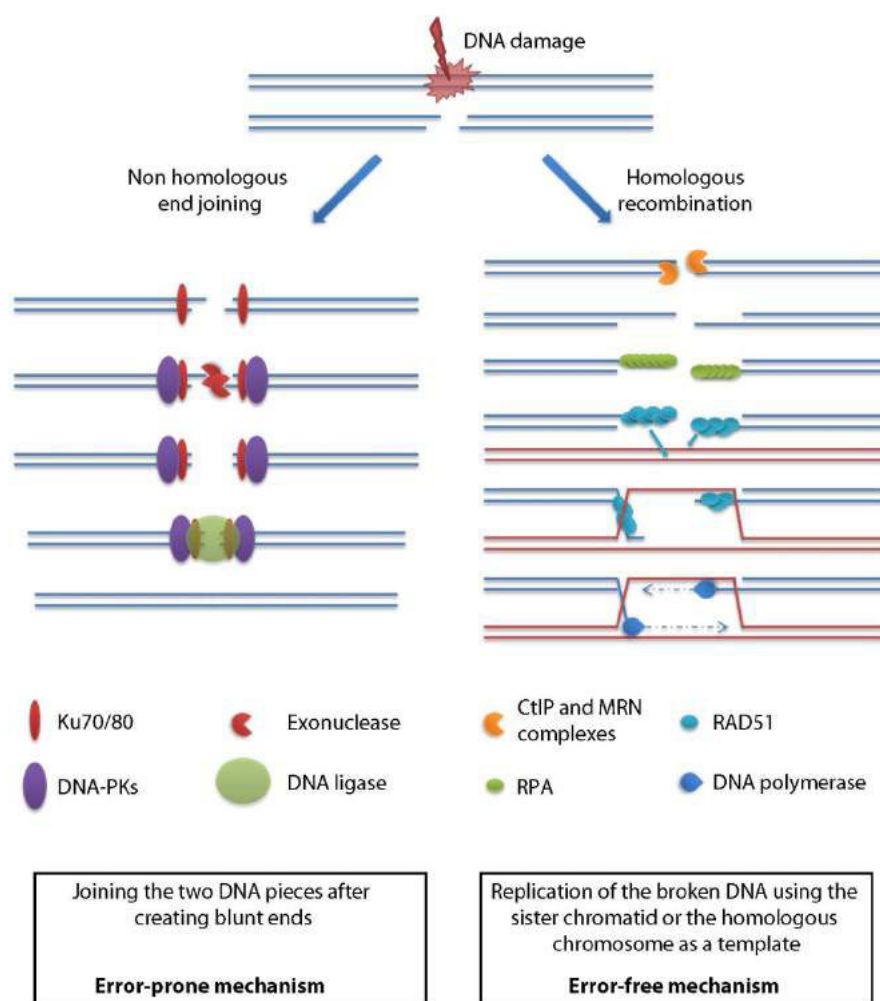


Figure 2. Schematic description of the two main pathways for repairing DNA double strand breaks.

In addition to the increased chromatin mobility, several studies describe the clustering of multiple DSBs. Lisby et al. showed the co-localization of DNA lesions in foci containing the repair factor Rad52 suggesting that these multiple DSBs are driven to a shared location, the so-called “repair centers” or “repair factories” [61]. When no homology is found and DSBs persist, Rad51, a protein involved in homology pairing, remains on the broken DNA indicating persistent homology search which ultimately leads to the relocation of the DSBs to the nuclear periphery [62,63]. Altogether, the different data obtained in yeast thus suggest a global picture in which the enhancement of the mobility of DNA breaks is a key step for their efficient repair (Figure 3).

5.2. The mammalian paradigm

While recent publications allowed us to draw a relatively clear picture of the modulation of chromatin dynamics in yeast upon induction of DSBs, the situation in mammalian nuclei appears much more complex. On the one hand, there are several findings similar to the yeast-like model in which damaged DNA gains mobility and, in some cases, relocates to repair-competent areas. After irradiation by α -particles, the damaged chromatin displays enhanced mobility compared to undamaged DNA [64] and tends to fuse into clusters [65]. Similarly increased dynamics was also found for uncapped telomeres, which can be recognized as DSBs [66]. Finally, damaged DNA in heterochromatin tends to move into euchromatin where γ H2AX foci are formed, suggesting that this relocation step is necessary for proper signaling and repair [67,68]. This mechanism, which is also observed in *Drosophila melanogaster* [69], may limit the risk of deleterious chromosomal rearrangements within the highly repetitive heterochromatin. However, there are numerous reports that do not observe pronounced changes in chromatin mobility upon damage induced by γ or UV-laser irradiation [29], X-ray irradiation [70], ion irradiation [71] or enzymatically-induced DSBs [72,73].

Besides the analysis of chromatin movements, many publications also investigated the modulation of the chromatin compaction state at DNA breaks. Smerdon and Lieberman showed in 1978 that UV-induced DNA damage gives rise to an increased sensitivity of chromatin to nucleases [74]. This higher accessibility at the nucleosomal level upon DNA damage is correlated with chromatin decondensation at the micrometer scales accessible by light microscopy [29,75], even though the causal link between these two remodeling events occurring at different scales is still unclear (Figure 3). Following this initial fast decondensation, the damaged chromatin area slowly recondenses [4], potentially reaching higher compaction levels than before damage induction [76].

Currently, we have no precise clue about the molecular mechanisms regulating chromatin packing upon DNA damage. Multiple proteins are recruited to the DNA breaks. Some of them, such as PARP1, promote chromatin decondensation [4,77], while others, such as HP1, induce the formation of a closed chromatin state [78,79]. It is unclear how the action of these proteins with opposite effects on chromatin packing is coordinated. Khurana and colleagues proposed that chromatin decondensation and compaction occur sequentially through a balance between the factors intervening in these two processes, this coordination being a key determinant of the choice of the repair pathway [4]. Alternatively, Hinde et al. suggested a model in which both chromatin expansion and compaction processes happen at the same time but in distinct regions of the chromatin in the vicinity of the DNA breaks [39].

6. Functional Roles of Chromatin Dynamics at the DNA Breaks

The data reviewed so far identify major changes in both chromatin mobility and compaction state during the DNA damage response. In this section, we will investigate the functional roles of these chromatin-remodeling processes.

Regarding the yeast model, it has been postulated that the increased mobility of DSBs may promote homology search, which is the limiting factor in HR (Figure 3). This is supported by the fact that the increased chromatin mobility upon DNA damage is absent in yeast depleted for proteins involved in homology search [59,60]. The increased chromatin movements may also promote the

merging of multiple DSBs in repair foci [61]. The formation of nuclear bodies is a classical cellular response to promote different functions due to the local accumulation of specific proteins [80].

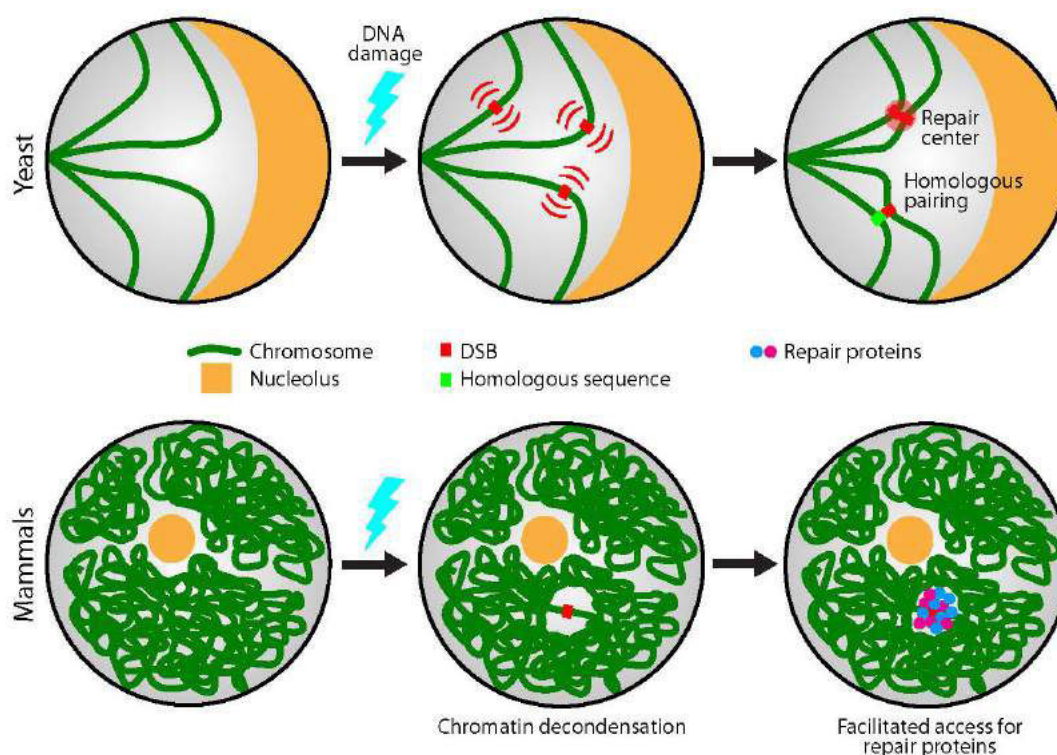


Figure 3. Schematic description of the changes in chromatin motions and compaction state observed at DNA breaks for yeast and mammals.

In this context, the DSB clusters may constitute areas highly favorable for efficient repair. However, it is interesting to note that increased chromatin mobility at the DNA breaks is not generally observed in mammalian nuclei. Two reasons may explain these differences between yeast and mammals. The first is the amplitude of the nuclear movements relative to the size of the nucleus. In the yeast nucleus characterized by a 2 μm diameter, chromatin loci already explore a significant fraction, about 10–20 %, of the nuclear volume in the absence of DNA damage [81]. Following a modest increase in chromatin mobility, this value increases to ~50 % upon DNA damage [59], allowing the efficient search for the intact homologous sequence required in HR. In mammalian nuclei, the amplitude of chromatin motions in the absence of damage is in the same range than in yeast but the volume to explore is two orders of magnitude bigger. Consequently, the efficient exploration of the nucleus for homologous pairing would require a strong increase in chromatin movements, which may only be achieved by major unfolding of the chromatin fiber. Nevertheless, long-range chromatin displacements can occur in mammalian nuclei as observed in the case of transcriptional activation [52]. Thus, rather than the potential inefficiency of the nuclear exploration for homologous pairing, chromatin may not display increased mobility at DNA breaks in mammalian cells to limit the risk of deleterious chromosome translocations, which could ultimately lead to cancer development [51,73,82]. Indeed, a recent genome-wide analysis of chromosomal rearrangements in mammalian nuclei shows that the physical proximity to the DSB is a key

determinant in the probability of translocation events [83]. Altogether, the potentially inefficient and risky pairing step with the homologous chromosome required for HR in unreplicated genomes may explain why mammalian cells rather use NHEJ to repair DSBs in G1 and only switch to HR when a close-by sister chromatid is available. It remains however unclear why the risk of ectopic translocation inherent to HR might be more tolerable in unicellular organisms such as yeast than in multicellular higher eukaryotes.

In addition to the modulation of chromatin movements, the activation of the DNA repair machinery is also associated with changes in chromatin compaction. It is assumed that chromatin decondensation following DNA damage is a necessary step and its impairment greatly inhibits the repair process [84]. A straightforward model is that chromatin decondensation facilitates DNA access to repair proteins (Figure 3) as illustrated by the hypersensitivity of the chromatin to nucleases at the DNA breaks. However, this simple model should be considered with caution because several studies reported that molecular tracers of sizes up to a few hundred kDa can easily diffuse through the nucleus and penetrate even the densely packed heterochromatin [85,86]. It was also proposed that the chromatin packing state may influence the way that proteins scan for binding sites, which correspond to DNA breaks in the case of repair proteins, along the chromatin fiber [86]. In addition, it was recently suggested that it is the over-compaction of chromatin at DNA breaks rather than its decondensation that may trigger the recruitment of some repair components [76]. The chromatin over-condensation or recondensation following DNA damage in association to the recruitment of heterochromatin proteins [4,76,79] may originate from the necessity to both inhibit transcription of the damaged DNA and keep the loose broken DNA ends in close proximity to facilitate repair.

To reconcile these different and sometimes contradictory observations, we will require a better understanding of the types of DNA lesions created with the different DNA damaging methods [87,88]. Other parameters such as the differential activation of distinct DNA repair pathways depending on the cell type or the cell cycle, or the time-window at which the chromatin movements are assessed, must be also analyzed carefully.

7. The Future Step: Relating the Changes in Chromatin Dynamics at DNA Breaks to the Activity of the DNA Repair Machinery

The changes in the chromatin architecture at DNA breaks described in the previous sections may be the direct consequence of the modifications of the physical properties of the DNA polymer upon damage. DSBs occurring in particular in the linker DNA could dramatically destabilize the chromatin fiber. Single and double strand DNA breaks may also lead to a local release of topological constraints, a key component of the chromatin packing state [89,90]. However, the fact that the chromatin remodeling mechanisms observed at DNA breaks are inhibited when impairing specific DNA repair pathways [59,60,77] suggest that these remodeling mechanisms are not the mere physical consequences of breaks along the DNA but are rather driven by the activity of the DNA repair machinery.

The DNA repair machinery directly acts on the chromatin fiber via three major mechanisms: i) nucleosome destabilization, ii) alteration of the nucleosome-nucleosome interactions within the fiber and iii) nucleosome repositioning [91]. These chromatin remodeling processes involve a complex choreography of molecular actors. The most canonical post-transcriptional modification found at DSBs is the phosphorylation of the H2AX histone variant, which is a major signal controlling the

recruitment of several members of the DNA repair machinery. It may also play a structural role by promoting chromatin relaxation [92] or nucleosome destabilization [93,94] at DNA breaks. The formation of negatively charged chains of poly-ADP-ribose, another post-transcriptional modification often found at DNA breaks [95], on the linker histone H1 is thought to induce the relaxation of the chromatin fiber due to the repulsion between the neighboring nucleosomes within the fiber [96,97]. The histone variant H2A.Z also appears as a key regulator of the nucleosome stability at DNA breaks [98]. Finally, multiple ATP-dependent chromatin remodeling enzymes are recruited at DNA damage sites. These enzymes are often part of multi-subunits complexes, fuelled by the energy provided by ATP hydrolysis to actively alter histone-DNA interactions leading to nucleosome sliding, eviction or histone exchange [99]. Altogether, these different molecular actors of the DNA repair machinery acting on the nucleosomes will have a major impact on the internal organization of the chromatin fiber, which we identified in the first section of this review as the primary structure of chromatin. It remains however largely unknown how these changes occurring on this primary structure will influence the higher hierarchical folding steps of the chromatin to ultimately lead to the modifications of the chromatin movements or compaction levels that were reviewed above. In the following, we will show how recent developments in high-resolution fluorescence microscopy and in the modeling of chromatin architecture by polymer physics may help in building an integrated description of the interplay between chromatin architecture and DNA repair mechanisms.

Chromatin dynamics in living nuclei is usually studied by tracking diffraction-limited fluorescent spots corresponding to defined tagged chromatin areas. This approach allows to assess chromatin movements as small as a few tens of nanometer, well below the nominal spatial resolution of optical microscopy, provided that the signal-to-noise ratio (SNR) of the tracked spots is sufficiently high [100]. For many years, reaching high SNR required the labeling of chromatin regions containing about 0.1 to 1 Mb of DNA, thus preventing the direct characterization of the dynamics of the smaller structural units of chromatin [54]. The recent progress in single-molecule imaging abolished this limitation since single fluorescently labeled nucleosomes [101] or single dyes incorporated in the DNA [102] can be detected in living cells, allowing to follow their local movements [103]. When used in fixed samples for ultrastructure reconstruction, these single-molecule imaging approaches also further our understanding of the fine-scale organization of chromatin [104,105,106]. These new methodologies will refine our description of the dynamic chromatin architecture in the absence of and following DNA breaks. To study the dynamic structural information of chromatin at an even smaller scale, the analysis of fluorescence resonance energy transfer (FRET) signals between fluorophores attached to chromatin components, such as histones, appears to be a promising method [107]. Because FRET is sensitive to variations of few nanometers in the distance between the two fluorophores, the recording of the variations of FRET signals upon DNA damage should help to identify subtle changes in the packing state of chromatin.

Given the complexity of chromatin architecture and the diversity of experimental approaches to study chromatin structure and dynamics, the precise understanding of the interplay between the chromatin state and DNA repair mechanisms would clearly benefit from an integrated multiscale model describing the spatial organization of chromatin in the interphase nucleus. In 2009, Emanuel et al. made the provocative statement that, with the resolution of the experimental methods available at the time, any of the structural models could fit the data [108]. Nevertheless, since then, we gained significant quantitative understanding about the dynamic chromatin architecture. Based on these new

findings, different models have been proposed. A very simple polymer model was introduced by Rouse in 1953 [109]. The polymer is modeled as a chain of beads connected with springs and the contributions of volume exclusion and hydrodynamic interactions are neglected. Surprisingly, this model agrees very well with the experimental data describing chromatin movements in bacteria [110] and yeast [44]. Nevertheless, fitting these data with the Rouse model leads to an unrealistic highly flexible chromatin fiber with a persistence length of only few nanometer [44]. In addition, while the subdiffusive motion displayed by chromatin in bacteria and yeast appears homogeneous over the assessed timescales in agreement with the predictions of the Rouse model [44,110], the situation in mammalian nuclei is more complex with different subdiffusive regimes depending on the timescales [47]. These different results call for polymer models more complex than the Rouse chain to describe the subdiffusive chromatin movements [111].

In 2009, based on the spatial proximity maps obtained by Hi-C methods (high throughput sequencing combined to chromosome conformation capture), it was proposed that chromatin adopts a particular metastable compact configuration: the fractal globule [15,112]. Noteworthy, this fractal feature nicely agrees with data obtained using different methods [113]. Yet, this model suffers from several limitations. In particular, it fails to predict the compact structure of chromosome territories [114]. To obtain this compact configuration, multiple models have been proposed to take into account the formation of dynamic chromatin loops [114,115,116]. One interesting feature associated with the presence of loops is that they allow the generation of chromatin structures that agree with the fluorescence in-situ hybridization (FISH) data, while limiting the formation of knots, which are thought to be deleterious for the cells [117]. Despite not being a necessary condition [43], these loops may also contribute to the spontaneous unmixing of chromosomes, which could explain the existence of chromosome territories [118,119]. It remains, however, unclear whether the loop formation requires specific interactions along the chromatin fiber [114,120] or if non-specific, entropy driven, contacts are sufficient [121]. Very recently, Zhang et al. have used Hi-C contact maps to define an effective energy landscape for the chromatin fiber [122]. Based on this energy function, they could simulate chromatin architectures that recapitulate the formation of loops and their assembling into topologically associated domains. Besides the chromatin polymer itself, a global model should also include its surrounding heterogeneous environment. For example, the crowding induced by the numerous macromolecules (proteins, RNA...) diffusing through the nucleus seems to have a major impact on chromatin architecture [113].

8. Conclusion

Even though if it is now clear that complex chromatin remodeling events occur at DNA breaks, we still have some difficulties to draw a clear picture of the interplay between the DNA repair processes and the dynamic chromatin architecture. Among others, two elements would help to make significant progress in this direction. First, we would need a global and integrated description of the chromatin architecture in the absence and upon DNA damage. Second, we should investigate more precisely the impact of the multiscale chromatin organization on the ability of DNA repair proteins navigating through the nucleus to find their target and bind to it. The recent technical breakthroughs achieved to investigate chromatin structure at high resolution and the development of complex polymer models of the chromatin will definitely help to answer these questions in the future. Altogether, we foresee that advances in the establishment of an integrated chromatin polymer model

together with the improving spatial and temporal resolution of the methods used to analyze chromatin architecture should greatly refine the description of chromatin organization. Once such a refined picture will be available, it will perhaps be possible to better understand how remodeling events occurring at the fiber level such as those induced by molecular actors of the DNA repair machinery, can influence chromatin architecture at multiple space scales.

Acknowledgments

This work was supported by grants from the Agence National de la Recherche (JCJC-SVSE2-2011, ChromaTranscript project) and from the European Union (FP7-PEOPLE-2011-CIG, ChromaTranscript project). G.T. acknowledges the financial support from the Deutsche Forschungsgemeinschaft (TI 817/2-1) and from the Worldwide Cancer Research (#14-1315).

Conflict of Interest

The authors declare no conflict of interest.

References

1. Woodcock CL, Ghosh RP (2010) Chromatin higher-order structure and dynamics. *Cold Spring Harb Perspect Biol* 2: a000596.
2. Sexton T, Cavalli G (2015) The role of chromosome domains in shaping the functional genome. *Cell* 160: 1049–1059.
3. Miné-Hattab J, Rothstein R (2012) Increased chromosome mobility facilitates homology search during recombination. *Nat Cell Biol* 14: 510–517.
4. Khurana S, Kruhlak MJ, Kim J, et al. (2014) A macrohistone variant links dynamic chromatin compaction to BRCA1-dependent genome maintenance. *Cell Rep* 8: 1049–1062.
5. Robinson PJJ, Fairall L, Huynh VAT, et al. (2006) EM measurements define the dimensions of the “30-nm” chromatin fiber: evidence for a compact, interdigitated structure. *Proc Natl Acad Sci U S A* 103: 6506–6511.
6. Schalch T, Duda S, Sargent DF, et al. (2005) X-ray structure of a tetranucleosome and its implications for the chromatin fibre. *Nature* 436: 138–141.
7. Joti Y, Hikima T, Nishino, et al. (2012) Chromosomes without a 30-nm chromatin fiber. *Nucl Austin Tex* 3: 404–410.
8. Fussner E, Ahmed K, Dehghani, et al. (2010) Changes in chromatin fiber density as a marker for pluripotency. *Cold Spring Harb Symp Quant. Biol* 75: 245–249.
9. Yokota H, van den Engh G, Hearst JE, et al. (1995) Evidence for the organization of chromatin in megabase pair-sized loops arranged along a random walk path in the human G0/G1 interphase nucleus. *J Cell Biol* 130: 1239–1249.
10. Petrascheck M, Escher D, Mahmoudi T et al. (2005) DNA looping induced by a transcriptional enhancer in vivo. *Nucleic Acids Res.* 33: 3743–3750.
11. Pombo A, Dillon N (2015) Three-dimensional genome architecture: players and mechanisms. *Nat Rev Mol Cell Biol* 16: 245–257.

12. Dixon JR, Selvaraj S, Yue F, et al. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485: 376–380.
13. Nora EP, Lajoie BR, Schulz EG, et al. (2012) Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 485: 381–385.
14. Sexton T, Yaffe E, Kenigsberg E, et al. (2012) Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell* 148: 458–472.
15. Lieberman-Aiden E, van Berkum NL, Williams L, et al. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326: 289–293.
16. Bolzer A, Kreth G, Solovei I, et al. (2005) Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. *PLoS Biol* 3: e157.
17. Cremer T, Cremer M (2010) Chromosome territories. *Cold Spring Harb Perspect Biol* 2: a003889.
18. Kinney NA, Onufriev AV, Sharakhov IV (2015) Quantified effects of chromosome-nuclear envelope attachments on 3D organization of chromosomes. *Nucl Austin Tex* 6: 212–224.
19. Heun P, Laroche T, Shimada K, et al. (2001) Chromosome dynamics in the yeast interphase nucleus. *Science* 294: 2181–2186.
20. Levi V, Ruan Q, Plutz M, et al. (2005) Chromatin dynamics in interphase cells revealed by tracking in a two-photon excitation microscope. *Biophys J* 89: 4275–4285.
21. Hubner M, Spector D (2010) Chromatin Dynamics. *Annu Rev Biophys* 39: 471–489.
22. Javer A, Long Z, Nugent E, et al. (2013) Short-time movement of E. coli chromosomal loci depends on coordinate and subcellular localization. *Nat Commun.* 4: 3003.
23. Gibcus JH, Dekker J (2013) The hierarchy of the 3D genome. *Mol Cell* 49: 773–782.
24. Weber SC, Spakowitz AJ, Theriot JA (2012) Nonthermal ATP-dependent fluctuations contribute to the in vivo motion of chromosomal loci. *Proc Natl Acad Sci U S A* 109: 7338–7343.
25. Pliss A, Malyavantham KS, Bhattacharya S, et al. (2013) Chromatin dynamics in living cells: identification of oscillatory motion. *J Cell Physiol* 228, 609–616.
26. Gerlich D, Beaudouin J, Kalbfuss B, et al. (2003) Global chromosome positions are transmitted through mitosis in mammalian cells. *Cell* 112: 751–764.
27. Walter J, Schermelleh L, Cremer M, et al. (2003) Chromosome order in HeLa cells changes during mitosis and early G1, but is stably maintained during subsequent interphase stages. *J Cell Biol* 160: 685–697.
28. Müller I, Boyle S, Singer RH, et al. (2010) Stable morphology, but dynamic internal reorganisation, of interphase human chromosomes in living cells. *PloS One* 5: e11560.
29. Kruhlak MJ, Celeste A, Dellaire G, et al. (2006) Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J Cell Biol* 172: 823–834.
30. Zink D, Cremer T, Saffrich R, et al. (1998) Structure and dynamics of human interphase chromosome territories in vivo. *Hum Genet* 102: 241–251.
31. Jackson DA, Pombo A (1998) Replicon clusters are stable units of chromosome structure: evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells. *J Cell Biol* 140: 1285–1295.
32. Robinett CC, Straight A, Li G, et al. (1996) In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J Cell Biol* 135: 1685–1700.

33. Jacome A, Fernandez-Capetillo O (2011) Lac operator repeats generate a traceable fragile site in mammalian cells. *EMBO Rep* 12: 1032–1038.
34. Dubarry M, Loiodice I, Chen CL, et al. (2011) Tight protein-DNA interactions favor gene silencing. *Genes Dev* 25: 1365–1370.
35. Saad H, Gallardo F, Dalvai M, et al. (2014) DNA dynamics during early double-strand break processing revealed by non-intrusive imaging of living cells. *PLoS Genet* 10: e1004187.
36. Chen B, Gilbert LA, Cimini BA, et al. (2013) Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* 155: 1479–1491.
37. Miyanari Y, Ziegler-Birling C, Torres-Padilla M-E (2013) Live visualization of chromatin dynamics with fluorescent TALEs. *Nat Struct Mol Biol* 20: 1321–1324.
38. Zidovska A, Weitz DA, Mitchison TJ (2013) Micron-scale coherence in interphase chromatin dynamics. *Proc Natl Acad Sci U S A* 110: 15555–15560.
39. Hinde E, Kong X, Yokomori K, et al. (2014) Chromatin dynamics during DNA repair revealed by pair correlation analysis of molecular flow in the nucleus. *Biophys J* 107: 55–65.
40. Marshall WF, Straight A, Marko JF, et al. (1997) Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr Biol CB* 7: 930–939.
41. Bornfleth H, Edelmann P, Zink D, et al. (1999) Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy. *Biophys J* 77: 2871–2886.
42. Qian H, Sheetz MP, Elson EL (1991) Single particle tracking. Analysis of diffusion and flow in two-dimensional systems. *Biophys J* 60: 910–921.
43. Rosa A, Everaers R (2008) Structure and dynamics of interphase chromosomes. *PLoS Comput Biol* 4: e1000153.
44. Hajjoul H, Mathon J, Ranchon H, et al. (2013) High-throughput chromatin motion tracking in living yeast reveals the flexibility of the fiber throughout the genome. *Genome Res* 23: 1829–1838.
45. Havlin S, Ben-Avraham D (2002) Diffusion in disordered media. *Adv Phys* 51: 187–292.
46. Doi M (1996) *Introduction to polymer physics* Oxford University Press.
47. Bronstein I, Israel Y, Kepten E, et al. (2009) Transient anomalous diffusion of telomeres in the nucleus of mammalian cells. *Phys Rev Lett* 103: 018102.
48. Dion V, Kalck V, Seeber A, et al. (2013) Cohesin and the nucleolus constrain the mobility of spontaneous repair foci. *EMBO Rep* 14: 984–991.
49. Gartenberg MR, Neumann FR, Laroche T, et al. (2004) Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. *Cell* 119: 955–967.
50. Hu Y, Kireev I, Plutz M, et al. (2009) Large-scale chromatin structure of inducible genes: transcription on a condensed, linear template. *J Cell Biol* 185: 87–100.
51. Neumann FR, Dion V, Gehlen LR, et al. (2012) Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. *Genes Dev* 26: 369–383.
52. Chuang C-H, Carpenter AE, Fuchsova B, et al. (2006) Long-range directional movement of an interphase chromosome site. *Curr Biol* 16: 825–831.
53. Khanna N, Hu Y, Belmont AS (2014) HSP70 transgene directed motion to nuclear speckles facilitates heat shock activation. *Curr Biol* 24: 1138–1144.
54. Chubb JR, Boyle S, Perry P, et al. (2002) Chromatin motion is constrained by association with nuclear compartments in human cells. *Curr Biol* 12: 439–445.

55. Lucas JS, Zhang Y, Dudko OK, et al. (2014) 3D trajectories adopted by coding and regulatory DNA elements: first-passage times for genomic interactions. *Cell* 158: 339–352.
56. Daley JM, Gaines WA, Kwon Y, et al. (2014) Regulation of DNA pairing in homologous recombination. *Cold Spring Harb Perspect Biol* 6: a017954.
57. Lieber MR (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem* 79: 181–211.
58. Sonoda E, Hohegger H, Saberi A, et al. (2006) Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. *DNA Repair* 5: 1021–1029.
59. Dion V, Kalck V, Horigome C, et al. (2012) Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat Cell Biol* 14: 502–509.
60. Seeber A, Dion V, Gasser SM (2013) Checkpoint kinases and the INO80 nucleosome remodeling complex enhance global chromatin mobility in response to DNA damage. *Genes Dev* 27: 1999–2008.
61. Lisby M, Mortensen UH, Rothstein R (2003) Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nat Cell Biol* 5: 572–577.
62. Nagai S, Dubrana K, Tsai-Pflugfelder M, et al. (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 322: 597–602.
63. Kalocsay M, Hiller NJ, Jentsch S (2009) Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol Cell* 33: 335–343.
64. Krawczyk PM, Borovski T, Stap J, et al. (2012) Chromatin mobility is increased at sites of DNA double-strand breaks. *J Cell Sci* 125: 2127–2133.
65. Aten JA, Stap J, Krawczyk PM, et al. (2004) Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* 303: 92–95.
66. Dimitrova N, Chen Y-CM, Spector DL, et al. (2008) 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 456: 524–528.
67. Jakob B, Splinter J, Conrad S, et al. (2011) DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. *Nucleic Acids Res* 39: 6489–6499.
68. Ježková L, Falk M, Falková I, et al. (2014) Function of chromatin structure and dynamics in DNA damage, repair and misrepair: γ -rays and protons in action. *Appl Radiat Isot Data Instrum Methods Use Agric Ind Med* 83: 128–136.
69. Chiolo I, Minoda A, Colmenares SU, et al. (2011) Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* 144: 732–744.
70. Nelms BE, Maser RS, MacKay JF, et al. (1998) In situ visualization of DNA double-strand break repair in human fibroblasts. *Science* 280: 590–592.
71. Jakob B, Splinter J, Durante M, et al. (2009) Live cell microscopy analysis of radiation-induced DNA double-strand break motion. *Proc Natl Acad Sci U S A* 106: 3172–3177.
72. Soutoglou E, Dorn JF, Sengupta K, et al. (2007) Positional stability of single double-strand breaks in mammalian cells. *Nat Cell Biol* 9: 675–682.
73. Roukos V, Voss TC, Schmidt CK, et al. (2013) Spatial dynamics of chromosome translocations in living cells. *Science* 341: 660–664.

74. Smerdon MJ, Lieberman MW (1978) Nucleosome rearrangement in human chromatin during UV-induced DNA- repair synthesis. *Proc Natl Acad Sci U S A* 75: 4238–4241.
75. Ziv Y, Bielopolski D, Galanty Y, et al. (2006) Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat Cell Biol* 8: 870–876.
76. Burgess RC, Burman B, Kruhlak MJ, et al. (2014) Activation of DNA damage response signaling by condensed chromatin. *Cell Rep* 9: 1703–1717.
77. Smeenk G, Wiegant WW, Marteijn JA, et al. (2013) Poly(ADP-ribosyl)ation links the chromatin remodeler SMARCA5/SNF2H to RNF168-dependent DNA damage signaling. *J Cell Sci* 126: 889–903.
78. Baldeyron C, Soria G, Roche D, et al. (2011) HP1alpha recruitment to DNA damage by p150CAF-1 promotes homologous recombination repair. *J Cell Biol* 193: 81–95.
79. Ayrapetov MK, Gursoy-Yuzugullu O, Xu C, et al. (2014) DNA double-strand breaks promote methylation of histone H3 on lysine 9 and transient formation of repressive chromatin. *Proc Natl Acad Sci U S A* 111: 9169–9174.
80. Zhu L, Brangwynne CP (2015) Nuclear bodies: the emerging biophysics of nucleoplasmic phases. *Curr Opin Cell Biol* 34: 23–30.
81. Chubb JR, Bickmore WA (2003) Considering nuclear compartmentalization in the light of nuclear dynamics. *Cell* 112: 403–406.
82. Lemaître C, Soutoglou E (2015) DSB (Im)mobility and DNA repair compartmentalization in mammalian cells. *J Mol Biol* 427: 652–658.
83. Klein IA, Resch W, Jankovic M, et al. (2011) Translocation-capture sequencing reveals the extent and nature of chromosomal rearrangements in B lymphocytes. *Cell* 147: 95–106.
84. Murr R, Loizou JI, Yang Y-G, et al. (2006) Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. *Nat Cell Biol* 8: 91–99.
85. Verschure PJ, van der Kraan I, Manders EMM, et al. (2003) Condensed chromatin domains in the mammalian nucleus are accessible to large macromolecules. *EMBO Rep* 4: 861–866.
86. Bancaud A, Huet S, Daigle N, et al. (2009) Molecular crowding affects diffusion and binding of nuclear proteins in heterochromatin and reveals the fractal organization of chromatin. *EMBO J* 28: 3785–3798.
87. Dinant C, de Jager M, Essers J, et al. (2007) Activation of multiple DNA repair pathways by sub-nuclear damage induction methods. *J Cell Sci* 120: 2731–2740.
88. Kong X, Mohanty SK, Stephens J, et al. (2009) Comparative analysis of different laser systems to study cellular responses to DNA damage in mammalian cells. *Nucleic Acids Res* 37: e68.
89. Gilbert N, Allan J (2014) Supercoiling in DNA and chromatin. *Curr Opin Genet Dev* 25: 15–21.
90. Elbel T, Langowski J (2015) The effect of DNA supercoiling on nucleosome structure and stability. *J Phys Condens Matter Inst Phys J* 27: 064105.
91. Polo SE (2015) Reshaping chromatin after DNA damage: the choreography of histone proteins. *J Mol Biol* 427: 626–636.
92. Downs JA, Lowndes NF, Jackson SP (2000) A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* 408: 1001–1004.
93. Heo K, Kim H, Choi SH, et al. (2008) FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. *Mol Cell* 30: 86–97.

94. Li A, Yu Y, Lee S-C, et al. (2010) Phosphorylation of histone H2A.X by DNA-dependent protein kinase is not affected by core histone acetylation, but it alters nucleosome stability and histone H1 binding. *J Biol Chem* 285: 17778–17788.
95. Golia B, Singh HR, Timinszky G (2015) Poly-ADP-ribosylation signaling during DNA damage repair. *Front Biosci Landmark Ed* 20: 440–457.
96. Poirier GG, de Murcia G, Jongstra-Bilen J, et al. (1982) Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. *Proc Natl Acad Sci U S A* 79: 3423–3427.
97. de Murcia G, Huletsky A, Lamarre D, et al. (1986) Modulation of chromatin superstructure induced by poly(ADP-ribose) synthesis and degradation. *J Biol Chem* 261: 7011–7017.
98. Xu Y, Ayrappetov MK, Xu C, et al. (2012) Histone H2A.Z controls a critical chromatin remodeling step required for DNA double-strand break repair. *Mol Cell* 48: 723–733.
99. Clapier CR, Cairns BR (2009) The biology of chromatin remodeling complexes. *Annu Rev Biochem* 78: 273–304.
100. Cheezum MK, Walker WF, Guilford WH (2001) Quantitative comparison of algorithms for tracking single fluorescent particles. *Biophys J* 81: 2378–2388.
101. Wombacher R, Heidbreder M, van de Linde S, et al. (2010) Live-cell super-resolution imaging with trimethoprim conjugates. *Nat Methods* 7: 717–719.
102. Benke A, Manley S (2012) Live-cell dSTORM of cellular DNA based on direct DNA labeling. *Chembiochem Eur J Chem Biol* 13: 298–301.
103. Hihara S, Pack C-G, Kaizu K, et al. (2012) Local nucleosome dynamics facilitate chromatin accessibility in living mammalian cells. *Cell Rep* 2: 1645–1656.
104. Récamier V, Izeddin I, Bosanac L, et al. (2014) Single cell correlation fractal dimension of chromatin: a framework to interpret 3D single molecule super-resolution. *Nucl Austin Tex* 5: 75–84.
105. Ricci MA, Manzo C, García-Parajo MF, et al. (2015) Chromatin fibers are formed by heterogeneous groups of nucleosomes in vivo. *Cell* 160: 1145–1158.
106. Zhang Y, Máté G, Müller P, et al. (2015) Radiation induced chromatin conformation changes analysed by fluorescent localization microscopy, statistical physics, and graph theory. *PloS One* 10: e0128555.
107. Llères D, James J, Swift S, et al. (2009) Quantitative analysis of chromatin compaction in living cells using FLIM-FRET. *J Cell Biol* 187: 481–496.
108. Emanuel M, Radja NH, Henriksson A, et al. (2009) The physics behind the larger scale organization of DNA in eukaryotes. *Phys Biol* 6: 025008.
109. Rouse P (1953) A Theory of the Linear Viscoelastic Properties of Dilute Solutions of Coiling Polymers. *J Chem Phys* 21: 1272–1280.
110. Weber SC, Spakowitz AJ, Theriot JA (2010) Bacterial chromosomal loci move subdiffusively through a viscoelastic cytoplasm. *Phys Rev Lett* 104: 238102.
111. Metzler R, Jeon J-H, Cherstvy AG, et al. (2014) Anomalous diffusion models and their properties: non-stationarity, non-ergodicity, and ageing at the centenary of single particle tracking. *Phys Chem Chem Phys* 16: 24128–24164.
112. Mirny LA (2011) The fractal globule as a model of chromatin architecture in the cell. *Chromosome Res Int J Mol Supramol Evol Asp Chromosome Biol* 19: 37–51.

113. Huet S, Lavelle C, Ranchon H, et al. (2014) Relevance and limitations of crowding, fractal, and polymer models to describe nuclear architecture. *Int Rev Cell Mol Biol* 307: 443–479.
114. Barbieri M, Chotalia M, Fraser J, et al. (2012) Complexity of chromatin folding is captured by the strings and binders switch model. *Proc Natl Acad Sci U S A* 109: 16173–16178.
115. Mateos-Langerak J, Bohn M, de Leeuw W, et al. (2009) Spatially confined folding of chromatin in the interphase nucleus. *Proc Natl Acad Sci U S A* 106: 3812–3817.
116. Bohn M, Heermann DW (2010) Diffusion-driven looping provides a consistent framework for chromatin organization. *PloS One* 5: e12218.
117. Jerabek H, Heermann DW (2014) How chromatin looping and nuclear envelope attachment affect genome organization in eukaryotic cell nuclei. *Int Rev Cell Mol Biol* 307: 351–381.
118. Cook PR, Marenduzzo D (2009) Entropic organization of interphase chromosomes. *J Cell Biol* 186: 825–834.
119. Bohn M, Heermann DW (2011) Repulsive forces between looping chromosomes induce entropy-driven segregation. *PloS One* 6: e14428.
120. Jost D, Carrivain P, Cavalli G, et al. (2014) Modeling epigenome folding: formation and dynamics of topologically associated chromatin domains. *Nucleic Acids Res* 42: 9553–9561.
121. Finan K, Cook PR, Marenduzzo D (2011) Non-specific (entropic) forces as major determinants of the structure of mammalian chromosomes. *Chromosome Res Int J Mol Supramol Evol Asp Chromosome Biol* 19: 53–61.
122. Zhang B, Wolynes PG (2015) Topology, structures, and energy landscapes of human chromosomes. *Proc Natl. Acad Sci U S A* 112: 6062–6067.



AIMS Press

© 2015 Sébastien Huet, et al., licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>)

The poly(ADP-ribose)-dependent chromatin remodeler Alc1 induces local chromatin relaxation upon DNA damage

Hafida Sellou,^{1-3,*} Théo Lebeaupin,^{1-3,*} Catherine Chapuis,^{1,2} Rebecca Smith,³ Anna Hegele,³ Hari R. Singh,³ Marek Kozlowski,³ Sebastian Bultmann,^{4,5} Andreas G. Ladurner,^{3,5,6} Gyula Timinszky,^{3,#} and Sébastien Huet^{1,2,#}

¹CNRS, UMR 6290, Institut Génétique et Développement de Rennes, Rennes, France

²Université de Rennes 1, Structure fédérative de recherche Biosit, Rennes, France

³Department of Physiological Chemistry, Biomedical Center Munich, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

⁴Department of Biology II, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

⁵Center for Integrated Protein Science Munich (CIPSM), Department of Chemistry and Biochemistry, Ludwig-Maximilians-Universität München, Munich, Germany

⁶Munich Cluster for Systems Neurology (SyNergy), Biomedical Center Munich, Ludwig-Maximilians-Universität München, Munich, Germany

*These authors contributed equally to this work

#Correspondence: gyula.timinszky@med.lmu.de; sebastien.huet@univ-rennes1.fr

Running title: Chromatin remodeling at DNA lesions

Character counts: 19675

ABSTRACT

Chromatin relaxation is one of the earliest cellular responses to DNA damage. However, what determines these structural changes, including their ATP requirement, is not well understood. Using live-cell imaging and laser micro-irradiation to induce DNA lesions, we show that the local chromatin relaxation at the DNA damage sites is regulated by PARP1 enzymatic activity. We also report that H1 is mobilized at the DNA damage sites but, since this mobilization is largely independent of poly(ADP-ribosylation), it cannot solely explain the chromatin relaxation. Finally, we demonstrate the involvement of Alc1, a poly(ADP-ribose)- and ATP-dependent remodeler, in the chromatin relaxation process. The deletion of Alc1 impairs chromatin relaxation after DNA damage, while its over-expression strongly enhances relaxation. Altogether, our results identify Alc1 as an important player in the fast kinetics of the NAD⁺- and ATP-dependent chromatin relaxation upon DNA damage *in vivo*.

INTRODUCTION

The complex multiscale architecture of chromatin poses a formidable challenge for the DNA repair machinery, which requires regulated access to DNA lesions. Early steps of the DNA damage response involve chromatin remodeling, leading to an increased sensitivity of chromatin to nucleases (Smerdon and Lieberman, 1978). Experiments in living cells have shown that DNA damage induced by laser micro-irradiation leads to an ATP-dependent, but ATM-independent, chromatin relaxation at sites of DNA damage (Kruhlak et al., 2006). While the dense packing of chromatin may hinder the efficiency of DNA repair (Schuster-Böckler and Lehner, 2012), recent reports also show that chromatin over-compaction at DNA lesions may also be important to inhibit transcription during repair and to keep the broken DNA ends in close proximity (Ayrapetov et al., 2014; Burgess et al., 2014).

One of the earliest events upon DNA damage is the recruitment and activation of poly(ADP-ribose) polymerase 1 (PARP1), a key regulator of chromatin structure during DNA repair and transcription (Lebeaupin et al., 2015; Kim et al., 2004). It is activated by DNA lesions and attaches poly(ADP-ribose) (PAR) to itself and other chromatin factors including histones. The binding of PARP1 to chromatin modifies its compaction state through multiple, sometimes opposite, mechanisms. Inactive PARP1 competes with the linker histone H1 leading to the formation of compact and transcriptionally repressed genomic regions (Kim et al., 2004). In contrast, PARylated polynucleosomes appear as a loose, beads-on-a-string fiber, on electron micrographs (Poirier et al., 1982). It was suggested that the PARylation of chromatinized H1 could counteract its ability to condense chromatin (Huletsky et al., 1989). Additionally, PARylation is also involved in the recruitment and the regulation of several chromatin remodeling enzymes whose ATP-dependent activity could promote chromatin relaxation (Chou et al., 2010; Polo et al., 2010; Smeenk et al., 2013).

In the present work, we sought to address the impact of PARP1 on chromatin structure and dynamics following DNA damage. Using photo-activated histones, live-cell imaging and laser micro-irradiation in human cells, we analyzed the contributions of PARylation, linker histone H1, ATP and the nucleosome remodeler Alc1 during the transient chromatin relaxation observed upon DNA damage.

RESULTS AND DISCUSSION

DNA damage induction by laser micro-irradiation induces a rapid chromatin relaxation at the DNA lesions

To assess large-scale chromatin reorganization at sites of DNA damage in living cells, we established an assay using human U2OS cells expressing the core histone H2B labeled with the photo-convertible dyes PAGFP or PAtagRFP. By irradiating a predefined nuclear area with a 405 nm laser, we simultaneously photo-convert the tagged histones and, if cells have been Hoechst-presensitized, induce DNA lesions, allowing us to compare chromatin dynamics in the presence or absence of DNA damage (Figure 1A-C).

Upon micro-irradiation at 405 nm of cells expressing photoactivatable H2B and pre-sensitized with Hoechst, we observed a rapid increase of the size of the photo-converted chromatin area (Figure 1B-C), indicating chromatin relaxation at DNA damage sites, as previously reported (Kruhlak et al., 2006). However, an alternative interpretation could be the local release of photo-converted H2B through nucleosome remodeling induced upon DNA damage (Polo, 2015). To distinguish between these two possibilities, we labeled DNA by incorporating fluorescent nucleotides (Schermelleh et al., 2001). Upon irradiation, we observed the directional movement of fluorescent spots away from the irradiated line

(Figure 1D-E and supplementary movie 1), with a speed similar to the one characterizing the expansion of the H2B photo-converted area (Figure 1F). These results indicate that the changes in the size of the photo-activated H2B area upon DNA damage reflect the relaxation of chromatinized DNA, rather than the local release of photo-activated H2B. This fast initial chromatin relaxation upon DNA damage is followed by a slow recondensation with chromatin recovering a compaction state close to its pre-damage level in about 20 minutes (Figure 1G).

Chromatin relaxation at DNA damage sites is controlled by PARP1 activation

In agreement with recent reports (Khurana et al., 2014; Strickfaden et al., 2016), we observed that chromatin relaxation at DNA lesions is PARylation dependent (Figures 2A and S1A-B). Interestingly, inhibiting PARylation not only abolished chromatin relaxation at DNA damage sites but also induced a small but significant chromatin over-compaction upon laser micro-irradiation (Figure 2A). The human PARP enzyme family has multiple members and we found that PARP1, PARP2 and PARP3 are all recruited at DNA damage sites (Figure S1C). Yet, chromatin relaxation at DNA lesions was dramatically reduced in PARP1 knockout cells (Figure 2A-B), highlighting the predominant role of PARP1 in DNA damage-induced PARylation. Remarkably, laser irradiation in the PARP1 knockouts did not lead to chromatin over-compaction, even after inhibition of PARylation (Figure 2A). Instead, we observed residual chromatin relaxation independent of PARylation activity. Since PARP inhibitors do not block the recruitment of PARP1 to DNA damage (Timinszky et al., 2009), our data suggest that chromatin over-compaction when inhibiting PARylation is due to PARP1 binding to DNA lesions, whereas its product, PAR, is responsible for chromatin relaxation. These findings reconcile oppositely reported effects of PARP1 on chromatin structure (Kim et al., 2004; Poirier et al., 1982).

Chromatin relaxation at DNA lesions is not directly triggered by the mobilization of linker histone H1

In vitro studies identified the linker histone H1 to be crucial for the formation of compact chromatin (Thoma et al., 1979). Since H1 is a substrate of PARP1, PARylation of H1 could trigger its dissociation from chromatin, as shown for regulated transcription (Ju et al., 2006), and lead to chromatin relaxation. To test this hypothesis, we analyzed H1 (H1.1 variant) dynamics at DNA lesions in cells co-expressing H2B-PATagRFP and H1-PAGFP, allowing us to simultaneously label the damaged chromatin area and follow the dynamics of the H1 proteins localized within this area at the time of irradiation (Figure 3A and supplemental movie 2). To quantify the redistribution of photo-activated H1 from the irradiated area independently of the co-occurring chromatin relaxation process, the integrated fluorescence signal for H1 was measured within the irradiated area defined by the segmentation of the H2B channel (Figure 3B).

We found that H1 initially localized within the irradiated area is released faster in the presence of DNA damage (Figure 3C). Knowing that most H1 molecules are bound to chromatin at any given time (Beaudouin et al., 2006), this can only reflect impaired binding to chromatin. Once the photoconverted H1 proteins are redistributed over the entire nucleus, the DNA damage area appears to be depleted for H1 (Figure S1D). This depletion progressively disappears as chromatin slowly recondenses. At the same time, we observed no significant release of the core histone H2B from the irradiated region (Figure S1E).

Inhibiting PARylation reduced H1 dynamics both in the presence and absence of DNA damage, while deleting PARP1 only slowed down H1 dynamics in the presence of DNA damage (Figure. 3C). These data are consistent with the observation that the PARylation of H1 increases its dynamics (Ju et al., 2006). Nevertheless, even in the presence of PARP inhibitor or in the PARP1 knockout cells, H1 dynamics was always much faster after DNA damage as compared to the dynamics observed when no damage was

induced (Figure 3C). Since, at the same time, chromatin relaxation was abolished in cells treated with PARP inhibitors and strongly reduced in the PARP1 knockouts (Figure 2A), it indicates that chromatin loosening at DNA lesions is not the direct consequence of PAR-driven H1 mobilization at DNA lesions, in contrast to a recent report (Strickfaden et al., 2016). However, we cannot exclude that the PARylation-dependent chromatin relaxation requires concomitant H1 mobilization, always observed upon DNA damage independently of PARP1 activation. Furthermore, it is possible that the DNA damage-induced H1 mobilization accounts for the observed residual chromatin relaxation observed in the PARP1 knockouts.

Contribution of ATP-dependent processes in chromatin relaxation at DNA lesions

In vitro PARP1 activation results in chromatin loosening in the absence of ATP (Poirier et al., 1982), whereas ATP depletion abolishes chromatin relaxation at DNA lesions in live cells (Kruhlak et al., 2006; Luijsterburg et al., 2012). In order to establish the role of ATP in our assays, we quantified chromatin relaxation and PARylation levels upon laser micro-irradiation in cells depleted for ATP. We found that ATP depletion significantly impaired chromatin relaxation upon DNA damage (Figure 4A) while not affecting the level of PARylation at the lesions, as shown by the similar accumulation of the PAR-binder WWE domain of RNF146 at DNA damage sites (Figure 4B) (Wang et al., 2012). Nevertheless, ATP depletion did not fully abolish chromatin relaxation, its amplitude corresponding to approximately half of the control situation, suggesting that PARylation acts on chromatin in both ATP-dependent and -independent ways. A confounding effect of ATP inhibition is chromatin condensation (Figure 4C). This, in turn, could affect chromatin relaxation at DNA lesions. We induced chromatin hyper-compaction similar to that of ATP depletion (Figure 4C) using hypertonic treatment (Walter et al., 2013). In hypertonic cells, relaxation was slightly increased as compared to isotonic controls (Figure 4D), while PARylation at the site of damage was unchanged (Figure 4E). Thus, tighter chromatin packing increases loosening, rather than reducing it, which is observed upon ATP depletion.

The ATP-dependent remodeler Alc1 contributes to chromatin relaxation at DNA damage sites

Several ATP-dependent chromatin-remodeling enzymes have been shown to be regulated by PARP activation (Chou et al., 2010; Polo et al., 2010; Smeenk et al., 2013). However, the only chromatin-remodeling enzyme with an ADP-ribose-binding domain actively remodeling nucleosomes upon PARP1 activation is Alc1 (Gottschalk et al., 2009; Pines et al., 2012). To address the role of Alc1 in chromatin relaxation, we generated an Alc1 knockout U2OS cell line (Figure 5A). By co-expressing a fluorescently tagged version of Alc1 together with H2B-PAGFP in these cells, we followed the recruitment of this protein at DNA damage sites together with the relaxation process (Figure 5B-C). The fast accumulation of Alc1 observed at the site of DNA damage, with a maximum recruitment a few seconds after laser micro-irradiation, is compatible with a role for Alc1 in chromatin relaxation at DNA breaks, a process that lasts approximately 60s. The recruitment of Alc1 at DNA damage sites was abolished by PARP inhibitor treatment or for Alc1 lacking the PAR-binding macrodomain (Figure S2A), indicating that Alc1 recruitment is fully controlled by PARP1 activation at DNA lesions.

The loss of Alc1 had no detectable effect on chromatin architecture in the absence of DNA damage (Figure S2B-D) but led to impaired chromatin relaxation upon laser irradiation (Figures 5D and S2E). Expression of wild-type Alc1, but not the ATPase-dead mutants (Alc1-E175Q or Alc1-K77R), fully restored chromatin relaxation at DNA lesions in Alc1 knockouts. Cells depleted for Alc1 using RNAi behaved in a similar manner (Figure S2F-G). While ATP depletion only slightly reduced chromatin relaxation in the Alc1 knockout cells (Figure 5E), the inhibition of PARylation completely suppressed the relaxation process (Figure 5F) suggesting that the remaining chromatin relaxation observed at DNA damage sites in the absence of Alc1 is mediated mainly by the ATP-independent loosening effect of

PARYlation. Importantly, the over-expression of Alc1 in wild-type cells strongly increased chromatin relaxation at DNA lesions, while over-expressing the ATPase-dead Alc1-E175Q had no effect (Figure 5G). Altogether, these results identify Alc1 as a mediator of PARYlation-dependent chromatin relaxation through its ATP-dependent remodeling activity. A recent publication also reported the role of the remodeler CHD2 in the chromatin relaxation at DNA lesions (Luijsterburg et al., 2016). Since CHD2 appears to be recruited at the DNA damage sites slightly later than Alc1, the two remodelers may act sequentially to allow chromatin loosening. Further work is required to understand how the activities of these two remodelers are coordinated.

In the classical view, supported by a recent report (Izhar et al., 2015), chromatin relaxation promotes accessibility of the DNA lesions for the repair machinery. Nevertheless, this straightforward model is also questioned by recent findings showing that several key members of the repair machinery are recruited during the slow chromatin recondensation phase rather than upon the initial relaxation (Burgess et al., 2014). It was also proposed that the transient chromatin loosening at DNA lesions could contribute to the choice of repair pathways (Khurana et al., 2014). The fact that both PARP1 and Alc1, two proteins that we identified as being involved in the chromatin relaxation at DNA lesions, are important for maintaining genomic integrity (Ahel et al., 2009) strongly argues for a key regulatory role of the modulation of the chromatin compaction state during the DNA damage response of the cells. In addition, we propose that the dramatic increase in chromatin relaxation observed in case of Alc1 over-expression might underlie the oncogenic potential of this remodeler, which has been shown to promote cancer progression and metastasis (Cheng et al., 2013).

MATERIAL AND METHODS

Plasmids

The core histone H2B, subcloned from the pH2B-mCherry vector (gift from J. Ellenberg, Neumann et al., 2010, Euroscarf accession number P30632), was cloned into pPAtagRFP-N1 using NdeI and BamHI restriction sites. pPAtagRFP-N1 was a gift from V. Verkhusha (Subach et al., 2010), Addgene plasmid # 31941). The histone H2B-PAGFP and histone H1.1-PAGFP were gifts from J. Ellenberg (Beaudouin et al., 2006), Euroscarf accession number P30499 and P30503, respectively. Another construct of H1.1-PAGFP was produced with the PAGFP tag on the other side of the protein to ensure that similar results could be obtained with both constructs (Hutchinson et al., 2015). H1.1 was PCR amplified from the H1.1-PAGFP plasmid and subcloned into pmEGFP-N1 using BglII and ApaI to obtain the H1.1-EGFP construct. Wild type Alc1 and E175Q Alc1 mutant fused to the C-terminus of EGFP or mCherry were obtained by exchanging YFP for the respective fluorescent protein in the constructs described previously (Gottschalk et al., 2009). The Alc1- Δ macro mutant fused to YFP was described previously (Gottschalk et al., 2009). The Alc1-K77R construct fused to mCherry was obtained by first mutating a wild type ALC1 construct fused to YFP (Gottschalk et al., 2009) using Quikchange *in-vitro* mutagenesis (Agilent), and then exchanging YFP for mCherry. The WWE domain of RNF146 (amino acids 99-183) was cloned into pmEGFP-C1 using BglII and EcoRI by PCR amplifying it from a cDNA library. For PARP1-EGFP, we exchanged mCherry with EGFP from a PARP1-mCherry construct published previously (Timinszky et al., 2009). PARP2-EGFP was generated by PCR cut with NheI/SmaI into pmEGFPC1 with NheI/SmaI. PARP3-EGFP (short isoform) is a gift from C. Prigent (Rouleau et al., 2007). Mammalian expression was under the control of CMV promoter. All constructs were sequence verified.

Cell culture, inhibitor treatments, and osmotic shocks

Cells used for this work were wild-type U2OS cells or knockout cells made from parental U2OS cells. Cells were routinely cultured in Dulbecco's modified Eagle's medium (with 4.5 g/L glucose) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μ g/mL penicillin, 100 U/mL

streptomycin in 5% CO₂ at 37 °C. For microscopy, cells were plated on Lab-Tek II chambered coverglass (Thermo Scientific). Pre-sensitization was achieved by bathing cells for 1h in culture medium containing 0.3 µg/mL Hoechst 33342 (Life Technologies). Immediately before imaging, the growth medium was replaced by Leibovitz's L-15 medium (Life Technologies) supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 µg/mL penicillin and 100 U/mL streptomycin. The PARP1 inhibitors AG14361 and Olaparib (Euromedex) were used at 30 µM and 50 µM, respectively. ATP depletion was achieved as described in (Platani et al., 2002). The osmotic shock procedure was previously described in (Walter et al., 2013).

Live cell DNA labeling with fluorescent nucleotides

U2OS cells expressing H2B-PATagRFP were synchronized at the G1/S phase transition by treating the cells with aphidicolin (Sigma) at 5 µg/mL for 18h. After aphidicolin release, the cell layer, bathed with growing medium containing 10 µM of dUTP-ATTO633 (Jena-Bioscience), was scraped using a silicon stick to allow nucleotide loading and integration to the DNA during replication (Schermelleh et al., 2001).

Transfections and generation of stable and knockout cell lines

Transient transfections were performed 12-24h after plating cells using XtremeGENE HP (Roche) or JetPRIME (Polyplus Transfection) according to manufacturer's instructions. Cells were imaged 48 to 72h after transfection.

To establish U2OS cell lines stably expressing H2B-PATagRFP, wild-type cells were transfected with the H2B-PATagRFP construct using XtremeGENE HP (Roche) and grown in culture medium containing Geneticin (PAA) for selection. Clones with stably integrated H2B-PATagRFP were picked after two weeks of Geneticin selection. Once selected, these cells were cultured in normal medium supplemented with 500 µg/mL Geneticin (PAA).

The knockout cell lines were made according to the protocol described by the Zhang lab (Ran et al., 2013). The target sequence for *ALC1* (5'-GACTTCCCTCAAGTACGTTAG-3') and PARP1 (5'-GTCCAACAGAAGTACGTGCAA-3') was chosen according to the web-based CRISPR design tool from Zhang lab (<http://www.genome-engineering.org>). The sgRNA oligos were introduced into pX458 expressing Cas9 nuclease fused to GFP (Addgene #48138). pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang (Addgene plasmid # 48138). We transfected the plasmids using the transfection reagent XtremeGENE HP (Roche) according to manufacturer's protocol. Single GFP positive cells were sorted into 96-well plates using FACS. The knockout cell lines grown up from the single cells were identified by western blot using specific antibodies against PARP1 or ALC1.

siRNA knock down

For RNAi-mediated knockdown, we used Silencer® Select Negative Control No. 2 (ref 4390846) and siRNA against ALC1 (CHD1L) (ref s18358) from Ambion (Thermo Fisher Scientific). Cells grown in normal culture medium were transfected with 500 nM siRNA using Oligofectamine (Life Technologies) according to the manufacturer's instructions. After 48h, cells were used for imaging or harvested for protein analysis.

Western blot

Cell lysates were separated using SDS-PAGE, and transferred to nitrocellulose membranes (GE Healthcare) and blocked in 5% (w/v) milk powder in 0.05% (v/v) PBS-Tween 20 at room temperature. The primary antibodies were diluted in 5% (w/v) milk powder in 0.05% (v/v) PBS-Tween 20 and used at the following concentrations: affinity purified anti-ALC1 rabbit polyclonal, 1:2500; anti-Actin (Sigma-Aldrich, A5060), 1:1000; anti-PARP1 rabbit polyclonal, 1:10000; the mouse monoclonal (DM1A) anti-

tubulin (Sigma-Aldrich T9026), 1:20000. To detect primary antibodies, HRP-conjugated secondary antibodies were used. The HRP-conjugated anti-rabbit IgG and anti-mouse IgG antibodies (Bio-Rad) were used at 1:10000 and the blot was developed using the ELC reagent (Merck Millipore).

Live cell imaging and laser micro-irradiation

Live cell imaging was performed on an inverted confocal spinning disk (imaging scan head CSU-X1 from Yokogawa and microscope body Ti-E from Nikon) equipped with a single-point scanning head to allow laser micro-irradiation and local photoactivation using a 405 nm laser (see the sketch on Fig. S1A). We used a Plan APO 63x oil immersion objective lens (O.N. 1.4) and a sCMOS ORCA Flash 4.0 camera (Hamamatsu) for imaging the cells. The pixel resolution at the object plane was 108 nm. The fluorescence of EGFP and the activated form of PAGFP was excited with a laser at 488 nm and the one of mCherry and the activated form of PAtagRFP was excited with a laser at 561 nm. For fluorescence detection, we used band pass filters adapted to the fluorophores. Laser powers were adjusted to minimize bleaching during the time-lapse acquisitions. Photoactivation and DNA damage were induced simultaneously using a 405 nm laser. The power of the 405 nm laser used for photoactivation and, for cells pre-sensitized with Hoechst, induction of DNA lesions, was set to 125 μ W at the sample level, unless stated otherwise. Cells were irradiated along a 16 μ m-long line crossing the nucleus. The microscope is equipped with a heating chamber to maintain cells at 37 °C during the imaging experiments.

For the images shown on Figures S1A and S2A, a different setup was used. The images were taken on an inverted AxioObserver Z1 confocal spinning-disk microscope (Zeiss) equipped with a single-point scanning head for laser micro-irradiation and local photoactivation using a 405 nm laser (Rapp OptoElectronic). We used a C-Apo 63 \times water immersion objective lens (O.N. 1.2) and the images were acquired on a AxioCam HRm CCD camera (Zeiss). The pixel resolution at the object plane was 171 nm. The fluorescence of EGFP and YFP was excited with a laser at 488 nm and the one of the activated form of PAtagRFP was excited with a laser at 561 nm. For fluorescence detection, we used band pass filters adapted to the fluorophores. The micro-irradiation conditions at 405 nm were adjusted to obtain amplitudes of the chromatin relaxation at DNA lesions which were similar to those obtained with the system described above. The cells were maintained at 37°C using a heating chamber.

Image analysis

The time-lapse sequences were analyzed automatically using custom-made routines written in MatLab (MathWorks) to quantify chromatin relaxation at DNA lesions. The chromatin area micro-irradiated at 405 nm and tagged with the photoactivatable H2B were segmented by k-means segmentation. An ellipsoid was fitted to the segmented area and its minor axis length was used to estimate the width of the micro-irradiated chromatin area and thus assess changes in the chromatin compaction level.

To analyze the release of the photoactivatable H1 proteins from the area irradiated at 405 nm, the H1 integrated intensity was measured inside the segmented micro-irradiated chromatin area in cells co-expressing H1 and H2B tagged with two different photoactivatable dyes. This intensity was divided by the H1 intensity integrated over the whole nucleus to correct for bleaching and small focus drifts. For this step, the whole nuclei were segmented using the low fluorescence signal coming from the non-activated tagged H2B proteins. The same approach was used to analyze the release of the H2B proteins from DNA lesions and to characterize Alc1 recruitment kinetics.

When necessary, nuclei movements occurring during the timelapse experiments were corrected using the ImageJ plugin StackReg (Thévenaz et al., 1998).

The accumulation of the fluorescently tagged WWE domain of RNF146 at the DNA lesions was quantified as follows. By manual segmentation, the mean fluorescence intensity in three areas was estimated: at the site of DNA damage (I_d), in a region of the nucleus not subjected to laser irradiation (I_{nd}) and outside the cells (I_{bg}). The accumulation of the WWE domain at the DNA lesions A_{WWE} was then calculated as:

$$A_{WWE} = \frac{I_d - I_{bg}}{I_{nd} - I_{bg}}$$

For chromatin texture analysis, wild-type and Alcl knockout U2OS cells were plated on Lab-TekII-chambered coverglass, fixed with 4% paraformaldehyde for 10 min at room temperature and stained with Hoechst 33342 (1 µg/mL) for 1h. Confocal images were captured on a Leica SP8 confocal microscope using a Plan APO 63x oil immersion objective lens (O.N. 1.4). Hoechst staining was excited with a 405-nm laser and the emission band was chosen to optimize fluorescence collection. The pinhole was set to one Airy unit and we used a pixel size of 60 nm. To analyze chromatin texture, the GLCM ImageJ texture plugin written by Julio E. Cabrera was used. The correlation and contrast parameters were chosen to characterize chromatin texture using a pixel-to-pixel distance of 7 pixels, which allowed maximizing the differences measured between the cells bathed with the isotonic medium and those subjected to osmotic stress.

In cells labeled with fluorescent nucleotides, the chromatin dynamics was assessed by tracking the fluorescently labeled DNA replication foci using the plugin Particle Tracker from Image J (Sbalzarini and Koumoutsakos, 2005).

Statistics

Unless stated otherwise, for all curves and boxplots shown on the figures, more than 15 cells per condition were analyzed. Boxplots were generated using a web-tool developed by the Tyers and Rappsilber labs (<http://boxplot.tyerslab.com/>). The box limits correspond to the 25th and 75th percentiles and the bold line indicates the median value. The whiskers extend 1.5 times the interquartile range and outliers are shown by dots. Unless stated otherwise, p values were calculated using unpaired Student's t-test assuming unequal variances. On the boxplots, * refers to $p < 0.05$, ** to $p < 0.01$, *** to $p < 0.001$, **** to $p < 0.0001$ and n.s. to non significant.

ACKNOWLEDGMENTS

We thank the Microscopy Rennes Imaging Center (BIOSIT, Université Rennes 1) for technical assistance. This work was supported by the Agence National de la Recherche (JCJC-SVSE2-2011, ChromaTranscript project, to S.H.), the Ligue contre le Cancer du Grand-Ouest (committees 35 and 72, to S.H.), the European Union (FP7-PEOPLE-2011-CIG, ChromaTranscript project, to S.H and Marie Curie Initial Training Network, Nucleosome4D to A.G.L.), the Deutsche Forschungsgemeinschaft (TI 817/2-1 to G.T. and SFB collaborative research center 1064 to A.G.L.) and the Worldwide Cancer Research (14-1315; to G.T.). H.S.'s PhD fellowship was funded by the Centre National de la Recherche Scientifique and the Région Bretagne. Our collaboration benefited from funding from the Hubert Curien partnership / German Academic Exchange Service – DAAD – (28486ZD, to S.H., 55934632; to G.T.) and the Deutsche Forschungsgemeinschaft CIPSM and SyNergy excellence clusters (to A.G.L.). The authors declare no competing financial interests.

REFERENCES

Ahel, D. et al. (2009) Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science* 325, 1240–1243.

Ayrapetov, M.K., Gursoy-Yuzugullu, O., Xu, C., Xu, Y., Price, B.D. (2014) DNA double-strand breaks promote methylation of histone H3 on lysine 9 and transient formation of repressive chromatin. *Proc. Natl. Acad. Sci. U. S. A.* 111, 9169–9174.

Beaudouin, J., Mora-Bermúdez, F., Klee, T., Daigle, N., Ellenberg, J. (2006) Dissecting the contribution of diffusion and interactions to the mobility of nuclear proteins. *Biophys. J.* 90, 1878–1894.

Burgess, R.C., Burman, B., Kruhlak, M.J., Misteli, T. (2014) Activation of DNA damage response signaling by condensed chromatin. *Cell Rep.* 9, 1703–1717.

Cheng, W., Su, Y., Xu, F. (2013) CHD1L: a novel oncogene. *Mol. Cancer* 12, 170.

Chou, D.M., Adamson, B., Dephoure, N.E., Tan, X., Nottke, A.C., Hurov, K.E., Gygi, S.P., Colaiácovo, M.P., Elledge, S.J. (2010) A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. U. S. A.* 107, 18475–18480.

Gottschalk, A.J. et al. (2009) Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. *Proc. Natl. Acad. Sci. U. S. A.* 106, 13770–13774.

Huletsky, A., de Murcia, G., Muller, S., Hengartner, M., Ménard, L., Lamarre, D., Poirier, G.G. (1989) The effect of poly(ADP-ribosyl)ation on native and H1-depleted chromatin. A role of poly(ADP-ribosyl)ation on core nucleosome structure. *J. Biol. Chem.* 264, 8878–8886.

Hutchinson, J.B., Cheema, M.S., Wang, J., Missiaen, K., Finn, R., Gonzalez Romero, R., Th'ng, J.P.H., Hendzel, M., Ausió, J. (2015) Interaction of chromatin with a histone H1 containing swapped N- and C-terminal domains. *Biosci. Rep.* 35.

Izhar, L., Adamson, B., Ciccio, A., Lewis, J., Pontano-Vaites, L., Leng, Y., Liang, A.C., Westbrook, T.F., Harper, J.W., Elledge, S.J. (2015) A Systematic Analysis of Factors Localized to Damaged Chromatin Reveals PARP-Dependent Recruitment of Transcription Factors. *Cell Rep.* 11, 1486–1500.

Ju, B.-G., Lunyak, V.V., Perissi, V., Garcia-Bassets, I., Rose, D.W., Glass, C.K., Rosenfeld, M.G. (2006) A topoisomerase II β -mediated dsDNA break required for regulated transcription. *Science* 312, 1798–1802.

Khurana, S. et al. (2014) A macrohistone variant links dynamic chromatin compaction to BRCA1-dependent genome maintenance. *Cell Rep.* 8, 1049–1062.

Kim, M.Y., Mauro, S., Gévry, N., Lis, J.T., Kraus, W.L. (2004) NAD⁺-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. *Cell* 119, 803–814.

Kruhlak, M.J., Celeste, A., Deltaille, G., Fernandez-Capetillo, O., Müller, W.G., McNally, J.G., Bazett-Jones, D.P., Nussenzweig, A. (2006) Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol.* 172, 823–834.

- Lebeaupin, T., Sellou, H., Timinszky, G., Huet, S. (2015) Chromatin dynamics at DNA breaks: what, how and why? *AIMS Biophys.* 2, 458–475.
- Luijsterburg, M.S., Lindh, M., Acs, K., Vrouwe, M.G., Pines, A., van Attikum, H., Mullenders, L.H., Dantuma, N.P. (2012) DDB2 promotes chromatin decondensation at UV-induced DNA damage. *J. Cell Biol.* 197, 267–281.
- Luijsterburg, M.S. et al. (2016) PARP1 Links CHD2-Mediated Chromatin Expansion and H3.3 Deposition to DNA Repair by Non-homologous End-Joining. *Mol. Cell* 61, 547–562.
- Neumann, B. et al. (2010) Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature* 464, 721–727.
- Pines, A. et al. (2012) PARP1 promotes nucleotide excision repair through DDB2 stabilization and recruitment of ALC1. *J. Cell Biol.* 199, 235–249.
- Platani, M., Goldberg, I., Lamond, A.I., Swedlow, J.R. (2002) Cajal body dynamics and association with chromatin are ATP-dependent. *Nat. Cell Biol.* 4, 502–508.
- Polo, S.E., Kaidi, A., Baskcomb, L., Galanty, Y., Jackson, S.P. (2010) Regulation of DNA-damage responses and cell-cycle progression by the chromatin remodelling factor CHD4. *EMBO J.* 29, 3130–3139.
- Polo, S.E. (2015) Reshaping chromatin after DNA damage: the choreography of histone proteins. *J. Mol. Biol.* 427, 626–636.
- Poirier, G.G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C., Mandel, P. (1982) Poly(ADP-ribosylation) of polynucleosomes causes relaxation of chromatin structure. *Proc. Natl. Acad. Sci. U. S. A.* 79, 3423–3427.
- Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F. (2013) Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.* 8, 2281–2308.
- Rouleau, M., McDonald, D., Gagné, P., Ouellet, M.-E., Droit, A., Hunter, J.M., Dutertre, S., Prigent, C., Hendzel, M.J., Poirier, G.G. (2007) PARP-3 associates with polycomb group bodies and with components of the DNA damage repair machinery. *J. Cell. Biochem.* 100, 385–401.
- Sbalzarini, I.F., Koumoutsakos, P. (2005) Feature point tracking and trajectory analysis for video imaging in cell biology. *J. Struct. Biol.* 151, 182–195.
- Schermelleh, L., Solovei, I., Zink, D., Cremer, T. (2001) Two-color fluorescence labeling of early and mid-to-late replicating chromatin in living cells. *Chromosome Res. Int. J. Mol. Supramol. Evol. Asp. Chromosome Biol.* 9, 77–80.
- Schuster-Böckler, B., Lehner, B. (2012) Chromatin organization is a major influence on regional mutation rates in human cancer cells. *Nature* 488, 504–507.
- Smeenk, G. et al. (2013) Poly(ADP-ribosylation) links the chromatin remodeler SMARCA5/SNF2H to RNF168-dependent DNA damage signaling. *J. Cell Sci.* 126, 889–903.

Smerdon, M.J., Lieberman, M.W. (1978) Nucleosome rearrangement in human chromatin during UV-induced DNA-repair synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 75, 4238–4241.

Strickfaden, H. et al. (2016) Poly(ADP-ribosyl)ation-dependent transient chromatin decondensation and histone displacement following laser micro-irradiation. *J. Biol. Chem.* 291, 1789–802.

Subach, F.V., Patterson, G.H., Renz, M., Lippincott-Schwartz, J., Verkhusha, V.V. (2010) Bright monomeric photoactivatable red fluorescent protein for two-color super-resolution sptPALM of live cells. *J. Am. Chem. Soc.* 132, 6481–6491.

Thévenaz, P., Ruttimann, U.E., Unser, M. (1998) A pyramid approach to subpixel registration based on intensity. *IEEE Trans. Image Process. Publ. IEEE Signal Process. Soc.* 7, 27–41.

Thoma, F., Koller, T., Klug, A. (1979) Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell Biol.* 83, 403–427.

Timinszky, G. et al. (2009) A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. *Nat. Struct. Mol. Biol.* 16, 923–929.

Walter, A., Chapuis, C., Huet, S., Ellenberg, J. (2013) Crowded chromatin is not sufficient for heterochromatin formation and not required for its maintenance. *J. Struct. Biol.* 184, 445–53.

Wang, Z., Michaud, G.A., Cheng, Z., Zhang, Y., Hinds, T.R., Fan, E., Cong, F., Xu, W. (2012) Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WWE domains suggests a general mechanism for poly(ADP-ribosyl)ation-dependent ubiquitination. *Genes Dev.* 26, 235–240.

FIGURE LEGENDS

Figure 1. DNA damage induced by laser microirradiation induces transient chromatin relaxation. (A) Recruitment of PARP1 at the micro-irradiated area in cells co-expressing PARP1-mCherry and H2B-PAGFP. Bar = 4 μm . In cells non pre-sensitized with Hoechst, the 405 nm irradiation induces local photoactivation of the H2B-PAGFP but no recruitment of PARP1-mCherry. In contrast, in case of Hoechst pre-sensitization, the 405 nm irradiation induces both photoactivation of the H2B-PAGFP and a marked recruitment of PARP1-mCherry, indicating the presence of DNA lesions. Similarly, we observed the recruitment of 53BP1 only in cells pre-sensitized with Hoechst (data not shown). (B) Confocal image sequence of a human U2OS nucleus expressing H2B-PAGFP. Bar = 4 μm . The automatic segmentation of the histone H2B channel is shown in red below the raw images. The average thickness of the segmented line can be plotted as a function of time after irradiation, as shown in (C) for cells pre-sensitized or not with Hoechst (mean \pm SEM). Based on this analysis, the ratio between the thicknesses of the photo-converted line at time = 60s and time = 0s can be calculated to estimate the relative relaxation of the irradiated region. (D) Confocal image sequence of a U2OS cell expressing H2B-PATagRFP (red) and labeled with fluorescent nucleotides dUTP-ATTO633 (green). Bar = 4 μm . (E) Enlarged view of the region overlaid in yellow on the previous panel. On the images are shown the segmentation of the photo-converted chromatin area (red outline) and trajectories of individual foci labeled with fluorescent nucleotides (green). For this experiment, the power of the 405 nm laser used for simultaneous photo-activation and micro-irradiation was set to 250 μW at the sample level, instead of 125 μW , to induce an enhanced chromatin relaxation allowing an easier identification of the phase of directed motion for the dUTP-labeled foci. (F) Comparison between the speed at which the width of the H2B labeled region is growing and the speed of the dUTP-labeled foci perpendicular to the irradiation line. We show the average speed for the 30s subsequent to laser micro-irradiation. 12 cells were analyzed. p values were calculated by paired t-test. (G) Dynamics of the chromatin compaction state at DNA damage sites over long time scales measured in wild type U2OS cell expressing H2B-PATagRFP (mean \pm SEM).

Figure 2. PARP1 activity controls chromatin relaxation at DNA damage sites. (A) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type and PARP1 knockout cells (clone 2C8) transfected with H2B-PAGFP and treated or not with the PARP1 inhibitor AG14361 (30 μM , 1h). Similar results were obtained with a second PARP1 knockout cell clone (clone 2C12, data not shown). (B) Western-blot of wild-type U2OS cells and two PARP1 knockout cell lines.

Figure 3. The linker histone H1 is mobilized at DNA lesions. (A) Confocal image sequence of a U2OS nucleus co-expressing H2B-PATagRFP and H1.1-PAGFP. For the H1 channel, the image contrast was enhanced to allow the visualization of H1 redistribution over the entire nucleus following laser micro-irradiation. This led to an apparent saturation of the image at time = 0s. Bar = 4 μm . (B) Kinetics of the release of the H1 proteins localized at the DNA lesions at the time of laser micro-irradiation in wild-type cells co-expressing H2B-PATagRFP and H1.1-PAGFP, pre-sensitized or not with Hoechst and treated or not with the PARP1 inhibitor AG14361 (30 μM , 1h) (mean \pm SEM). (C) Characteristic release time for H1, measured at half fluorescence decay, in wild type and PARP1 knockout cells.

Figure 4. Chromatin relaxation at DNA damage sites partially depends on ATP. (A) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type cells expressing H2B-PAGFP and depleted or not for ATP. (B) Accumulation of the WWE domain of RNF146 at the DNA lesions estimated 60s after laser micro-irradiation in wild type cells expressing an EGFP tagged version of WWE and depleted or not for ATP. (C) Confocal image of U2OS cell nuclei stained with Hoechst and left untreated, depleted for ATP or bathed with hypertonic medium. Bar = 4 μm . (D) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type cells expressing H2B-PAGFP and bathed in isotonic or hypertonic media.

(E) Accumulation of the WWE domain of RNF146 at the DNA lesions estimated 60s after laser micro-irradiation in wild type cells bathed in isotonic or hypertonic media.

Figure 5. The chromatin remodeler Alc1 contributes to chromatin relaxation upon DNA damage. (A) Western-blot of wild type U2OS cells and the Alc1 knockout cell line. (B) Confocal image sequence of a U2OS nucleus co-expressing H2B-PAGFP and Alc1-mCherry. Bar = 4 μ m. (C) Kinetics of Alc1 recruitment and chromatin relaxation at the DNA lesions measured in Alc1 knockout cells co-expressing H2B-PAGFP and Alc1-mCherry (mean \pm SEM). (D) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells versus Alc1 knockout cells co-transfected with H2B-PAGFP and an empty plasmid (\emptyset), wild type Alc1 or the catalytic-dead mutant Alc1 E175Q, both fused to mCherry. (E) Relative chromatin relaxation at 60s after laser micro-irradiation in Alc1 knockout cells expressing H2B-PAGFP and depleted or not for ATP. (F) Relative chromatin relaxation at 60s after laser micro-irradiation in Alc1 knockout cells expressing H2B-PAGFP and treated or not with the PARP1 inhibitor AG14361 (30 μ M, 1h) (G) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells expressing H2B-PAGFP and transfected with uncoupled mCherry, wild type Alc1 fused to mCherry or the catalytic-dead mutant Alc1 E175Q fused to mCherry.

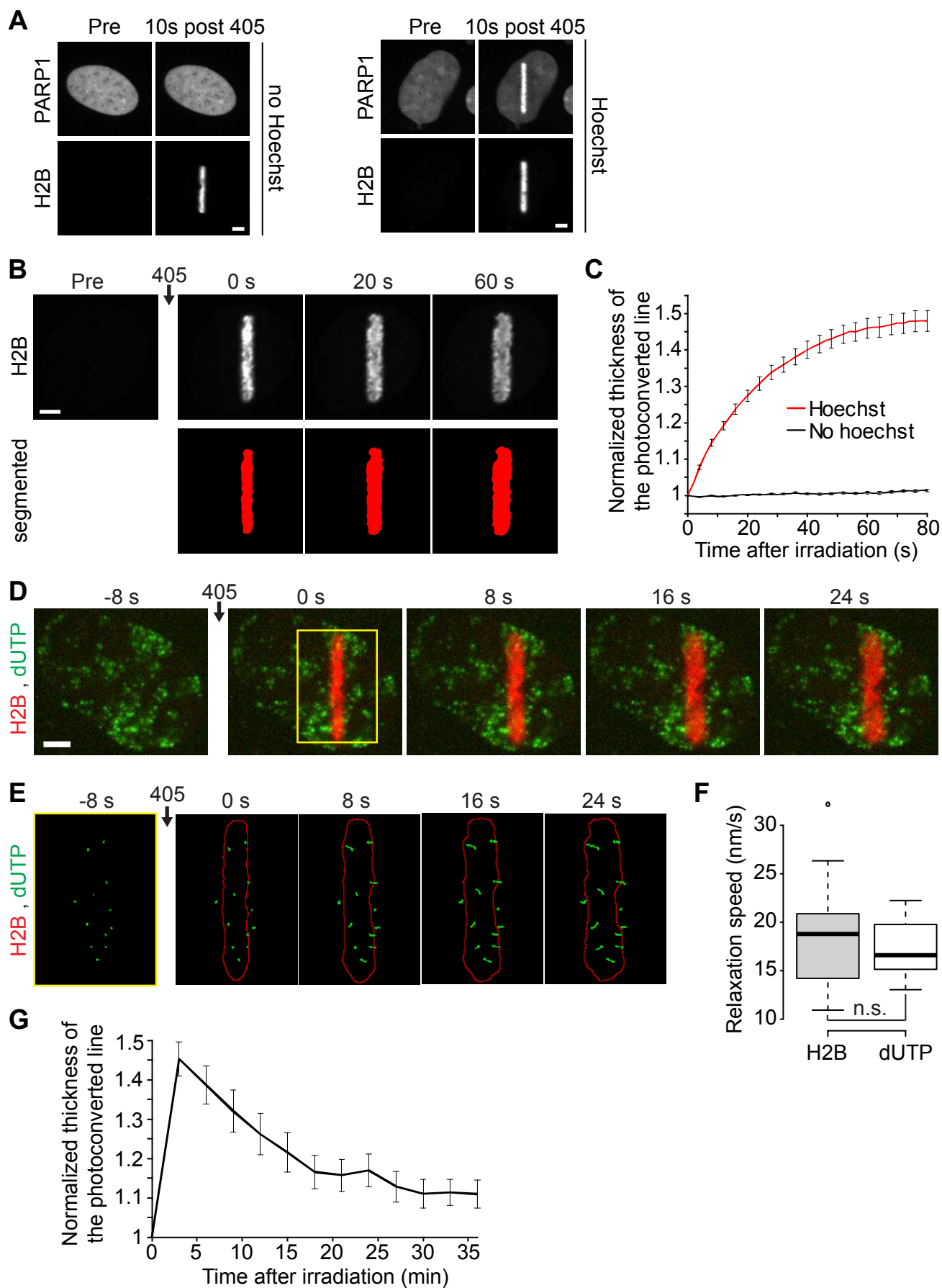


Figure 1. See next page for the figure legend.

Figure 1. DNA damage induced by laser microirradiation induces transient chromatin relaxation. (A) Recruitment of PARP1 at the micro-irradiated area in cells co-expressing PARP1-mCherry and H2B-PAGFP. Bar = 4 μ m. In cells non pre-sensitized with Hoechst, the 405 nm irradiation induces local photoactivation of the H2B-PAGFP but no recruitment of PARP1-mCherry. In contrast, in case of Hoechst pre-sensitization, the 405 nm irradiation induces both photoactivation of the H2B-PAGFP and a marked recruitment of PARP1-mCherry, indicating the presence of DNA lesions. Similarly, we observed the recruitment of 53BP1 only in cells pre-sensitized with Hoechst (data not shown). (B) Confocal image sequence of a human U2OS nucleus expressing H2B-PAGFP. Bar = 4 μ m. The automatic segmentation of the histone H2B channel is shown in red below the raw images. The average thickness of the segmented line can be plotted as a function of time after irradiation, as shown in (C) for cells pre-sensitized or not with Hoechst (mean \pm SEM). Based on this analysis, the ratio between the thicknesses of the photo-converted line at time = 60s and time = 0s can be calculated to estimate the relative relaxation of the irradiated region. (D) Confocal image sequence of a U2OS cell expressing H2B-PATagRFP (red) and labeled with fluorescent nucleotides dUTP-ATTO633 (green). Bar = 4 μ m. (E) Enlarged view of the region overlaid in yellow on the previous panel. On the images are shown the segmentation of the photo-converted chromatin area (red outline) and trajectories of individual foci labeled with fluorescent nucleotides (green). For this experiment, the power of the 405 nm laser used for simultaneous photo-activation and micro-irradiation was set to 250 μ W at the sample level, instead of 125 μ W, to induce an enhanced chromatin relaxation allowing an easier identification of the phase of directed motion for the dUTP-labeled foci. (F) Comparison between the speed at which the width of the H2B labeled region is growing and the speed of the dUTP-labeled foci perpendicular to the irradiation line. We show the average speed for the 30s subsequent to laser micro-irradiation. 12 cells were analyzed. p values were calculated by paired t-test. (G) Dynamics of the chromatin compaction state at DNA damage sites over long time scales measured in wild type U2OS cell expressing H2B-PATagRFP (mean \pm SEM).

Sellou et al., Figure 1

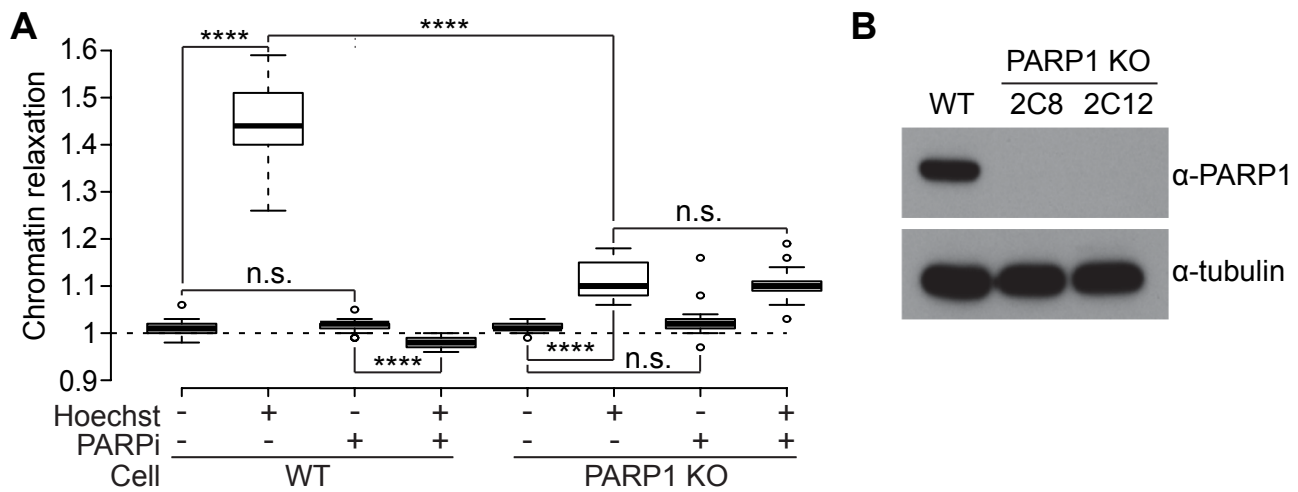


Figure 2. PARP1 activity controls chromatin relaxation at DNA damage sites. (A) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type and PARP1 knockout cells (clone 2C8) transfected with H2B-PAGFP and treated or not with the PARP1 inhibitor AG14361 (30 μ M, 1h). Similar results were obtained with a second PARP1 knockout cell clone (clone 2C12, data not shown). (B) Western-blot of wild-type U2OS cells and two PARP1 knockout cell lines.

Sellou et al., Figure 2

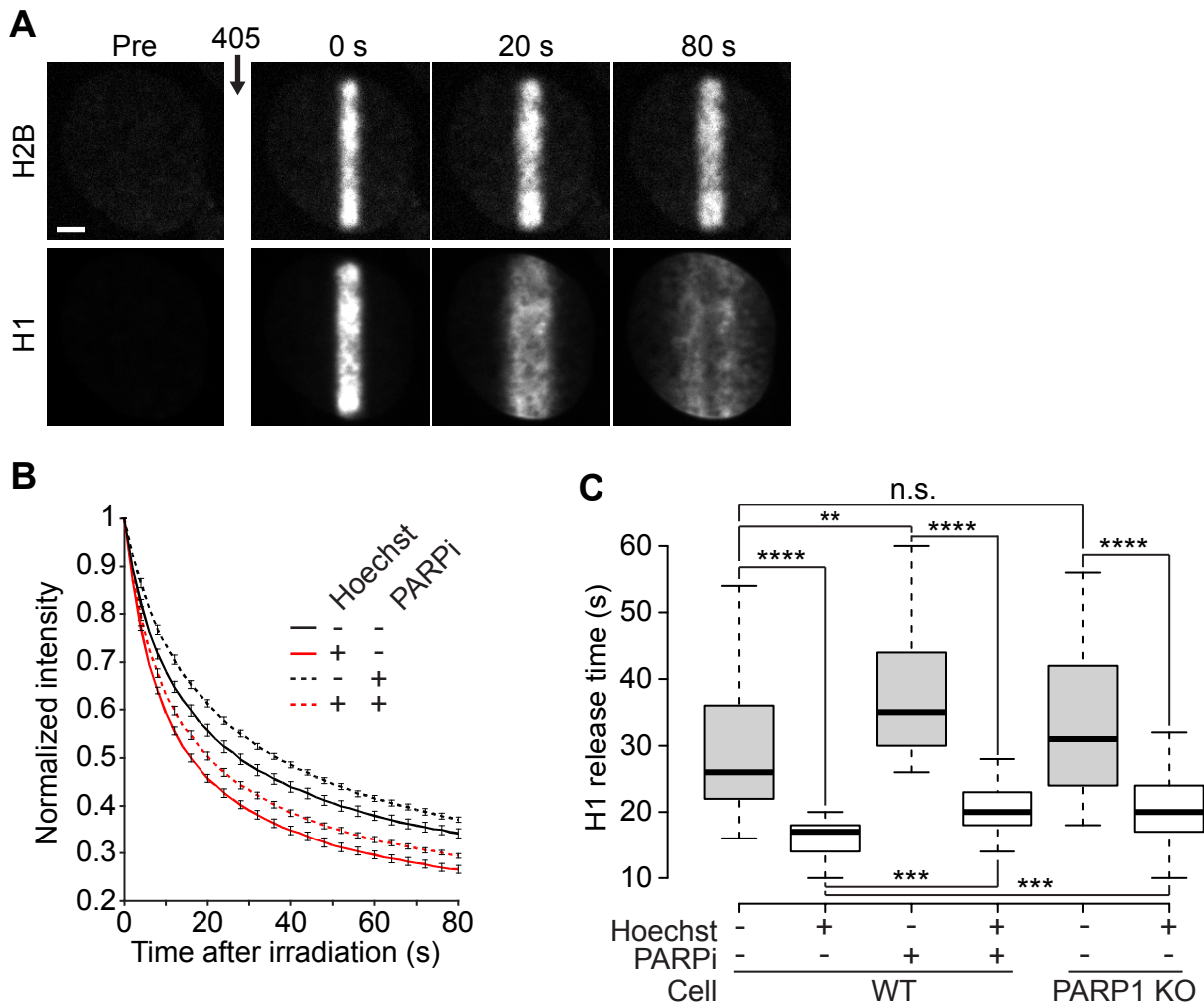


Figure 3. The linker histone H1 is mobilized at DNA lesions. (A) Confocal image sequence of a U2OS nucleus co-expressing H2B-PATagRFP and H1.1-PAGFP. For the H1 channel, the image contrast was enhanced to allow the visualization of H1 redistribution over the entire nucleus following laser micro-irradiation. This led to an apparent saturation of the image at time = 0s. Bar = 4 μ m. (B) Kinetics of the release of the H1 proteins localized at the DNA lesions at the time of laser micro-irradiation in wild-type cells co-expressing H2B-PATagRFP and H1.1-PAGFP, pre-sensitized or not with Hoechst and treated or not with the PARP1 inhibitor AG14361 (30 μ M, 1h) (mean \pm SEM). (C) Characteristic release time for H1, measured at half fluorescence decay, in wild type and PARP1 knockout cells.

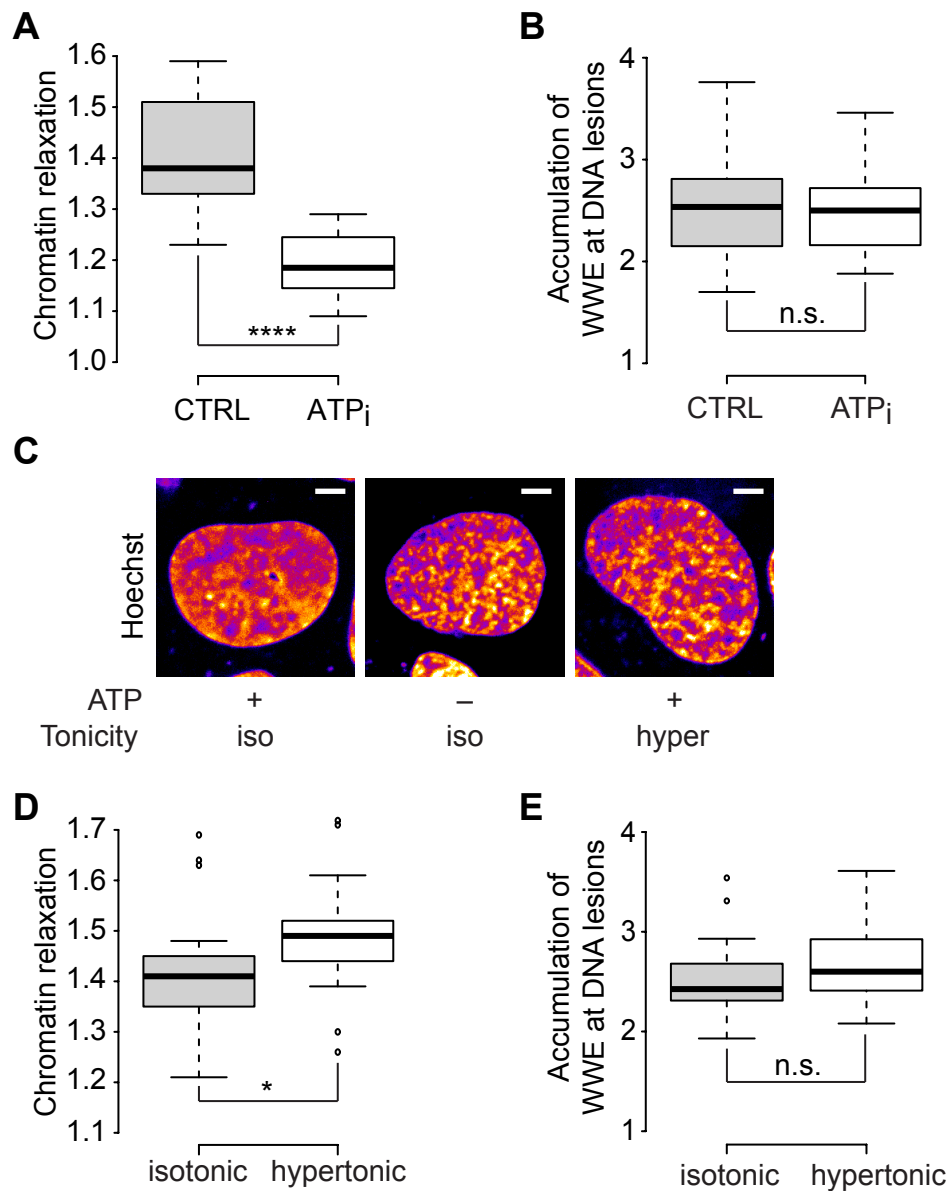


Figure 4. Chromatin relaxation at DNA damage sites partially depends on ATP. (A) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type cells expressing H2B-PAGFP and depleted or not for ATP. (B) Accumulation of the WWE domain of RNF146 at the DNA lesions estimated 60s after laser micro-irradiation in wild type cells expressing an EGFP tagged version of WWE and depleted or not for ATP. (C) Confocal image of U2OS cell nuclei stained with Hoechst and left untreated, depleted for ATP or bathed with hypertonic medium. Bar = 4 μ m. (D) Relative chromatin relaxation at 60s after laser micro-irradiation in wild type cells expressing H2B-PAGFP and bathed in isotonic or hypertonic media. (E) Accumulation of the WWE domain of RNF146 at the DNA lesions estimated 60s after laser micro-irradiation in wild type cells bathed in isotonic or hypertonic media.

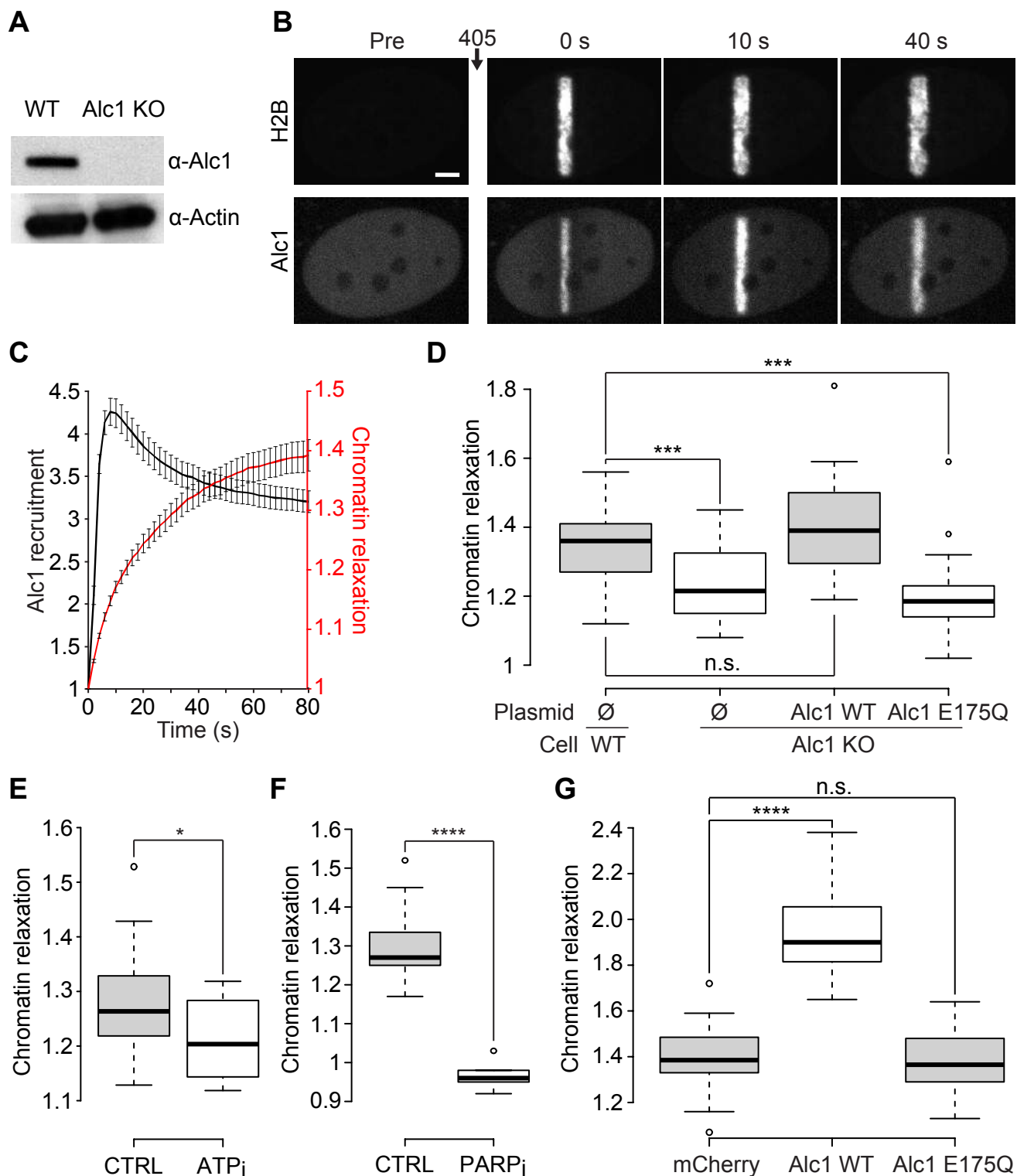


Figure 5. The chromatin remodeler Alc1 contributes to chromatin relaxation upon DNA damage. (A) Western-blot of wild type U2OS cells and the Alc1 knockout cell line. (B) Confocal image sequence of a U2OS nucleus co-expressing H2B-PAGFP and Alc1-mCherry. Bar = 4 μ m. (C) Kinetics of Alc1 recruitment and chromatin relaxation at the DNA lesions measured in Alc1 knockout cells co-expressing H2B-PAGFP and Alc1-mCherry (mean \pm SEM). (D) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells versus Alc1 knockout cells co-transfected with H2B-PAGFP and an empty plasmid (\emptyset), wild type Alc1 or the catalytic-dead mutant Alc1 E175Q, both fused to mCherry. (E) Relative chromatin relaxation at 60s after laser micro-irradiation in Alc1 knockout cells expressing H2B-PAGFP and depleted or not for ATP. (F) Relative chromatin relaxation at 60s after laser micro-irradiation in Alc1 knockout cells expressing H2B-PAGFP and treated or not with the PARP1 inhibitor AG14361 (30 μ M, 1h) (G) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells expressing H2B-PAGFP and transfected with uncoupled mCherry, wild type Alc1 fused to mCherry or the catalytic-dead mutant Alc1 E175Q fused to mCherry.

The poly(ADP-ribose)-dependent chromatin remodeler Alc1 induces local chromatin relaxation upon DNA damage

Hafida Sellou, Théo Lebeaupin, Catherine Chapuis, Rebecca Smith, Anna Hegele, Hari R. Singh, Marek Kozlowski, Sebastian Bultmann, Andreas G. Ladurner, Gyula Timinszky, and Sébastien Huet

SUPPLEMENTAL INFORMATION

SUPPLEMENTARY FIGURES

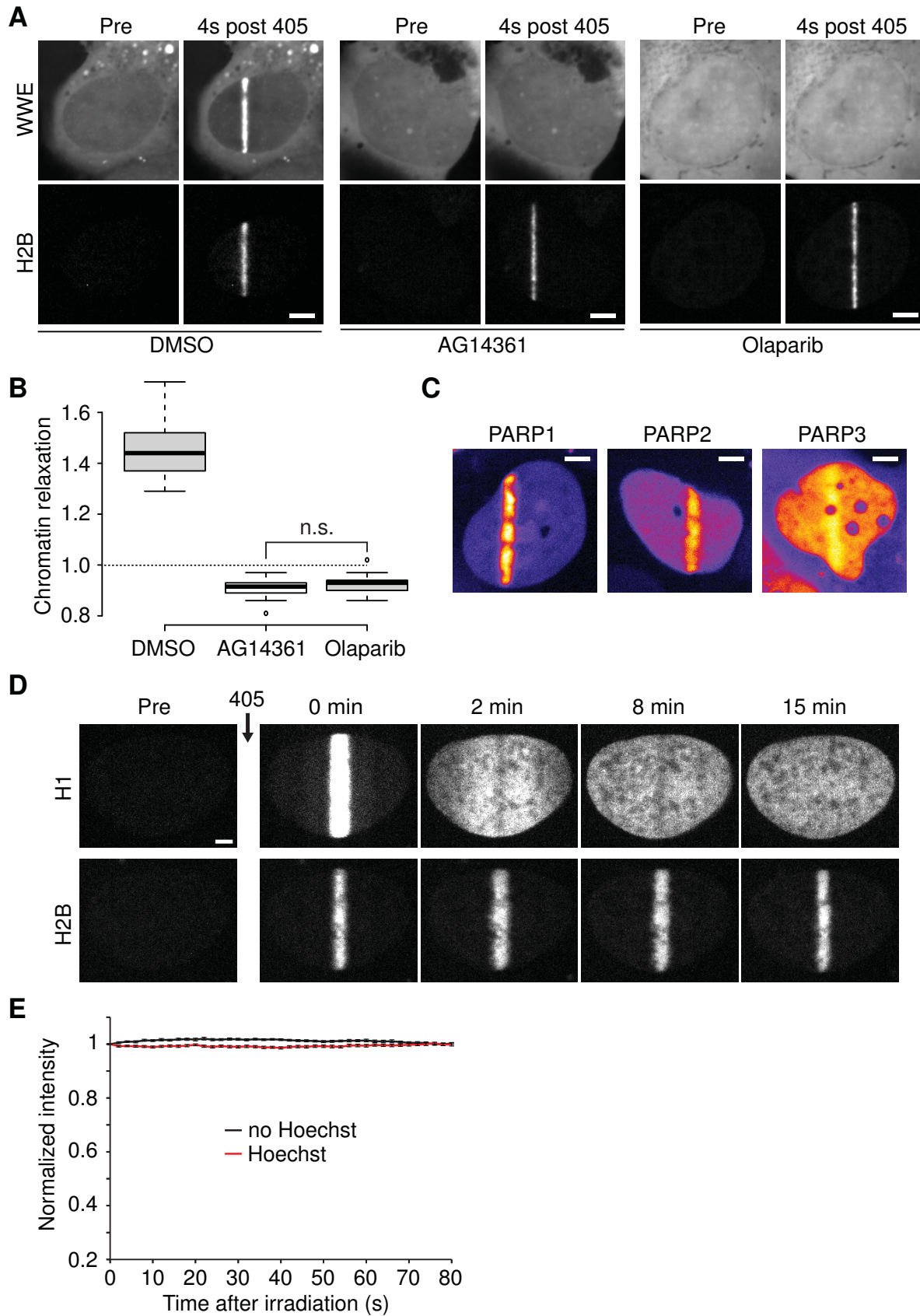
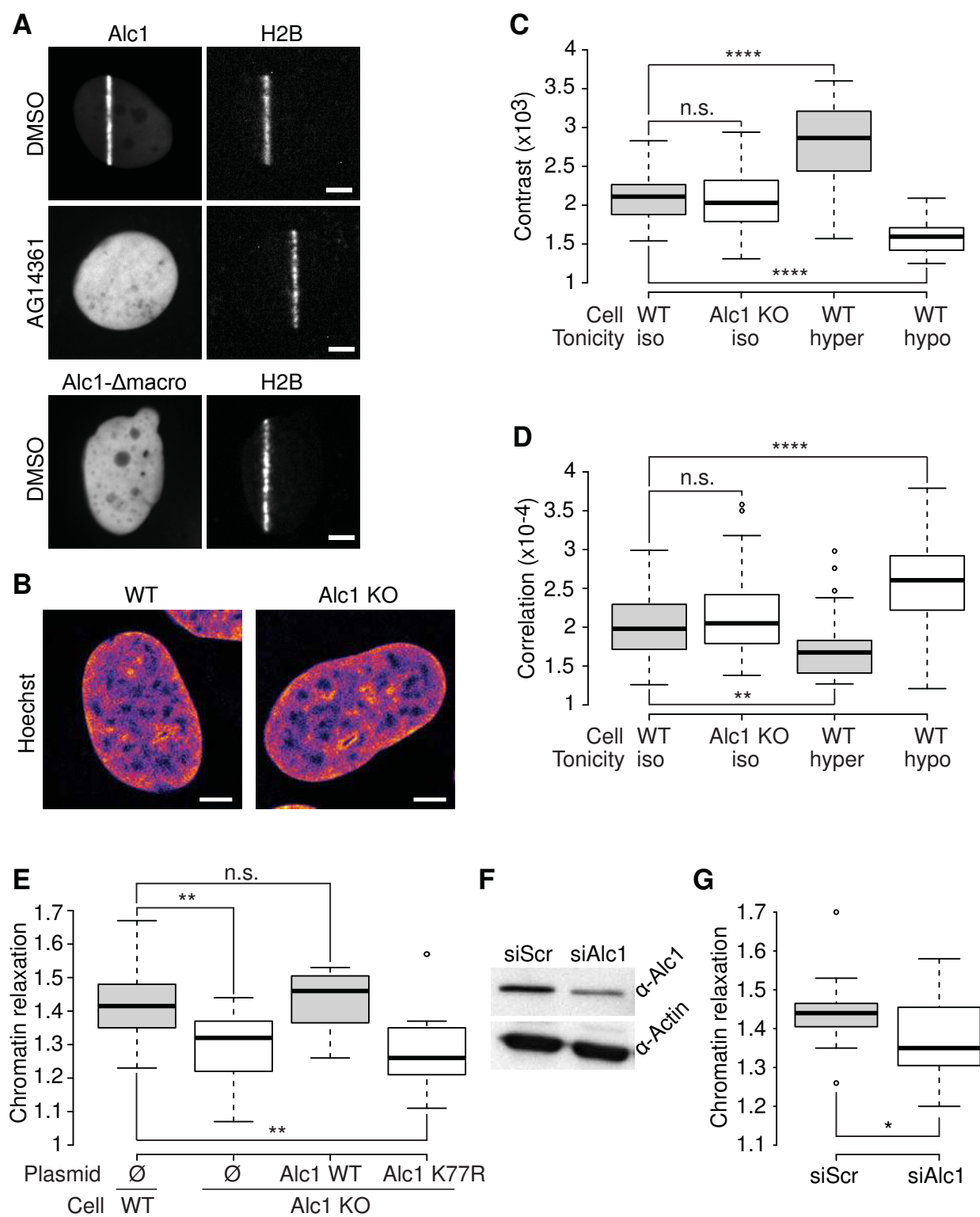


Figure S1. PARP1-dependent chromatin dynamics at DNA damage sites. (A) Recruitment at the DNA damage sites of the PAR-binder WWE domain of RNF146 in cells co-expressing WWE-EGFP and H2B-PATagRFP, pre-sensitized with Hoechst and treated or not with the PARP inhibitors AG14361 (30 μ M, 1h) or Olaparib (50 μ M, 1h). Bar = 4 μ m. The strong recruitment of the WWE domain observed at DNA lesions in the control cells was completely abolished upon treatment with AG14361 or Olaparib, demonstrating the efficiency of these inhibitors. (B) Relative chromatin relaxation at 60s after laser micro-irradiation in U2OS cells expressing H2B-PAGFP and treated or not with the PARP1 inhibitors AG14361 (30 μ M, 1h) or Olaparib (50 μ M, 1h). (C) Recruitment of human PARP1, PARP2 and PARP3 at DNA damage sites induced by laser micro-irradiation. U2OS cells were transfected with GFP-tagged PARP proteins. Images were taken 2 minutes after laser irradiation. Bar = 4 μ m. (D) Confocal image sequence acquired at long time scales for a U2OS cell co-expressing H2B-PATagRFP and H1-PAGFP. For the H1 channel, the image contrast was enhanced to allow the visualization of H1 redistribution over the entire nucleus following laser micro-irradiation. This led to an apparent saturation of the image at time = 0s. Bar = 4 μ m. (E) Kinetics of H2B release from the irradiated line in wild-type U2OS cells expressing H2B-PATagRFP and pre-sensitized or not with Hoechst (mean \pm SEM).



Sellou et al., Figure S2

Figure S2. The chromatin remodeler Alc1 is involved in chromatin relaxation at DNA damage sites. (A) Recruitment of Alc1 at the DNA damage sites in cells co-expressing the wild-type version of Alc1 fused to EGFP or a mutant lacking the macro domain (Alc1- Δ macro) fused to YFP, and H2B-PATagRFP. Cells were pre-sensitized with Hoechst and treated or not with the PARP inhibitor AG14361 (30 μ M, 1h). Images were acquired 4 s after laser micro-irradiation Bar = 4 μ m. The recruitment of Alc1 at DNA lesions was fully abolished upon treatment with AG14361 or for an Alc1 mutant lacking the macro domain. (B) Confocal images of wild-type and Alc knockout U2OS cells labeled with Hoechst. Bar = 4 μ m. (C-D) Quantitative analysis of the Hoechst patterns in wild-type and Alc knockout U2OS cells. Two parameters were assessed to characterize the chromatin compaction state: the contrast (C) and the pixel-to-pixel correlation (D). As positive controls, we analyzed the chromatin patterns in cells bathed with hypertonic or hypotonic medium to induce chromatin hyper-compaction or decompaction, respectively. More than 70 cells conditions for the wild-type and Alc1 knockout cells. More than 25 cells conditions for the wild-type cells subjected to osmotic stresses. (E) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type cells versus Alc1 knockout cells co-transfected with H2B-PAGFP and an empty plasmid (\emptyset), wild type Alc1 or the catalytic-dead mutant Alc1 K77R, both fused to mCherry. More than 12 cells per condition were imaged. (F) Western-blot of U2OS cells treated with a scrambled siRNA or with a siRNA directed against Alc1. (G) Relative chromatin relaxation at 60s after laser micro-irradiation for wild type U2OS cells stably expressing H2B-PATagRFP and transfected with a scrambled siRNA or a siRNA directed against Alc1.

SUPPLEMENTAL MOVIE LEGENDS

Supplemental movie 1.

Complete time-lapse acquisition corresponding to the sequence shown on Figure 1D. The displayed cell expresses H2B-PATagRFP (red) and is labeled with fluorescent nucleotides dUTP-ATTO633 (green). The trajectories of individual dUTP-labeled foci labeled are shown in white. Time = 0s corresponds to laser micro-irradiation. Bar = 4 μ m.

Supplemental movie 2.

Time-lapse confocal sequence for a wild type U2OS cell co-expressing H2B-PATagRFP (top) and H1-PAGFP (bottom). Time = 0s corresponds to laser micro-irradiation. Bar = 4 μ m.