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The centrin-binding protein Sfi1 : functions in fission yeast and human

Imen Bouhleb Bougdhira

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The Centrin-binding Protein Sfi1: Functions in Fission Yeast and Human

Thèse de doctorat de l'Université Paris-Saclay
Préparée à Université Paris-Sud

École doctorale n°577 structure et dynamique des systèmes vivants
(SDSV)

Spécialité de doctorat: sciences de la vie et de la santé

Thèse présentée et soutenue à Paris, le 7 Décembre 2017, par

Imen Bouhlel Bougdhira

Composition du Jury :

Frédéric Bocard Directeur de Recherche, I2BC, Orsay	Président
Paul Guichard Professeur Assistant, Université de Genève, Suisse	Rapporteur
Bénédicte Delaval Chercheur CR1, CRBM, Montpellier	Rapporteur
Iain Hagan Professeur, CRUK Institute, Manchester	Examineur
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Anne Paoletti Directrice de Recherche, Institut Curie, Paris	Directrice de thèse

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LIST OF ABBREVIATIONS

aa	amino acids
APC/C	Anaphase Promoting Complex/Cyclosome
ATP	Adenine Triphosphate
AS	Analogue sensitive
BB	Basal Body
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cas9	CRISPR-associated nuclease
DA	Distal Apendages
DNA	Deoxyribonucleic Acid
EM	Electron microscopy
GCP	γ -Tubulin Complex Protein
GDP	Guanosine Diphosphate
GFP	Green Fluorescent Protein
CP	Central Plaque
GTP	Guanosine Triphosphate
HB	Half Bridge
ICL	Infraciliary lattice
INM	Inner Nuclear Membrane
IP	Inner Plaque
KO	Knock-out
MAP	Microtubule Associated Protein
MT	Microtubule
MTOC	Microtubule Organizing Centre
OP	Outer Plaque
ONM	Outer Nuclear Membrane
PCM	Pericentrosomal Matrix
Plk	Polo Like Kinase
PinB	Pinhead Body
PinF	Pinhead Feet

RPE1	Retinal Pigmentation epithelial cells 1
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
<i>S. pombe</i>	<i>Schizosaccharomyces pombe</i>
SD	Subdistal appendages
SFR	Sfi1 Repeats
SPB	Spindle Pole Body
STORM	stochastic optical reconstruction microscopy
WT	Wild type
3DSIM	3Dimensial Structured-illumination Microscopy

Chapter I: INTRODUCTION

The centrosome is the main microtubule-organizing centre. It was first described more than a century ago independently by Edouard Van Beneden and Theodor Boveri who named it *corpuscule centrale* and *centrosome* respectively (Boveri, 1887; van Beneden, 1887). The central position of this organelle in the cell, the fact that it seemed to be self-replicating and transmitted through generations from mother to daughter cells generated a great excitement. In his 1887 communication Boveri wrote “The centrosome represents the dynamic centre of the cell; its division creates the centre of forming daughter cells, around which all other cellular components arrange themselves symmetrically” (Boveri, 1887; see also the translated and annotated version Boveri, 2008).

Nowadays, centrosome study represents an entire field of research in biology. Electron microscopy and molecular and cellular biology allowed scientists to unravel its particular structure and its very specific nine-fold symmetry in addition to its role in nucleating and organizing microtubules. In the last decade, super resolution microscopy and new electron microscopy techniques helped to unravel more secrets of the structure and the establishment of the nine fold symmetry. It has also become very clear that in addition to its role as microtubule organizing centre, the centrosome is a central signalling hub in the cell.

During my thesis, I examined the centrosome assembly and regulations in two organisms: the fission yeast *Schizosaccharomyces pombe* and Human. In this introduction, I will introduce the centrosome in mammalian cells. I will shortly introduce the microtubules, their dynamics and functions. Then I will detail the centrosome organization and architecture. I will also describe the centrosome cycle and its role in cell cycle regulation. In the second section, I will describe the yeast spindle pole body, the functional homolog of centrosome, and highlight the

important contributions of this model to the centrosome field. In the final part of this introduction, I will describe Centrins and Sfil functions across evolution.

1. The Centrosome in mammalian cells

1.1 Microtubules dynamics and organization

Microtubules are one of the three cytoskeletal filaments of the cell. They are essential for cellular organization and survival.

1.1.1 Microtubules structure

Microtubules (MTs) are composed of heterodimers of α and β -tubulin (Desai and Mitchison, 1997). The two globular subunits share a 40% homology (Burns, 1991). They interact together through non-covalent bounds (Nogales et al., 1998) to form a linear protofilament. A MT is composed of several protofilaments that interact laterally, leading to the assembly of a hollow helical cylinder of 25 nm diameter and up to 20 μ m in length (Evans et al., 1985). *In vivo*, one MT is generally assembled from 13 protofilaments, but this number can vary in different organisms or during cell cycle phases (Figure 1-1). *In vitro*, the number of protofilaments can vary from 10 to 16 (Desai and Mitchison, 1997).

MTs are polarized due to the organization of the tubulin heterodimers. The less dynamic end of the MT, called the minus end, is formed with α -tubulin, whereas the highly dynamic plus end is formed with β -tubulin. Each tubulin unit binds a molecule of guanosine triphosphate (GTP) at its N-terminus (Nogales et al., 1998). Therefore each dimer is associated with two GTP molecules, one is non-hydrolysable and bound to α -tubulin and one is hydrolysable and bound to the β -tubulin. The β -tubulin GTP is hydrolysed to a guanosine diphosphate (GDP) upon incorporation of the tubulin dimer into the lattice (Desai and Mitchison, 1997).

The GTP composition of MTs is essential for their dynamics. GTP hydrolysis will lead to the depolymerisation of the MT, while GDP-bound dimers are more stable. During growth, the GTP hydrolysis rate is lower than the tubulin assembly rate, and this results in the formation of the “GTP cap” at the plus tip of the MT, allowing growth to continue (Drechsel and Kirschner, 1994). However, when the tubulin incorporation rate is low, GTP hydrolysis lead to the removal of the GTP cap, exposing the GDP bound β -tubulin and leading to the depolymerisation of the MT. This phenomenon is called “catastrophe”. During the shrinking phase that coincides with depolymerisation, the MT can switch back to growth, in an event known as “rescue”. The rescue events are more likely to happen at sites known as “GTP islands” where GTP molecules are found within the MT lattice (Dimitrov et al., 2008). These GTP sites are thought to be remnants of former catastrophes. When depolymerisation reaches a GTP island, it will pause (or dwell) and may switch back to growth at these sites (Dimitrov et al., 2008). This alternation of phases of catastrophe and rescue confers the typical dynamic instability of MTs (Walker et al., 1988).

More recently, MTs dynamics regulation has been shown to be modulated by a mechanical and structural mechanism by which a damaged MT can acquire a capacity to prevent its catastrophic depolymerization through the incorporation of new dimers of tubulin. Tubulin incorporation could occur not only at MT ends but also along the MT lattice. Moreover, tubulin incorporation was observed to be preferentially located in regions where the lattice is submitted to geometrical and mechanical constraints such as MT crossover, bundle and bending site (Aumeier et al., 2016).

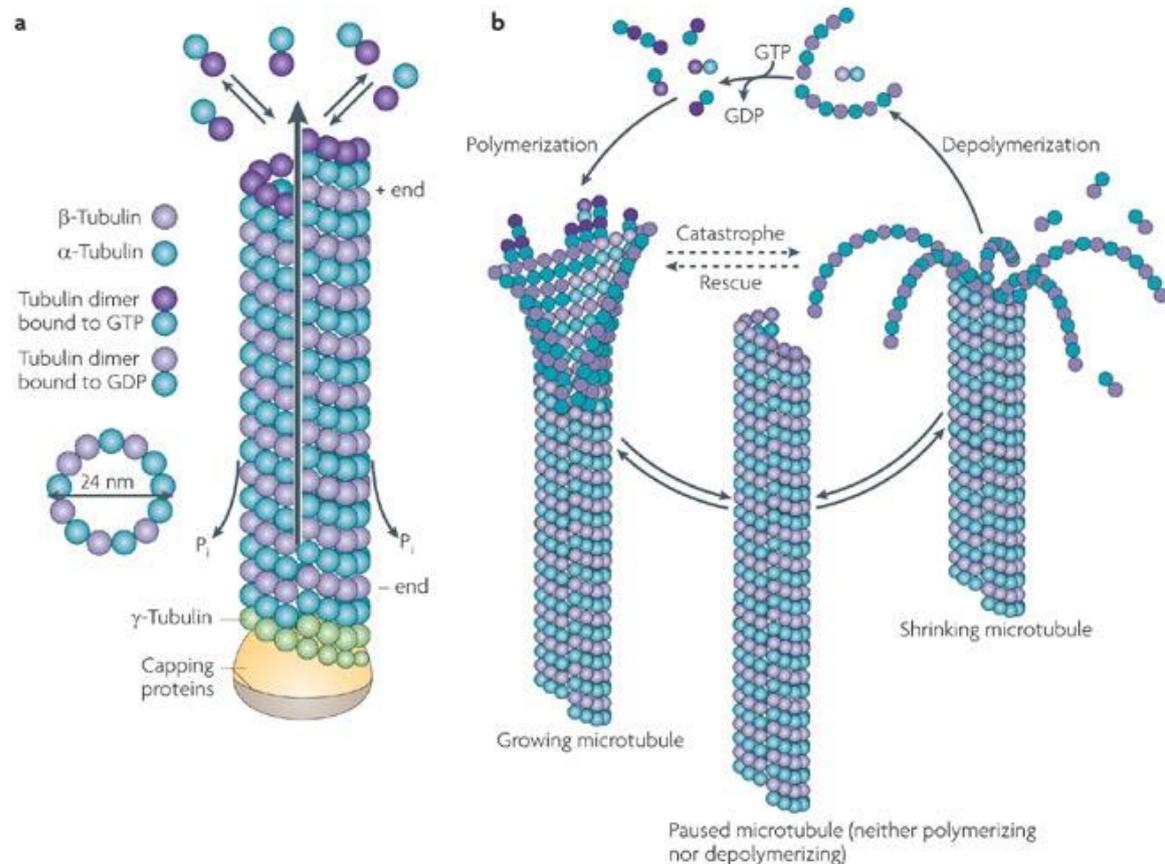


Figure 1-1: Microtubules Assembly and Dynamics

From Rochlin, M. W., Dailey, M. E. & Bridgman, P. C – Nature Reviews

1.1.2 Microtubules nucleation

Existing MTs can grow and shrink but new MTs can also be assembled *de novo* at nucleation sites. These sites are called microtubule organizing centres (MTOC). Centrosomes are the main MTOC in animal cells, but other non centrosomal MTOCs exist. For instance, the Golgi apparatus and the nucleus are also known to nucleate MTs (reviewed in Sanchez and Feldman, 2017 and Nishita et al., 2017). A general requirement for these assembly sites is the presence of γ -tubulin which is part of the γ -Tubulin Ring Complex (γ -TuRC) (Moritz et al., 1995; Zheng et al., 1995).

The γ -TuRC is composed of γ -tubulin and γ -tubulin complex proteins (GCPs). The γ -tubulin isoform is a homologue of α and β -tubulin and has been shown to be essential for MT nucleation in all studied organisms (Kollman et al., 2011). GCP proteins share highly conserved motifs: the GRIP1 and GRIP2 motifs, which are unique to the GCP proteins. Although the exact function of these motifs remains unclear, it has been speculated that they may play a role in protein-protein interaction (Murphy et al., 2001). In addition to γ -Tubulin and the GCPs, MOZART1 (for mitotic-spindle organizing protein associated with a ring or γ -tubulin 1) has also been identified as an integral component of the γ -TuRC. MOZART1 is conserved in all eukaryotes and seems to play a role in recruiting the γ -TuRC to MTOCs (Hutchins et al., 2010).

Prior to the assembly of the complete γ -TuRC, a smaller Y shaped complex is formed: the γ -Tubulin Small Complex (γ TuSC; Figure 1-2a). It is composed of GCP2 and GCP3 and two γ -tubulin molecules. Then several γ -TuSC are arranged into a helix by GCP4, GCP5 and GCP6 (Kollman et al., 2010; Kollman et al., 2008). The γ -TuRC is formed into β -fold symmetric rings, which act as platform and initiate MTs assembly (Figure 1-2b).

Different mechanisms for MT assembly have been proposed: the template model and the protofilament model. The template model is the most accepted model and proposes that the α/β -tubulin dimer interacts with the ring like shape of the γ -TuRC through α -tubulin. The new MT will then grow using this base as a template. The second is the protofilament model and proposes that the tubulin dimer would interact laterally with the open end of the γ -TuRC. According to this model, the γ -TuRC would not be a template ring but a protofilament that curves due to intrinsic curvature of monomers interactions (reviewed in Kollman et al., 2011).

Fission yeast cells generally have around four MTs bundles in interphase that run mostly parallel to the long axis of the cell (Hagan, 1998; Hagan and Hyams, 1988) and all components of the γ -TuRC are conserved (see Sawin and Tran, 2006). During interphase, MTs can be nucleated not only from the spindle pole body (SPB), the

functional homolog of the centrosome but also from additional sites on the nuclear surface, on MTs themselves, and in the cytoplasm (Drummond and Cross, 2000; Sawin and Tran, 2006). These non-SPB sites are generally known collectively as interphase MTOCs (iMTOCs). During mitosis, the cytoplasmic face of the SPB nucleates astral MTs. At the end of mitosis, MTs are nucleated from an equatorial MTOC (eMTOC) at the cell division site (the septum), forming a transient structure, the post-anaphase array (PAA). Importantly, all these MTOCs are able to recruit the fission yeast γ -TuRC complex, simply referred to as the γ -Tubulin Complex (γ -TuC). Interestingly, in budding yeast, the γ -TuSC does not assemble into a γ -TuRC and the γ -TuSC is sufficient to nucleate MTs (Kollman et al., 2011). The γ -TuSC is activated upon its assembly, but requires Spc110 to be anchored to the SPB.

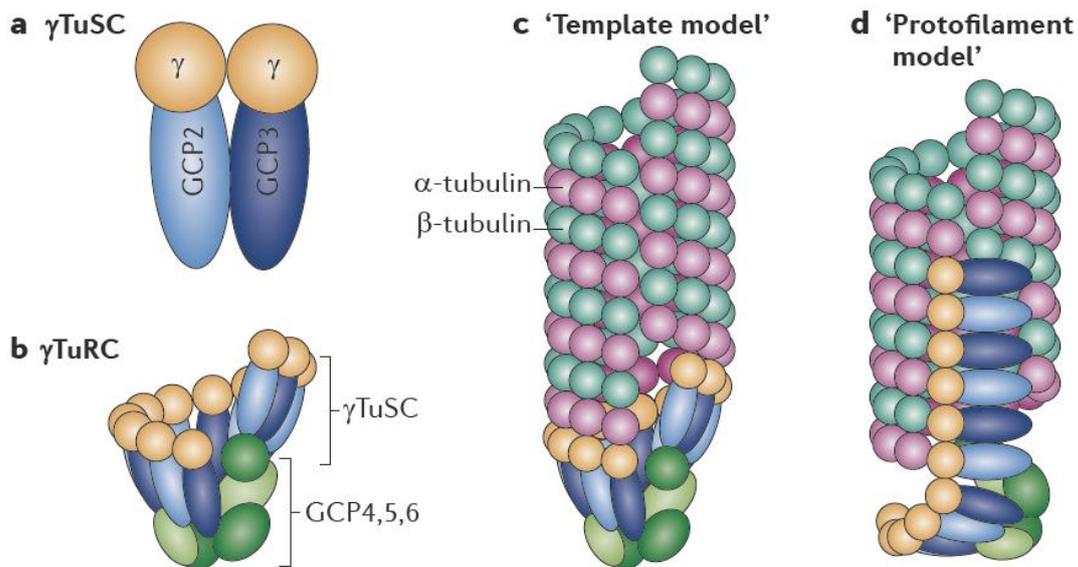


Figure 1-2: The γ -TuRC complex and MT nucleation models

From Kollman et al 2011.

Similarly, the γ -TuRC nucleating activity requires attachment to the site of nucleation to be activated (Stearns and Kirschner, 1994). Most of the γ -tubulin is cytoplasmic and devoid of microtubule-nucleation activity before being recruited to the centrosome (Moudjou et al., 1996; Stearns and Kirschner, 1994). In animal cells,

several centrosomal proteins are involved in the γ -TuRC attachment to the centrosome, including pericentrin, ninein, AKAP-450 (also known as Kendrin) and Cep192 (Delgehyr et al., 2005; Takahashi et al., 2002; Zimmerman et al., 2004) (Gomez-Ferreria et al., 2007).

MT nucleation can also be achieved in absence of centrosomes. In non-centrosomal MTOCs, various proteins can recruit the γ -TuRC (for review see Sulimenko et al., 2017). For example, in mammalian and *Drosophila* S2 cells, the augmin complex recruits the γ -tubulin to existing MTs of the mitotic spindle, and contributes to the spindle robustness. This interaction allows the nucleation of new MTs and participates in efficient chromosome capture via the kinetochores (Goshima et al., 2008). In plants, where there is no centrosome, the augmin complex is the major regulator the mitotic spindle assembly (Hotta et al., 2012).

In the fission yeast *S.pombe*, it has been shown that Mto1/2 complex is required for the attachment and activation of the γ -TuRC at all the MTOCs of the cell. Deletion of Mto2 leads to decreasing number of MTs bundles in the cell, which in turn results in cell morphology and nuclear positioning defects (Janson et al., 2005). Moreover, during interphase, Mto1/2 recruits the γ -TuRC to pre-existing MTs, and to the nuclear envelope, leading to MT nucleation from all these sites (Samejima et al., 2010). Moreover, when Mto1 is deleted, *de novo* MTs nucleation is completely abolished (Samejima et al., 2008).

In addition to its role in MTs nucleation the γ -TuRC complex is also important for capping and stabilizing the MT minus end independently of its nucleation activity (Anders and Sawin, 2011; Wiese and Zheng, 2000), and in controlling plus end dynamics (Bouissou et al., 2009).

1.1.3 Modulation of MTs dynamics by MAPS

Even though MT assembly and GTP/GDP turnover confers an intrinsic MT dynamics, a plethora of actors also regulate MT dynamics. These actors are grouped under the term of MT associated proteins (MAPs) and form a large heterogeneous

group. MAPs functions span from stabilizing the MT lattice and increasing MT assembly to severing the lattice and inducing catastrophe (For reviews see Alfaro-Aco and Petry, 2015 and Akhmanova and Steinmetz, 2015).

MT depolymerases destabilize MTs by promoting catastrophe to regulate MT stability and length (Howard and Hyman, 2007). Although many MT depolymerases exist in the cell, one of the best-characterized destabilizing MAP *in vitro* is the kinesin-13 MCAK/XKCM1, which increases catastrophe rates in cells and regulates the size of MT structures (Kline-Smith and Walczak, 2002). MT polymerases oppose depolymerases and promote growth or rescue depolymerizing MTs (Howard and Hyman, 2007). One example of MT stabilizing agent is the MT polymerase XMAP215/ch-TOG (Brouhard et al., 2008), which enhances MT growth rates up to 10-fold *in vitro*.

Another class of MAPs that play an essential role for MT dynamics are the plus TIP proteins. They interact with the plus end of MTs. This family includes EB1 and EB3 that promote the stable and continuous growth of MTs by preventing catastrophe (Schuyler and Pellman, 2001).

A particular group of MAPs that appear not to directly influence MTs dynamics is the family of MAP65/Ase1/PRC1. These MAPs have been shown to crosslink MTs into parallel bundles (Chang-Jie and Sonobe, 1993; Walczak and Shaw, 2010). These crosslinkers contain a MT-binding domain and a domain mediating homodimerization, which in turn allows the simultaneous interaction with two MTs (Schuyler et al., 2003). The function of these MAPs is for instance essential during mitosis where it bundles antiparallel MTs in the spindle midzone (Courtheoux et al., 2009; Kotwaliwale et al., 2007; Loiodice et al., 2005; Meadows and Millar, 2008; Rincon et al., 2017b; Yamashita et al., 2005).

Thus, MAPs establish, maintain, and disassemble MTs to influence their dynamics and organization in the cell, which allows for modulating MTs functions and organization.

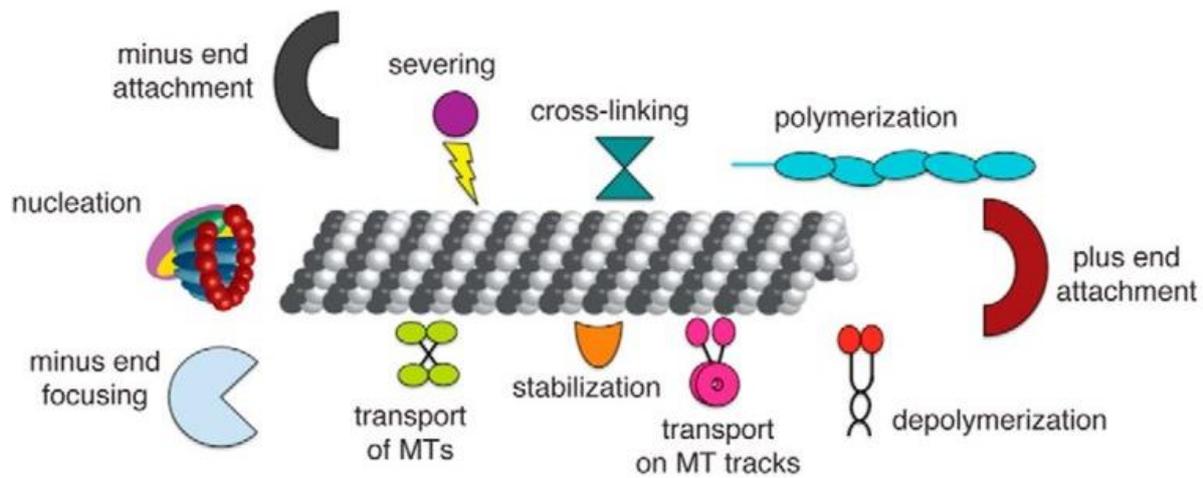


Figure 1-3: Activities of microtubules associated proteins (MAPs)

From Kollman et al 2011.

1.1.4 The tubulin code

Another level of MTs dynamics regulations are the post-translational (PTM) modifications of tubulin. These modifications include polyglutmylation, polyglycylation, detyrosination, acetylation and phosphorylation (Westermann and Weber, 2003). In most cases, the modifications preferentially target tubulin subunits that are incorporated in the MT lattice, thus generating local marks on tubulin. PTMs are also enriched on stable MTs as defined by MTs increased half-life (reviewed in Gadadhar et al., 2017 and Janke, 2014). The modifications can either target the tubulin body (acetylation occurs on lysine 40 of α -tubulin) or the C-terminal tail of tubulin (polyglutamylolation or detyrosination), leading to the formation of lateral chains. Different enzymes are involved in the generation and removal of the modifications (see Figure 1-4). Although research over the last few years has identified most of the PTM actors, few enzymes remain yet to be characterized, such as the enzyme responsible for the removal of glycine(s) from polyglycyolated tubulin.

As the modifications very often occur at the C-terminal tail of tubulin, this creates side chains along the MT lattice. These tails are exposed to the outer surface of the MTs and regulate their interaction with many MAPs. For instance, detyrosination of

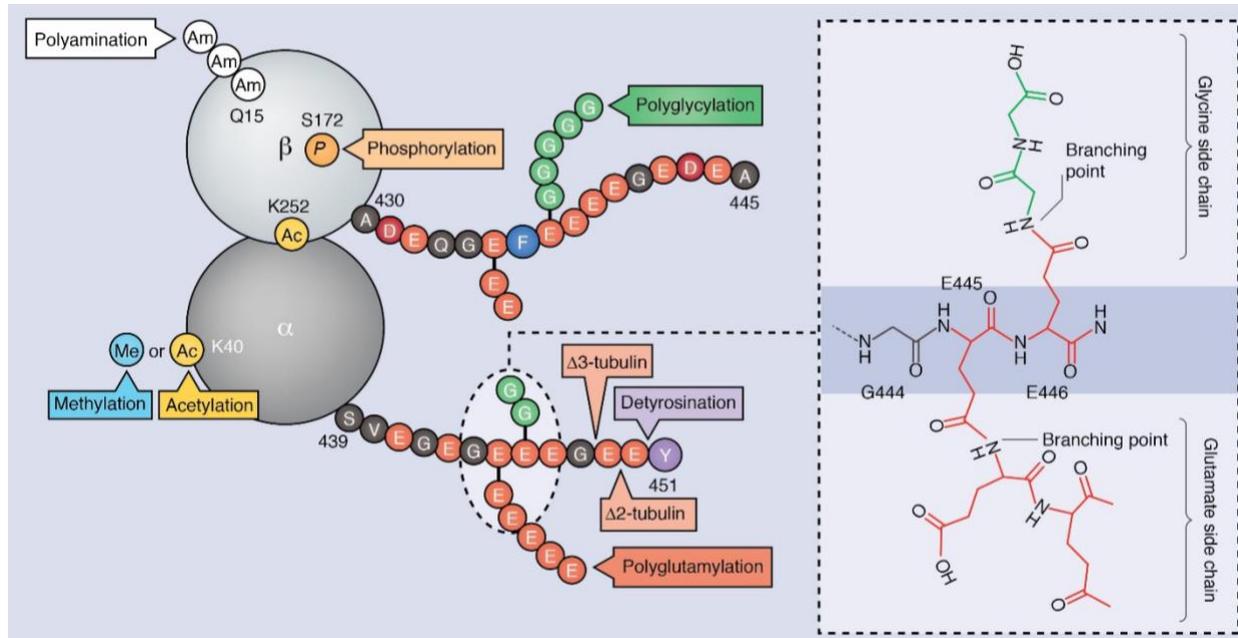


Figure 1-4: Tubulin Posttranslational modifications

From (Gadadhar et al., 2017)

α -tubulin leads to a decrease of CLIP-170 recruitment at the plus end of MT and prevent the molecular motors Kif2C and Kif2A from disassembling MTs (Peris et al., 2009). Moreover, tyrosination has a strong effect on the processivity of the dynein motor (McKenney et al., 2016).

The tubulin acetylation is the only modification that is located in the lumen of the MT lattice and is often associated with stable MTs. It is still unclear if this PTM is a cause or a consequence of MT stability. Nonetheless, it has been shown that tubulin acetylation is required for proper mitotic spindle dynamics (Patel et al., 2016; Wang et al., 2016) and for intracellular transport (Dompierre et al., 2007; Reed et al., 2006). Additionally, it has been shown recently, that tubulin acetylation protects MTs from aging induced fragility and mechanical stress and increases their resistance. Moreover, depletion of the tubulin acetyltransferase TAT1 led to a significant increase in the frequency of MT breakage (Portran et al., 2017; Xu et al.,

2017). Interestingly, it has been proposed that TAT1 could access the lumen of MTs when tubulin dimers are detached from the MT lattice. Acetylated MTs are thought to have increased plasticity because tubulin acetylation weakens lateral interactions of the MTs protofilaments (Portran et al., 2017).

1.2 The centrosome: two centrioles surrounded by the PCM

In animal cells, a centrosome is composed of two centrioles surrounded by the pericentriolar matrix (PCM). The centrioles are MT based structures that undergo duplication once per cell cycle. This process is tightly regulated and relies on the recruitment of essential players to the PCM. In this section, I will first describe the structure and organization of the centriole and PCM. Then, I will depict the maturation of the PCM, a prerequisite for bipolar spindle assembly and centriole duplication at the next cell cycle. At the end of this section, I will detail the duplication process of the centriole in human cells.

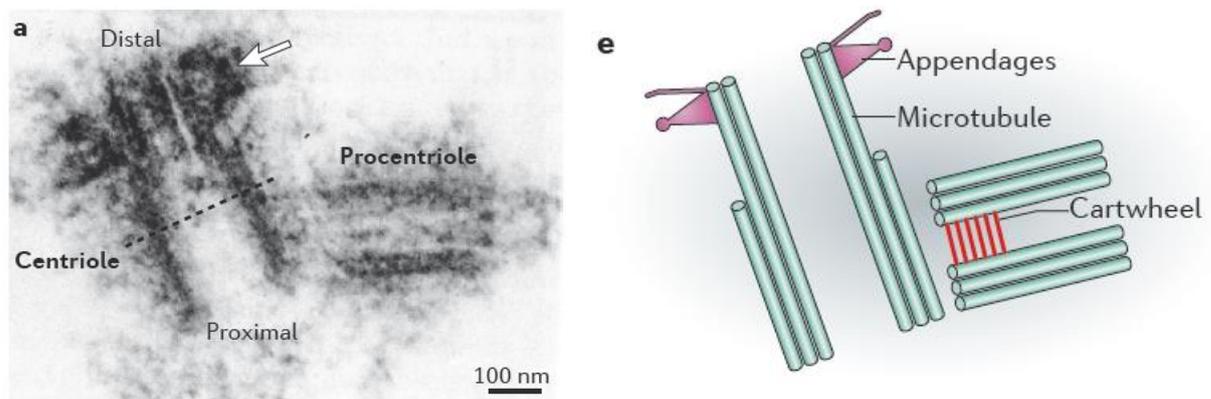


Figure 1-5: Centrioles Structure

From Gönczy, 2012

1.2.1 Centriole remarkable architecture

A centrosome is composed of two centrioles surrounded by the PCM. The centrioles are cylindrical organelles composed of MTs organized in a nine-fold radial symmetry and are approximately 200 nm wide and 500 nm long. The two centrioles are attached with a linker that keeps them in an orthogonal configuration for most of the cell cycle.

The centriole is a polarized organelle, with proximal and distal regions that differ in the number of MTs that they harbour. Indeed, nine MT triplets, the A-, B, and C-MT

within each triplet, are present in the proximal region. In mammalian cells, the C-MT disappears from the distal end which is thus composed of nine set of microtubule doublets (Bernhard and De Harven, 1956). The A-MT is composed of 13 tubulin protofilaments, while the B-MT is fused to the A-MT and is only composed of 10 protofilaments. Similarly, the C-MT is fused to B-MT and is also composed of 10 protofilaments (Figure 1-6). At the proximal part of the centriole, the A-MT of one triplet is connected to the C-of the previous triplet through the A-C linker. At the distal part, this linker disappears and it is unclear how the A- /B-MT connection is maintained (Guichard et al., 2013).

As mentioned above, the centriole wall is composed of nine triplets of MTs in most organisms, but this number can vary in some cases. For instance, *D. melanogaster* centrioles are composed of nine doublets of MTs (Lattao et al., 2017).

Centrioles are transmitted through cell generations, which require a high stability. Consistently, centriolar MTs are highly enriched with PTMs, notably polyglutamylation and acetylation, and these PTM are required for centriole maintenance (Bobinnec et al., 1998). Although the high stability of centrioles was generally thought to be an intrinsic property, PCM is required for their stability. For instance, the complete absence of PCM leads to the loss of centriole (Izquierdo et al., 2014).

The two centrioles are different in age and are characterized by different features. The mother centriole is older and possesses distal (DA) and subdistal appendages (SDA) (Anderson, 1972). The distal appendages are essential for centriole docking at the plasma membrane when the assembly of a cilium is initiated (Ishikawa et al., 2005). The subdistal appendages play a role in MTs anchoring to the PCM during interphase (Bornens, 2002; Piel et al., 2001). For instance, Ninein, a γ -TuRC anchoring protein, is localized at the centriole subdistal appendages (Mogensen et al., 2000).

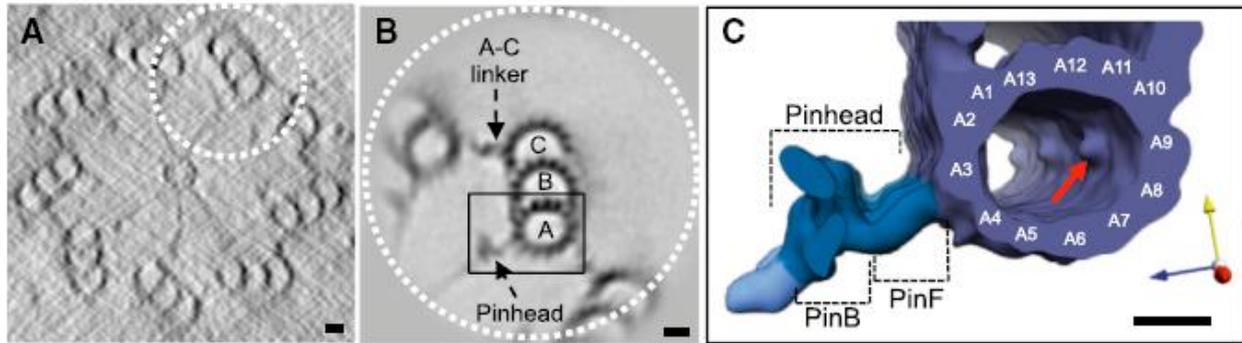


Figure I-6: Organization of the MTs triplets

From Guichard et al. 2013

The cartwheel is a structure localized at the proximal region of the daughter centriole and is characterized with approximately ~100 nm height. It is assembled during early steps of centriole assembly (reviewed in Hirono, 2014). Indeed, the cartwheel can be seen in some organisms before the recruitment of centriolar MTs (Cavalier-Smith, 1974) and is essential for centriole biogenesis in most organisms. The cartwheel is also assembled with a nine-fold symmetry, suggesting that it may impart the signature nine-fold radial symmetry to the centriole (reviewed in Hirono, 2014).

Cartwheel structure has been described in detail in several unicellular organisms like *Paramecium tetraurelia*, *Chlamydomonas reinhardtii* and *Trichonympha* (Cavalier-Smith, 1974; Dippell, 1968; Guichard et al., 2012; Guichard et al., 2013). Recent studies took advantage of the giant cartwheel of *Trichonympha* to perform cryoelectron tomography and subtomogram averaging. In this organism, the cartwheel is ~1500 nm high and its overall structure is very similar to that of other organisms, which made it a suitable model to elucidate the architecture of the cartwheel. This study confirmed the existence of a cartwheel displaying a central hub of ~22-nm-diameter from which nine spokes emanate to connect to MTs. The extremely high resolution achieved in this study allowed the identification of a new structure within the central hub. Indeed, they observed that the central hub

comprises densities within the external ring. These regions were called Cartwheel Inner Densities or CID (Guichard et al., 2013).

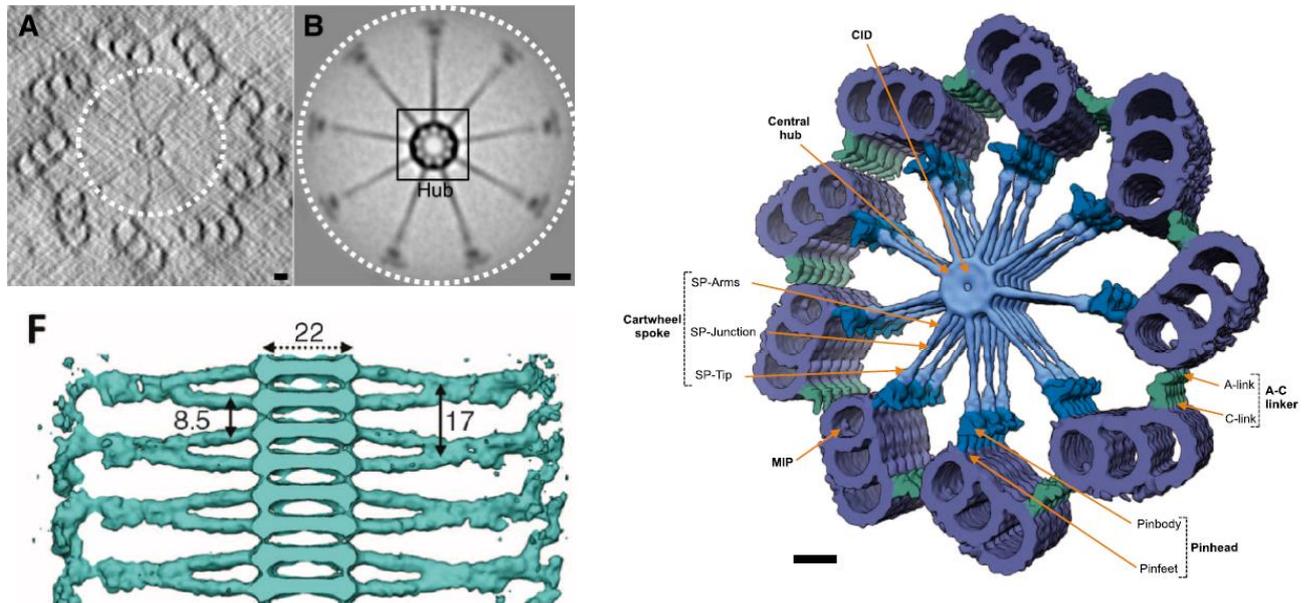


Figure 1-7: Cartwheel Structure

From Guichard et al. 2013

The authors also observed that the MT connection to the cartwheel was achieved through a pinhead region. The Pinhead is a 15 nm long structure that connects the cartwheel to the MTs. It interacts with the third protofilament of the A-MT and can be subdivided into two regions: the Pinbody (PinB) and the Pinfeet (PinF) (Figure 1-6). PinB is a 5 nm long structure that interacts with the cartwheel. The PinF consists of two densities of ~10 nm long that exhibit alternating vertical spacing of 8 and 9 nm and are responsible for MT interaction (Guichard et al., 2013). Interestingly, this study also confirmed the presence of the Pinhead in *C. reinhardtii* centriole.

A previous study of the same group (Guichard et al., 2012) revealed that the Sas6 rings forming the cartwheel (see centriole duplication section for details on Sas6 assembly into rings) were stacked and not forming helices, therefore solving a long standing riddle (Cottee et al., 2011). Each ring is composed of nine homodimers of

Sas6, and one layer of the cartwheel is composed of two rings spaced by ~8.5 nm. The spokes of the rings are connected at the periphery generating a ~17 nm interval at the external side of the cartwheel (Figure 1-7) (Guichard et al., 2012).

To summarize, these two studies allowed the assembly of a 3D map of the cartwheel in *Trypanosoma brucei* and allowed the identification of new features like the A-C linker, the Pinhead and the CID (Figure 1-7) (Guichard et al., 2012; Guichard et al., 2013).

1.2.2 The PCM, amorphous no more

The PCM serves as a platform for protein complexes that regulate MT nucleation, anchoring and organization. Indeed, the γ -TuRC complex is recruited at the PCM and this allows the centrosome to nucleate MTs. It is also essential for centrosome maturation and centriole duplication (for review see Nigg and Stearns, 2011).

In the earliest electron micrographs of centrosomes, the PCM appeared as a densely amorphous mass surrounding the centriole (Robbins et al., 1968). This was probably due to the fact that the PCM does not behave like most ordered proteinaceous assemblies. Thus, since its first depictions the PCM was always described as an amorphous mass surrounding the centriole. It was nevertheless clear that the PCM anchored and nucleated MTs (Gould and Borisy, 1977). In this section, I will describe PCM organization based on the most recent studies, using the human names of proteins to limit confusion (refer to table 1 for homologs in *H. sapiens*, *D. melanogaster* and *C. elegans*).

H.sapiens	D. melanogaster	C.elegans
PLK4	SAK	zyg-1
CEP192	DSpd-2	spd2
CEP152	Asterless	
Pericentrin	D-PLP	
CEP215/ CDK5RAP2	Cnn	Spd-5
STIL	Ana2	Sas-5
SAS-6	DSas6	sas6
CPAP	DSas4	sas4
CEP135	DBld-10	

Table 1 : Protein homology in H.sapiens, D.melanogaster and C.elegans

The resolution required to distinguish subdomains in the PCM was not achieved before the implementation of super-resolution microscopy techniques such as 3D-structured illumination (3D-SIM) or stochastic optical reconstruction microscopy (STORM). These techniques combined with labelling of individual proteins (or domains of the same protein) allowed the mapping of the PCM organization. Four independent studies in human and *Drosophila* cells combined systematic characterization and super resolution to show the existence of PCM layers at the proximity of the centriole (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012; Sonnen et al., 2013). The PCM layers are divided in two domains with different characteristics: at the proximity of the centriole wall, a first layer forms a toroid while a second layer extends away from the centriole and forms a matrix (Figure 1-8).

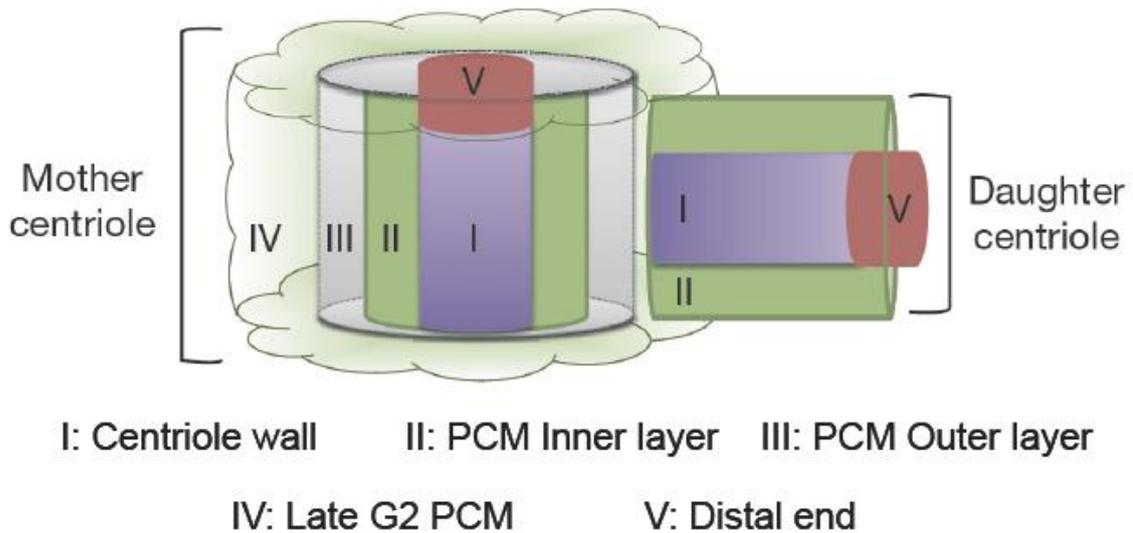


Figure 1-8: organization of the PCM

Adapted From Fu and Glover 2012.

PCM only accumulates at the mother centriole (Conduit et al., 2010; Fu and Glover, 2012). Pericentrin localizes at the pericentriolar region (Region II and III in Figure 1-8). Its C-terminal region contains the PACT domain and is oriented toward the centriole wall. The N-terminal region projects outwards to the periphery. Probing of different PCM proteins domains followed by 3D subvolume alignment and averaging revealed toroids of varying diameters (Doxsey et al., 1994; Lawo et al., 2012; Mennella et al., 2012). Moreover, STORM experiments showed that the pericentrin homolog in *Drosophila* (D-PLP) forms clusters in the PCM region, which might follow the nine-fold symmetry of the centriole (Lawo et al., 2012). These findings suggest that pericentrin/D-PLP forms elongated fibres in the PCM and support the notion that centriole symmetry acts as an organizing principle that extends into the PCM.

Cep152 follows the same organization as pericentrin (Fu and Glover, 2012). Subdiffraction imaging shows that pericentrin and Cep152 are mostly organized in an interleaved fashion with few areas of overlap, largely excluding the formation of hetero-oligomeric structures through their coiled-coil regions (Fu and Glover, 2012).

At the outer layer of PCM (zone IV, Figure 1-8), Cep192 is distributed rather homogeneously around the centriole wall. Analysis with multiple antibodies against different regions of Cep192 showed no clear polarity, suggesting that it is organized in a tightly packed matrix. CDK5RAP2 and γ -tubulin are also found in the matrix and show similar organization to Cep192 (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012; Sonnen et al., 2013).

Thus, in the interphasic centrosome, two layers of organization of the PCM are present: a proximal layer of pericentrin and Cep152, which form fibres extending up to hundreds of nanometres away from the centriole wall; and, in contrast, a matrix of interspersed CDK5RAP2, γ -tubulin, and Cep192 molecules.

1.2.3 Centrosome maturation in mitosis

At the onset of mitosis, the centrosome undergoes maturation, which is characterized by a drastic expansion of the PCM and a robust increase in microtubule-nucleating and organizing activity caused by the increased recruitment of γ -TuRC complex (Khodjakov and Rieder, 1999). During centrosome maturation, the PCM proximal layer acts as a scaffold for PCM expansion. Failure of this process causes defects in bipolar spindle formation and chromosome congression. PCM components, including pericentrin, ninein and CEPI92 are known to be critical for γ -tubulin recruitment to the centrosome (Delgehyr et al., 2005; Takahashi et al., 2002; Zimmerman et al., 2004); Gomez-Ferreria et al., 2007; Joukov et al., 2014).

Pericentrin is required for centrosome maturation and its centrosomal level increases at the onset of mitosis. Moreover, its depletion results in a significant reduction of PCM components at spindle poles and leads to monopolar spindles (Chen et al., 2004; Purohit et al., 1999; Zimmerman et al., 2004). CDK5RAP2 interaction with pericentrin is critical for efficient PCM accumulation during centrosome maturation (Kim and Rhee, 2014). A phospho-mutant of the drosophila homolog of CDK5RAP2 has been shown to impair PCM expansion in mitosis (Conduit et al., 2014). Consistently, overexpression of Pericentrin or CDK5RAP2 has

been shown to artificially promote PCM expansion in interphase arrested mammalian tissue culture cells (Lawo et al., 2012; Loncarek et al., 2008).

Furthermore, Plk1 directly phosphorylates pericentrin at the onset of mitosis. This phosphorylation is essential for the expansion of the PCM (Dzhindzhev et al., 2010; Lee and Rhee, 2011). Pericentrin then recruits a module composed of CEP192, Plk1 and Aurora A to the inner layers of the PCM. This module is essential for the γ -TuRC recruitment of and MTs assembly (Joukov et al., 2014). Indeed, depletion of CEP192 or mutations that impair its interaction with Aurora A or Plk1 impair MTs assembly and bipolar spindle assembly. Although it is not clear if CEP192 interacts directly with the γ -TuRC, the phosphorylation of the N-terminal domain of CEP192 is essential for γ -TuRC localization at the mitotic centrosome. Moreover, depletion of CEP192 in HeLa cells leads to the failure in centrosome separation in mitosis and appearance of monopolar mitotic spindle. This is due to failure of CEP192-associated Plk1 to activate Eg5 by phosphorylation. Eg5 is a molecular motor and is essential for centrosomes separation and bipolar spindle assembly (Joukov et al., 2014).

The kinase Aurora A also plays additional roles in PCM expansion. Aurora A and CDK5RAP2 are interdependent for their centrosomal localization at the centrosome in mitosis (Terada et al., 2003). At mitosis onset, Aurora A and CDK5RAP2 physically interact and this interaction allows Aurora A phosphorylates CDK5RAP2 (Barros et al., 2005; Hannak et al., 2001; Terada et al., 2003).

Centrosome maturation seems therefore to rely on Plk1 and Aurora A driving the assembly of the core structure of mitotic PCM scaffold including pericentrin, CEP192 and CDK5RAP2.

1.2.4 Centriole duplication

The centriole undergoes duplication once, and only once, per cell cycle. The number of centrioles in a cell depends on a tightly controlled centriole cycle: (1) it occurs once per cell cycle during S-phase (temporal control) and (2) only one centriole (litter control) forms per and near each existing centriole (spatial control). Centriole

number defects can arise from cytokinesis failure, simultaneous assembly of many procentrioles around a mother centriole, or multiple duplication rounds during one cell cycle.

Centrosome amplification is a common cancer hallmark. Although multiple centrioles can cluster to nucleate bipolar spindles, the increased incidence of unbalanced merotelic attachments can compromise chromosome segregation fidelity and generate genomic instability (Reviewed in Rhys and Godinho, 2017). It is therefore vital that the cells control the centrosome number by tightly regulating its duplication process.

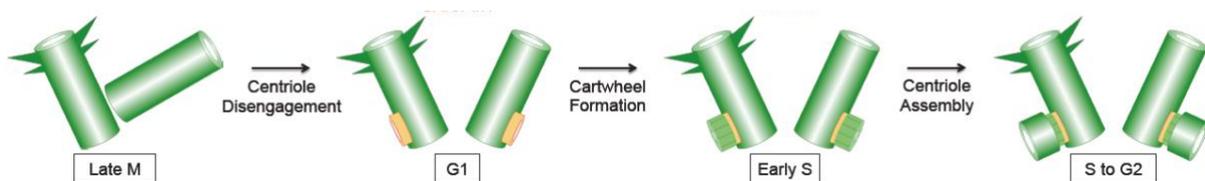


Figure 1-9: Centriole duplication steps

Adapted From D. Gambarotto.

Centriole duplication is a multi-step process with many molecular players (detailed below, Figure 1-9). It starts with the disengagement of the centrioles which is thought to be the first licensing signal for centriole duplication. Later steps define the site of centriole duplication in G1. The cartwheel is assembled at the G1/S transition. The final stage includes elongation of centrioles by addition of tubulin dimers.

The control of centriole assembly and number is tightly coordinated with the DNA replication cycle. At the end of mitosis/beginning of G1-phase, DNA strands are unwound for licensing factors to prime replication origins. The replication machinery is only able to access to DNA after licensing, and then bind DNA and stimulate replication during the following S-phase. Similarly, centriole disengagement in mitosis was suggested as the 'licensing' step for S-phase centriole

biogenesis (Tsou et al., 2009). Both cycles rely on a temporal biphasic division where licensing and new structure synthesis are mutually exclusive.

Centriole duplication process have been described through a series of pioneering studies performed in *C. elegans* embryos where centrosome duplication defects were easy to follow during the first divisions. Indeed, as with many species, *C. elegans* oocytes lack centrioles and the two centrioles that are brought by the sperm after fertilization are duplicated during the first mitosis (Kirkham et al., 2003). If the centriole duplication is impaired, a monopolar spindle will be assembled in the second mitosis. A genome- wide screen in *C. elegans* has brought to light five essential proteins for centriole duplication: ZYG1, Spd-2, Sas4, Sas5 and Sas6 (Dammermann et al., 2004; Delattre et al., 2006; Kemp et al., 2004; Kirkham et al., 2003; Leidel et al., 2005; O'Connell et al., 2001; Pelletier et al., 2004).

Following the *C. elegans* studies, homolog proteins of the five initial components and additional components have been identified in several organisms including *Drosophila* and *H. sapiens*. In this part, I will mainly focus on the mammalian mechanisms of centriole assembly. I will occasionally refer to other organisms when necessary, using the human names of proteins to limit confusions (see table 1 for protein homology in *H. sapiens*, *D. melanogaster* and *C. elegans*).

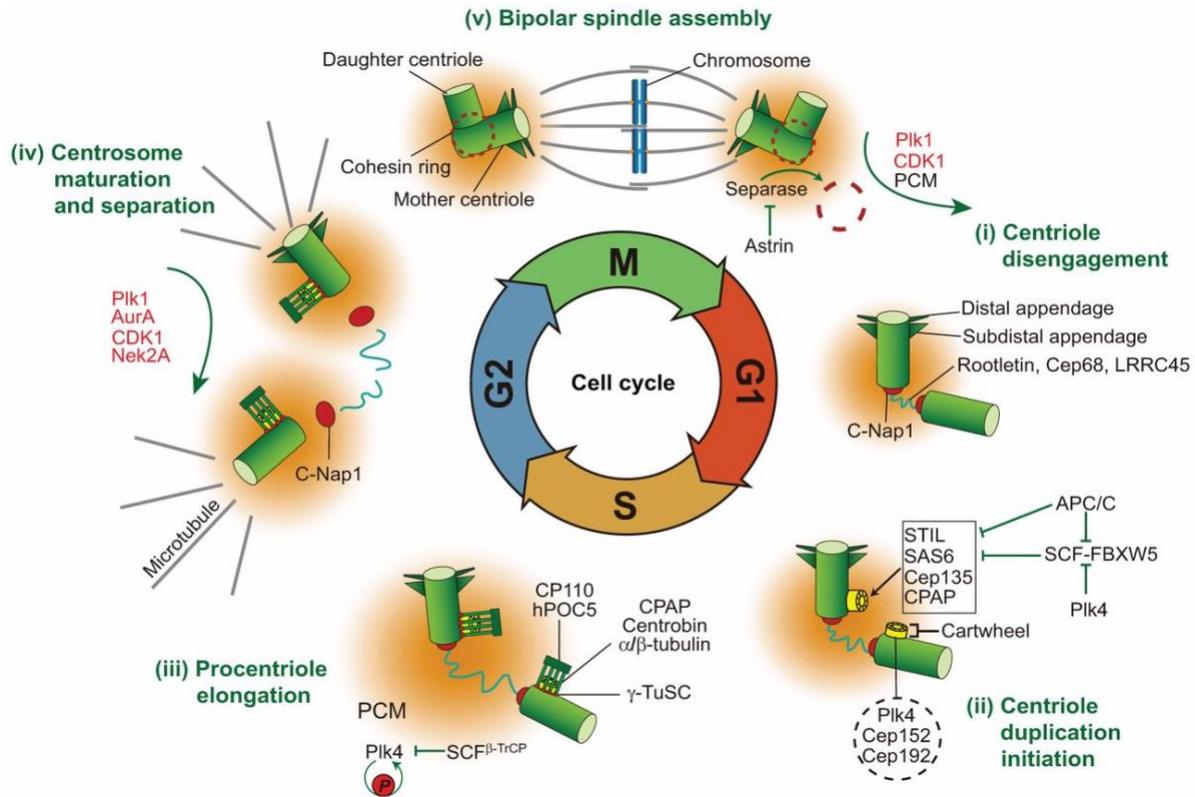


Figure 1-110 Interplay between the centrosome cycle and the cell

Adapted From Wang et al, 2014.

a. Centriole disengagement

Towards the end of mitosis, centrioles lose their orthogonal arrangement and become disengaged (Kuriyama and Borisy, 1981). This process involves the disorientation and physical separation of mother and daughter centrioles. Disengagement is an important licensing step for the next round of centrosome replication, preventing reduplication within one cell cycle (Tsou and Stearns, 2006). Engagement is thought to be a critical block to reduplication inherent to the centriole. Consistent with this, physical removal of the daughter centriole by laser ablation induces reduplication of the daughter at the proximal end of the mother centriole (Loncarek et al., 2008).

The mechanism of centriole disengagement is similar to that of sister chromatid separation during anaphase. Sister chromatids are held together by the ring cohesin complex; dissociation of this complex by separase-mediated cleavage of the cohesion subunit Scc1 allows segregation of sister chromatids. The cohesin complex also localizes to the junction of engaged centrioles and is cleaved there by separase-mediated Scc1 proteolysis (Schockel et al., 2011; Tsou et al., 2009). Separase is activated when its inhibitor securin is targeted for degradation by the E3 ligase anaphase promoting complex/cyclosome (APC/C)–Cdc20 and thus contributes to centriole disengagement (Prosser et al., 2012).

The polo-like kinase 1 (Plk1) functions in cooperation with separase to trigger centriole disengagement (Tsou et al., 2009), and also mediates an APC/C–Cdc20-independent pathway of disengagement (Hatano and Sluder, 2012; Prosser et al., 2012). Plk1 interacts with the smaller variant of Shugoshin 1 (Sgo1), sSgo1, which localizes to the centrosome in a Plk1-dependent manner and functions in the protection of centriole cohesion (Wang et al., 2008). Furthermore, cleavage of cohesin is insufficient for centriole disengagement in *Drosophila* (Oliveira and Nasmyth, 2013). In addition to cohesin, pericentrin is a crucial target of separase at the centrosome and is important for centriole disengagement as it protects the engaged centrioles from premature disengagement (Lee and Rhee, 2011; Matsuo et al., 2012). Recent studies in *C. elegans* showed that MT-dependent forces also promote centriole disengagement (Cabral et al., 2013). In addition to disengagement, Plk1-dependent phosphorylation of daughter centrioles components in early mitosis is also a licensing step for centriole duplication in the next cell cycle (Kong et al., 2014; Loncarek et al., 2010; Wang and Zheng, 2011).

After centriole disengagement, a proteinaceous linker composed of C-Napl and the filamentous protein rootletin is established between the two centrioles and physically connects them during the next interphase until entry into mitosis (Mardin and Schiebel, 2012). This proteinaceous linker is referred to as centrosome cohesion or the G1-G2 tether (Graser et al., 2007; Nigg and Stearns, 2011).

b. Centriole duplication initiation

Plk4 is the master regulator of centriole duplication (Bettencourt-Dias et al., 2005; Habedanck et al., 2005). Many studies have shown that its depletion lead to centriole loss and its overexpression lead to supernumerary centrosomes (Kleylein-Sohn et al 2007 ; Rodrigues-Martins et al., 2007). Interestingly, in this context, instead of one daughter centriole at the tip of each mother, a number of 5 to 6 centrioles surrounds the mother centriole. This conformation resembles a flower petal organization and is called the 'rosette' conformation (Kleylein-Sohn et al., 2007). The centrioles are assembled simultaneously around the mother centriole and require a number of centriole components. This study shows that Sas6, CPAP, CP110 and Cep135 are essential for centriole assembly.

Plk4 recruitment to the mother centriole occurs in late G1. At first, Plk4 is localized all around the centriole. Its recruitment depends on Cep192 and Cep152 and is regulated in space and time. Cep192 forms a torus around the centriole and interacts with Plk4 through its Polo Box domain forming a scaffold around the centriole (Kim et al., 2013; Sonnen et al., 2013). Cep152 is also recruited to the centriole in a Cep192-dependant mechanism. It stochastically interacts with Cep192-bound Plk4. This interaction leads to a scaffold switching where Plk4 is relocated to the edge of the Cep152 ring, resulting in Plk4 localization in a larger Plk4 ring (See Figure 1-II; Park et al., 2014). The competition between Cep192 and Cep152 interaction with Plk4 is the consequence of the binding of the two proteins to the same domain of Plk4 (Park et al., 2014). Moreover, molecule counting has shown that the number of Cep192 molecules is much lower than the number of Cep152 molecules. Additionally, Plk4 interacts with 2/2 stoichiometry with Cep192 while one Cep152 molecule interacts with 2 Plk4 molecules. Thus, once Cep152 is recruited at the centriole, Plk4 is more likely to interact with Cep152 leading to the scaffold switch of its localization (Bauer et al., 2016; Park et al., 2014).

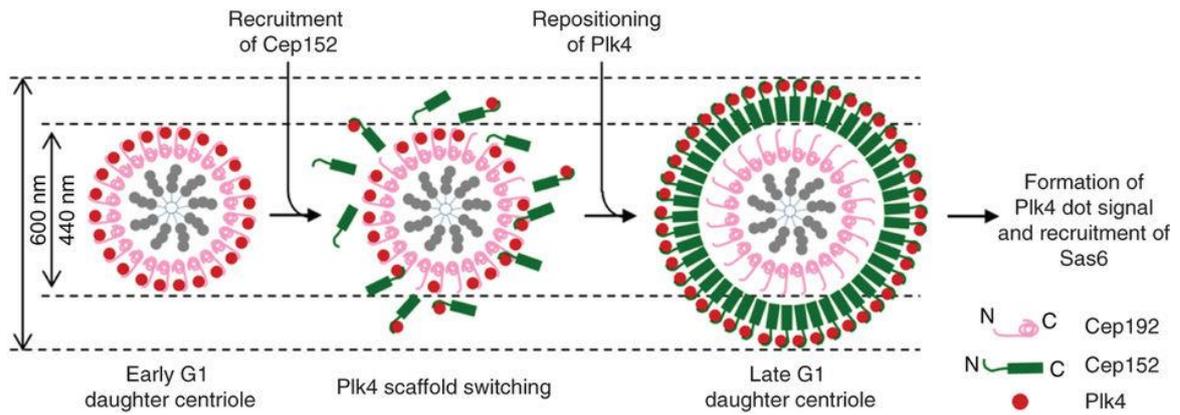


Figure 1-II: Spatial and temporal recruitment of Plk4 to the centriole during centrosome maturation.

From (Park et al., 2014)

In order to duplicate the centriole at one site at the proximal end of the mother centriole, Plk4 is focused to only one foci at the proximal end. This event is initiated in G1 to S transition and precedes cartwheel assembly (Park et al., 2014; Sonnen et al., 2013). Plk4 focusing is achieved through the degradation of Plk4 around the centriole. Indeed, Plk4, present as a dimer, trans-auto phosphorylates at the serine 305 in its T-loop (Lopes et al., 2015) which leads to its degradation via the proteasome (Sillibourne et al., 2010). Interestingly, Plk4 does not undergo degradation at one specific site: the centriole duplication site. How is Plk4 “protected” from its own destruction at only one specific site? Plk4 interaction with STIL protects Plk4 from being degraded (Arquint et al., 2015; Klebba et al., 2015; Moyer et al., 2015). STIL binds to Plk4 through the Polo Box3 domain. This interaction is thought to release the inhibition of Plk4 activity, allowing its full activation (Moyer et al., 2015). Once activated, Plk4 auto-phosphorylates two additional residues, thus increasing the level of prevention of auto inhibition (Klebba et al., 2015).

Moreover, STIL is able to oligomerize, which allows its enrichment to one specific site and inhibits its recruitment to other centriolar sites. This mechanism controls

new centriole number by ensuring that Plk4 is only maintained at one site of the centriole. Consistently, overexpression of Plk4 can promote the recruitment of STIL and Sas6 and leads to supernumerary centrioles (Kleylein-Sohn et al., 2007; Stevens et al., 2010).

Interestingly, the active form of Plk4 is only found at the centrosome, suggesting a local regulation (Sillibourne et al., 2010). Plk4 is also found in the cytoplasm, and artificially increased levels of Plk4 in the cytoplasm lead to *de novo* centriole duplication (Lopes et al., 2015). This result suggests that Plk4 levels may need to reach a certain threshold to induce centriole assembly and that the centrosome acts as a concentrator of Plk4 leading eventually to its activation and to the assembly of a new centriole.

c. Cartwheel assembly

STIL is phosphorylated upon its interaction with Plk4 and this phosphorylation is essential for Sas6 recruitment to the new procentriole site (Kratz et al., 2015). Sas6 is a key component of the cartwheel and has been shown to be essential for centriole biogenesis in numerous organisms. It is composed of globular N-terminal domain followed by a long coiled coil region and an unstructured C-terminal domain (Leidel et al., 2005). Sas6 forms dimers through its coiled coil domain and is abundant in the cytoplasm (Figure 1-12; Bauer et al., 2016). In human cells, Sas6 is predominantly found as dimers in the cytoplasm (Keller et al., 2014). Structural and biophysical experiments have shown that Sas6 is able to oligomerize and form heterodimers that

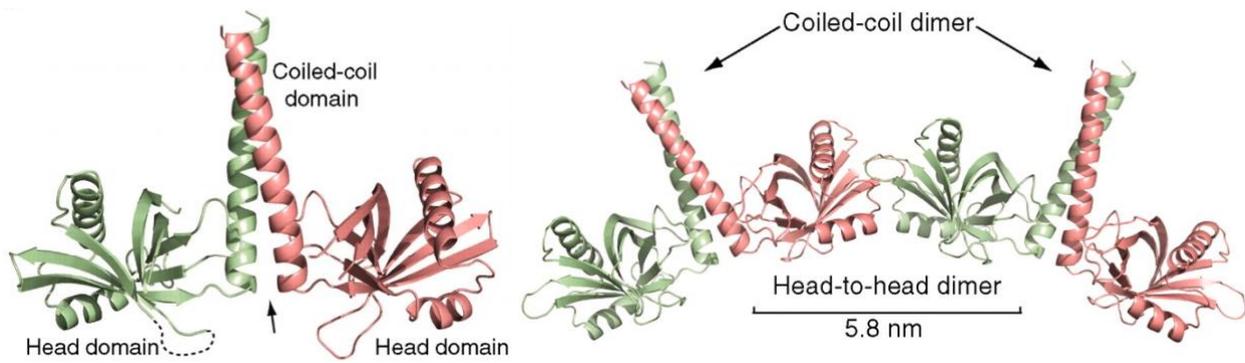


Figure 1-12: Sas6 structure and mechanisms of its oligomerization

From (van Breugel et al., 2011)

assemble in a ring-containing structure. These rings displayed a high proportion of nine dimers of Sas6 (Kitagawa et al., 2011). The inner circle of Sas6 rings is formed by the N-termini interacting together. The inner circle of these structures has a ~22 nm diameter, which is analogous to the hub of the *in vivo* structure. Moreover, spokes emanate every 40° from the ring forming *in vitro*, reminiscent of the *in vivo* structure. (Kitagawa et al., 2011; van Breugel et al., 2011). These observations suggest that the self-assembly of Sas6 could be at the origin of the nine fold symmetry.

Since Sas6 is abundant in the cytoplasm and is able to self-assemble, one puzzling question is why cartwheels are only assembled once per cell cycle and at one specific location. One answer to this question comes from *in vitro* analysis of the affinity of the N-termini interaction (Kitagawa et al., 2011; van Breugel et al., 2011). The affinity of the interaction is very weak (50-100 μ M) so Sas6 assembly is very unlikely to happen in the cytoplasm. On the other hand, the number of Sas6 molecules recruited by the Plk4-STIL complex is high enough to induce Sas6 assembly into rings. Again, like for Plk4, the centrosome seems to act like a concentrator to achieve its own duplication cycle.

Another interesting observation comes from theoretical experiments (Klein et al., 2016) which predict that diffusing molecules of Sas6 are very unlikely to self-assemble into ring like structure. But if Sas6 molecules are now constrained onto a

surface, Sas6 oligomerization is predicted to happen with high synergy and occur orthogonally to the surface. *In vivo*, this surface is thought to be the Cep152 toroid, but the exact mechanism of how the rings of the cartwheel are assembled is still unknown.

Therefore Plk4-STIL and Sas6 are recruited to the centriole in a multi-step process that will allow the establishment of the duplication site and the assembly of the cartwheel.

d. Procentriole elongation

After the formation of the procentriole, a new centriole start elongating in S phase, followed by elongation of the distal region during G2 phase. A number of conserved molecules are involved in the regulation of centriole elongation.

Cep135 is a highly conserved centrosomal protein that is involved in cartwheel assembly. Cep135 directly interacts with SAS-6 via its C-terminal region and with MTs via its N-terminal region, acting as the physical link between SAS-6 and MTs (Lin et al., 2013). Depletion of Cep135 results in the formation of abnormal centriole structures with altered MT triplet numbers and a short centriole.

Cep135 interacts with CPAP via its N-terminal region and is involved in CPAP-induced centriole elongation (Lin et al., 2013). CPAP stabilizes the cartwheel structure and plays an important role in recruiting MTs to the cartwheel. Consistently, overexpression of CPAP results in elongated centrioles (Kohlmaier et al., 2009; Schmidt et al., 2009; Tang et al., 2009).

Cep120, which localizes to daughter centriole, is essential for centriole assembly, (Mahjoub et al., 2010) and also interacts with CPAP and Spicel to positively regulate centriole elongation (Comartin et al., 2013; Lin et al., 2013). In addition, Plk2 phosphorylation is critical for the role of CPAP in procentriole formation and centriole elongation (Chang et al., 2010). A daughter centriole protein, centrobilin, is recruited to the centrosome in the early steps of centriole duplication, where it

interacts with CPAP and α/β -tubulin dimers and promotes the elongation and stability of the centrioles (Gudi et al., 2015; Gudi et al., 2014).

Distinct proximal and distal elongation steps have been identified. The centrin-binding protein, POC5, and OFDI localize to the distal portion of the centriole and are required for distal elongation (Azimzadeh et al., 2009; Singla et al., 2010). Moreover, Pocl play a role in the early steps of centriole duplication and the later step of elongation (Keller et al., 2009).

CPII0 and its interacting proteins Cep97 and Kif24 act as capping structures that determine the final length of the centriole. CPII0 localizes to the distal end of the centriole and its depletion impairs the regulation of centriole length, resulting in a long centriole (Schmidt et al., 2009). Cep97 recruits CPII0 to the centrosome; depletion of Cep97 also results in centriole elongation (Spektor et al., 2007). Moreover, loss of Kif24 leads to the disappearance of CPII0 from the mother centriole but not from abnormally long centrioles (Kobayashi et al., 2011).

1.3 Cellular functions of the centrosome

1.3.1 General principles of cell cycle progression

The cell division cycle allows one cell to become two. It is used by all cellular life forms. It allows growth in cell number while conserving the genetic identity of the mother cell. We can divide the cell division cycle in two parts: mitosis and interphase. Interphase is devoted to growth and duplication of the genetic material. Mitosis includes the separation of the two copies of the genetic material and is followed by cytokinesis. The progression of the cell cycle is controlled by the cell cycle machinery and the fidelity of the whole process is ensured by checkpoints mechanisms that halt cell cycle progression when their requirements are not fulfilled.

a. Interphase: getting ready to divide

Before a cell divides, it must undergo a series of events that will ensure genetic integrity and cell viability in the next generation. The cell needs to grow enough so that after division the daughter cells are about the same size as the mother cell. The cell also needs to duplicate its genomic material and make sure that there are no mistakes when copying the DNA, or correct them, in order to preserve the genomic integrity. In parallel to DNA replication, the main microtubule organizing center, the centrosome starts duplicating. Its duplication will be completed by the time the cells start cell division (For review see (Bartek and Lukas, 2001; Harper and Brooks, 2005).

Interphase is thus classically divided in three phases which in a chronological order are: G1, S and G2. In G1 the newborn cell grows, synthesizes the necessary proteins and takes the decision of engaging into another cell cycle or not. If the cell decides to engage into a cell cycle, G1 phase will be followed by the S phase during which the genetic material is duplicated. Finally, in G2 phase the cell checks that the duplication went right and no mistakes were made, if necessary it will engage correction mechanisms.

b. Mitosis: segregating cellular components in two equal sets

Once faithfully replicated, the genomic material needs to be segregated in two identical sets during mitosis. The separation of two copies of genetic material during mitosis is a multistep process mediated by the mitotic spindle. The spindle starts assembling in prometaphase while chromosomes condense. This is followed by nuclear envelope breakdown (NEBD) in prometaphase, chromosome capture by the spindle during metaphase and segregation of sister chromatids, each containing one copy of the genetic material in anaphase.

The activation of Cdk1 promotes chromosome condensation and the separation of the duplicated centrosomes which will form the poles of the spindle. The complete formation of the spindle and the above mentioned rearrangements of the nuclear envelope happen during Prometaphase.

During Metaphase the chromosomes are correctly attached by their centromeres which will build up protein platforms, the kinetochores. The role of the kinetochores is to attach the chromosomes to MTs of the spindle which will pull them apart. Until all the chromosomes are bi-oriented, unattached kinetochores produce a checkpoint signal that prevents the metaphase/anaphase transition. This control mechanism is the spindle assembly checkpoint (SAC) which assembles at the kinetochore. The SAC remains active until all kinetochores have been correctly attached and there is a uniform tension in each pair of sister chromatids. At this stage the cell has established the metaphase plate. Once the SAC is satisfied, another regulatory complex is switched on, the anaphase promoting complex (APC). The APC as its name states allows the cells to enter Anaphase by cleavage of the cohesin link that holds sister chromatid together. The tension exerted by MTs on kinetochores separate chromosomes in two movements: anaphase A, in which chromosomes are pulled towards the spindle poles by contraction of the kinetochore Mts; and anaphase B, in which the spindles are further separated from each other by the elongation of interpolar MTs. The chromosomes then start decondensing and the

nuclear envelope reassembles. This last phase corresponds to Telophase (Figure 1-13; for reviews see Gheghiani and Gavet, 2014; Rhind and Russell, 2012).

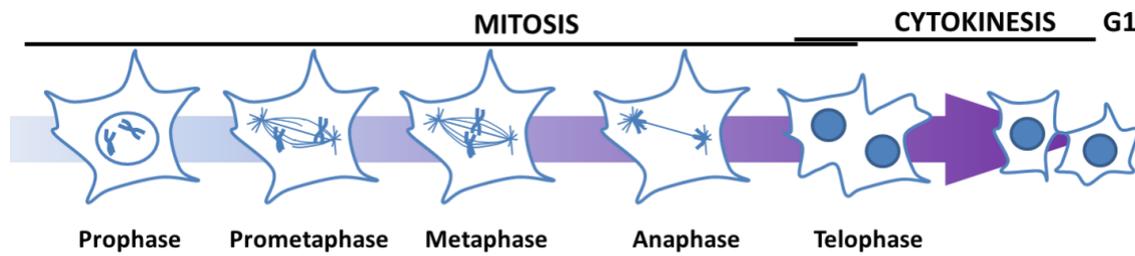


Figure 1-13 Mitotic phases

Adapted from Rhind and Russel, 2012.

Molecular mechanisms controlling Mitosis Commitment and exit

Mitosis initiation is driven by extensive protein phosphorylation and dephosphorylation events. Historically, these events were thought to be the direct consequence of Cdk1/CyclinB activity, but the last years have brought the role of the phosphatases in light. In addition to Cdk1/CyclinB activity, the phosphatases PPI and PP2A have also been shown to control mitotic progression and exit (detailed later).

The start of mitosis is triggered by the activation of the mitotic kinase Cyclin-dependent kinase (Cdk1) in prophase. Cdk1 is the key regulator of mitotic transition. Activation of the kinase drives entry into mitosis (Gould and Nurse, 1989; Nurse, 1990), and its inactivation drives exit from mitosis (Murray, 1989). Cdk1 kinase requires an activating partner—a cyclin. Cyclin B is a pivotal activator of Cdk1 in mitosis. The Cdk1-Cyclin B complex is also known as MPF (mitosis promoting factor) as it was first discovered for its role as main mitotic inducer. During mitotic exit, cyclin B is degraded by the ubiquitin-proteasome pathway (Glotzer et al., 1991). Cyclin B degradation causes irreversible inactivation of Cdk1 and therefore triggers the mitotic exit. In addition to the activation by cyclins, Cdk1 activity can be

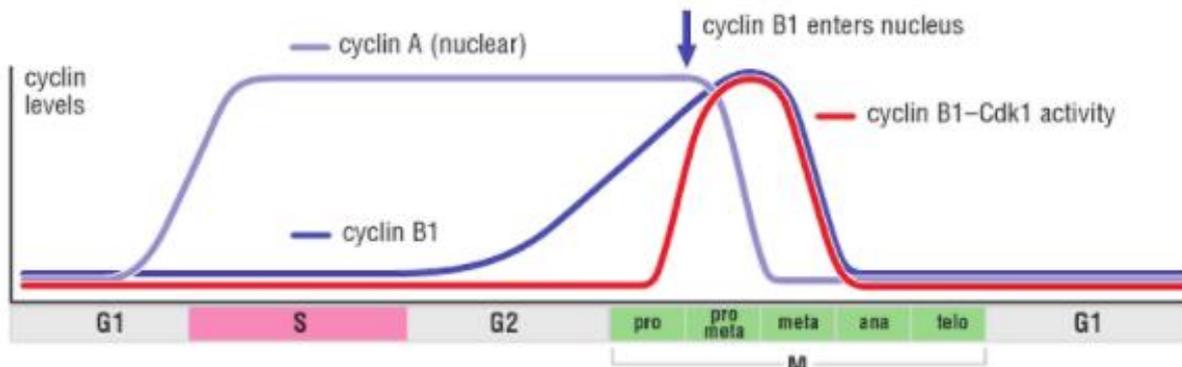


Figure 1-14: Cyclin levels in mammalian cells

negatively regulated upon phosphorylation by the tyrosine kinase Wee1 (Parker and Piwnica-Worms, 1992). This inhibitory phosphorylation is removed by Cdc25 phosphatases. Polo-like kinase 1 (Plk1) has been reported to phosphorylate, at least in vitro, Cdc25 and Wee1 (Lobjois et al., 2011; Nakajima et al., 2003; Roshak et al., 2000; Watanabe et al., 2005), and is required for Cdk1-CyclinB1 activation and mitotic entry (Gheghiani et al., 2017).

As mentioned above, several studies have shown that the phosphatases PPI and PP2A are key regulators of mitosis. PP2A complexes comprise a catalytic and scaffolding subunit and a regulatory B subunit. It has been shown recently that PPI can interact with two PP2A complexes that are associated with different B subunits B55 and B56 (Grallert et al., 2015).

At mitotic onset, PPI, PP2A-B55 and PP2A-B56 are maintained inactive through their phosphorylation by Cdk1/CyclinB1 (Figure 1-15). As mitosis progresses, Cdk1/CyclinB1 levels decrease, which allows dephosphorylation of PPI and its activation. This, in turn, allows PPI to interact with PP2A-B55 and to dephosphorylate it. The activated PP2A-B55 complex subsequently interacts with PP2A-B56. However, there is slight delay between the interaction of PPI with PP2A-B56 and its activation since PP2A-B56 is also phosphorylated by Plk1. Consistently, when Plk1 levels decrease at the end of mitosis, PP2A-B56 can now be activated, reinforcing mitotic exit (Grallert et al., 2015). This elegant relay of phosphatases activations has been characterized in fission yeast, and it is thought to be conserved

in human cells as these interaction could be reproduced *in vitro* using human proteins (Figure I-15; Grallert et al., 2015).

Another level of regulation depends on PPI and PP2A-B56. Recent data in human cells demonstrated that both PPI and PP2A-B55 participate in the dephosphorylation of the kinase Greatwall (Gwl) on its activating sites during meiotic and mitotic exit. Greatwall is a kinase that is activated upon mitotic entry

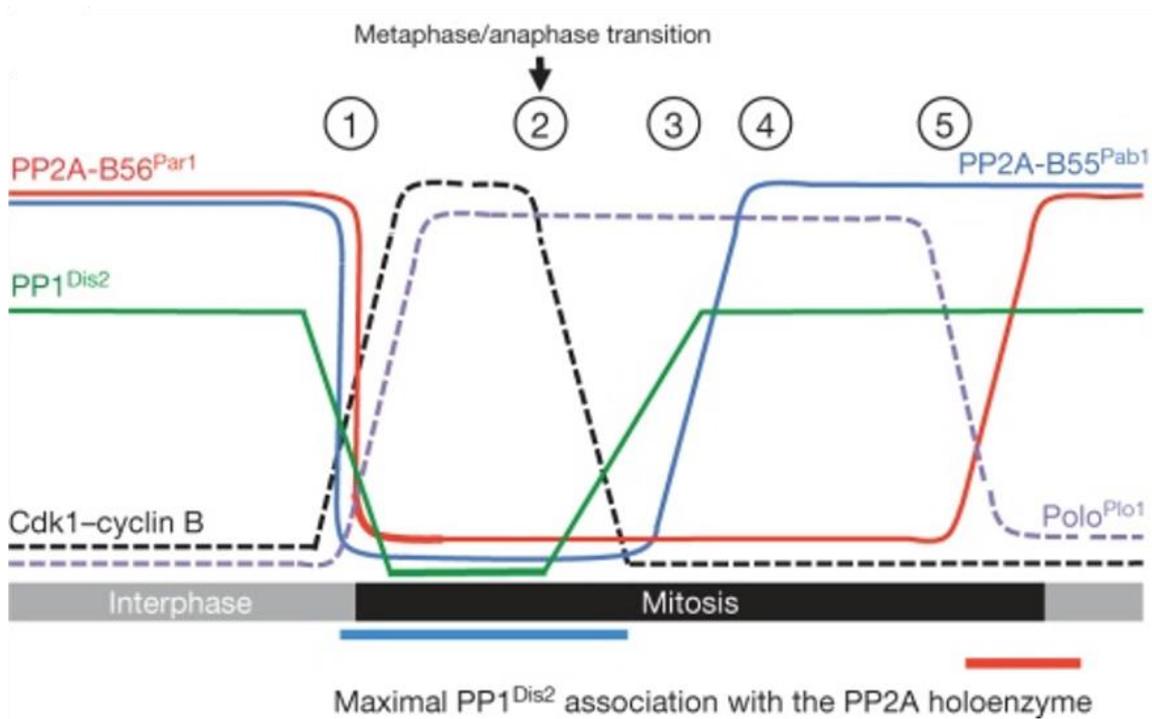


Figure I-15: Mitotic kinases and phosphatases levels variation in mitosis.

From (Grallert et al., 2015).

and is essential for PP2A-B55 inhibition (Alvarez-Fernandez et al., 2013; Gharbi-Ayachi et al., 2010; Mochida et al., 2010; Vigneron et al., 2009). This inhibition results in the stable phosphorylation of Cdk1-Cyclin-B substrates at mitotic entry. A recent study has shown that Gwl inactivation relies on its phosphorylation by PPI, which in turn allow the activation of PP2A-B56 (Ma et al., 2016).

In summary, mitosis entry and exit are key events and their regulation relies on parallel and interdependent phosphorylations and dephosphorylations events.

These events ensure the correct timing and therefore the correct completion of such an important step for cell survival and proliferation (Figure 1-15).

c. Cytokinesis: making two independent cellular entities

Cytokinesis is the final step of cell division which physically and irreversibly separates the mother cell in two daughter cells. Since it is irreversible, cytokinesis has to occur at the proper time and in a properly defined position and orientation in order to avoid chromosome segregation defects.

Cytokinesis in fungal amoeboid and animal cells takes place in four steps. First the cell determines the division site; it is an important step since the division plane needs to take into account the spindle axis and other factors in order to avoid cut phenotypes. Once the division site has been chosen, the contractile ring assembles. Its main components are actin cables and myosin II. The third step is the constriction of the actomyosin ring and formation of the cleavage furrow. Finally the separation of the daughter cells occurs. In metazoans it happens in a process called abscission and in fission yeast it requires the digestion of the cell wall that separates the two daughter cells (Glotzer, 2017; Oliferenko et al., 2009; Pollard, 2017).

1.3.2 The centrosome functions in cell cycle progression

The first link between the centrosomes and cell cycle progression was made after injection of purified centrosomes into *Xenopus* or starfish oocytes mimicked fertilization and led to initiation of parthenogenetic development (Klotz et al., 1990; Picard et al., 1987). These two initial observations were rapidly followed by many other studies suggesting that the centrosome could be an important hub for mitotic kinases, as many were found to localize to the centrosome at G2/M transition. Indeed, Cdk1/CyclinB, Cdc25, Plk1 and Aurora A were all localized to the centrosome (Bailly et al., 1989; Debec and Montmory, 1992; Dutertre et al., 2002; Golsteyn et al., 1995; Gopalan et al., 1997; Kimura et al., 1997; Roghi et al., 1998). Importantly, the initial activation of Cdk1/CyclinB complex was observed at the centrosome and it

was shown that Plk1 phosphorylates cyclin B1 on centrosomes in prophase (Gavet and Pines, 2010; Jackman et al., 2003). Accordingly, Cdc25 was also found to act specifically on Cdk1/CyclinB at the centrosome (Dutertre et al., 2002; Lindqvist et al., 2005). These localizations and activations strongly suggested that the centrosome facilitates the activation of Cdk1/CyclinB by bringing the required regulatory components in close proximity. Although, some cells can progress through mitosis despite centrosome loss, cell cycle progression was impaired in DT40 B cells where centrosomes were genetically removed (Basto et al., 2006; Khodjakov and Rieder, 2001; Sir et al., 2013). These apparently contradictory results indicate that in some organisms additional mechanisms or components could rescue centrosome loss.

Additionally, centrosome has been linked to cytokinesis in mammalian cells and in yeast. Indeed, DT40 B cells (mentioned above, Sir et al., 2013) display cytokinesis defects in addition to cell cycle progression defects. Cytokinesis defects have also been observed in cells where centrosome was laser ablated (Khodjakov and Rieder, 2001).

Moreover, the mother centriole has been shown to move towards the midbody at the end of mitosis and to localize to the mitotic bridge (Piel et al., 2000). Finally, a growing list of centrosomal proteins were found to localize to the midbody towards the end of mitosis (Jakobsen et al., 2011). However, unlike in yeast (see section 2.4) the centrosomal function in cytokinesis is not clearly established.

1.3.3 The centrosome surveillance pathway

Early studies showed that cell cycle progression from G1 to S was impaired when centrosomes were removed from certain cell types by microsurgery or laser ablation (Hinchcliffe et al., 2001; Khodjakov and Rieder, 2001; Maniotis and Schliwa, 1991). Importantly, depletion of a large number of centriole components also arrested cells in G1 and was concomitant with an accumulation of p53 in the nucleus. Remarkably,

cell lines with compromised p53 like HeLa cells did not undergo G1 arrest (Mikulec et al., 2007) and deletion of p53 in non-cancerous cell line alleviated the cell cycle block caused by the centrosome (Wong et al., 2015). These results led to the hypothesis that a G1/S checkpoint might monitor centrosome status, to ensure that only cells with the correct number of centriole can progress through the cell cycle. This checkpoint would require p53 to protect against genome instability by preventing the growth of cells with too few centrosomes.

One difficulty in deciphering the mechanisms of this pathway is the various roles of p53 in cell maintenance. Evidences for a direct effect of centrosome loss on p53 activation was difficult to distinguish from other cellular mechanisms. A long standing question in the centrosome field, was if the cell cycle arrest is a consequence of centrosome defects or loss, or if it is an indirect consequence of a prolonged mitosis or DNA damage in the cell. Indeed, centrosome loss induces a prolongation of mitosis duration and an increase in chromosome missegregation (Wong et al., 2015). It has also been observed in RPE1 cells (non-cancerous cell line) that at a threshold of 90 min of mitosis duration, the cells would arrest in the next cell cycle (Wong et al., 2015). The mechanisms of p53 activation in these cells remained uncharacterized until very recently.

Two new technologies have allowed the characterization of the players involved in the centrosome surveillance pathway. First, the development of a chemical inhibitor of Plk4 called centrinone (Wong et al., 2015). Centrinone allowed the reversible depletion of centrosomes in cells. The complete loss of centrosomes requires several cycles of cell division without centrosome duplication. Remarkably, if the drug is removed, the cells can assemble centrosomes *de novo*. This drug thus provided a tool to study the potential existence of mechanisms sensing the centrioles number.

A second technological breakthrough came with the wide-scale implementation of CRISPR/Cas9 technology. This technique allows modification of endogenous genes by tagging/mutating the target gene or by performing conditional knock-outs.

Three studies have identified almost simultaneously the first effectors of p53 activation after centrosome loss induced by Plk4 inhibition and a genome wide screen for suppressors of the cell cycle arrest in absence of centrosomes. One study used the centrinone drug (Fong et al., 2016) while another combined chemical genetic approaches to specifically inhibit Plk4 kinase activity. Mutation of a single amino acid in the ATP-binding pocket of Plk4 creates an analogue-sensitive (AS) kinase that can be inhibited with non-hydrolysable ATP analogues (Lambrus et al., 2016). CRISPR/Cas9 was used to knock in the Plk4 AS mutation into the endogenous Plk4 locus in non-transformed RPE1 cells. The third study (Meitinger et al., 2016), established a conditional KO of Plk4.

The three groups identified 53BP1, described below as well as the deubiquitinase USP28 as two essential components acting upstream of p53, as deletion of either of these components impairs induction of p53 and G1 arrest after centrosome depletion.

53BP1 is a key scaffold protein recruited to damaged chromatin after DNA double-strand breaks and has been shown to interact with p53 in the DNA repair pathway (Panier and Boulton, 2014). In these studies, 53BP1 was shown to be the centrosomal activator of p53 in response to centrosome loss. USP28 was shown to stabilize 53BP1. 53BP1 was also recently reported to be a centrosomal protein and a binding partner of mitotic Plk1. During mitosis, 53BP1 is stabilized through phosphorylation by Plk1, and deubiquitinated by the ubiquitin-specific protease 7 (USP7). Moreover, 53BP1 depletion induces mitotic defects such as spindle misorientation attributed to the presence of extra centrosomes or mispositioning of centrosomes (Yim et al., 2017). These defects are often seen in cancer tissues and are considered as hallmark of cancer.

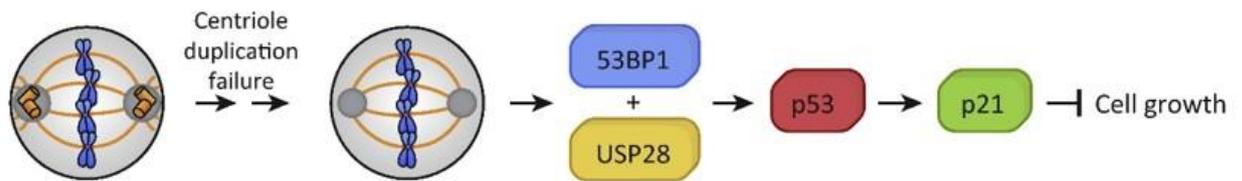


Figure 1-16: The centrosome surveillance pathway

From Lambrus and Holland 2017

However, one difficult question to assess was whether 53BP1 activation of p53 in the centrosome surveillance pathway was independent of its role in DNA repair. The answer came from two different studies. First, another independent screen showed that the p53 arrest in cells lacking centrosomes is different from other pathway leading to the activation of p53 (McKinley and Cheeseman, 2017). Indeed, inactivation of centrosomal proteins triggering a cell cycle arrest did not induce DNA damage as observed with the DNA damage marker phosphorylated histone H2AX. Moreover, as confirmed by previous studies (Fong et al., 2016; Lambrus et al., 2016; Meitinger et al., 2016), the knockout of 53BP1 restored cell growth of RPE1 cells in the presence of centrinone. However, it did not restore the cell cycle progression caused by DNA damage or mitotic defects induced cell cycle arrest. Second, Cuella-Martin and colleagues brought additional data that demonstrate that 53BP1 DNA repair and p53-regulatory roles are independent of one another and rely on their association with distinct interaction partners (Cuella-Martin et al., 2016). They show that 53BP1-dependent, p53-regulatory and DNA-repair activities can be entirely separated. (Figure 1-16)

Altogether, these studies show the emergence of a new signalling function of the centrosome by controlling cell cycle progression into the next cycle only in the presence of the correct number of centrosomes. Thus, this pathway has been named the centrosome surveillance pathway (Lambrus et al., 2016).

1.3.4 The centrosome is a Microtubule Organizing centre

In mammalian cells and many other eukaryotic cells, the centrosome is the main MT organizing centre in mammalian cells. In addition to its role of nucleating MT courtesy of the anchoring of the γ -TuRC complex, it also organizes the MT network.

d. Interphasic MT organization

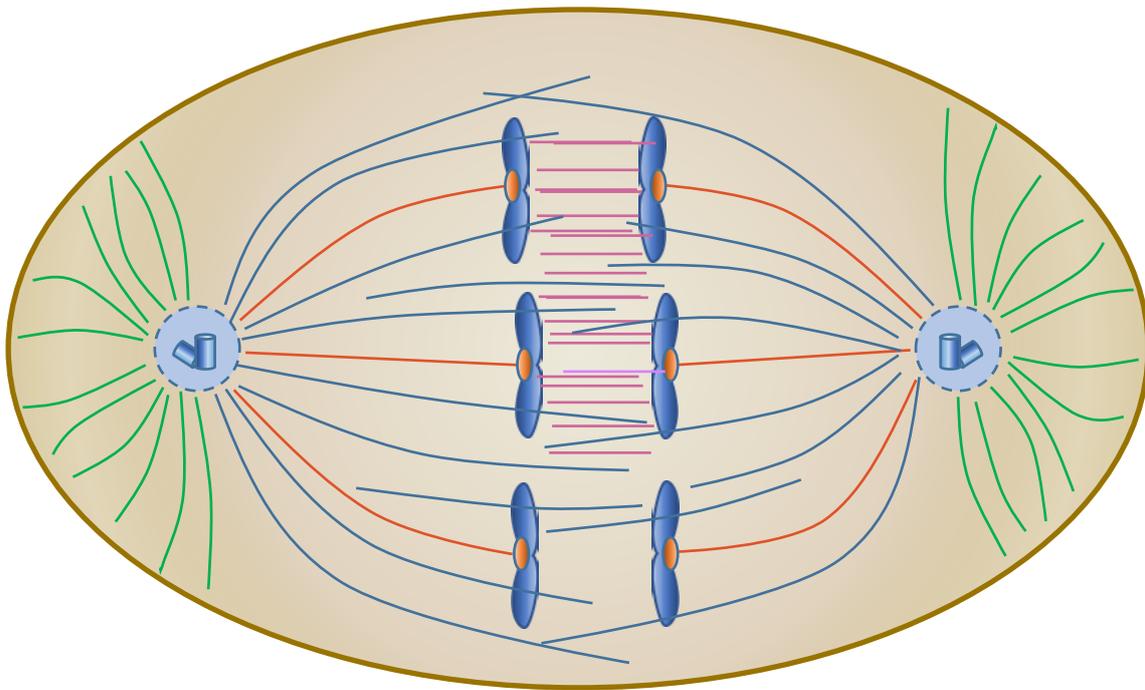
In interphase, centrosomes anchor MTs to form a radial array that provides cells with support and rigidity. This array also establishes intracellular polarity, with MT minus ends concentrated at the MTOC and plus ends extending toward the cell cortex. MT polarity then provides directionality to traffic a variety of cargo toward and away from the nucleus. Other examples of centrosome-based intracellular polarity include the apical to basal MTs found in columnar epithelial cells and the MT arrays oriented toward the leading edge of migrating cells (de Forges et al., 2012).

Furthermore, the interphase array can be remodelled to become non-centrosomal depending on the cell's needs (Bartolini and Gundersen, 2006). For example, non-centrosomal MTs are arranged along the axons of neurons to facilitate trafficking of mitochondria (Hollenbeck and Saxton, 2005), messenger RNA (Sossin and DesGroseillers, 2006) and essential proteins to and from the growth cone. In cells with non-centrosomal MT arrays, centrosomes are thought to serve as the main MT nucleation site, as is the case in neurons, producing MTs and then releasing or severing them to allow for their transport (Wang and Brown, 2002).

e. Mitotic spindle assembly

The mitotic spindle is assembled at mitosis onset and is essential for chromosome segregation during cell division. It is mainly nucleated by the two centrosomes located at the two opposite poles of the dividing cell. Mitotic spindle is composed of different populations of MTs. Astral MTs emanate from the centrosome and are in contact with the cell cortex. They are essential for anchoring the spindle to the cell cortex and play important functions in spindle orientation (Reber and Hyman,

2015; Wittmann et al., 2001). Interpolar MTs emanate from centrosomes and grow towards the center of the cell, overlapping the kinetochore and central spindle MTs. Kinetochore MTs connect the spindle to the chromosomes through the proteinaceous kinetochore supercomplex. The central spindle is formed in anaphase between the segregating chromosomes, and signals where the cell should divide.



Astral Interpolar Kinetochore Central Spindle

Figure 1-17: Mitotic Spindle Microtubule Populations

Although spindle assembly can proceed in complete absence of centrosomes (Azimzadeh et al., 2012; Basto et al., 2006; Khodjakov et al., 2002), they clearly contribute to spindle assembly efficiency and robustness. Centrosomes, are for instance, essential during fast cycles of early development (Stevens et al., 2007). Moreover, mutations in the *C. elegans* centrosomal protein Spd-5 lead to defects in centrosome function and spindle formation, highlighting the importance of centrosomes in *C. elegans* embryos (Hamill et al., 2002). In addition, these results suggest that non-centrosomal spindle assembly pathways cannot compensate for defects in centrosome function in this system (Hamill et al., 2002).

Furthermore, in absence of centrosomes in mitosis, a general increase of chromosome segregation defects is observed and mitosis duration is prolonged (Hayward et al., 2014; Sir et al., 2013). Two recent studies in human and chicken cells confirmed that a delay in anaphase onset is a characteristic for of cells lacking centrosomes, as both studies found a delay in bipolar spindle formation that resulted in a prolonged prometaphase/metaphase (Sir et al., 2013; Wong et al., 2015). In some cases, this will lead to cell cycle arrest in the next G1 phase (Lambrus et al., 2016; Sir et al., 2013; Wong et al., 2015). It is now generally accepted that even if centrosomes are not absolutely essential for bipolar spindle formation, they ensure rapid and accurate formation of a bipolar spindle.

Another important requirement for the centrosome in mitosis is its role in positioning the mitotic spindle, since cytokinesis always occurs perpendicular to the spindle. Many cells divide asymmetrically and this is key in cell fate determination, tissue organization and morphogenesis (Morin and Bellaiche, 2011). Asymmetric cell division requires two coupled events. First, the establishment of a polarity axis through asymmetric distribution of polarity factors at the cell cortex or via neighbouring cells and second, the alignment of the mitotic spindle along this axis (Knoblich, 2010). For this, an efficient communication and synchronization of both machineries is required. A very well conserved mechanism of spindle orientation has been characterized in different organisms and cells. The polarity complex, composed by Par-3/Par-6/aPKC, has been shown to localize at the cell cortex and to interact with the 'spindle orientation complex' composed of Pins/NuMA/Gai. NuMA functions as an adaptor between astral MTs and the polarity complex via its association with the cortical pool of the minus end direct MT motor Dynein. Correct positioning is therefore obtained via anchoring the spindle to the cell cortex and pulling forces generated by Dynein on MTs (Couwenbergs et al., 2007; Kiyomitsu, 2016; Merdes et al., 1996; Morin and Bellaiche, 2011; Nguyen-Ngoc et al., 2007; Wang and Zheng, 2011).

1.3.5 Basal body and ciliogenesis

In cycling cells, the centrosome acts as an MTOC. However, when cells differentiate or exit the cell cycle, the PCM is disassembled and centrioles move towards the cell periphery to assemble the cilium. Centrosome movement towards the cell membrane requires MTs network densification and bundling, with the transient formation of an array of cold-resistant MTs. Additional cellular changes have also been observed during early steps of ciliogenesis such as actin cytoskeleton reorganization and nucleus displacement. (Pitaval et al., 2017).

Cilia are composed of an axoneme, a MT-based structure that protrudes from the cell membrane. Two types of cilia exist: motile cilia, required for cell movement or for flow generation, and primary cilia that are found in the majority of the human body cells and function in sensing and signal transduction. Motile cilia usually have a central pair of MTs and nine outer doublet MTs, whereas primary cilia lack the central pair (Nigg and Raff, 2009). Primary cilia are involved in several signalling pathways required for cell proliferation, differentiation, migration and polarization (Anderson, 1972). For instance, primary cilium defects lead to a diverse range of diseases grouped under the term ciliopathies. These defects could lead to organ specific diseases like the polycystic kidney disease, which is characterized by formation of clusters of cysts causing kidneys to enlarge and lose function over time. On the other hand, various ciliopathies like Bardet Biedl Syndrome or Joubert Syndrome can affect several organs of differing severity, and symptoms including liver fibrosis, deafness, infertility, obesity and diabetes (for review see (Fry et al., 2014)).

At the basis of the cilium lies the basal body (BB) which is composed of the mother centriole of the centrosome. Transition from centriole to BB requires several steps and components. At cell cycle exit, the PCM disassembles and the centriole pair associated with a Golgi-derived vesicle moves toward the plasma membrane (PM). Subsequently, it will then be docked to the PM via the distal appendages (Sorokin, 1968; Anderson, 1972). The distal (DA) and subdistal (SDA) appendages of the

mother centriole play essential roles in ciliogenesis. The DA component CEP164 has been shown to orchestrate several steps in early ciliogenesis (Cajane and Nigg, 2014; Graser et al., 2007; Schmidt et al., 2012). For instance, CEP164 is essential for the recruitment of tau tubulin kinase-2 (TTBK2) to the distal end of the centriole (Cajane and Nigg, 2014). TTBK2, then in turn, initiates the removal of C110 from the mother centriole- an important prerequisite for ciliogenesis initiation (Goetz et al., 2012; Spektor et al., 2007).

In multi-ciliated cells, the canonical way of centriole duplication is not enough to produce hundreds of BB required for ciliogenesis. In these conditions, the centriole intrinsic block is alleviated and centrioles can assemble *de novo* around electron-dense structures called deuterosomes (Sorokin, 1968; Zhao et al., 2013). Until recently, it was believed that these centrioles assemble in a centriole-independent fashion, i.e. without using any centriolar template. Live cell imaging in ependymal differentiated cells combined with super-resolution microscopy have allowed to determine that this massive duplication occurred around the pair of pre-existing centrioles (Al Jord et al., 2014). The duplicated procentrioles first appeared as ‘halos’ that progressively adopted a flower-like shape, reminiscent of the ‘rosettes’ configuration observed in cells overexpressing Plk4 (Kleylein-Sohn et al., 2007). Surprisingly, in these cells, the daughter centriole served as a template in the deuterosome duplication pathway for the assembly of new procentrioles (Al Jord et al., 2014).

The presence of a primary cilium is incompatible with cell division, as the basal body must be released from the cell surface to function in mitosis. When cells re-enter the cell cycle, primary cilia are disassembled, and basal bodies migrate to a position near the nucleus to act as a mitotic apparatus, indicating that the conversion between centrioles and basal bodies/primary cilia is reversible and linked to the cell cycle.

Interestingly, Plk1 and AuroraA are key players in cilium disassembly. AuroraA is recruited to the BB prior to cell cycle re-entry by HEF1, a transducer of integrin

initiated attachment (Pugacheva et al., 2007). Subsequently, AuroraA phosphorylates HDAC6. This phosphorylation activates HDAC6 which, in turn, deacetylates MTs leading to the disassembly of the cilium (Pugacheva et al., 2007). Plkl also interact with HDAC6 and promotes MTs deacetylation independently of AuroraA (Wang et al., 2013).



Figure 1-18: Assembly steps of the primary cilium

From Moser et al 2009.

Over the past 10–15 years, cilia loss and/or dysfunction have been linked to numerous human disorders, collectively termed ciliopathies. Phenotypes associated with cilia dysfunction are often syndromic and include cystic kidneys, polydactyly, and obesity, to name a few (reviewed in (Gerdes et al., 2009)).

Interestingly, *Drosophila* mutants that lack centrioles nevertheless die soon after birth (Basto et al., 2006). Rather than reflecting the absence of centrosomes, however, this death appears to result from the lack of cilia that are essential for the function of certain mechano- and chemosensory neurons. These observations support the view that centrioles, may have originally acquired the ability to form centrosomes not to increase the efficiency of cell division but rather to ensure that the centrioles associate with the spindle poles and are thereby equally partitioned between the two daughter cells (Marshall, 2009).

2. The spindle pole body in yeast

Budding and Fission yeast have been used as model organisms since decades. Their study has provided significant insights not only into cell cycle control and damage responses, but to numerous aspects of cell behaviour, from chromosome segregation to protein secretion. These two organisms provide sophisticated genetic and molecular tools, as well as genome-level strategies to examine gene regulation and cellular responses. Despite superficial similarities, these species are significantly diverged from one another. Moreover, they are morphologically different. *S.cerevisiae* are round cells, and divide by budding. The new daughter cell grows as a bud, and separation occurs when the daughter cell reaches a certain size, differing from that of the mother cell. Moreover, the bud appears in G1 and the mitotic spindle starts assembling in G2. On the other hand, Fission yeast, is a rod shaped organism. Cells grow longitudinally and double their size before dividing by fission. The two daughter cells are equal in size (reviewed in (Humphrey and Pearce, 2005)).

In yeast, the functional analogue of the centrosome is the spindle pole body (SPB). Since most fungi undergo closed mitosis, the nuclear envelope (NE) remains intact. Therefore, the SPB must be able to nucleate nuclear MTs like interpolar MTs to assemble the mitotic spindle, but also cytoplasmic MTs such as astral MTs. Moreover, during mitosis, the SPB is the only functional MTOC. To achieve this dual function, the SPB must be in tight contact with the NE. For instance, in budding yeast the SPB is inserted into the nuclear envelope during the first steps of its assembly. In contrast, in fission yeast, the SPB is only inserted into the nuclear envelope during mitosis.

In this section, I will describe the SPB in budding yeast (*S.cerevisiae*) and in fission yeast (*S. pombe*) referring to them as ScSPB and SpSPB respectively. In these two models, most of the components of the SPB are conserved and play similar function.

2.1.1 The Spindle Pole Body ultrastructure

The structure of SPB has been described first by electron microscopy (Byers and Goetsch, 1975; Ding et al., 1997; Giddings et al., 2001). Although SPB are associated with NE like centrosomes they differ from the metazoan centrosome since they lack centrioles and PCM, and are composed of lamellar structures instead (Figure 2-1).

ScSPB are composed of at least five layers (Figure 2-1): the outer (OP), inner (IP) and central (CP) plaques. Two intermediate layers separate the central and outer layers (IL1 and IL2 in Figure 2-1). The outer and inner layers are located at the cytoplasmic and nucleoplasmic sides of the scSPB respectively. They play a role in nucleating MTs. The central plaque is connected to the NE throughout the cell cycle. Moreover, the core SPB, formed of the five layers is connected to an adjacent structure called the half-bridge (HB). The HB is also tightly linked to the NE on its cytoplasmic surface but is not inserted into it (Byers and Goetsch, 1975; Giddings et al., 2001). Nuclear and cytoplasmic MTs are anchored perpendicular to the inner and outer plaques.

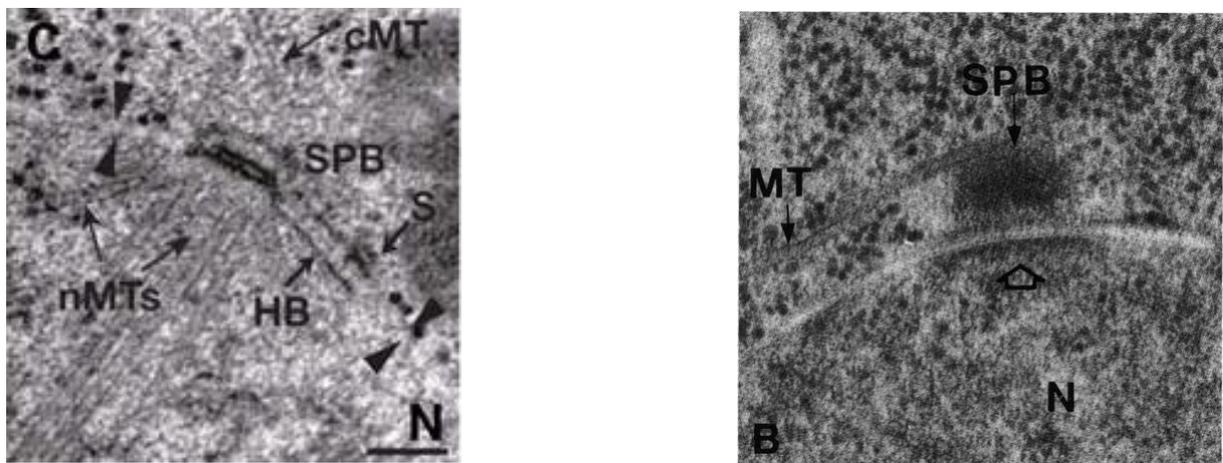


Figure 2-1: Ultrastructure of the SPB

(left, budding yeast (Giddings et al., 2001) and fission yeast of the SPB (right, (Ding et al., 1997)

In SpSPB plaques exist but are less easily distinguishable apart from a central dark layer (Figure 2-1 B (Ding et al., 1997; Uzawa et al., 2004). SpSPB also possesses a HB that is similar to that found in budding yeast. A recent study has shown a reconstruction of a duplicated SPB, revealing the central bridge as an oblique structure wider than the flanking SPB (Hoog et al., 2013). The two central plaques are circular discs extending from the two upper ends of the central bridge. Each disc is slightly curved, concave to the nuclear envelope (Hoog et al., 2013).

As mentioned above, the SpSPB is only inserted into the NE shortly before mitosis. This process is known as polar fenestration and starts by the appearance of an invagination under the SpSBP. At the end of mitosis, the SpSPB is extruded from the NE (Tanaka and Kanbe, 1986).

Another difference between *S. pombe* and *S. cerevisiae* SPB is related to MT attachment: during interphase or mitosis, cytoplasmic MTs attach to the SpSPB in parallel to the outer surface, and not perpendicular to it like in *S. cerevisiae*. This organization has impact on spindle positioning during mitosis and nuclear positioning during interphase.

2.1.2 Molecular Organization of the SPB

In budding yeast, Spc42 is the main component of the CP of scSPB. As many SPB proteins, Spc42 is a large coiled-coil protein. It is able to form dimers and trimers and has been shown to form hexagonal crystal lattice. At the central plaque, the number of Spc42 molecules has been estimated to ~ 1000 molecules (Bullitt et al., 1997).

The N-terminus of Spc42 associates with the C-terminus of Spc110 and Spc29, two other essential coiled-coil proteins that localize to the nuclear face of the CP (Adams and Kilmartin, 1999; Elliott et al., 1999). The C-terminus of Spc110 localizes to the central plaque and binds to Spc29 and calmodulin (Cmd1) through unique but partially overlapping binding sites (Elliott et al., 1999; Kilmartin and Goh, 1996; Spang et al., 1996; Stirling et al., 1994; Sundberg et al., 1996). Cmd1 is thought to

regulate the binding of Spc110 to Spc29 (Elliott et al., 1999; Geiser et al., 1993; Geiser et al., 1991).

The length of Spc110 coiled-coils determines the distance between the CP and IP (Kilmartin et al., 1993; O'Toole et al., 1999). The N-terminus of Spc110 localizes to the IP and recruits Spc98, a yeast component of the γ -TuSC (Kilmartin et al., 1993; Knop and Schiebel, 1997; Nguyen et al., 1998; O'Toole et al., 1999; Sundberg and Davis, 1997; Vinh et al., 2002).

The C-terminus of Spc42 faces the cytoplasm and binds to the C-terminus of Cnm67 (Adams and Kilmartin, 1999). Cnm67 coiled-coil region facilitates its dimerization and functions as a spacer between IL2 and IL1 (Schaerer et al., 2001). The N-terminus of Cnm67 binds to the outer plaque protein Nud1 (Adams and Kilmartin, 1999), Elliott et al. 1999).

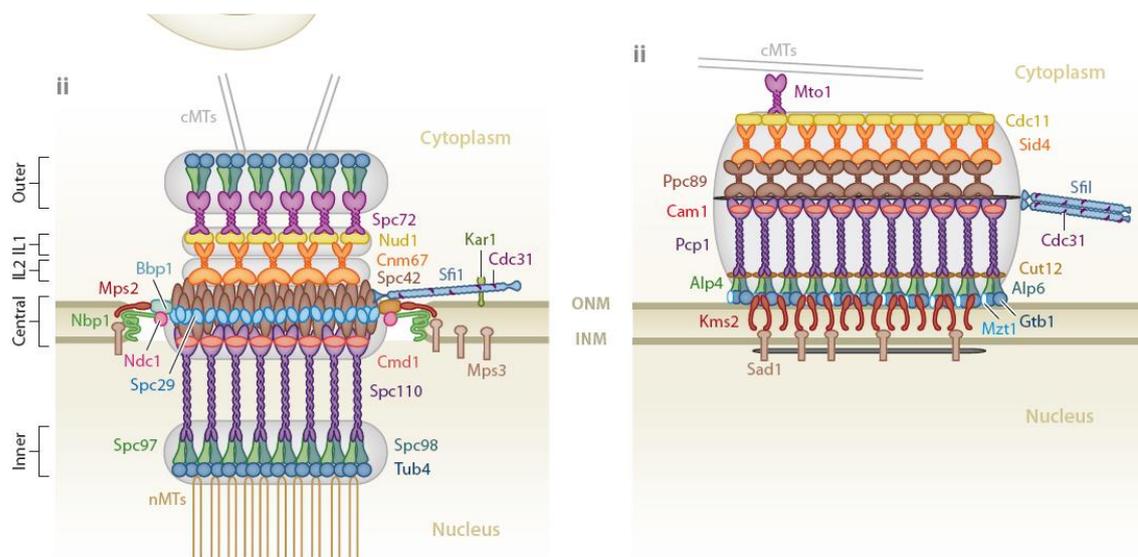


Figure 2-2: The molecular organization of the SPB

Left: budding yeast and right: fission yeast From Cavanaugh and Jaspersen, 2017.

Another coiled-coil protein, Spc72, is found in the OP (Chen et al., 1998; Knop and Schiebel, 1997; Wigge et al., 1998). The C-terminal domain of Spc72 binds to the C-terminus of Nud1 (Gruneberg et al., 2000). The N-terminal domain of Spc72 recruits the γ -TuSC to the cytoplasmic side of the scSPB (Knop and Schiebel, 1997).

The CP is linked to the NE to a site when the inner (INM) and outer (ONM) nuclear membranes are fused. This anchorage is mediated by the membrane proteins Ndc1, Mps2, Bbp1 and Npb1 and Mps3 (Araki et al., 2006; Kupke et al., 2011; Kupke et al., 2017; Schramm et al., 2000). Interestingly, Ndc1 is also an essential protein for nuclear pore complexes biogenesis (Chial et al., 1998), suggesting the existence of similar mechanisms for SPB and NPCs insertion into the NE (reviewed in (Cavanaugh and Jaspersen, 2017))

The half bridge (HB) is also connected to the CP of scSPB and is composed of Cdc31, Karl, Mps3, and Sfil and is the site of new SPB assembly (Biggins and Rose, 1994; Jaspersen et al., 2002; Kilmartin, 2003 Spang,et al 1993). The two membrane proteins, Karl and Mps3, localize to the half-bridge and are required to maintain its structure (Jaspersen et al., 2002; Rose and Fink, 1987; Spang et al., 1995; Vallen et al., 1994). Both proteins bind to Cdc31, the yeast centrin homolog, which is also required for half-bridge integrity (Baum et al., 1986; Biggins and Rose, 1994; Jaspersen et al., 2002; Spang et al., 1995; Vallen et al., 1994). An additional half-bridge component, Sfil, was identified for its ability to bind to Cdc31 and has been shown to be essential for HB assembly and SPB duplication (Kilmartin, 2003). Sfil functions in budding and fission yeast will be detailed later in this manuscript.

Until recently, aside from their interactions with Cdc31, little was known about how these proteins come together to form the HB. However, a recent study has characterized the mechanism of Karl interaction with Sfil and defined its role in HB and SPB maintenance (Seybold et al., 2015). Using super-resolution microscopy, Karl was found to localize to the central region of the bridge between the SPB and its satellite. This localization is consistent with the finding of a direct interaction of Karl with Sfil C-terminus as well as the last Sfil repeats found close to the C-terminus of Sfil (Seybold et al., 2015). Karl was shown to be required to align Sfil arrays along the NE. This conclusion is based on the arched bridge phenotype observed in mutant lacking Karl (and cdc31 mutant that supresses the lethality of Karl deletion). Binding of Karl to the Sfil C-terminus (CT) and to the neighbouring Sfil repeats seems

therefore to promote Sfil-C-C-Sfil interactions. This is supported by the finding that deletion of Sfil-CT does not lead to collapse of the bridge and that Karl still localizes at the bridge centre in *sfilΔct* cells. Remarkably, elevated Karl at SPB led to an extraordinarily elongated bridge without a satellite, suggesting a defect in the antiparallel organization of Sfil molecules (Seybold et al., 2015). This could be due to Karl recruiting an excess of Sfil in a random fashion accompanied by bridge elongation. This study brings important insights into the functions and regulations of the HB in budding yeast.

Nevertheless, the mechanisms regulating the link between HB and SPB are not fully understood. Karl has been shown to be involved in connecting the half-bridge to the core SPB via its interaction with Bbpl (Schramm et al., 2000). However, recent findings on Karl localization to the central region of the HB suggest the existence of at least two different pools, one at the centre of the HB and the other towards the N-terminus of Sfil and the core SPB. Another component of the HB, the transmembrane protein Mps3, has been shown to be important for anchoring the of the SPB into the NE. This is achieved via its interactions with Bbpl-Mps2 complexes as well as via its ability to induce membrane remodelling required for SPB insertion (Friederichs et al., 2011; Kupke et al., 2017). Therefore, Mps3 seems to act as a linker between the SPB, the HB and the NE.

SpSPB molecular architecture is not as well characterized as scSPB. However, regional connections between SpSPB proteins have been established such as the interaction between Pcp89 Sid4 and Cut11 (Schramm et al., 2000). Although Spc42 and Spc29 does not seem to be conserved in fission yeast, accumulating evidence suggests that Pcp89 is the functional homolog of Spc42 and is sufficient to fulfil similar functions as the two budding yeast proteins (Bestul et al., 2017; Rosenberg et al., 2006). Moreover, Cut11 is the Ndc1 homolog and localizes to the SpSPB only during its insertion into the NE (West et al., 1998). Additionally, Brr6 a NPC protein in budding yeast is essential for SpSPB insertion into the NE during mitosis (Tamm et al., 2011).

Pcp1 is the homolog of Spc110 (and pericentrin) and binds Calmodulin. It has been shown to link the γ -TuRC complex to the nuclear face of the SpSPB. Moreover, overexpression of Pcp1 produces multiple, abnormal, SPB-like structures that are reminiscent of supernumerary centrosomes (Flory et al., 2002).

On the cytoplasmic side of the SpSPB, Cdc11 and Sid4, two members of the SIN pathway (see later section) recruit Mto1, the anchoring protein of the γ -TuRC complex (Morrell et al., 2004; Samejima et al., 2010; Tomlin et al., 2002).

It is possible that, like Mps3, Sad1 links the interface of SPB and half-bridge (Jaspersen et al., 2002) and plays a role in SPB insertion into the nuclear envelope (Araki et al., 2006; Friederichs et al., 2011; Jaspersen et al., 2002).

2.2 Spindle Pole Body duplication

The steps that ultimately lead to ScSPB duplication have been known since the analysis of the SPB duplication cycle by electron microscopy (EM) in the 1970s (Byers and Goetsch, 1975). However, the molecular understanding of budding yeast SPB duplication was achieved only with the discovery of HB/bridge components.

SPB duplication can be divided into three steps: (1) HB elongation, (2) assembly of the satellite and its extension into a duplication plaque and retraction of the bridge, and (3) insertion of the duplication plaque into the nuclear envelope and assembly of the IP.

HB elongation is the initial step of SPB duplication in both budding and fission yeast. Mechanisms of HB elongation are detailed in a later section. (see section 3-2). Briefly, the HB extension starts in G1 and consists of the assembly of anti-parallel arrays of Sfi1 and Cdc31, to create a new site for the assembly of the satellite (Kilmartin, 2003).

Satellite assembly can be detected by EM in G₁ phase. At this stage, it consists of a small electron-dense structure that appears on the cytoplasmic side of the HB, distal to the existing scSPB (Fig. 2-3). This satellite has an apparently spherical morphology in haploid cells but a plaque-like structure in larger tetraploid cells (Adams and

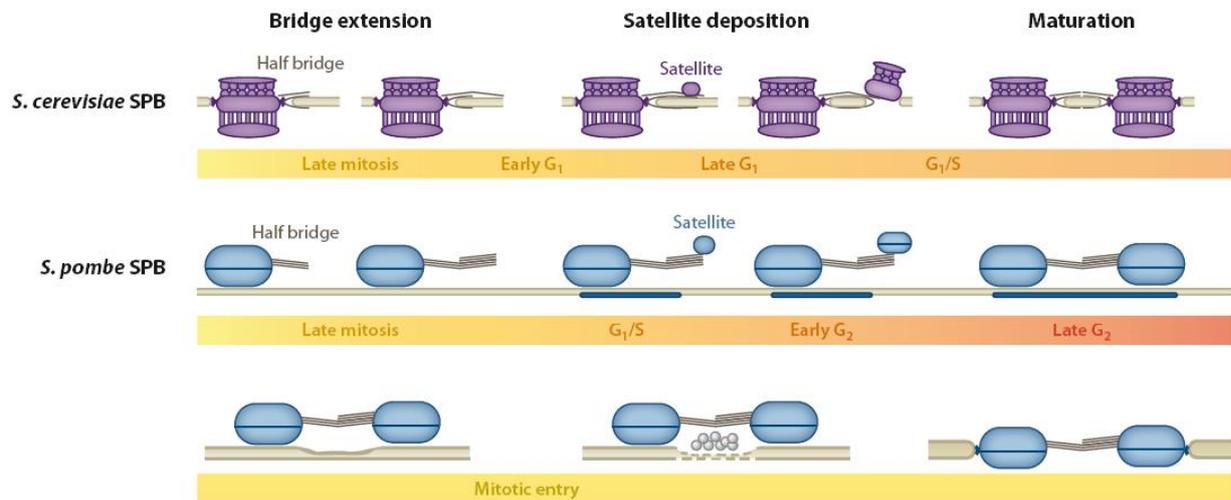


Figure 2-3: the duplication process in budding and fission yeast

Top: budding yeast and bottom (2 lines): fission yeast

From Cavanaugh and Jaspersen, 2017.

Kilmartin, 1999). The satellite contains the main cytoplasmic scSPB components: Nud1, Cnm67, Spc42 and Spc29. Thus, the satellite can be considered a miniature version of the cytoplasmic core of the scSPB, but is not yet inserted into the nuclear envelope, and does not contain the nuclear scSPB component Spc110 and cannot organize MTs (Adams and Kilmartin, 1999).

In S phase, the satellite is transformed into a duplication plaque similar in size and composition to the CP. Immuno-EM showed that it contains the same SPB components as the satellite (Adams and Kilmartin, 1999) suggesting that assembly of these cytoplasmic scSPB components onto the satellite, thereby enlarging its diameter, might simply form the duplication plaque. A characteristic bend in the half-bridge is also observed at the proximal end of the duplication plaque (Fig. 2-3). This suggests that the half-bridge is under tension at this time. Mechanistically, SPB insertion requires formation of a NE pore, a highly curved membrane where the INM

and ONM are contiguous (Figure 2-3) (reviewed in (Jaspersen and Ghosh, 2012)). The bilayers of the NE fuse at the distal end of the HB and this is often associated with the presence of a pore structure similar in morphology to a nuclear pore complex.

In budding yeast, NPCs are frequently observed near the duplicating ScSPB, resulting in the idea that NE pores at the NPC might facilitate membrane incorporation (Adams and Kilmartin, 1999). Mps3 has been shown to be involved in SPB insertion thanks to its capacity of modelling the type of lipids that are present into the NE. This function is mediated by its transmembrane domain (Friederichs et al., 2011). Moreover, studies of Ndc1 and Mps3 suggest that the distribution of Ndc1 between the NPC and ScSPB is controlled by Mps3; in cells lacking the NPC subunit Pom152, more Ndc1 is available to the ScSPB to recruit Nbp1 (Chen et al., 2014; Chial et al., 1998; Katta et al., 2015). The role of Mps3 in Ndc1 binding is particularly interesting in light of recent observation of Sad1 rings at the fission yeast SpSPB prior to mitosis (Bestul et al., 2017). Intriguingly, this introduces the possibility that these proteins may function as a scaffold to recruit proteins involved in membrane remodelling required for SPB insertion. Accordingly, Mps3 have also been shown to be present in complexes with Bp1 and Mps2 and these complexes are required for SPB anchoring to the NE (Kupke et al., 2017).

To form a bipolar mitotic spindle, the duplicated SPB must separate, and the two SPB move to opposite sides of the NE. During separation, it is important that both SPB inherit a half bridge, so that they are competent for duplication in the next cell cycle. It is generally assumed that Sfi1 C termini of the full bridge must detach for this to occur. Moreover, mutants of C-terminal domain of Sfi1 induced a cell cycle arrest with duplicated but unseparated scSPB, strongly suggesting a function of this domain in SPB separation (Anderson et al., 2007).

The SpSPB duplication process in fission yeast is much less well characterized than in budding yeast because SPB substructures and duplication intermediates are not resolved as well by electron microscopy as they are in budding yeast. Nevertheless, immunoelectron microscopy revealed the localization of fission yeast Cdc31 to the

bridge of side-by-side SPBs or to a site next to the SPB in an anaphase cell (Paoletti et al., 2003). However, Electron microscopy studies have so far failed to pinpoint the time at which half-bridge elongation takes place in fission yeast.

Moreover, there has been a long-standing debate about fission yeast SPB duplication timing. Ding and colleagues (Ding et al., 1997) observed that appearance of the duplicated daughter spSBP occurs during G2, employing elutriation-synchronized cycling cell culture. However, Uzawa and colleagues (Uzawa et al., 2004) observed that the duplication had already occurred in G1/S arrested cells using a *cdc10* mutant and that HU-arrested cells had duplicated SPB. They concluded that the SPB duplication occurs before S phase. This apparent discrepancy could come from the fact that Uzawa and colleagues used cells which were originally arrested in G1 by nitrogen starvation prior to release into rich media to be re-arrested at the *cdc10* arrest point in G1/S. These cells would have spent longer time to reach G1/S compared to the cycling cells and may have made an advance in the SPB duplication cycle. If the SPB duplication cycle is uncoupled with the DNA synthesis cycle, which is the case for mammalian cells as well as meiotic fission yeast cells (Funaya et al., 2012), this could explain why duplicated SpSPB have been observed in these cells.

In a recent study aiming to characterize the effects of Mal3 and Tip1 deletion (EB1 and CLIP-1 homologs) on MTs anchoring at the SpSPB, the authors compared the morphology of wild-type (WT) and Mal3 deleted cells using electron microscopy and tomography (Hoog et al., 2013). Twenty WT SpSPB were analysed in early G2 cells. The tomograms revealed that one SPB from a septating cell had an unduplicated SPB. From cells in early G2, 26% had not yet started to duplicate the SPB. 53% of the SPB had a clear secondary SPB 'bud' on the opposite side of the central bridge and 21% were not clearly identifiable as either single or duplicated SpSPB. The authors noted that the SpSPB volume does not directly correlate to its duplication state, indicating that the SPB grows by first initiating a bud, and then increases in volume. Consequently indicating the existence of a satellite equivalent structure in *S.pombe*

(Hoog et al., 2013). Moreover, this study brings additional proof that duplication of the SpSPB takes place in G2.

2.3 The SPB and cell cycle control

In addition to its role as an MTOC, the SPB is also a template for cell cycle control and progression. The two best examples are the roles of Plol in mitosis promotion when interacting with Cut12, and its role in controlling cytokinesis via its interaction with Sid4.

Cell cycle progression and mitotic entry is known to be regulated by the CDK1 activity balance. In fission yeast, as in many organisms, several physiological cellular inputs modulate mitotic entry by acting on the equilibrium between Cdc25 and Wee1 to activate or block CDK1 activity. Mitotic entry is also influenced by the recruitment of Plol to the SPB that modulates Cdc2-Cdc13 activation feedback loop. Cut12 recruits Plol to the SPB for this function. Accordingly, an hyperactive mutation of Cut12 (*cut12-s11*) enables Cdc25-defective cells such as *cdc25-22* to enter mitosis (Hudson, Feilotter et al. 1990; Bridge, Morphew et al. 1998; Grallert, Chan et al. 2013).

A negative regulator of Plol recruitment to SPB is the protein phosphatase 1 Dis2. Dis2 binds to Cut12 through a PPI docking site (PDS), and the presence of the phosphatase inhibits Plol recruitment, most likely by dephosphorylation. However, Cdc2 and the NIMA kinase Fin1 phosphorylate Cut12 during G2 to impair Dis2 binding, allowing Plol recruitment to the SPB (Grallert et al., 2013).

Importantly, Plol receives inputs from an upstream signalling pathway, the stress response pathway (SRP), itself regulated by TOR (target of rapamycin) that modulates cell size at division in response to a variety of environmental cues including stress and nutritional availability (Davie and Petersen, 2012). Upon TOR activation, Gcn2, a kinase which affects transcriptions levels of Pyp2 (Nemoto et al., 2010) gets inactivated. This leads to an increase of Pyp2 levels. The phosphatase

Pyp2 inhibits the MAPK Sty1. This delays mitosis onset by inhibiting Plol recruitment to SPB (Petersen, 2009; Petersen and Nurse, 2007) that is otherwise favoured by Sty1 phosphorylation of Plol on Serine 402. This phosphorylation leads to Plol association with Cut12 (Grallert et al., 2013; Petersen, 2009; Petersen and Hagan, 2005; Petersen and Nurse, 2007).

The link between the SPB and cytokinesis was made when two regulators of cytokinesis in fission yeast were shown to localise at the SpSPB. This study has shown that the septum initiation network (SIN) is regulated from one SPB that harbours active Spgl GTPase (Sohrmann et al., 1998). In budding yeast, the orthologous pathway is called the mitotic exit network (MEN). The MEN and the SIN are also related to the Hippo pathways in metazoan. Although the outcomes of the SIN and MEN are different, the core signalling cascade is highly conserved and include upstream regulation by Polo kinase (reviewed in (Hergovich and Hemmings, 2012) and (Simanis, 2015)). Components of the MEN and SIN pathways are recruited to the cytoplasmic face of the ScSPB/SpSPB by Nud1/Cdc11. However, duplicated mitotic poles are not equivalent: whereas upstream MEN/SIN components are recruited to both, downstream components only localize at one of the SPB. Interestingly, when the daughter spSPB is ablated, the mother SPB can take over to preserve the activation of the SIN. Additionally, the SPB integrity is required for the SIN to function (Magidson et al., 2006).

Classically it has been considered that the SIN was activated at the exit of mitosis, but more and more studies show evidence of activity of SIN kinases as early as mid G2, with different roles in cell cycle regulation and mitosis commitment as explained earlier in this manuscript (Grallert et al., 2012). Moreover, it has been shown recently that the removal of the SAD kinase Cdr2 from the cortex during cytokinesis resets the division plane for the next cell cycle. Cdr2 removal is induced by its phosphorylation by the SIN kinase Sid2, which is required for its removal from the

cell cortex. Failure to remove Cdr2 from the cell cortex leads to defects in division plan positioning (Rincon et al., 2017a).

SIN inactivation in fission yeast lead to two types of phenotypes (Krapp and Simanis, 2008). Mutants of activator elements (*cdc7*, *cdc11*, *cdc14*, *etd1*, *mob1*, *sid1*, *sid2*, *sid4* and *spg1*) which block the pathway give rise to multinucleated cell. These mutants assemble rings that will fragment afterwards due to their inability to contract (Creanor and Mitchison, 1990; Fankhauser et al., 1993; Fankhauser and Simanis, 1994; Guertin et al., 2000; Jimenez and Oballe, 1994; Nurse et al., 1976; Salimova et al., 2000; Schmidt et al., 1997; Sparks et al., 1999). On the other hand, mutations of negative regulators of SIN (*Cdc16* and *Byr4*) result in several cytokinesis rounds giving rise to multiseptated cells (Fankhauser et al., 1993; Minet et al., 1979; Song et al., 1996).

A large amount of work has been done in fission yeast to characterize and determine components of the SIN (Balasubramanian et al., 2004; Krapp et al., 2004; Wolfe and Gould, 2005). The proteins of the SIN pathway are sequentially recruited during the cell cycle to the SPB where they are anchored through the platform made by the scaffolds *Cdc11* and *Sid4*. The SIN activity is controlled by the GTPase *Spg1* that can switch from the inactive GDP bound form to the active GTP bound form. *Spg1* activity is negatively regulated by a GAP composed of *Byr4* and *Cdc16*. *Spg1* activation triggers a kinase cascade composed of *Cdc7*, the *Sid1/Cdc14* complex, and the *Sid2/Mob1* complex. (Roberts-Galbraith and Gould, 2008).

Importantly, a major upstream regulator of the SIN is the kinase *Plol* that associates with *Sid4* (Tanaka et al., 2001). Interestingly, *Plol* association with *Sid4* is blocked by ubiquitination of *Sid4* by the ubiquitin ligase *Dmal* when the mitotic spindle checkpoint is engaged, in order to delay cytokinesis onset and avoid damaging the DNA (Johnson et al., 2012).

3. Centrins and Sfil in centrosome biogenesis

Sfil and Centrin are well conserved binding partners, associated with centrosomes throughout evolution. In spite of this conservation, they have been assigned a variety of functions at centrosomes or beyond and a united vision of their cellular function is still lacking at this stage. Interestingly, Centrin functions in SBP/BB duplication and maintenance have been heavily documented. In contrast, its exact function at the centrosomes in human cells remains unknown and it seems to be clearly dispensable for centriole duplication. Consistently, Sfil functions in yeast and protists also show that it is essential for SPB and BB duplication and maintenance, but its functions in humans have not been reported yet.

3.1 Functional diversity and specialization of Centrins

Centrin is an ancient protein thought to have arisen within the ancestor of all eukaryotes via gene duplication (Aubusson-Fleury et al., 2017; Bornens and Azimzadeh, 2007). Centrins are EF-hand calcium-binding proteins closely related to Calmodulin. Members of the EF-hand superfamily carry distinct helix-loop-helix structures that chelate Ca²⁺ (Figure 3-1). Centrins possess four EF-hand domains. These domains are highly evolutionary conserved and are responsible for the calcium contraction of Centrin fibres (Hu and Chazin, 2003; Matei et al., 2003; Thompson et al., 2006; Veeraraghavan et al., 2002)

The number of centrin isoform varies depending on the organism. While only one centrin gene has been identified in budding and fission yeast (Baum et al., 1986; Paoletti et al., 2003), four centrin genes have been discovered in mammals (Errabolu et al., 1994; Gavet et al., 2003; Hart et al., 1999; Lee and Huang, 1993; Middendorp et al., 1997); five centrin genes are found in *Trypanosoma brucei* (Berriman et al., 2005), more than 10 in *Tetrahymena* and 48 in *Paramecium* (Gogendeau et al., 2007). The amplified number of centrins in protists may reflect the variety of centrins functions and the importance of centrin composed structures.

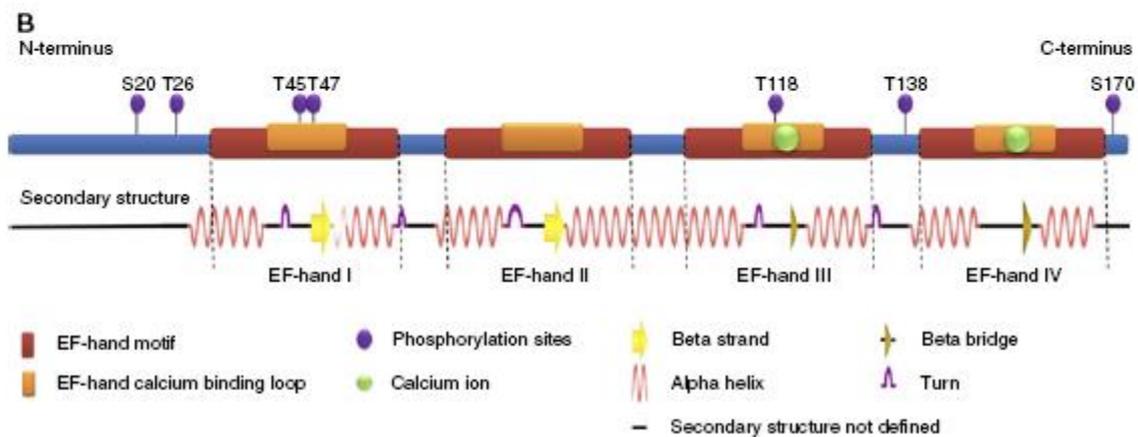


Figure 3-1: Centrins domains

From (Thompson et al., 2006)

Based on sequence comparison, two subfamilies of centrins have traditionally been proposed: one includes mammalian Centrin 1, 2 and 4 and is more closely related to centrin from the green algae *Chlamydomonas reinhardtii* expressed from *vlf2* gene (variable flagella number 2), which controls the number of flagella (Errabolu et al., 1994; Huang et al., 1988; Paoletti et al., 1996). The second contains human Centrin 3 closer to Cdc31 that controls budding yeast SPB duplication (Baum et al., 1986). However, a recent study revisited the evolutionary story of Centrins and showed that they are rather subdivided in five families (Aubusson-Fleury et al., 2017). The third family is comprised of centrins that are functionally related to Calmodulin. The fourth family includes centrins that mainly play functions in cilia. A new family of Centrins has been identified in *Paramecium*. This family is composed of Centrins that are associated with the ciliate contractile filaments and termed ICL subfamily (Aubusson-Fleury et al., 2017).

Centrins are localized in the nucleus, at the centrosome and at contractile structures and basal bodies. Their functions span from nucleotide excision repair (Araki et al., 2001), mRNA export (Fischer et al., 2004), cellular morphogenesis (Ivanovska and

Rose, 2001), spindle pole body duplication (Baum et al., 1986) and BB maintenance (Wright et al., 1985). Part of this diversity stem from the fact that centrins have multiple binding partners, at centrosomes and BB or in other cellular compartments or organelles. This is the case for nucleotide excision repair for which centrins partners with XPC or for mRNA export for which centrins interact with mRNA export factors.

I will describe below the major function of centrins at centrosomes or BB in ciliated unicellular organisms and animal cells only.

3.1.1 Centrins in green algae and ciliates

Centrin has first been described as a major component of the striated flagellar roots (SFR) of the green alga *Tertraselmis striata* (Salisbury et al., 1984). It was shown to be essential for the contraction of the SFR in presence of high concentrations of Ca²⁺. Later investigations in a wide range of protists have shown that centrin is found in different contractile structures. In example, Centrin was found at cilia rootlets (Guerra et al., 2003; Lemullois et al., 2004), at the nuclear-BB connector and inter BB distal fibres in *Chlamydomonas reinhardtii* (Salisbury et al., 1988; Sanders and Salisbury, 1989).

Contractile structures have been involved in different processes, like response to external stimuli (Febre, 1981), food ingestion (Tucker, 1968), flagellar beat (Melkonian, 1980) and organelle/cytoskeleton organization (Wright et al. 1885; Hu et al 2008) and are often enriched in Centrins. Indeed, Centrin have been shown to be implicated in the formation of contractile fibres ranging from 3 to 8nm (Gogendeau et al., 2008) and Centrin inhibition often led to inhibition Ca²⁺ dependant contraction (Sanders and Salisbury, 1994), for review see (Zhang and He, 2012) and (Dantas et al., 2012).

The centrosomal functions of Centrins have been studied in many organisms including *Chlamydomonas reinhardtii*, *Tetrahymena thermophila* and *Paramecium*

tetraurelia (Koblenz et al., 2003; Ruiz et al., 2005; Stemm-Wolf et al., 2005). In *C.reinhardtii* and *Tetrahymena thermophila*, Centrin was found to be essential for BB duplication and organization (Koblenz et al., 2003; Stemm-Wolf et al., 2005). Depletion of Centrin lead to the decrease of the number of BB in the cells. Moreover, Centrin was essential for BB organization and segregation in these organisms (Koblenz et al., 2003; Stemm-Wolf et al., 2005). In *Paramecium*, Centrin was found to be dispensable for initial steps of BB assembly, but the two Centrin isoforms (PtCen2 and PtCen3) were required for either later steps of BB duplication or for the detachment of the newly assembled BB (Ruiz et al., 2005). Electron microscopy analyses have shown that in PtCen2 depleted cells, nascent BB are observed, but their orientation was incorrect. Moreover, these BB had structural defects as they sometimes lacked MTs at the BB wall. Some BB were also described as 'loose' as the connection between the triplet MTs appeared to be less tight than in a WT cell. In contrast, depletion of PtCen3 did not affect the duplication process, but the newly duplicated BB were unable to detach from the mother BB, and therefore impaired the BB organization of the cell (Ruiz et al., 2005). Interestingly, both isoforms were essential for cell survival as cells arrested growth and eventually died. Slow growth and cell cycle arrest leading to cell death was also observed in *Chlamydomonas* and *Tetrahymena* (Koblenz et al., 2003; Stemm-Wolf et al., 2005). Even though centrin is essential for BB assembly and maintenance, it is interesting to point out the variety of functions even at BB/centrosomal level.

A recent study has identified a new family of Centrins in *Paramecium*. Genome analyses in *Paramecium* revealed that the ICL subfamily comprises seven genes which for centrins that corresponds to three ohnolog pairs, i.e. gene duplicates originating from whole genome duplication (ICL1e and ICL1g; Cen8 and Cen10; Cen12 and Cen15) and a single gene, Cen18. This study showed that the ICL1e complex localizes to and duplicates along with the BB. Additionally, the sequential recruitment of the Centrin2, ICL1e and Centrin3 is required for correct duplication and positioning of the new BB (Aubusson-Fleury et al., 2017).

3.1.2 Centrosomal functions of Centrin in animal cells

Four Centrin isoforms were identified in mammalian cells (Errabolu et al., 1994; Gavet et al., 2003; Hart et al., 1999; Lee and Huang, 1993; Middendorp et al., 1997). Centrin 2 and Centrin 3 are ubiquitously expressed (see (Dantas et al., 2012) for review), while Centrin 1 is only expressed in the testes (Hart et al., 1999) and Centrin 4 expression is restricted to murine ciliated cells and has not been detected in human cells (Gavet et al., 2003). Endogenous or overexpressed Centrins all localize to centrioles, but seem to have different functions in animal cells (Paoletti et al., 1996); (Middendorp et al., 2000; Middendorp et al., 1997).

Levels of expression of Centrin isoforms have been investigated in human tracheal epithelial cells (LeDizet et al., 1998). This study found that Centrin 1 was not expressed in these cells, confirming its cellular specificity. Moreover, Centrin 2 levels increased when ciliogenesis was initiated, while Centrin 3 levels were unchanged. The differential levels of expression of Centrin 2 and Centrin 3 suggest that these isoforms are not interchangeable and that they each fulfil unique functions (LeDizet et al., 1998).

As mentioned above, Centrin1 expression is restricted to testes and has been shown to play an essential role in late steps of spermatogenesis and spermatid maturation. Mice carrying a deletion of Centrin did not show any developmental defect. However, while female mice were fertile, the males were found to be sterile. Spermatid features were analysed and were found to lack a sperm flagellum in most cases. Moreover, when the sperm flagellum was present it displayed severe disorganization in axonemal MTs (Avasthi et al., 2013).

Centrin 1 has been first expressed in HeLa cells to follow centrioles and is since often ectopically expressed in many cells lines as a marker for centrioles (Piel et al., 2000; White et al., 2000).

Centrin 2 is the most studied isoform. Initial studies suggested a role in the control of cell division (Middendorp et al., 2000; Paoletti et al., 1996). Since then, Centrin2

has then been proposed to be essential for centriole duplication in U2OS cells (Salisbury et al., 2002). To date, this result remains controversial since other studies have shown that Centrin 2 is dispensable for centriole duplication (Dantas et al., 2011; Strnad et al., 2007). Indeed, Dantas and colleague showed that centriole biogenesis occurs normally in DT40 cells in absence of all centrin isoforms (Dantas et al., 2011).

In zebrafish, Centrin2 has been shown to play an essential function in ciliogenesis (Delaval et al., 2011). In this study, centrin-depleted animals lack cilia and display morphological defects. More recently, Centrin 2 was also found to have a role in the regulation of gene expression and embryonic development in *Xenopus* (Shi et al., 2015).

Centrin2 has also been shown to undergo cell cycle regulated (Lukasiewicz et al., 2011) by Aurora A. During the cell cycle, levels of centrin will vary according to its phosphorylation state. Centrin2 amount starts to increase in G2/M, peaks in anaphase and is degraded by the APC/C complex. Phosphorylated centrin was mostly detectable during mitosis (at the centrioles and in the cytoplasm) and disappeared after anaphase onset. This study further showed that phosphorylated Centrin and Aurora A are enriched in breast cancer tissues. Two centrin mutants were generated to investigate the role of centrin phosphorylation in centrosome amplification in cancer tissues. When Centrin S170A was expressed in the absence of the endogenous protein and in cells overexpressing Aurora A, the percentage of cells with aberrant number of centrosomes dropped to 7%. In the Auroral OE cells and in the presence of a WT centrin this rate was of 84%. This study suggests a role for centrin in centrosome amplification, although the mechanism of this contribution is unclear.

Centrin2 has also been shown to be required for CPII0 removal from the distal cap of the centriole. In this study, the CRISPR/Cas9 mediated deletion of centrin2 gene induced a stabilization of CPII0 at the mother centriole, which impaired ciliogenesis (Prosser and Morrison, 2015).

Centrin 3 is related to the yeast CDC31 gene and has been shown to localize to the centrosome in human cells (Middendorp et al., 2000; Middendorp et al., 1997). Centrin3 transient overexpression did not alter the cell growth or mitosis. Unlike Centrin 1 and Centrin2, no stable cell line overexpressing Centrin 3 could be established. This may indicate that overexpression of Centrin3 may be deleterious to the cell after a few cell cycles. Centrin 3 function was then studied in *Xenopus* early embryos. Injections of Centrin 3 recombinant protein induced an increase of undercleaved embryos. In later stages, higher occurrence of cell lysis was observed. Most of the undersegmented blastomeres contained two asters indicating that Centrin3 could impair cell cleavage directly or by impairing centrosome duplication. Moreover, overexpression of Centrin3 in budding yeast had a dominant negative effect and caused a block in a cell cycle at G2/M. In these cells, SPB duplication was impaired. This suggests that Centrin 3 may sequester a Cdc31 partner and that this complex is essential for SPB duplication. Karl, a known partner of Cdc31 was found to interact with Centrin 3, but this interaction was lost when Cdc31 was overexpressed. But this was not the cause of the SPB duplication defect since Centrin 2 is also able to interact with Karl without impairing SPB duplication. The authors of this study discussed that Centrin may be binding an unidentified interactor of Cdc31. One may speculate that Centrin3 was sequestering Sfil molecules causing a defect in SPB duplication. At that time, Sfil was still unknown.

Another study has strengthened the idea that centrins are dispensable in centriole biogenesis. In this study, the authors induced centrosome amplification by overexpressing Plk4. In these conditions, Centrins were not among the essential proteins for centriole assembly. For instance, even in the presence of high amount of Plk4, Sas6, CPAP (Sas4), Cep135 and CPl10 were required for the assembly of new procentrioles. This assembly could occur in centrin 2 and 3 depleted cells (Kleylein-Sohn et al., 2007). Importantly, Centrin depletion in these cells did not alter the recruitment of CPl10, Cep135 and CPAP, indicating that centrins do not play a role in Plk4-induced centriole biogenesis.

To sum up, Centrioles functions are as diverse in animal cells as they are in protists. Although early studies pointed toward a role in centriole duplication, this function is still a matter of debate. Nevertheless, it is widely accepted that Centrioles are essential for centrioles' functions as well as for BB and in ciliogenesis.

3.2 Sfil and Cdc31 in yeast: Key regulators of SPB duplication

The yeast centrin CDC31 gene was identified in the Hartwell screen for cell cycle mutants in budding yeast (Hartwell et al., 1974). Cdc31 is a component of the half bridge and is essential for bipolar spindle assembly and SPB duplication (Baum et al., 1986; Spang et al., 1993). Indeed, temperature sensitive mutants arrest with a single large SPB (Baum et al., 1986) and fail to form a satellite (Winey et al., 1991).

Sfil was initially identified in budding yeast as a suppressor of the heat sensitivity associated with a particular mutation in adenylyl-cyclase (Ma et al., 1999). In this study, Sfil was partly characterized and found to be an essential protein whose depletion causes a G2/M arrest with failure to form a mitotic spindle (Ma et al., 1999).

Jonh Kilmartin independently undertook the identification of new Cdc31 interactors using a functional tagged version of Cdc31. The purified complexes contained Sfil and Cdc31. In this study, he showed that Sfil has a series of striking internal repeats in its central region. Twenty-one repeats of the following sequence AX₇LLX₃F/LX₂WK/R were identified in the Sfil sequence of *S. cerevisiae*. Moreover, 23 to 35 amino acids separated each repetition motif (Kilmartin, 2003; Li et al., 2006). Although Sfil sequence was globally divergent, the repeats were found to be highly conserved in two uncharacterized proteins in *S. pombe* and human. The same study showed that these repetitions constitute a centrin-binding motif and that mutations affecting one or two repeats impaired Sfil functions. These results suggest that all the repeats are required for a functional Sfil (Kilmartin, 2003).

Later crystallographic studies performed on *S. cerevisiae* Sfi1 and Cdc31 have established that Cdc31 wraps around the repeats containing central region of Sfi1 molecules that folds into a long α -helix (Figure 3-2). Two structures of complexes of Sfi1 and Centrin were purified and analysed. These complexes contained two or three repeats of Sfi1, and Centrin molecules were found to interact with each repeat in absence or presence of Ca^{2+} . One of the most interesting features of the two solved crystal structures was that centrin molecules bound to adjacent repeats interact with one another (Figures 3-2 and 3-3). Interactions occur between the C-terminal domain of one centrin and the N-terminal domain of the next, with impact on the position of the second centrin molecule rotated $\sim 65^\circ$ clockwise around the Sfi1 helix compared to the first. This organization leads to the formation of an elongated rigid filament that nicely matches the expected length of the half bridge (~ 60 nm, (Li et al., 2006)). Moreover, based on electron microscopy studies, it has been proposed that Sfi1 assemble in a single layer of parallel arrays on top of the NE.

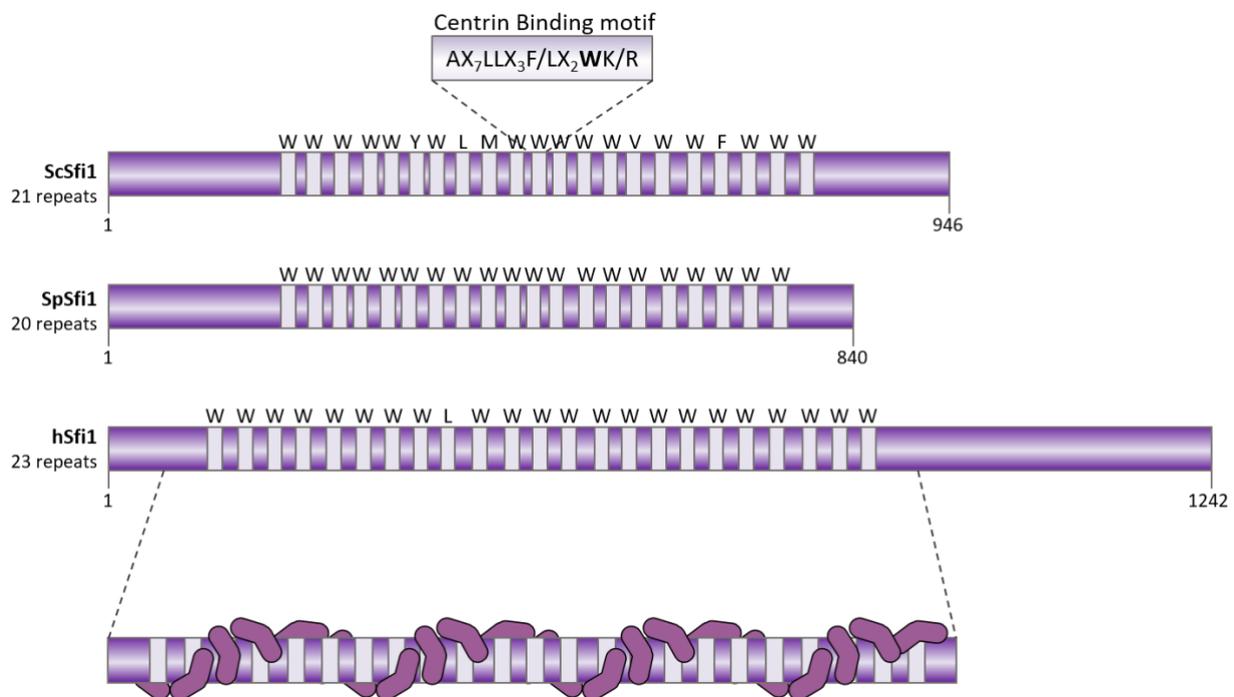


Figure 3-2: Alignments of yeast and human Sfi1 domains.

Scheme adapted from (Ruthnick et al., 2017).

Moreover, the authors suggested that upon initiation of SPB duplication, additional Sfi1 molecules are recruited to the half-bridge where they interact end-on with the existing Sfi1 molecules, via their C-termini. This end-on interaction results in a doubling of the bridge length, and the Sfi1 C termini are now positioned at the center of the bridge. This model was strongly supported by immuno-EM analysis of strains expressing Sfi1 fused to N- or C-terminal GFP tags. In duplicated SPB, the N-terminus is positioned at the junction between the SPB, whereas the C-terminal tag was found to be in the center of the bridge (Li et al., 2006). Thus, the Sfi1p C-termini are ideally placed to promote HB elongation but also to control SPB separation upon

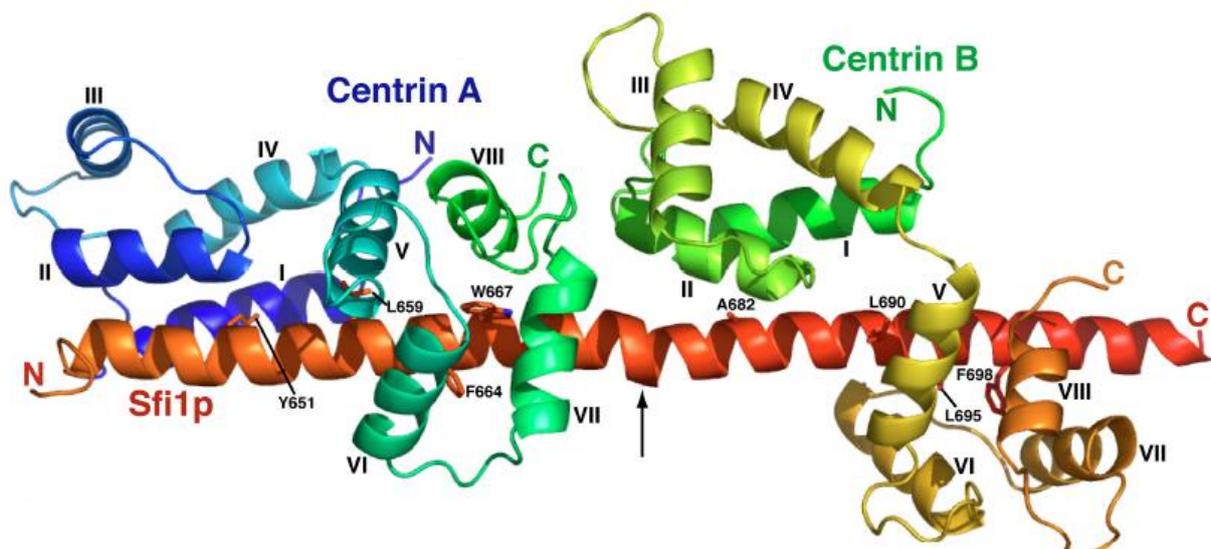


Figure 3-3: Sfi1 repeats and Centrin interactions

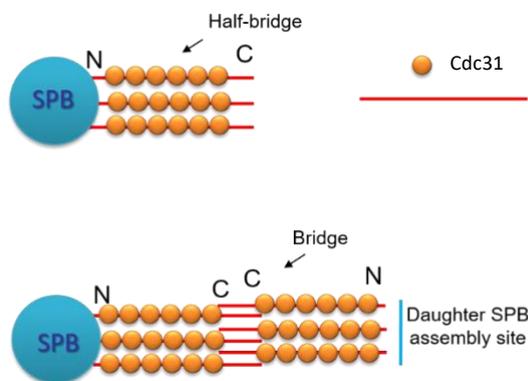
Ribbon diagram of the Sfi1-centrin complex containing two Sfi1 repeats and two centrins at low Ca^{2+} concentration. **From (Li et al., 2006)**

mitotic entry. Accordingly, the authors suggested that an important role for the Sfi1 C-terminus might be to mediate the cleavage of the bridge, either alone or in combination with other proteins.

Anderson and colleagues confirmed this hypothesis in a later study (Anderson et al., 2007). They identified new mutants carrying mutations in the C-terminus of Sfi1. These mutants were arrested in mitosis with an enrichment of cells with two labeled SPB with GFP-Spc42. Intriguingly; the distance between the two SPB was shorter

than expected in the presence of a short bipolar spindle. These observations suggested that Sfi1-65 and Sfi1-120 mutants could have duplicated their SPB but were unable to separate them pointing toward a new function of the C-terminus of Sfi1. EM analysis confirmed that in Sfi1-65 and Sfi1-120 mutants, two well-assembled SPB could be observed next to each other. However, there was a clear defect in SPB separation since they remained attached in mitotic cells.

Interestingly, this phenotype could be rescued with the overexpression of the Kinesin5 Cin8, suggesting that additional forces were required to separate the two SPBs in these Sfi1 mutants.



**Figure 3-4: Model of Sfi1/Cdc31 arrays
In budding yeast**

Scheme adapted from (Kilmartin, 2003)

3.3 Sfi1 in other organisms

Following Sfi1 identification in budding yeast, sequence homology analyses allowed the identification of a large α -helical protein in *Paramecium tetraurelia* that forms the infraciliary lattice (ICL) together with centrin (Figure 3-5). The ICL is a structure at the cell surface of *P. tetraurelia* that was known to contract in presence of Ca^{2+} . Previous biochemical and genetic studies have shown that the ICL is composed of diverse centrin isoforms and a high-molecular-mass centrin-associated protein. The identified protein was named PtCenBP1 (*P. tetraurelia* Centrin Binding Protein1) and was shown to contain 89 Sfi1 like repeats and to localise with Centrin. PtCenBP1 forms fibres connected to the basal bodies across the cell body of *Paramecium*. Depletion of PtCenBP1 lead to a failure of cellular contraction in presence of Ca^{2+} and later on to complete disassembly of the ICL (Gogendeau et al., 2007). Even

though Centriins have already been observed in contractile structures, this study is so far the first and only to show that Sfil-like proteins can form contractile fibres *in vivo*.

Tetrahymena thermophila is a ciliate with a highly organized MT based cytoskeleton. Each cell contains approximately 750 BBs, either in cortical rows or in the oral apparatus (Frankel, 2000; Wloga and Frankel, 2012). Two centriins reside primarily at BBs and both proteins localize asymmetrically to the anterior side of the basal body where new basal body assembly is initiated (Stemm-Wolf et al., 2005). In this organism, 13 Sfil-like proteins were identified and named Sfr1-13 (for Sfil like Repeats, (Stemm-Wolf et al., 2013). The amino acid sequences of these proteins vary in length (Sfr1 is the shortest and Sfr13 is the longest) and in the number of Sfil repeats (3 repeats for Sfr1 and 44 repeats in Sfr13). In this study, the authors confirmed the localization of all Sfr proteins to asymmetric sites around the BBs. Only Sfr1 localized to the BBs properly. Sfr proteins were thus proposed to be good candidates for collaborating with centriin in establishing the geometry and subsequent organization of BBs, with individual Sfr proteins potentially playing specific roles in the various steps of these processes. They specifically showed that Sfr13 is required for BB separation and stability. A second study showed that Sfr1 controls the density of BBs at the cortical rows. Indeed, Sfr1 depletion lead to an increase of the number of BB in the cell, which also increased the BB density on the cortical rows suggesting that Sfr1 could be acting through an inhibitory pathway of BB duplication (Heydeck et al., 2016). Interestingly, the increased number of BBs was only observed in growth phase of the cells. Indeed, WT or Sfr1 depleted cells grown under starvation conditions had the same number and BB densities. This result suggests different regulation of BB number and distribution. This result is unexpected since all reports of Sfil-like proteins seem to point towards a role in promoting duplication rather inhibiting it. Thus Sfr1, and potentially centriins, seem

to have an unexpected inhibitory role in *Tetrahymena* basal body production (Heydeck et al., 2016).

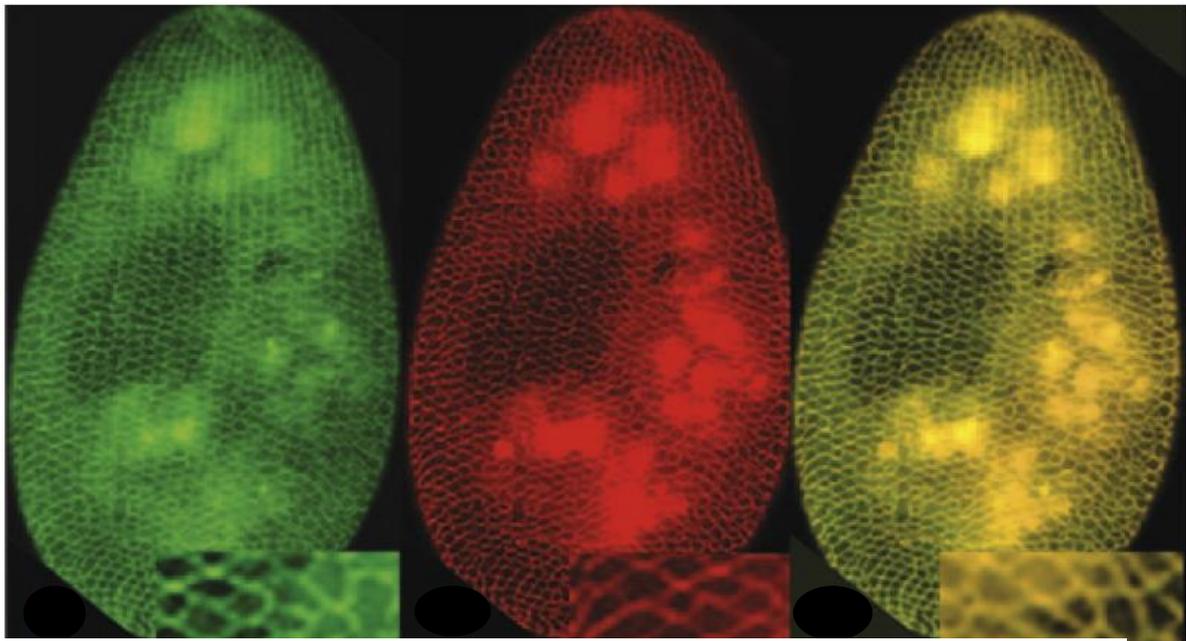


Figure 3-5: ICL organization visualised with Centrin and PtCenBPI labelling

From Gogendeau et al. 2007

The initial characterization study of ScSfil showed that Sfil is also conserved in human cells (Kilmartin, 2003). Interestingly, in this study, the authors mention other potential homologs in humans, where a small number of repeats is found, making them less similar to the yeast proteins. However, a two-hybrid approach to identify proteins interacting with human centrin proteins Centrin 2 or Centrin 3 lead to the identification of a protein with only 3 Sfil repeats in its sequence (Azimzadeh et al., 2009). This protein was formerly found to be a putative component of human centrosomes by mass spectrometry analysis (Andersen et al., 2003). Moreover, a *C. reinhardtii* homologue called POC5 (proteome of centriole 5) has also been found in centriole fractions (Keller et al., 2005).

In hPOC5 study, the authors demonstrated that, the first Sfil-repeat (also referred to as CBR for Centrin Binding Region 1) was not able to bind centrin. In contrast,

CBR2+3 bound Centrin 2 and Centrin 3 independently of calcium concentrations. Moreover, the interaction between hPOC5 and centrin proteins appears to be specific, as hPOC5 does not bind Calmodulin, a protein closely related to centrin. Fluorescence and electron microscopy also confirmed that hPOC5 was a centriolar protein which is recruited to the centrioles in G2 (Azimzadeh et al., 2009).

RNAi mediated depletion of hPOC5 only reduced the proteins levels at the daughter centriole, indicating a stable incorporation of hPOC5 at the mother centriole. Depletion of hPOC5 also lead to a cell cycle arrest in G1 in RPE1 cells, but HeLa cells have an extended S phase before undergoing cell death. Interestingly, RPE1 cell cycle arrest triggered p21 activation as a consequence of p53 activation (Azimzadeh et al., 2009). This could possibly result from the activation of the centrosome surveillance pathway (Fong et al., 2016; Lambrus et al., 2016; Meitinger et al., 2016; Wong et al., 2015)

Finally, the authors have shown that hPOC5-depleted HeLa cells arrested in S phase possessed duplicated centrosomes, each containing one mature mother centriole and one procentriole. EM of serial-sectioned cells revealed that the procentrioles are approximately 40% as long as full-length centrioles. In addition, the procentrioles were similar to those observed during early initiation of centriole duplication as they were composed of doublet MTs instead of triplets and retained the cartwheel. The authors confirmed by fluorescence microscopy that procentrioles in hPOC5-depleted cells are similar in length to those found during early steps of centrosome duplication. These observations confirm that hPOC5 is dispensable for centriole duplication but is required for centriole elongation in G2 and for the assembly of the distal region of centrioles (Azimzadeh et al., 2009).

hPOC5 variants have recently been identified in a cohort of patients suffering from Idiopathic scoliosis. Although these variants are functional, their expression in zebrafish induce the appearance of distorted spines. Moreover, in the context of this highly heterogeneous disorder with possible digenic inheritance for a subgroup of patients, identifying POC5 as the first IS-causing gene is a major step toward

deciphering the genetic causes of IS (Patten et al., 2015). This result indicates that Sfil like protein could play a role in human disease like idiopathic scoliosis which point to the importance of a better understanding of their functions in human.

4. Thesis Outline

The aim of my PhD was to understand the functions of Sfil and Centrin, in two model organisms. First, I investigated the role of Sfil in SPB duplication and separation using *S. pombe* as a model organism. In this project, I tried to understand the cell cycle regulations that control Sfil and Centrin functions. Using different techniques of fluorescence microscopy, we have confirmed that Sfil is a SPB component and that is required for SPB duplication and separation.

In the second part of my PhD, I got interested in Sfil function in human cells. Evidence from our results in fission yeast and from other studies suggested that Sfil might be required for centriole duplication. Our results show that this is not the case. Sfil seems instead to be required for ciliogenesis.

The results section is organized around two articles; the first article describing Sfil functions in *S. pombe* was published in the Journal of Cell science in 2015. The second paper concerns Sfil characterization in human cells and is in preparation.

Moreover, I am also an author of a protocol article published in Methods in Cell Biology, included in “Centrosome and Centrioles” series and published in 2015. This article described the methods used in the first article. I added this article to this manuscript as an appendix.

Chapter II: RESULTS

1. Article 1: Cell Cycle Control of Spindle Poles Bodies Duplication and Splitting by Sfil and Cdc31

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RESEARCH ARTICLE

Cell cycle control of spindle pole body duplication and splitting by Sfi1 and Cdc31 in fission yeast

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ABSTRACT

Spindle pole biogenesis and segregation are tightly coordinated to produce a bipolar mitotic spindle. In yeasts, the spindle pole body (SPB) half-bridge composed of Sfi1 and Cdc31 duplicates to promote the biogenesis of a second SPB. Sfi1 accumulates at the half-bridge in two phases in *Schizosaccharomyces pombe*, from anaphase to early septation and throughout G2 phase. We found that the function of Sfi1–Cdc31 in SPB duplication is accomplished before septation ends and G2 accumulation starts. Thus, Sfi1 early accumulation at mitotic exit might correspond to half-bridge duplication. We further show that Cdc31 phosphorylation on serine 15 in a Cdk1 (encoded by *cdc2*) consensus site is required for the dissociation of a significant pool of Sfi1 from the bridge and timely segregation of SPBs at mitotic onset. This suggests that the Cdc31 N-terminus modulates the stability of Sfi1–Cdc31 arrays in fission yeast, and impacts on the timing of establishment of spindle bipolarity.

KEY WORDS: Cell Cycle, Centrosome, Mitotic spindle, SPB

INTRODUCTION

The mitotic spindle is a complex microtubule-based structure responsible for the accurate segregation of chromosomes. Its assembly and function are therefore under strict and robust regulatory mechanisms. Spindle assembly starts during prophase when duplicated centrosomes move apart to establish a bipolar structure of antiparallel overlapping interpolar microtubules. Although spindle assembly can proceed in the complete absence of centrosomes in animal cells (Khodjakov et al., 2000; Basto et al., 2006), centrosomes, which nucleate and anchor microtubules, contribute to spindle assembly efficiency and robustness. They are, for instance, essential during fast cycles of early development (Stevens et al., 2007). They are generated by conservative duplication of pre-existing single-copy centrosomes to limit their number to exactly two per cell after duplication and prevent deleterious effects of supernumerary centrosomes in spindle assembly, organ morphogenesis and tumor formation (Basto et al., 2008; D'Assoro et al., 2002; Marthiens et al., 2013).

Spindle pole bodies (SPBs) are yeast plaque-like organelles attached to the nuclear envelope that are functionally similar to centrosomes of animal cells. Like centrosomes, they are generated by conservative duplication of pre-existing SPBs. In budding as well as fission yeast, the daughter SPB assembles at the tip of a mother SPB appendage called the bridge, which maintains the association of the duplicated SPBs until mitotic onset. Duplicated SPBs inserted in the nuclear envelope nucleate intranuclear microtubules to initiate bipolar spindle assembly (Adams and Kilmartin, 2000; Ding et al., 1997; Jaspersen and Ghosh, 2012; Jaspersen and Winey, 2004; Lim et al., 2009; Uzawa et al., 2004).

In *Saccharomyces cerevisiae*, the SPB bridge was shown to contain two major evolutionarily conserved proteins, Sfi1 and Cdc31 (yeast centrin), that are essential for SPB duplication and bipolar spindle formation (Baum et al., 1986; Kilmartin, 2003; Li et al., 2006; Paoletti et al., 2003; Spang et al., 1993). Their orthologs play important roles at centrosomes and cilia in a variety of eukaryotes (Avasthi et al., 2013; Azimzadeh et al., 2009; Azimzadeh et al., 2012; Balestra et al., 2013; Dantas et al., 2012; Delaval et al., 2011; Gogondeau et al., 2007; Gogondeau et al., 2008; Jerka-Dziadosz et al., 2013; Stemm-Wolf et al., 2013; Wei et al., 2014; Zhang and He, 2012).

Crystallographic studies performed on Sfi1 and Cdc31 have established that Sfi1 molecules form an extended α -helix containing repeats on which the Ca^{2+} -binding calmodulin-like Cdc31 molecules bind. Moreover, based on electron microscopy studies, it has been proposed that, to form a half-bridge, these molecules assemble into a parallel array with Sfi1 N-termini attached to the mother SPB. In this model, daughter SPB assembly is initiated by half-bridge duplication, achieved by assembly of a second array of Sfi1 molecules, creating a new half-bridge, anti-parallel to the parental half-bridge and connected to it by interactions between Sfi1 C-termini (Anderson et al., 2007; Li et al., 2006).

Very recent studies further show that the coordination of half-bridge duplication and bridge splitting with cell cycle progression is controlled by the cyclin-dependent kinase Cdk1 (encoded by *cdc2*). Cdk1 phosphorylates the Sfi1 C-terminus to promote the segregation of the two half-bridges at mitotic entry (Anderson et al., 2007; Avena et al., 2014; Elserafy et al., 2014). This event is also controlled to a lesser extent by the polo-like kinase Cdc5 that targets additional sites on the Sfi1 C-terminus (Elserafy et al., 2014). Importantly, Cdk1-dependent phosphorylation of Sfi1 C-terminus was also shown to prevent SPB reduplication after half-bridge splitting (Avena et al., 2014; Elserafy et al., 2014). This inhibition is relieved by the Cdc14 phosphatase that dephosphorylates the Sfi1 C-terminus at mitotic exit (Avena

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et al., 2014; Elserafy et al., 2014). Finally, SPB duplication is also regulated by Mps1 kinase, the activity of which is necessary for half-bridge duplication (Elserafy et al., 2014). Interestingly, one G1 target of Mps1 is Cdc31. Phosphorylation of Cdc31 by Mps1 was shown to be necessary for SPB duplication. Whether this phosphorylation is sufficient to promote half-bridge duplication remains unknown (Araki et al., 2010).

Like budding yeast Cdc31, fission yeast Cdc31 is a component of the SPB half-bridge and bridge, and is essential for SPB duplication and bipolar spindle assembly (Paoletti et al., 2003). Sfi1 is also conserved in fission yeast (Kilmartin et al., 2003), and a very recent study showed that Sfi1 is required for SPB duplication (Lee et al., 2014). Nevertheless, when half-bridge duplication takes place or how half-bridge duplication and bridge splitting are coordinated with cell cycle progression in fission yeast remains largely unknown. In this study, we have analyzed how Sfi1 and Cdc31 function is regulated in fission yeast. We show that Cdc31 is necessary for the assembly of Sfi1 parallel arrays and that it functions at mitotic exit when Sfi1 accumulates quickly at the SPB to promote SPB duplication. In addition, Cdc31 is phosphorylated on an N-terminal Cdk1 consensus site to promote bridge splitting and SPB separation at mitotic entry.

RESULTS

Sfi1 associates with the half-bridge in a Cdc31-dependent manner

Fission yeast Sfi1 localizes to SPBs like its budding yeast counterpart (Kilmartin, 2003; Lee et al., 2014). Because we could not determine Sfi1 ultrastructural localization by immunoelectron microscopy (immuno-EM), we developed a fluorescent light microscopy method to verify whether Sfi1 associates with the SPB half-bridge and bridge like Cdc31 (Paoletti et al., 2003); cells expressing Sfi1 tagged C-terminally with mRFP, a fully functional fusion that can replace Sfi1 at its endogenous locus, and another SPB component tagged with GFP were fixed with methanol to prevent SPB movements. Sample stability was assessed by performing two successive acquisitions of red and green signal and checking the perfect superimposition of the two sets of red or green images. We finally used dual color fluorescent beads to check the registration of red and green signals in the microscopy setup used. Spatial shifts between red and green channels were less than 1 pixel in the field of view.

Using this method, we could observe that in short G2 or mitotic cells, Sfi1-mRFP staining was always closely apposed to but never perfectly colocalized with calmodulin (Cam1), which binds the nuclear side SPB component Pcp1 (Flory et al., 2002; Fong et al., 2010; Moser et al., 1997) or other SPB core components such as Sid2, which associates with the external surface of the SPB (Sparks et al., 1999), or the transmembrane SUN domain protein Sad1 (Hagan and Yanagida, 1995) (Fig. 1A,C,D,G). We then measured fluorescence intensity across SPBs using the linescan tool of Metamorph software. In early G2, peak intensities of Sfi1 never coincided with the peak intensities of core SPB markers and were generally separated by 2 pixels representing ~130 nm theoretically (supplementary material Fig. S1A–D).

At later stages of G2, as judged by cell length, two dots corresponding to duplicated SPBs could be discriminated with the three SPB core markers. These dots corresponded to two peaks generally separated by 4 pixels on fluorescence intensity profiles measured by using the Metamorph linescan tool, representing ~260 nm theoretically. In contrast, a single peak was observed

with Sfi1-mRFP, between the peaks of the SPB core markers, consistent with Sfi1 localization at the bridge connecting duplicated SPBs (Fig. 1A,C,D,G).

Immunodetection of Cdc31 further showed that Sfi1 colocalized perfectly with this half-bridge component throughout the cell cycle and that its peak intensity coincided perfectly with that of Cdc31 on fluorescence intensity profiles across SPBs (Fig. 1B,G; supplementary material Fig. S1B).

To confirm these results, we finally performed colocalization studies between Sfi1-mCherry and Sid4-GFP or between Sfi1-GFP and Sid4-mCherry, inverting the color channels used to visualize the two proteins (Fig. 1E,F; supplementary material Fig. S1E,F). This experiment confirmed that Sfi1 does not perfectly colocalize with SPB core components and sits between duplicated SPBs after duplication, independent of fluorescence wavelength. We therefore conclude that Sfi1 is a genuine half-bridge and/or bridge component in *S. pombe*.

We next determined how Sfi1 targeting to the half-bridge is controlled. To do so, we produced a series of Sfi1 constructs fused to GFP at the C-terminus that we expressed in wild-type cells in addition to endogenous Sfi1 (Fig. 2A). We found that deleting the Sfi1 N-terminus (amino acids 1–188), but not Sfi1 C-terminus (amino acids 766–840) strongly reduced Sfi1 targeting to the half-bridge but did not abolish it completely. When expressed alone, the central region of Sfi1 containing Cdc31-binding repeats (amino acids 189–765) was targeted to the half-bridge at similar low levels. Finally, the N-terminal or C-terminal domains did not associate with the half-bridge on their own. Thus, efficient targeting of Sfi1 to the half-bridge depends on the Cdc31-binding central domain combined with the Sfi1 N-terminus.

This result suggested that Cdc31 might be required for Sfi1 association with the half-bridge. To test this further, we used the *pnmt*-E147K-cdc31* mutant in which E147K-Cdc31 can be depleted upon addition of thiamine at 36°C, blocking SPB duplication and inducing the assembly of monopolar spindles (Paoletti et al., 2003). We found that Sfi1-GFP, now expressed from the endogenous Sfi1 locus, was lost from half-bridges upon Cdc31 depletion, whereas control cells maintained strong Sfi1-GFP staining (Fig. 2B). This demonstrates that Cdc31 is essential for the stability of Sfi1 half-bridge arrays.

We next wanted to test whether Cdc31 localization to the half-bridge reciprocally requires Sfi1. Given that *sfi1* is essential for cell survival, we produced a thermo-sensitive mutant of *sfi1* by random mutagenesis of the Sfi1 N-terminus. This mutant carries a frame shift at valine 10 and a point mutation changing asparagine 171 to aspartic acid. Why this mutant is thermosensitive is unclear. One possibility is that it might initiate translation at methionine 17 more efficiently at 25°C than at 36°C.

Immunostaining of *sfi1-1* cells grown for 4 hours at 36°C revealed some G2 cells lacking Cdc31 staining as well as mitotic cells with monopolar spindles lacking Cdc31 on the single spindle pole (Fig. 3A). We conclude that there is a co-requirement for Cdc31 and Sfi1 to assemble into stable half-bridge arrays.

To better characterize the *sfi1-1* mutant, we performed time-lapse imaging in *sfi1-1* cells expressing GFP-tagged tubulin. This confirmed that ~90% of total spindles were monopolar after 4 hours at 36°C (Fig. 3B,C). These monopolar spindles led to a prolonged mitosis, likely as a result of spindle checkpoint alert (Weiss and Winey, 1996), but eventually disassembled (Fig. 3C). Septation started before spindle disassembly and gave rise to cut

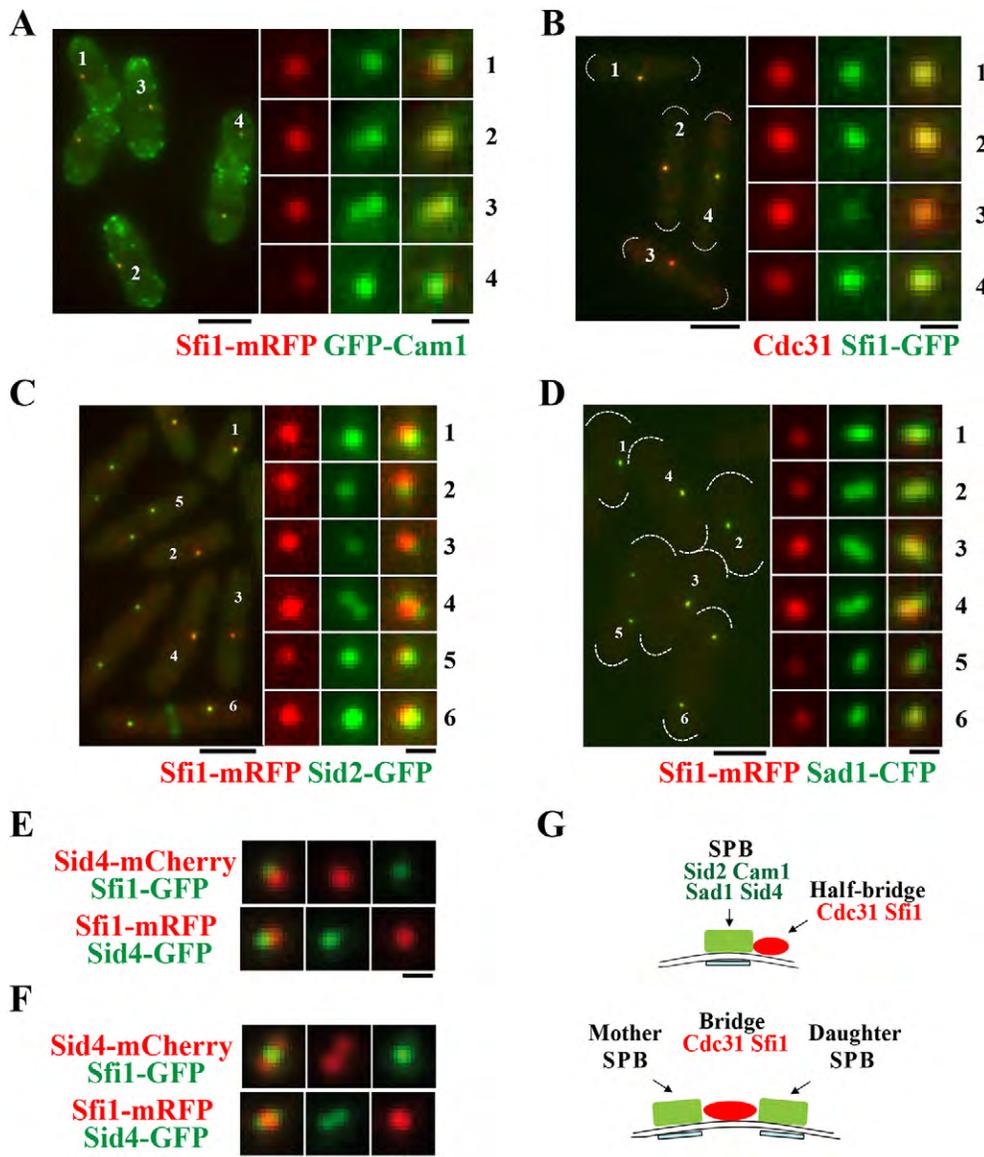


Fig. 1. Sfi1 localizes to the SPB half-bridge or bridge. (A–D) Localization at SPBs of Sfi1–mRFP and GFP–Cam1 (A), immunostained Cdc31 and Sfi1–GFP (B), Sfi1–mRFP and Sid2–GFP (C) or Sfi1–mRFP and Sad1–CFP (D). Insets show magnification of SPBs from cells numbered according to cell cycle progression in left panels. For B,D, the white lines indicate the ends of each cell. Scale bars: 5 μm (main image), 500 nm (insets). (E,F) Localization at unduplicated (E) or duplicated (F) SPBs of Sfi1–mRFP and Sid4–GFP or Sfi1–GFP and Sid4–mCherry. Scale bars: 500 nm. These SPBs correspond to SPBs 1 and 4 shown in supplementary material Fig. S1E,F, where a field view of several cells is also shown. (G) Schematic localization of Cam1, Sid2, Sad1, Sid4, Cdc31 and Sfi1 on non-duplicated SPB and half-bridge (left) or on duplicated SPBs linked by the bridge (right).

and multi-septation phenotypes (supplementary material Fig. S2A), similar to the phenotype of the *pnmt*-cdc31-E147K* mutant (Paoletti et al., 2003).

We next used Ppc89–GFP, a key structural component of the SPB core (Rosenberg et al., 2006) to verify the duplication status of the SPB. With this marker, we could observe the gradual accumulation of duplicated SPB pairs in the population of wild-type cells of increasing length that progress through G2 (supplementary material Fig. S2B). SPB pairs also accumulated gradually with cell size in the *cdc10-V50* mutant blocked at the G1/S transition for 3 or 5 hours at 36°C, or in cells blocked in S phase by treatment with hydroxyurea for 5 hours at 25°C (supplementary material Fig. S2B). These results are in agreement with recent electron microscopy observations showing that single and duplicated SPBs can be found in short G2 cells (Höög et al., 2013) but also confirm that SPB duplication does not require S phase entry (Uzawa et al., 2004). They show that Ppc89–GFP constitutes a good tool to monitor SPB duplication.

Analysis of mitotic cells expressing Ppc89–GFP and mCherry-tagged tubulin (Fig. 3D) showed that *sfi1-1* mutant cells failed to

duplicate their SPBs at 36°C; more than 90% of mitotic *sfi1-1* cells with monopolar spindles presented a single SPB labeled with Ppc89–GFP. In contrast, in the *sad1-1* mutant that also makes monopolar spindles (Hagan and Yanagida, 1995), we observed two Ppc89–GFP-stained SPBs at the single pole at high frequency. This confirmed that duplicated but not segregated SPBs can be distinguished easily using this marker in mitosis and that SPB duplication fails in *sfi1*-deficient cells. It also indicates that Sad1, in contrast to Sfi1, might contribute to the SPB cycle after its duplication phase. Finally, wild-type cells expressing Ppc89–GFP showed two segregated single SPBs at the poles of bipolar spindles. We also observed some cells with more than two Ppc89–GFP stained structures and multipolar spindles at low frequency (<4% mitotic cells). This suggests that tagging Ppc89 with GFP might not be fully innocuous at 36°C. The incidence of this type of defect was increased in *sfi1-1* mutants (~9% mitotic cells) suggesting that Ppc89–GFP might be synthetic lethal with the *sfi1-1* mutant.

SPB duplication defects in the *sfi1-1* mutant were confirmed by analyzing SPB status in late G2, as judged by the presence of

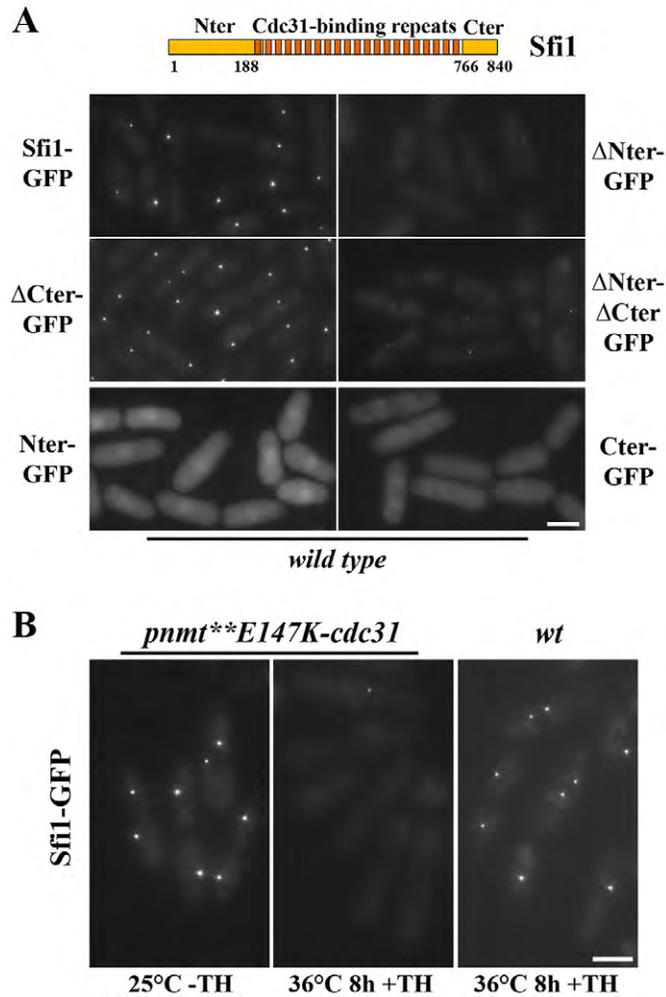


Fig. 2. Sfi1 targeting to the SPB is controlled by Cdc31. (A) Localization of C-terminally GFP-tagged Sfi1 or Sfi1 fragments expressed from the *leu1* locus in a wild-type strain. Sfi1-GFP targeting to the half-bridge requires the N-terminal and central Cdc31-binding regions of Sfi1. Nter, N-terminal; Cter, C-terminal. (B) Sfi1-GFP localization in the *pnm1**E147K-Cdc31* mutant grown at 25°C without thiamine (TH) or for 8 hours at 36°C after

interphase microtubules and a cell length ranging between 10 and 12 μ m. Whereas side-by-side duplicated SPBs could be distinguished in the vast majority of wild-type cells (~75%), they could be identified in a much reduced proportion of *sfi1-1* cells (~27%), confirming the results obtained in mitotic cells (supplementary material Fig. S2C).

We finally analyzed the SPB status in the *sfi1-1* mutant in post-mitotic cells using Sid2-GFP as a marker for the SPB (Fig. 3E; supplementary material Fig. S2D). Indeed, Sid2 also localizes to the division site in post-mitotic cells, allowing their identification. We found that 80% of post-mitotic *sfi1-1* mutant cells grown at 36°C for at least 3 hours presented a single SPB, in contrast to control cells that showed duplicated and segregated SPBs. The phenotype of the *sfi1-1* mutant was again distinct from that of the *sad1-1* mutant, where two juxtaposed Sid2-GFP-labeled SPBs could be easily discriminated (Fig. 3E; supplementary material Fig. S2D).

We conclude that Sfi1 is essential for SPB duplication in fission yeast, in agreement with its Cdc31-dependent localization

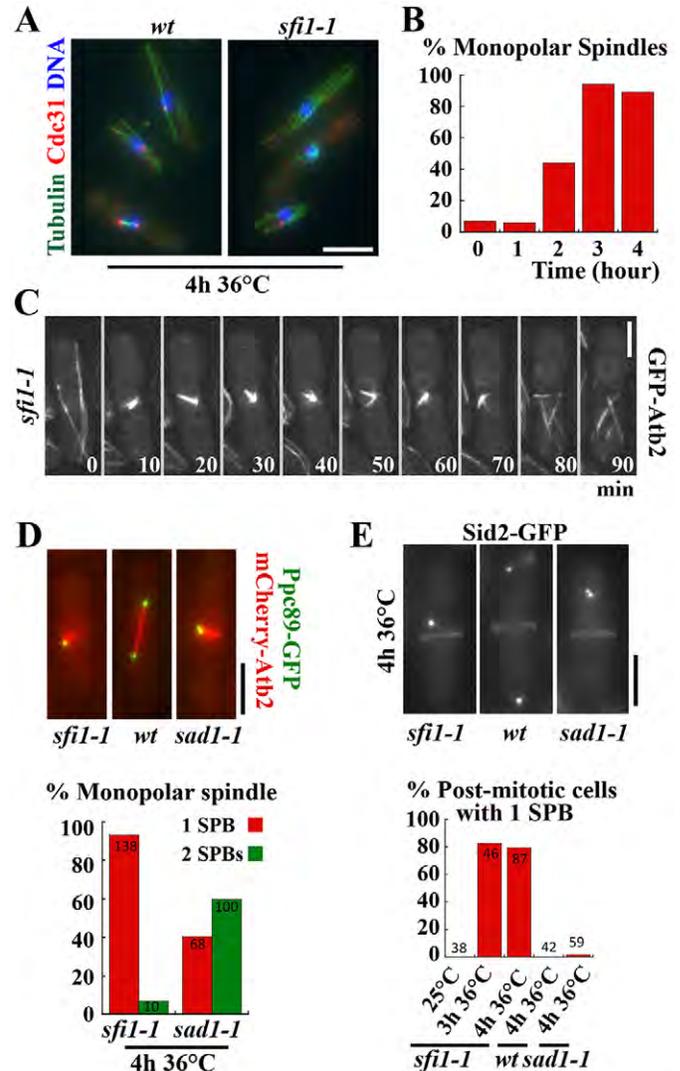


Fig. 3. Sfi1 is required for Cdc31 targeting to the SPB and is essential for SPB duplication. (A) Microtubule organization in *sfi1-1* mutant and wild-type (wt) cells. Cells were immunostained for tubulin, Cdc31 and DNA after 4 hours at 36°C. Scale bar: 5 μ m. (B) Percentage of mitotic *sfi1-1* cells expressing GFP-Atb2 with monopolar spindles after shift to 36°C. (C) Time-lapse movie of an *sfi1-1* cell expressing GFP-Atb2 grown at 36°C. Scale bar: 5 μ m. (D) Top, Ppc89-GFP distribution in typical mitotic *sfi1-1*, wild-type or *sad1-1* cells after 3 hours at 36°C. Scale bar: 5 μ m. Bottom, percentage of mitotic *sfi1-1* or *sad1-1* cells with monopolar spindles exhibiting one or two Ppc89-GFP-stained SPBs. *n*-values are shown on the graph. (E) Top, Sid2-GFP distribution in typical post-mitotic *sfi1-1*, wild-type or *sad1-1* cells after 4 hours at 36°C. Scale bar: 5 μ m. Bottom, percentage of post-mitotic *sfi1-1*, wild-type or *sad1-1* cells exhibiting a single Sid2-GFP-stained SPB.

to the half-bridge. This result is in agreement with the new SPB assembly defects identified in the *sfi1-M46* mutant when cells inherit insufficient Sfi1 (Lee et al., 2014).

Half-bridge duplication might be initiated during anaphase

We next wondered when half-bridge duplication takes place during cell cycle progression to allow the assembly of the daughter SPB. To address this question, we first used a quantitative approach to detect variations in Sfi1 amounts at the SPB that might signal half-bridge duplication. Live-cell imaging

of cells expressing Sfi1-GFP from the *sfi1* endogenous locus by spinning-disc confocal microscopy with low laser power, combined with SPB automatic tracking, allowed us to record Sfi1 intensity at SPBs for more than a complete cell cycle (Fig. 4A–C).

In agreement with observations made recently by Lee et al. (Lee et al., 2014), two phases of Sfi1-GFP intensity increase were detected after SPB segregation at mitotic entry. The first phase started just a few minutes after mitotic entry, in early anaphase, and lasted until mid-septation. Accordingly, we could measure that by the end of anaphase, Sfi1-GFP intensity had already increased by ~ 1.5 -fold compared to mitotic entry. In contrast, GFP-Cam1 intensity, used as a control, remained constant during the same period (Fig. 4D). Similarly, Sid4-GFP or Ppc89-GFP, two central components of the SPB core, displayed a limited increase during this period, showing that the fast intensity

increase observed at mitotic exit is specific to Sfi1. The second phase started after septation and lasted throughout G2 phase until the next round of mitosis.

Interestingly, Sfi1-GFP kept accumulating at the SPB when G2 phase lasted longer in a *cdc25-22* mutant grown at 36°C (Fig. 4E). Sfi1-GFP accumulation was also maintained upon overexpression of Cdc18 (Fig. 4F), which blocks cells in an abortive S phase and prevents SPB early maturation, i.e. extension of SPB plaques after duplication (Uzawa et al., 2004). This indicates that Sfi1-GFP accumulates at SPBs independently of SPB growth in size.

To determine which phase of Sfi1 accumulation corresponds to half-bridge duplication, we defined when Sfi1-Cdc31 function is necessary for SPB duplication. Instead of the *sfi1-1* mutant, which still exhibits monopolar spindles at low frequency under non-restrictive conditions and precludes determination of when Sfi1

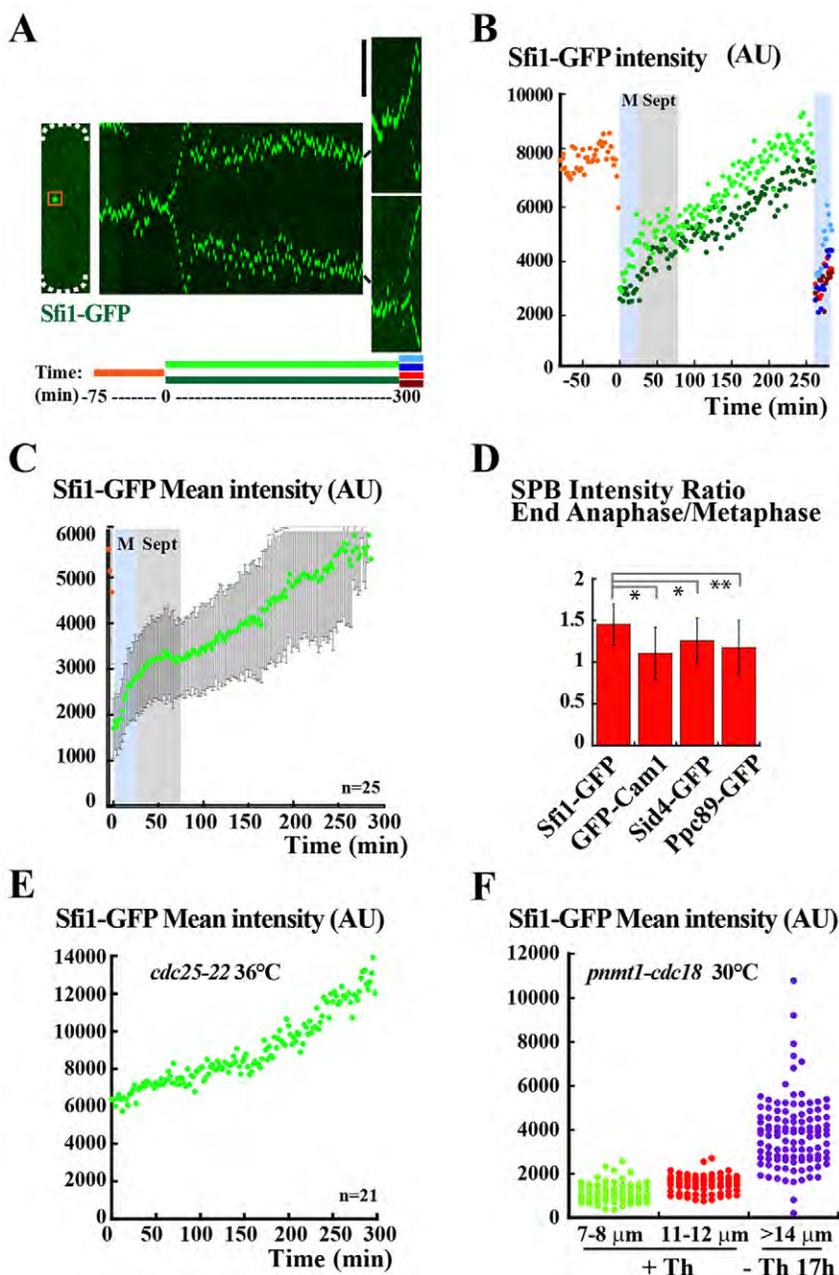


Fig. 4. Dynamics of Sfi1-GFP accumulation at the SPB half-bridge during cell cycle progression. (A,B) Kymograph of SPBs in a wild-type cell expressing Sfi1-GFP (A) and quantitative analysis of Sfi1-GFP intensity at SPBs during cell cycle progression (B). Bars in A (bottom) represent SPBs tracked in B with a similar color code. Scale bar: 5 μ m. AU, arbitrary units; $t=0$, SPB segregation; M, mitosis (blue zone); Sept, septation (gray zone). (C) Mean Sfi1-GFP intensity at SPB before (orange) and after segregation (green) at $t=0$. $n=25$. Error bars show \pm s.d. (D) Mean intensity ratio of Sfi1-GFP, GFP-Cam1, Ppc89-GFP or Sid4-GFP at SPBs in individual cells between metaphase and the end of anaphase (mean over four time-points). $n=19, 16, 39$ and 43 , respectively. Error bars show \pm s.d. $*P\leq 10^{-2}$; $**P\leq 10^{-3}$ (Student's t -test). (E) Mean Sfi1-GFP intensity at SPB in *cdc25-22* cells blocked in G2 at 36°C. $n=21$. (F) Sfi1-GFP intensity at SPBs in short (7–8 μ m) or long (11–12 μ m) *nmt1-cdc18* cells grown in the presence of thiamine (+Th), or in cells longer than 14 μ m after induction of Cdc18 overexpression for 17 hours at 30°C (–Th). $n=62, 64$ and 109 , respectively.

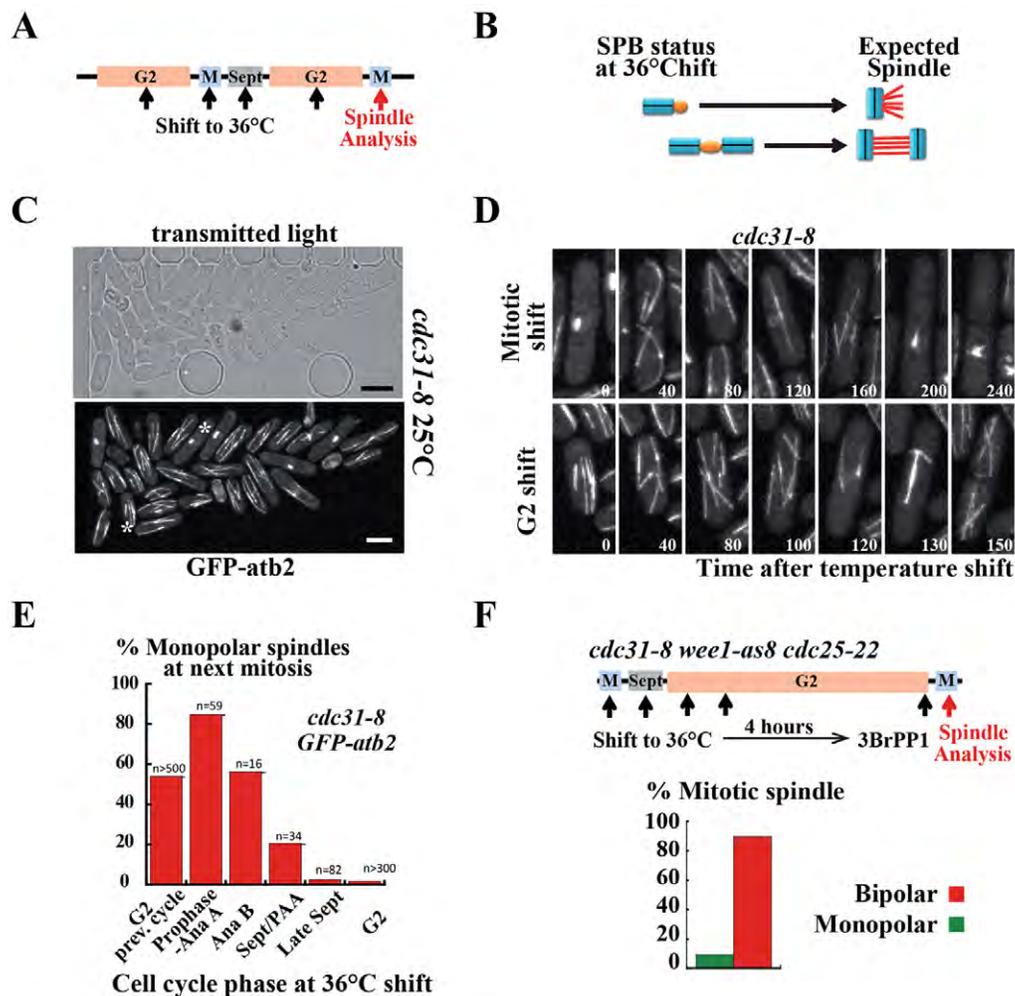


Fig. 5. Cdc31 promotes SPB duplication at mitotic exit. (A–C) Experimental design to determine when *cdc31* exerts its function. A *cdc31-8* mutant expressing GFP–Atb2 was subjected to fast temperature shift from 25°C to 36°C in microfluidic chambers under time-lapse imaging and further imaged for up to 5 hours to monitor mitotic spindles during the next round of mitosis (A). Shift to the restrictive temperature before half-bridge duplication (i.e. *cdc31-8* execution point) is expected to produce a monopolar spindle, whereas shift after the execution point is expected to produce a bipolar spindle (B). The cell cycle stage is defined based on microtubule organization at the time of temperature shift as shown in C. Transmitted light is used to identify septating cells. Scale bar: 5 μm. (D) Time-lapse analysis of spindle assembly after temperature shift for the two cells marked by an asterisk in C. Top cell, temperature shift in metaphase ($t=0$) leads to a monopolar spindle at $t=200$ minutes. Bottom cell, temperature shift during early G2 ($t=0$) leads to a bipolar spindle at $t=130$ minutes. Scale bar: 5 μm. (E) Percentage of monopolar spindles in a *cdc31-8* mutant expressing GFP–Atb2 and subjected to fast temperature shift from 25°C to 36°C under time-lapse imaging at various cell cycle stages, judged by microtubule organization and further imaged until the next mitosis. G2, interphase microtubules; Prophase–Ana A, short spindle; Ana B, long spindle; Sept/PAA, septating cells with post-anaphase array; Late Sept, septated cells without PAA. (F) Experimental scheme (top) and percentage of monopolar or bipolar spindles (bottom) in *cdc31-8 wee1-as8 cdc25-22* mutants expressing GFP–Atb2 and subjected to temperature shift at 36°C for 4 hours to block cells in G2, released from the block at 36°C by addition of 3BrPP1 under time-lapse imaging and

function is accomplished, we used the *cdc31-8* thermo-sensitive mutant isolated as described previously (Ohta et al., 2012), in which the *cdc31* open reading frame (ORF) carries a substitution changing arginine 134 to glycine and the 3'UTR is replaced by the ADH terminator.

Fast temperature shift of the *cdc31-8* mutant was performed under the microscope (Velve Casquillas et al., 2011). The cell cycle stage at the time of temperature shift was defined according to microtubule organization, using the fluorescent tubulin marker GFP–Atb2 (Atb2 is also known as Tub1) (Fig. 5C). This experimental setup allowed us to define at which point of the cell cycle *cdc31-8* inactivation led to a monopolar spindle phenotype during the next round of mitosis (Fig. 5A,B).

We found that cells shifted during late septation or G2 (i.e. during the second phase of Sfi1 accumulation) always produced bipolar spindles in the following mitosis, indicating that the half-bridge was already duplicated at these stages (Fig. 5D,E). Cells shifted in early septation with a post-anaphase array of microtubules showed only a low percentage of monopolar spindles during the next mitosis. In contrast, cells shifted during mitosis (i.e. before or concomitant to the first phase of Sfi1 accumulation) showed high levels of monopolar spindles during the next mitosis (Fig. 5D,E).

In a control experiment, we used the *cdc25-22 wee1-as8 cdc31-8* strain (Tay et al., 2013). This strain can be blocked in G2 for several hours at the restrictive temperature of 36°C, due to

cdc25 inactivation, and released synchronously into mitosis by addition of the ATP analog 3Br-PP1 that inactivates the *wee1-as8* allele and allows the cells to overcome the *cdc25-22* block. We observed that >90% of cells blocked in G2 at 36°C for up to 4 hours showed bipolar spindles when released into mitosis at 36°C (Fig. 5F). This shows that the differential outcomes in mitotic spindle organization observed upon shift of the *cdc31-8* mutant to 36°C are not due to long term incubation at the restrictive temperature but are truly specific to the cell cycle stage at which the shift was performed. This data strongly supports the hypothesis that the first phase of Sfi1 accumulation in late mitosis/early septation represents half-bridge duplication.

S phase takes place during septation in wild-type fission yeast, such that daughter cells are already in G2 when the septum is cleaved. To determine whether half-bridge duplication occurs

before S phase onset, we combined *cdc31-8* with the *wee1-as8* strain to delay S phase onset by addition of 3Br-PP1. Indeed, *wee1* inactivation generates very short cells following division that need to grow before committing to a new cell cycle and entering S phase (Fantes and Nurse, 1978; Sveiczer et al., 1996). In this experiment, owing to the incompatibility between 3Br-PP1 and polydimethylsiloxane (PDMS) used to build microfluidic devices for fast temperature shift, cells were instead transferred to lectin-coated glass-bottomed dishes, treated with 3Br-PP1 for 6 hours at 25°C to inactivate Wee1 and reduce cell size, then transferred to the microscope incubator heated at 36°C where imaging was started within a few minutes, followed by addition of hot medium to ensure fast completion of the temperature shift. Cell cycle stage was recorded at this point according to microtubule organization. With this experimental setup, cells

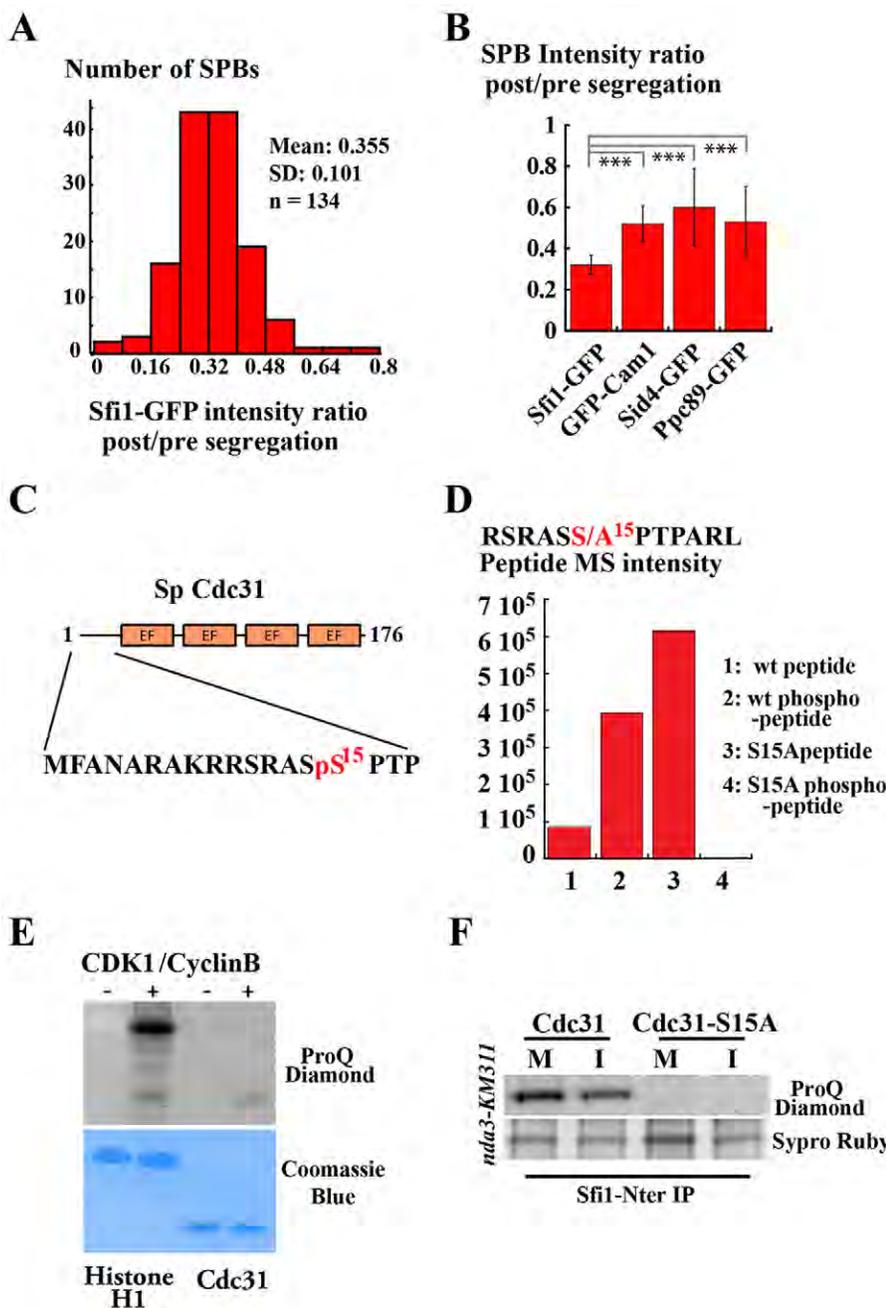


Fig. 6. Sfi1–Cdc31 arrays are destabilized at mitotic entry and Cdc31 is phosphorylated on a CDK1 consensus site. (A) Distribution of Sfi1–GFP intensity ratios before and after SPB segregation. (B) Mean intensity ratio of Sfi1–GFP, GFP–Cam1, Sid4–GFP or Ppc89–GFP at SPBs before and after segregation (means over four time-points). $n=12, 9, 41$ and 44 , respectively. Error bars show \pm s.d. $***P\leq 10^{-4}$ (Student's *t*-test). (C) Position of phospho-serine 15 detected by mass spectrometry on a Cdk1 consensus site in Cdc31. EF, EF-hand domains; Sp, *Schizosaccharomyces pombe*. (D) MS signal in LC-MS/MS sample of Cdc31 signature peptide for serine 15 phosphorylation RSRASSPTPARL [phospho-peptide $460.23^{(3+)}m/z$; non phosphorylated peptide: $433.58^{(3+)}m/z$] and of signature peptide RSRASAPTPARL after mutation of serine 15 to alanine [phospho-peptide $454.9^{(3+)}m/z$; non phosphorylated peptide $428.24^{(3+)}m/z$]. wt, wild type. (E) *In vitro* phosphorylation assay of Cdc31 purified from bacteria with the mammalian cyclin-B–CDK1 complex. Phospho-proteins are stained with ProQ Diamond. Histone H1 is used as a positive control. (F) Cdc31 and Cdc31-S15A were co-immunoprecipitated (IP) with Sfi1-Nter–GFP from *nda3-KM311* cells blocked in mitosis or released from block for 1 hour and analyzed for phosphorylation with the ProQ Diamond dye. Sypro Ruby reveals total Cdc31 protein in gel. M, mitosis; I, interphase.

might have experienced temperature shift slightly earlier than recorded. Nevertheless, >90% of cells shifted at 36°C after completion of septation displayed bipolar spindles during the next mitosis (supplementary material Fig. S2E,F), indicating that *cdc31* function is already accomplished in short G1 cells. We conclude that half-bridge duplication takes place immediately at mitotic exit when Sfi1-GFP accumulates quickly at the SPB.

Cdc31 phosphorylation on a consensus Cdk1 site promotes Sfi1 partial dissociation from the bridge and bridge splitting

Another striking observation from the quantitative analysis of Sfi1-GFP intensity, also reported by Lee et al. (Lee et al., 2014), is that upon segregation at mitotic onset, the two SPBs exhibit ~30% fluorescence intensity of the pre-mitotic SPB, instead of the expected 50% (Fig. 6A,B). This feature is specific for Sfi1 because we did not observe it with GFP-Cam1, for which the fluorescence intensity of segregated SPBs after mitotic entry was ~50% of the pre-mitotic SPB, nor for Sid4-GFP or Ppc89-GFP (Fig. 6B). This indicates that approximately a third of Sfi1 molecules dissociate from the bridge at mitotic onset. Whether

Sfi1 molecules are dispersed in the cytoplasm or are degraded is unclear.

Interestingly, we noticed that Sfi1-GFP intensity started to decrease a few minutes before bridge splitting (Fig. 4B,C). This suggested that the partial dissociation of Sfi1 from the half-bridge might be triggered by a mitotic kinase and could destabilize the bridge to favor bridge splitting. Searching for potential Cdk1 sites, we found that Cdc31 contains two Cdk1 consensus phosphorylation sites in its N-terminal part (serine 15 and threonine 17). To determine whether these sites were phosphorylated *in vivo* during mitosis, Cdc31 was co-immunoprecipitated from a mitotic cell extract with a soluble N-terminal fragment of Sfi1 that contains a single Cdc31 binding repeat and is more favorable for quantitative immunoprecipitation than full-length Sfi1. Mass spectrometric analysis showed that the serine 15 in a Cdk1 consensus site was phosphorylated *in vivo* during mitosis (Fig. 6C; supplementary material Fig. S3).

A serine-to-alanine mutation was introduced at position 15 of Cdc31 at the *cdc31* locus to inhibit phosphorylation. The *cdc31-S15A* strain was viable, indicating that this mutation does not

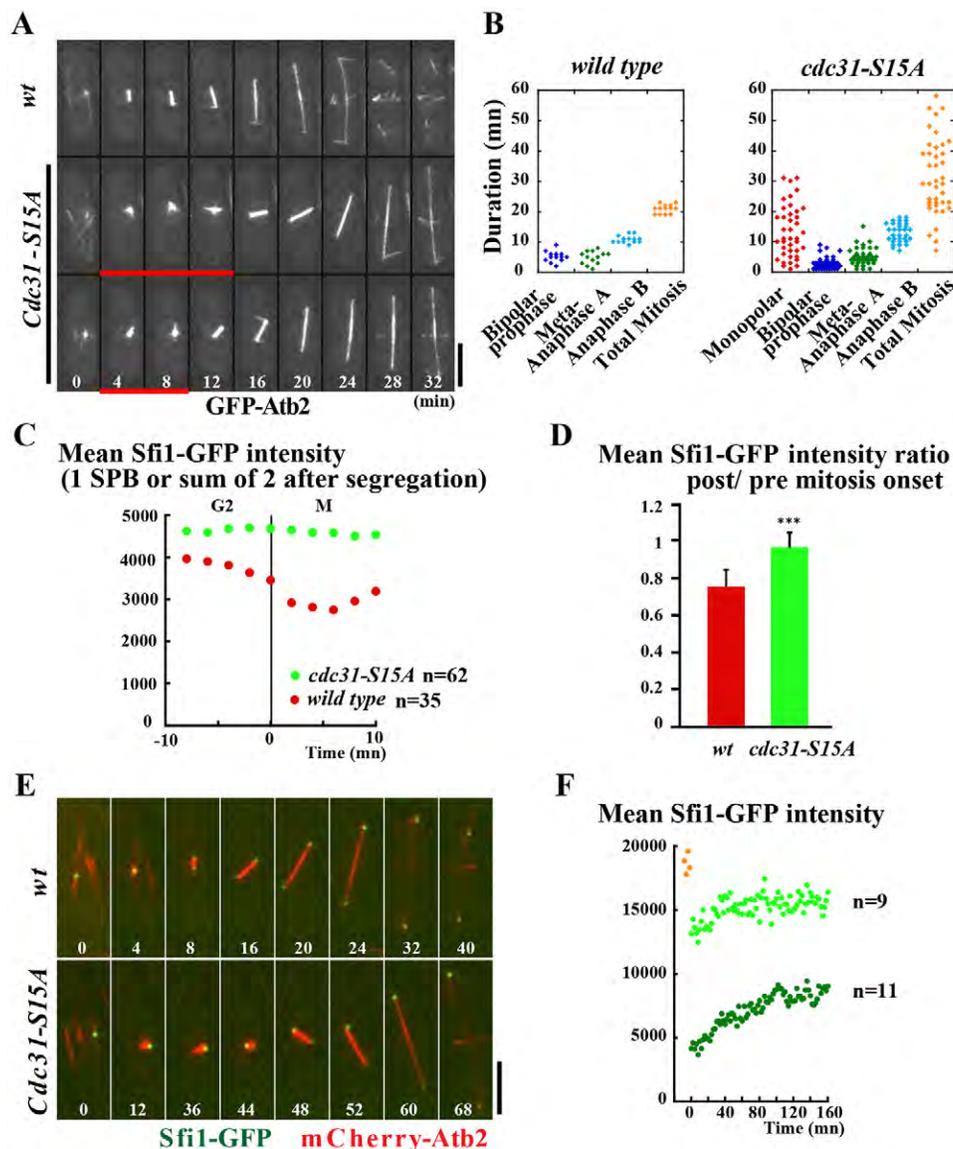


Fig. 7. Spindle pole segregation is delayed in the *cdc31-S15A* mutant and Sfi1 is stabilized at the bridge at mitotic onset. (A) Microtubule organization in *cdc31-S15A* mutant or wild-type (*wt*) cells expressing GFP-Atb2. Scale bar: 5 μ m. *t*=0, mitotic entry. (B) Duration of mitotic phases in similar cells as in A. *n*=13 for wild-type and 40 for *cdc31-S15A* strains. (C) Mean Sfi1-GFP intensity at SPB before segregation or sum of intensities of the two segregated SPBs. The black line at *t*=0 indicates mitotic onset. (D) Mean ratio of Sfi1-GFP intensity at one SPB or the sum of two segregated SPBs (mean of three time-points). Error bars show \pm s.d. ****P* ≤ 10⁻¹⁰ (Student's *t*-test). (E) Time-lapse analysis of wild-type and *cdc31-S15A* cells expressing Sfi1-GFP and mCherry-Atb2. Time after mitosis entry in minutes. Scale bar: 5 μ m. (F) Mean Sfi1-GFP intensity in the *cdc31-S15A* mutant. *n*=9 for premitotic (orange) and high-intensity SPBs (light green) and 11 for low-intensity SPBs (dark green).

abolish Cdc31 function. Phosphorylation of the Cdc31 peptide containing Cdk1 consensus sites was abolished (Fig. 6D), confirming that serine 15 is phosphorylated *in vivo* but threonine 17 is not. Furthermore, Cdc31 purified from bacteria was a substrate of mammalian cyclin-B–CDK1 *in vitro* (Fig. 6E). Finally, Cdc31 phosphorylation was fully abolished *in vivo* upon mutation of serine 15 to alanine (Fig. 6F). These results indicate that Cdc31 might be a target of mitotic Cdk1. We noted, however, that serine 15 remains phosphorylated 1 hour after release of the mitotic block, indicating that Cdc31 phosphorylation is not strictly restricted to mitosis.

Interestingly, analysis of microtubules using the fluorescent marker GFP–Atb2 revealed that *cdc31-S15A* cells assembled transient monopolar spindles (Fig. 7A,B). The monopolar spindle phase lasted for variable amounts of time (Fig. 7B) but resolved into a bipolar spindle. Accordingly, >50% of monopolar spindles were resolved into bipolar spindles in 1-hour-long movies (supplementary material Fig. S4C). This result was confirmed using Plo1–GFP that strongly associates with spindle poles during mitosis (Mulvihill et al., 1999). Plo1–GFP staining showed two juxtaposed dots in early mitosis, eventually segregating after a variable delay when the spindle became bipolar (supplementary material Fig. S4A,B).

The phenotype of the *cdc31-S15A* mutant is clearly distinct from that of *cdc31-8* or *sfi1-1* mutant phenotypes where SPB duplication is compromised. Accordingly, analysis of Sid2–GFP revealed two segregated SPBs in 97% of post-mitotic *cdc31-S15A* cells (supplementary material Fig. S4D,E). We thus conclude that the *cdc31-S15A* mutant is not defective for SPB duplication but displays a delay in separation of duplicated SPBs at mitotic onset.

Finally, quantitative analysis of Sfi1–GFP in the *cdc31-S15A* mutant revealed that close to 100% of the Sfi1 fluorescence intensity remained associated with SPBs at mitotic entry compared to only around two thirds in the wild-type strain (Fig. 7C,D). Surprisingly, an unequal distribution of Sfi1–GFP between the two SPBs was also evident after delayed SPB segregation (Fig. 7E). This asymmetry could result from an asymmetrical rupture of the bridge when proper regulation of bridge splitting is compromised or from an asymmetry in bridge biogenesis.

Analysis of Sfi1–GFP fluorescence intensity in the *cdc31-S15A* mutant showed a first increase at mitotic exit that might correspond to half-bridge duplication. Remarkably, although GFP intensity also increased during G2 on low-intensity SPBs, GFP intensity remained stable on high-intensity SPBs (Fig. 7F). Thus, the asymmetry in half-bridges inherited by the two daughter cells is compensated for at least in part by a differential accumulation of Sfi1 molecules in the bridge during the next G2 phase. Taken together, our data reveals that Cdc31

phosphorylation on serine 15 regulates Sfi1 dissociation from the bridge at mitotic onset to promote SPB separation and allow immediate assembly of a bipolar spindle.

DISCUSSION

In this study, we have shown that Sfi1 and its partner protein Cdc31 together control two key steps of the SPB duplication cycle in fission yeast: half-bridge duplication, which permits in turn the assembly of the daughter SPB, as well as SPB segregation at mitotic onset, necessary for bipolar spindle assembly (see Fig. 8). Sfi1–Cdc31 function is therefore largely conserved between budding and fission yeast (Anderson et al., 2007; Avena et al., 2014; Elserafy et al., 2014). Whereas the SPB duplication function was reported previously (Lee et al., 2014; Paoletti et al., 2003), our data provides the first evidence for a role of Cdc31 in the temporal control of SPB segregation and spindle bipolarity establishment.

We found that Cdc31 and Sfi1 are both required for the assembly of half-bridge arrays. Furthermore, our analysis of a new *cdc31* mutant, *cdc31-8*, using a fast microfluidic-based temperature shift system, combined with quantitative analysis of Sfi1 amounts at the SPB along cell cycle progression, indicates that half-bridge duplication is initiated during anaphase when the first phase of Sfi1 accumulation starts. Whether an SPB precursor or satellite assembles immediately upon half-bridge duplication remains to be determined.

Using Ppc89–GFP as a marker to follow daughter SPB biogenesis, we could also confirm that SPBs are committed to duplication before S phase entry because duplication was not blocked in the *cdc10-V50* mutant, as reported previously (Uzawa et al., 2004). Nevertheless, side-by-side duplicated SPBs were rarely detected in early G2 using this tool, and they gradually accumulated in the cell population during G2 phase. Although some duplicated SPBs might not be recognized using light microscopy owing to their orientation or when the daughter SPB size is too small, this result is in good agreement with electron microscopy data showing the presence of duplicated and nonduplicated SPBs in short G2 cells (Höög et al., 2013). It might also explain why duplication figures were initially only identified in long G2 cells (Ding et al., 1997).

A second phase of Sfi1 accumulation lasts all along G2 phase. We found that it persists longer if progression into mitosis is blocked. This accumulation does not require S phase exit and is therefore independent of SPB growth in size or maturation because it persisted upon overexpression of Cdc18 (Uzawa et al., 2004). Thus, it seems that the bridge and the SPB core are submitted to distinct regulations during cell cycle progression.

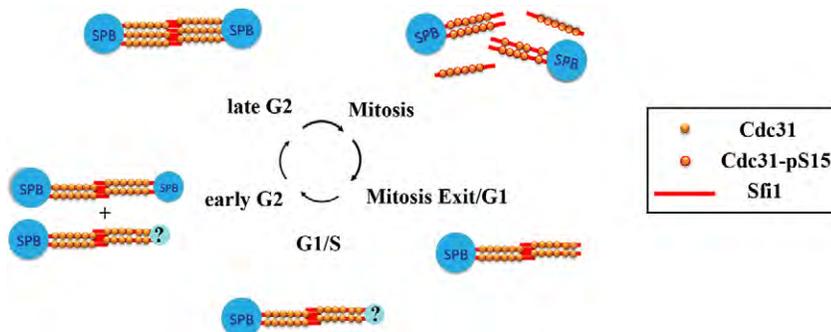


Fig. 8. Model for Cdc31–Sfi1 control of bridge duplication and splitting. Cdc31 phosphorylation on serine 15 allows partial dissociation of Sfi1 from the bridge during mitosis to favor SPB separation, a necessary step for bipolar spindle assembly. Note, however, that this phosphorylation might not be restricted to mitosis. Half-bridge duplication might occur at mitotic exit when a first phase of Sfi1 accumulation at the SPB takes place. Whether a satellite is formed at this stage remains unknown. The SPB is committed to duplication by G1/S but the daughter SPB only becomes visible gradually during G2 phase. Further accumulation of Sfi1 on the bridge occurs all along G2.

We have also shown that SPB separation at mitotic entry is regulated by Cdc31 phosphorylation on a Cdk1 consensus site on serine 15. This phosphorylation is necessary for the partial dissociation of Sfi1 from the bridge and immediate SPB separation at mitotic entry. Although we could show that Cdc31 is a substrate for CDK1–cyclin-B *in vitro*, serine 15 phosphorylation does not appear to be restricted to mitosis *in vivo* because we could observe this phosphorylation in post-mitotic extracts. New tools will be necessary to establish the precise pattern of phosphorylation of serine 15 according to cell cycle progression. At this stage, we can only speculate that serine 15 could either be protected from dephosphorylation by the Cdc14-like phosphatase Clp1 (also known as Flp1) that antagonizes CDK1 phosphorylation at mitotic exit (Cueille et al., 2001; Trautmann et al., 2001) or that it could be the target of additional kinases.

Another point that remains to be elucidated is the exact effect of serine 15 phosphorylation on Sfi1–Cdc31 arrays. Because Sfi1 association with the half-bridge requires Cdc31, phosphorylation of Cdc31 on serine 15 could modulate its interaction with Sfi1 to destabilize the half-bridge at mitotic entry. However, the Cdc31 N-terminus might not participate in the Sfi1–Cdc31 interaction in budding yeast, as judged by the crystal structure obtained for a fragment of budding yeast Sfi1 in complex with budding yeast Cdc31, where the Cdc31 N-terminus was not visible and hence not engaged in interactions with the Sfi1 helix (Li et al., 2006). In addition, a Cdc31 mutant lacking N-terminal residues can replace wild-type Cdc31 in *S. cerevisiae* (Li et al., 2006), similar to the *cdc31S15A* mutant in *S. pombe*. However, the timing of SPB separation was not reported in this budding yeast mutant.

An alternative role for the phosphorylation of fission yeast Cdc31 N-terminus is to modulate the oligomerization of Sfi1–Cdc31 complexes in parallel arrays. Additional biochemical work might solve this question if fission yeast Sfi1 can be purified in a soluble form. Interestingly, in contrast to the budding yeast half-bridge, the fission yeast half-bridge is a thick three-dimensional (3D) appendage (Ding et al., 1997; Paoletti et al., 2003; Uzawa et al., 2004). Moreover, the Cdc31 N-terminus is longer in *S. pombe* than in *S. cerevisiae*, where it lacks a Cdk1 consensus site. It is therefore possible that interactions between Sfi1–Cdc31 complexes involve additional interactions and regulations in fission yeast compared to *S. cerevisiae*.

It is also worth noting that SPB separation is delayed but not fully blocked when Cdc31 phosphorylation on serine 15 is inhibited. Thus, other regulatory mechanisms might function in parallel to Cdc31 phosphorylation to promote bridge splitting and release the two duplicated SPBs. One possibility is that it might involve the phosphorylation of the Sfi1 C-terminus as demonstrated recently in budding yeast (Avena et al., 2014; Elserafy et al., 2014). Sfi1 C-terminus contains a Cdk1 consensus site but its mutation to alanine led to SPB duplication defects (A.M., A.P., unpublished data). Because the Sfi1 C-terminus is very short in fission yeast (74 amino acids), these defects could possibly stem from global folding defects that might prevent the establishment of interactions between Sfi1 C-termini. In any case, this precludes drawing conclusions on the possible role of this consensus Cdk1 site in controlling SPB separation.

Alternatively, bridge splitting in the *cdc31-S15A* mutant could occur by non-regulated rupture when the kinesin Cut7 (a homolog of mammalian Eg5, also known as KIF11), associated with SPBs (Hagan and Yanagida, 1992), starts exerting the forces necessary to slide antiparallel microtubules apart to separate the two spindle

poles. Such a mechanism would be conceptually similar to the process of centriole disengagement during the development of *Caenorhabditis elegans* embryos, where separase-driven centriole separation becomes dispensable when the forces exerted by microtubule asters are strong enough to induce disengagement (Cbral et al., 2013).

To conclude, we have shown that fission yeast Cdc31 is an important regulator of Sfi1 arrays and is the target of modulation by phosphorylation with an important impact on spindle bipolarity and cell division. Cdc31 orthologs, the centrins, do not contain an N-terminal CDK1 consensus site but are subject to phosphorylation in physiological and pathological situations such as cancer (Araki et al., 2010; Lutz et al., 2001; Yang et al., 2010). It will be of great interest to determine whether centrin phosphorylation by CDKs or alternative kinases can modulate the assembly of arrays of Sfi1 orthologs to control their functions in centrosomes in other eukaryotes.

MATERIALS AND METHODS

Yeast genetics, strains and plasmids

All *S. pombe* strains are isogenic to 972 and are listed in supplementary material Table S1. Standard *S. pombe* molecular genetics techniques and media were used (http://research.stowers.org/baumannlab/documents/Nurselab_fissionyeasthandbook.pdf). Sfi1 deletion and C-terminal tagging with GFP or mRFP at the endogenous locus was performed as described previously (Bähler et al., 1998). Sid4 tagging with mCherry was performed similarly.

The S65T-GFP-Cam1 strain is a generous gift from Trisha Davis (University of Washington). The Sid2-GFP and Sid4-GFP strains were obtained from Dannel Mc Collum, University of Massachusetts. Ppc89-GFP and Plo1-GFP strains were given by Kathleen Gould (Vanderbilt University). The *nmt1-cdc18* strain was obtained from Paul Nurse (Cancer Research UK, London Research Institute). The *sad1-1*, *wee1-as8* and *wee1-as8 cdc25-22* mutants are generous gifts from Iain Hagan, Cancer Research UK, Manchester Institute. GFP–Atb2- and mCherry–Atb2-expressing strains have been described previously (Sato et al., 2009). These strains were crossed using the random spore method to obtain the strains listed in supplementary material Table S1.

Most plasmids used in this study derive from the integrative vector pJK148 (Keeney and Boeke, 1994). pAP244 (*pJK148-psfi1-sfi1-tnmt1, leu1+*) was obtained by insertion into pJK148 of a *KpnI-SmaI* PCR fragment encoding the *sfi1* promoter and ORF amplified from genomic DNA and a *SmaI-SacI* fragment encoding the *nmt1* terminator digested from pREP3X.

pAP246 [*pJK148-psfi1-sfi1-GFP-Tnmt1, leu1+*], pAP248 [*pJK148-psfi1-sfi1(1-765)-GFP-Tnmt1, leu1+*], pAP249 [*pJK148-psfi1-sfi1(1-188)-GFP-Tnmt1, leu1+*], pAP250 [*pJK148-psfi1-sfi1(766-840)-GFP-Tnmt1, leu1+*], pAP251 [*pJK148-psfi1-sfi1(189-765)-GFP-Tnmt1, leu1+*], pAP254 [*pJK148-psfi1-sfi1(189-840)-GFP-Tnmt1, leu1+*] were obtained by insertion of *SalI-NotI* fragments encoding part of the *sfi1* ORF obtained by PCR amplification and a *NotI-SmaI* fragment from pSGP572a encoding S65T-GFP in pAP244 digested by *SalI* and *SmaI*, except for pAP246 and pAP248, where *NsiI* and *NcoI* were used, respectively, instead of *SalI*. These plasmids were integrated at the *leu1* locus into a *leu1-32* strain using the lithium acetate-DMSO method (Bähler et al., 1998) after digestion by *NruI*.

To produce Cdc31 in bacteria, the Cdc31 ORF was subcloned between *BamHI* and *XmaI* sites in pEGEX-6P1 (Amersham; pIB14). To isolate the *sfi1-1* mutant, a *SalI-NarI* fragment encoding the Sfi1 N-terminus was amplified by PCR under mutagenic conditions and ligated into a *pJK148-psfi1-sfi1-tnmt1, leu1+* plasmid (pAP244) digested by similar enzymes. Mutagenized pAP244 was integrated at the *leu1* locus in a *leu1-32/leu1-32 sfi1+/sfi1Δ::kanMX6* diploid strain by homologous recombination after digestion by *NruI*. *Leu1+* colonies grown at 33°C on EMM medium without leucine were sporulated on ME and the *sfi1-1* temperature-sensitive mutant selected at 36°C on plates containing phloxin B after spore germination. Sequencing revealed a frame shift in the codon of

valine 10, a silent mutation in the codon of alanine 63 and a substitution of asparagine 171 to aspartic acid. The *Cdc31-ts8* mutant was isolated as described previously (Ohta et al., 2012).

To produce the *cdc31-S15A* mutant, *cdc31-Tadh-KanMX6* sequences, flanked by 0.5-kb sequences up- and down-stream of the *cdc31* ORF were cloned into pCR2.1-TOPO (Invitrogen) and subjected to site-directed mutagenesis. The mutant sequence was introduced at the *cdc31* locus in a diploid *cdc31A::ura4+/cdc31+* strain. Haploid G418-resistant *cdc31S15A* colonies were recovered after sporulation.

Immunolabeling and microscopy

Immunolabeling of Cdc31 with an anti-Cen3 rabbit antibody, a generous gift of Michel Bornens (Institut Curie) (Paoletti et al., 2003) of tubulin with TAT1 monoclonal antibody (mAb) obtained from Keith Gull (University of Oxford), and DNA with DAPI was performed on cells fixed with methanol at -20°C for 2 minutes as described previously (Paoletti et al., 2003).

Epi-fluorescence images shown in Fig. 1A–D, Fig. 2, Fig. 3A,D,E, supplementary material Fig. S1A–D, Fig. S2B–D; and Fig. 1E,F, supplementary material Fig. S1E,F were acquired, respectively, on a DMRXA2 and DM 5000 B upright microscope (Leica Microsystems), equipped with a 100 \times /1.4NA oil-immersion PlanApo objective, a PIFOC objective stepper and a Coolsnap HQ CCD camera (Photometrics) on cells fixed with -20°C methanol for 2 minutes to stop SPB movement and rehydrated in PEM buffer (100 mM PIPES pH 6.9, 1 mM EGTA, 1 mM MgCl_2) and mounted between slide and coverslip. Stacks of nine planes spaced by 0.5 μm were acquired, and maximum projections were performed using Metamorph software.

Spinning-disc confocal images shown in Fig. 3C, Fig. 4A, Fig. 5C,D, supplementary material Fig. S2A,E and Fig. 7A,E, supplementary material Fig. S4A,D were acquired, respectively, on a Nikon Eclipse TE2000-U microscope equipped with a 100 \times 1.45NA, oil-immersion objective, a PIFOC objective stepper, a Yokogawa CSU22 confocal unit and a Roper HQ2 CCD camera and a laser bench (Errol) with 491–561-nm diode laser, 50 mW each (Cobolt) and a Nikon Eclipse Ti-E microscope equipped with the Perfect Focus System, a 100 \times /1.45NA PlanApo oil-immersion objective, a Mad City Lab piezo stage, a Yokogawa CSUX1 confocal unit a Photometrics HQ2 CCD camera and a laser bench (Errol) with 491–561-nm diode laser, 100 mW each (Cobolt).

Cells were mounted between slides or coverslips or, for movies, mounted on YE5S agarose pads (Tran et al., 2004) (Fig. 3C; Fig. 4A) or in microfluidic devices (Velve-Casquillas et al., 2010) (Fig. 5C,D; Fig. 7A–E; supplementary material Fig. S2A; Fig. S4A–D). Stacks of 9–11 planes spaced by 0.5 μm were acquired and maximum projections were performed using Metamorph software. For time-lapse movies of *sfi1-1*, GFP–Atb2 images were acquired every 10 minutes for 5 hours (binning 2 gain 3, 300 ms at 30% GFP laser power). For Sfi1–GFP time-lapse movies shown in Fig. 4A, images were acquired every 2 minutes for 5 hours (binning 2 gain 3, 300 ms at 10% GFP laser power). For time-lapse movies of Cam1–GFP (Fig. 4D; Fig. 6B) images were acquired every 2 minutes for 5 hours (binning 2 gain 3; 300 ms at 20% GFP laser power). The *Cdc31-S15A* mutant expressing GFP–Atb2 was imaged every minute for 1 hour (Fig. 5D, binning 2 gain 3; 200 ms at 10% GFP laser power). Plo1–GFP- and Sid4–mCherry-expressing strains were imaged every 2 minutes for 2 hours (supplementary material Fig. S3C, binning 2 gain 3; 200 ms at 10% GFP laser power and 300 ms at 15% mCherry laser power). Strains expressing Sid2–GFP and mCherry–Atb2 were imaged for 1 hour at 1-minute intervals (supplementary material Fig. S3E, binning 2 gain 3; 200 ms at 10% GFP laser power and 300 ms at 12% mCherry laser power). Strains expressing Sfi1–GFP and mCherry–Atb2 were imaged for five hours with 2-minute intervals for GFP and 4-minute intervals for mCherry (supplementary material Fig. S3F,G, binning 2 gain 3; 400 ms at 15% mCherry laser power and 200 ms at 10% GFP laser power).

G1 block in the *cdc10-V50* mutant and S phase block with hydroxyurea

The *cdc10-V50* mutant expressing Ppc89–GFP was grown exponentially at 25°C in liquid YE5S then transferred at 36°C for 3.5 hours or 5 hours

(Uzawa et al., 2004). Control cells expressing only Ppc89–GFP were grown under the same conditions. To block cells in S phase, cells expressing Ppc89–GFP were grown exponentially in YE5S at 25°C and treated for 5 hours with 11 mM hydroxyurea (HU). Cells were fixed in methanol at -20°C for 2 minutes as described previously (Paoletti et al., 2003) prior to imaging. Cell size was measured in Metamorph from DIC images.

SPB tracking and intensity measurements

For intensity measurements shown in Fig. 4B,C, SPBs were tracked automatically on 3D spinning-disc confocal stacks using MIA software developed on site (Racine et al., 2006; Théry et al., 2006) or using the tracking object tool in Metamorph. Sfi1–GFP fluorescence intensity was corrected for background and computed over time in Matlab. In other figures, SPBs were either tracked manually or using the Tracking Objects tool of Metamorph (Molecular Devices) using regions of 6 \times 6 pixels. Fluorescence ratios shown in Fig. 6A,B are derived from intensities measured immediately before and after SPB segregation at mitotic entry. In Fig. 7C,D, mean SPB fluorescence intensity was recorded on regions of 5 \times 5 or 6 \times 6 pixels using Metamorph on maximum projections of spinning-disc confocal z-stacks and corrected for background inside the same cell.

Sfi1–GFP fluorescence analyses were performed similarly in *cdc25-22* cells grown in YE5S and blocked in G2 by shift to 36°C (Fig. 4E) or in *pnmt1-cdc18* cells grown overnight in EMM medium containing 5 $\mu\text{g/ml}$ thiamine and then blocked in S phase by overexpression of Cdc18 by growth for 17 hours at 30°C in the absence of thiamine (Fig. 4F).

Fast temperature shift

Fast temperature shift experiments shown in Fig. 5C–E were performed using an in-house built fast temperature control system (Velve Casquillas et al., 2011), a prototype of Tempocell (Elvesys). Briefly, cells were set at 25°C under a spinning-disc confocal microscopy setup described previously (Almonacid et al., 2011) and shifted to 36°C after recording a first image to determine the cell cycle stage. Cells were then imaged every 10 minutes for 5 hours.

G2 block in *cdc31-8 cdc25-22 wee1-as8* and release with 3Br-PP1 at 36°C

We crossed the *cdc31-8* mutant expressing GFP–Atb2 with *cdc25-22 wee1-as8* (Tay et al., 2013). The *cdc31-8 cdc25-22 wee1-as8* mutant expressing GFP–Atb2 (AP4831) was grown exponentially in liquid YE5S and transferred to glass-bottomed Petri dishes (WPI) coated with soybean lectin (0.1 mg/ml, Sigma) for 4 hours at 36°C to block cells in G2 and inactivate Cdc31. Imaging was then started at 36°C and the G2 block was released at frame 2 by the addition of 10 μM 3BrPP1. Spindle morphology was recorded during the next mitosis.

Cdc31-8 *wee1-as8* temperature shift

We also isolated a *cdc31-8 wee1-as8* strain expressing GFP–Atb2 (AP4833). These cells were grown exponentially in liquid YE5S and transferred to glass-bottomed Petri dishes coated with lectin (0.5 mg/ml, Sigma) for 6 hours at 25°C in the presence of 10 μM 3BrPP1 to produce short cells following division. Imaging was then started within a few minutes at 36°C and preheated medium containing 10 μM 3BrPP1 was added immediately after to ensure complete temperature shift to 36°C . Cell cycle stage was recorded immediately before the addition of preheated medium and spindle morphology was recorded at the next round of mitosis.

Co-immunoprecipitation and mass spectrometric analysis of Cdc31

A volume of 1.5 l of cells was grown to 4×10^7 cells at 30°C in YE5S medium concentrated two times as compared to regular YE5S medium (YE5S 2X). AP4100 and AP4110 cells were shifted for 7 hours at 18°C to block cells in mitosis before extract preparation. The cells were first washed with 50 ml of STOP buffer (150 mM NaCl, 50 mM NaF, 10 mM NaEDTA, 1 mM Na_3N_3), then resuspended in 6 ml of 1D buffer (50 mM

HEPES pH 7.5, 100 mM NaCl, 1 mM EDTA, 1% NP40, 20 mM β -glycerophosphate, 50 mM NaF, 0.1 mM Na_3VO_4 , 1 mM PMSF complemented with complete EDTA-free anti-protease tablets from Roche). Cell aliquots of 600 μl were added to 600 μl of glass beads and lysed using a FastPrep FP120A instrument (Qbiogene; two cycles of 40 s at maximum speed). Lysates were then spun at 10,000 g for 10 minutes at 4°C, and supernatants were recovered. Soluble extracts were incubated with anti-mouse-IgG magnetic beads (M-280 DYNAL, Invitrogen) coupled to 18 μg of anti-GFP mAb (Roche) for 2 hours at 4°C; then, the beads were washed seven times with 1D buffer, and were resuspended in SDS-PAGE sample buffer and heated at 95°C for 5 minutes. The immunoprecipitation samples were subjected to SDS-PAGE, and the Cdc31 band stained with Colloidal Blue was excised, reduced with 10 mM DTT and alkylated with 55 mM iodoacetamide. After washing and shrinking of the gel pieces with 100% acetonitrile, in-gel digestion was performed using chymotrypsin (Sequencing Grade, Promega) overnight in 25 mM ammonium bicarbonate at 30°C. The extracted peptides were analyzed by nano-LC-MS/MS using an Ultimate 3000 system (Dionex S.A.) coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Samples were loaded on a C18 precolumn (300 μm inner diameter \times 5 mm; Dionex) at 20 $\mu\text{l}/\text{min}$ in 5% acetonitrile, 0.1% trifluoroacetic acid (TFA). After 3 minutes of desalting, the precolumn was switched on line with the analytical C18 column (75 μm inner diameter \times 50 cm; C18 PepMapTM, Dionex) equilibrated in solvent A (2% acetonitrile, 0.1% formic acid). Bound peptides were eluted using a 160-minute linear gradient [from 0 to 30% (v/v)] of solvent B (80% acetonitrile, 0.085% formic acid) at a 150 nl/min flow rate and an oven temperature of 40°C. Data-dependent acquisition was performed on the LTQ-Orbitrap mass spectrometer in the positive-ion mode. Survey MS scans were acquired in the Orbitrap on the 350–1000 m/z range with the resolution set to a value of 100,000. Each scan was recalibrated in real time by co-injecting an internal standard from ambient air into the C-trap ('lock mass option'). The five most intense ions per survey scan were selected for collision-induced dissociation (CID) fragmentation and the resulting fragments were analyzed in the linear trap (LTQ). Target ions already selected for MS/MS were dynamically excluded for 30 seconds. Data were recorded using the Xcalibur software (version 2.2) and the resulting spectra were then analyzed using the MascotTM Software created with Proteome Discoverer (version 1.4, Thermo Scientific) using the SwissProt *Schizosaccharomyces pombe* (fission yeast) database, containing 5089 protein sequences. Carbamidomethylation of cysteines, oxidation of methionine, protein N-terminal acetylation and phosphorylated serine, threonine and tyrosine were set as variable modifications for all Mascot searches. Specificity of chymotrypsin and trypsin digestion was set, and four missed cleavage sites were allowed. The mass tolerances in MS and MS/MS were set to 2 ppm and 0.8 Da, respectively. Phosphorylated peptides that have their non-phosphorylated counterparts were manually validated.

Cyclin-B-CDK1 in vitro kinase assay and detection of phospho-proteins in immunoprecipitation assays

Cdc31 was produced in fusion with GST from pIB14 as described above in BL21/DE3 bacteria. Expression was induced by addition of 0.5 mM IPTG for 16 hours at 20°C. Bacteria were lysed in lysis buffer [PBS containing 0.5% Triton X-100, 10 mM MgCl_2 , 1 mg/ml lysozyme (Euromedex), 1 mg/ml DNase I (Roche), 1 mM PMSF (Sigma), 5 mg/ml Pepstatin A (Sigma), Complete Mini-EDTA-free Protease Inhibitor Cocktail Tablet (Roche)]. GST-Cdc31 was purified on glutathione beads and eluted from beads by cleavage with PreScission protease between GST and Cdc31. Purified Cdc31 was subjected to an *in vitro* kinase assay with cyclin-B-CDK1 (BioLabs), in parallel to histone H1 (positive control, BioLabs), according to the manufacturer's instructions. Proteins were then subjected to SDS-PAGE and the gel was stained with ProQ Diamond (Life Technologies) to detect phospho-proteins, according to the manufacturer's instructions, and Coomassie Blue to detect all proteins.

Similarly, Cdc31 was purified from nda3-KM311 cells expressing Sfi1 tagged at the N-terminus with GFP and blocked in mitosis for 7 hours at 16°C or released from the block for 1 hour at 32°C using an anti-GFP

mAb (Roche) as described above. Immunoprecipitates were subjected to SDS-PAGE and the gel was stained with ProQ Diamond to detect phospho-proteins and Sypro Ruby to detect all proteins according to the manufacturer's instructions.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

I.B.B., A.M. and N.B. conceived, performed and analyzed experiments, F.D. and D.L. performed mass spectrometry analysis, J.B. developed automated tracking and SPB signal analysis, G.V.C. and P.T. designed and produced the fast temperature-shift control device, M.O. and M.S. created the cdc31-8 and cdc31-S15A mutants, A.P. conceived and performed experiments and supervised the study.

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Supplementary material

Supplementary material available online at <http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.159657/-DC1>

References

- Adams, I. R. and Kilmartin, J. V. (2000). Spindle pole body duplication: a model for centrosome duplication? *Trends Cell Biol.* **10**, 329–335.
- Almonacid, M., Celton-Morizur, S., Jakubowski, J. L., Dingli, F., Loew, D., Mayeux, A., Chen, J. S., Gould, K. L., Clifford, D. M. and Paoletti, A. (2011). Temporal control of contractile ring assembly by Plo1 regulation of myosin II recruitment by Mid1/anillin. *Curr. Biol.* **21**, 473–479.
- Anderson, V. E., Prudden, J., Prochnik, S., Giddings, T. H., Jr and Hardwick, K. G. (2007). Novel sfi1 alleles uncover additional functions for Sfi1p in bipolar spindle assembly and function. *Mol. Biol. Cell* **18**, 2047–2056.
- Araki, Y., Gombos, L., Migueletti, S. P., Sivashanmugam, L., Antony, C. and Schiebel, E. (2010). N-terminal regions of Mps1 kinase determine functional bifurcation. *J. Cell Biol.* **189**, 41–56.
- Avasthi, P., Scheel, J. F., Ying, G., Frederick, J. M., Baehr, W. and Wolfrum, U. (2013). Germline deletion of Cetrn1 causes infertility in male mice. *J. Cell Sci.* **126**, 3204–3213.
- Avena, J. S., Burns, S., Yu, Z., Ebmeier, C. C., Old, W. M., Jaspersen, S. L. and Winey, M. (2014). Licensing of yeast centrosome duplication requires phosphoregulation of sfi1. *PLoS Genet.* **10**, e1004666.
- Azimzadeh, J., Hergert, P., Delouvé, A., Euteneuer, U., Formstecher, E., Khodjakov, A. and Bornens, M. (2009). hPOC5 is a centrin-binding protein required for assembly of full-length centrioles. *J. Cell Biol.* **185**, 101–114.
- Azimzadeh, J., Wong, M. L., Downhour, D. M., Sánchez Alvarado, A. and Marshall, W. F. (2012). Centrosome loss in the evolution of planarians. *Science* **335**, 461–463.
- Bähler, J., Wu, J. Q., Longtine, M. S., Shah, N. G., McKenzie, A., III, Steever, A. B., Wach, A., Philippsen, P. and Pringle, J. R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14**, 943–951.
- Balestra, F. R., Strnad, P., Flückiger, I. and Gönczy, P. (2013). Discovering regulators of centriole biogenesis through siRNA-based functional genomics in human cells. *Dev. Cell* **25**, 555–571.
- Basto, R., Brunk, K., Vinadogrova, T., Peel, N., Franz, A., Khodjakov, A. and Raff, J. W. (2008). Centrosome amplification can initiate tumorigenesis in flies. *Cell* **133**, 1032–1042.
- Basto, R., Lau, J., Vinogradova, T., Gardiol, A., Woods, C. G., Khodjakov, A. and Raff, J. W. (2006). Flies without centrioles. *Cell* **125**, 1375–1386.
- Baum, P., Furlong, C. and Byers, B. (1986). Yeast gene required for spindle pole body duplication: homology of its product with Ca^{2+} -binding proteins. *Proc. Natl. Acad. Sci. USA* **83**, 5512–5516.

- Cabral, G., Sans, S. S., Cowan, C. R. and Dammermann, A. (2013). Multiple mechanisms contribute to centriole separation in *C. elegans*. *Curr. Biol.* **23**, 1380–1387.
- Cueille, N., Salimova, E., Esteban, V., Blanco, M., Moreno, S., Bueno, A. and Simanis, V. (2001). Flp1, a fission yeast orthologue of the *S. cerevisiae* CDC14 gene, is not required for cyclin degradation or rum1p stabilisation at the end of mitosis. *J. Cell Sci.* **114**, 2649–2664.
- D'Assoro, A. B., Lingle, W. L. and Salisbury, J. L. (2002). Centrosome amplification and the development of cancer. *Oncogene* **21**, 6146–6153.
- Dantas, T. J., Daly, O. M. and Morrison, C. G. (2012). Such small hands: the roles of centrins/caltractins in the centriole and in genome maintenance. *Cell. Mol. Life Sci.* **69**, 2979–2997.
- Delaval, B., Covassin, L., Lawson, N. D. and Doxsey, S. (2011). Centrin depletion causes cyst formation and other ciliopathy-related phenotypes in zebrafish. *Cell Cycle* **10**, 3964–3972.
- Ding, R., West, R. R., Morphew, D. M., Oakley, B. R. and McIntosh, J. R. (1997). The spindle pole body of *Schizosaccharomyces pombe* enters and leaves the nuclear envelope as the cell cycle proceeds. *Mol. Biol. Cell* **8**, 1461–1479.
- Elserafy, M., Šarić, M., Neuner, A., Lin, T. C., Zhang, W., Seybold, C., Sivashanmugam, L. and Schiebel, E. (2014). Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Curr. Biol.* **24**, 1456–1466.
- Fantes, P. A. and Nurse, P. (1978). Control of the timing of cell division in fission yeast. Cell size mutants reveal a second control pathway. *Exp. Cell Res.* **115**, 317–329.
- Flory, M. R., Morphew, M., Joseph, J. D., Means, A. R. and Davis, T. N. (2002). Pcp1p, an Spc110p-related calmodulin target at the centrosome of the fission yeast *Schizosaccharomyces pombe*. *Cell Growth Differ.* **13**, 47–58.
- Fong, C. S., Sato, M. and Toda, T. (2010). Fission yeast Pcp1 links polo kinase-mediated mitotic entry to gamma-tubulin-dependent spindle formation. *EMBO J.* **29**, 120–130.
- Gogondeau, D., Beisson, J., de Loubresse, N. G., Le Caer, J. P., Ruiz, F., Cohen, J., Sperling, L., Koll, F. and Klotz, C. (2007). An Sfi1p-like centrin-binding protein mediates centrin-based Ca²⁺-dependent contractility in *Paramecium tetraurelia*. *Eukaryot. Cell* **6**, 1992–2000.
- Gogondeau, D., Klotz, C., Arnaiz, O., Malinowska, A., Dadlez, M., de Loubresse, N. G., Ruiz, F., Koll, F. and Beisson, J. (2008). Functional diversification of centrins and cell morphological complexity. *J. Cell Sci.* **121**, 65–74.
- Hagan, I. and Yanagida, M. (1992). Kinesin-related cut7 protein associates with mitotic and meiotic spindles in fission yeast. *Nature* **356**, 74–76.
- Hagan, I. and Yanagida, M. (1995). The product of the spindle formation gene *sad1+* associates with the fission yeast spindle pole body and is essential for viability. *J. Cell Biol.* **129**, 1033–1047.
- Höög, J. L., Huisman, S. M., Brunner, D. and Antony, C. (2013). Electron tomography reveals novel microtubule lattice and microtubule organizing centre defects in +TIP mutants. *PLoS ONE* **8**, e61698.
- Jaspersen, S. L. and Ghosh, S. (2012). Nuclear envelope insertion of spindle pole bodies and nuclear pore complexes. *Nucleus* **3**, 226–236.
- Jaspersen, S. L. and Winey, M. (2004). The budding yeast spindle pole body: structure, duplication, and function. *Annu. Rev. Cell Dev. Biol.* **20**, 1–28.
- Jerka-Dziadosz, M., Koll, F., Włoga, D., Gogondeau, D., Garreau de Loubresse, N., Ruiz, F., Fabczak, S. and Beisson, J. (2013). A Centrin3-dependent, transient, appendage of the mother basal body guides the positioning of the daughter basal body in *Paramecium*. *Protist* **164**, 352–368.
- Keeney, J. B. and Boeke, J. D. (1994). Efficient targeted integration at *leu1-32* and *ura4-294* in *Schizosaccharomyces pombe*. *Genetics* **136**, 849–856.
- Khodjakov, A., Cole, R. W., Oakley, B. R. and Rieder, C. L. (2000). Centrosome-independent mitotic spindle formation in vertebrates. *Curr. Biol.* **10**, 59–67.
- Kilmartin, J. V. (2003). Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. *J. Cell Biol.* **162**, 1211–1221.
- Lee, I. J., Wang, N., Hu, W., Schott, K., Bähler, J., Giddings, T. H., Jr, Pringle, J. R., Du, L. L. and Wu, J. Q. (2014). Regulation of spindle pole body assembly and cytokinesis by the centrin-binding protein Sfi1 in fission yeast. *Mol. Biol. Cell* **25**, 2735–2749.
- Li, S., Sandercock, A. M., Conduit, P., Robinson, C. V., Williams, R. L. and Kilmartin, J. V. (2006). Structural role of Sfi1p-centrin filaments in budding yeast spindle pole body duplication. *J. Cell Biol.* **173**, 867–877.
- Lim, H. H., Zhang, T. and Surana, U. (2009). Regulation of centrosome separation in yeast and vertebrates: common threads. *Trends Cell Biol.* **19**, 325–333.
- Lutz, W., Lingle, W. L., McCormick, D., Greenwood, T. M. and Salisbury, J. L. (2001). Phosphorylation of centrin during the cell cycle and its role in centriole separation preceding centrosome duplication. *J. Biol. Chem.* **276**, 20774–20780.
- Marthiens, V., Rujano, M. A., Pennetier, C., Tessier, S., Paul-Gilloteaux, P. and Basto, R. (2013). Centrosome amplification causes microcephaly. *Nat. Cell Biol.* **15**, 731–740.
- Moser, M. J., Flory, M. R. and Davis, T. N. (1997). Calmodulin localizes to the spindle pole body of *Schizosaccharomyces pombe* and performs an essential function in chromosome segregation. *J. Cell Sci.* **110**, 1805–1812.
- Mulvihill, D. P., Petersen, J., Ohkura, H., Glover, D. M. and Hagan, I. M. (1999). Plo1 kinase recruitment to the spindle pole body and its role in cell division in *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **10**, 2771–2785.
- Ohta, M., Sato, M. and Yamamoto, M. (2012). Spindle pole body components are reorganized during fission yeast meiosis. *Mol. Biol. Cell* **23**, 1799–1811.
- Paoletti, A., Bordes, N., Haddad, R., Schwartz, C. L., Chang, F. and Bornens, M. (2003). Fission yeast *cdc31p* is a component of the half-bridge and controls SPB duplication. *Mol. Biol. Cell* **14**, 2793–2808.
- Racine, V., Hertzog, A., Jouanneau, J., Salamer, J., Kervrann, C. and Sibarita, J.-B. (2006). Multiple-target tracking of 3D fluorescent objects based on simulated annealing. In *Proceedings of the IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, pp. 1020–1023.
- Rosenberg, J. A., Tomlin, G. C., McDonald, W. H., Snysman, B. E., Muller, E. G., Yates, J. R., III and Gould, K. L. (2006). Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. *Mol. Biol. Cell* **17**, 3793–3805.
- Sato, M., Toya, M. and Toda, T. (2009). Visualization of fluorescence-tagged proteins in fission yeast: the analysis of mitotic spindle dynamics using GFP-tubulin under the native promoter. *Methods Mol. Biol.* **545**, 185–203.
- Spang, A., Courtney, I., Fackler, U., Matzner, M. and Schiebel, E. (1993). The calcium-binding protein cell division cycle 31 of *Saccharomyces cerevisiae* is a component of the half bridge of the spindle pole body. *J. Cell Biol.* **123**, 405–416.
- Sparks, C. A., Morphew, M. and McCollum, D. (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. *J. Cell Biol.* **146**, 777–790.
- Stemm-Wolf, A. J., Meehl, J. B. and Winey, M. (2013). Sfr13, a member of a large family of asymmetrically localized Sfi1-repeat proteins, is important for basal body separation and stability in *Tetrahymena thermophila*. *J. Cell Sci.* **126**, 1659–1671.
- Stevens, N. R., Raposo, A. A., Basto, R., St Johnston, D. and Raff, J. W. (2007). From stem cell to embryo without centrioles. *Curr. Biol.* **17**, 1498–1503.
- Sveiczzer, A., Novak, B. and Mitchison, J. M. (1996). The size control of fission yeast revisited. *J. Cell Sci.* **109**, 2947–2957.
- Tay, Y. D., Patel, A., Kaemena, D. F. and Hagan, I. M. (2013). Mutation of a conserved residue enhances the sensitivity of analogue-sensitised kinases to generate a novel approach to the study of mitosis in fission yeast. *J. Cell Sci.* **126**, 5052–5061.
- Théry, M., Racine, V., Piel, M., Pépin, A., Dimitrov, A., Chen, Y., Sibarita, J. B. and Bornens, M. (2006). Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc. Natl. Acad. Sci. USA* **103**, 19771–19776.
- Tran, P. T., Paoletti, A. and Chang, F. (2004). Imaging green fluorescent protein fusions in living fission yeast cells. *Methods* **33**, 220–225.
- Trautmann, S., Wolfe, B. A., Jorgensen, P., Tyers, M., Gould, K. L. and McCollum, D. (2001). Fission yeast Clp1p phosphatase regulates G2/M transition and coordination of cytokinesis with cell cycle progression. *Curr. Biol.* **11**, 931–940.
- Uzawa, S., Li, F., Jin, Y., McDonald, K. L., Braunfeld, M. B., Agard, D. A. and Cande, W. Z. (2004). Spindle pole body duplication in fission yeast occurs at the G1/S boundary but maturation is blocked until exit from S by an event downstream of *cdc10+*. *Mol. Biol. Cell* **15**, 5219–5230.
- Velve-Casquillas, G., Le Berre, M., Piel, M. and Tran, P. T. (2010). Microfluidic tools for cell biological research. *Nano Today* **5**, 28–47.
- Velve Casquillas, G., Fu, C., Le Berre, M., Cramer, J., Meance, S., Plecis, A., Baigl, D., Greffet, J. J., Chen, Y., Piel, M. et al. (2011). Fast microfluidic temperature control for high resolution live cell imaging. *Lab Chip* **11**, 484–489.
- Wei, Y., Hu, H., Lun, Z. R. and Li, Z. (2014). Centrin3 in trypanosomes maintains the stability of a flagellar inner-arm dynein for cell motility. *Nat. Commun.* **5**, 4060.
- Weiss, E. and Winey, M. (1996). The *Saccharomyces cerevisiae* spindle pole body duplication gene *MPS1* is part of a mitotic checkpoint. *J. Cell Biol.* **132**, 111–123.
- Yang, C. H., Kasbek, C., Majumder, S., Yusof, A. M. and Fisk, H. A. (2010). Mps1 phosphorylation sites regulate the function of centrin 2 in centriole assembly. *Mol. Biol. Cell* **21**, 4361–4372.
- Zhang, Y. and He, C. Y. (2012). Centrins in unicellular organisms: functional diversity and specialization. *Protoplasma* **249**, 459–467.

Supplementary figure legends

Figure S1: Sfi1 localization and targeting to the SPB half-bridge and time-lapse analysis of *sfi1-1* mutant

A-D: Fluorescence intensity profiles across SPBs shown in insets from cells expressing Sfi1-mRFP and GFP-Cam1 (A), or expressing Sfi1-GFP and immuno-stained for Cdc31 (B) or expressing Sfi1-mRFP and Sid2-GFP (C) or Sfi1-mRFP and Sad1-CFP (D). SPBs from insets are shown in Figure 1A-D and numbered similarly. Bars: 500 nm. Note that Sfi1 maximum intensity never coincides with Cam1, Sid2 or Sad1 on SPBs (left) or localizes between the two maxima of intensity after SPB duplication (right). **E-F.** Localization at SPBs of Sfi1-GFP and Sid4-mCherry (E) or Sfi1-mRFP and Sid4-GFP (F). Bars: 5 μ m. Insets: magnification of SPBs from cells numbered according to cell cycle progression in left panels. Bars: 500 nm.

Figure S2: *Sfi1-1* mutant phenotype

A: *Sfi1-1* mutant expressing the microtubule marker GFP-Atb2 was imaged by time-lapse microscopy after shift to the restrictive temperature of 36°C to monitor mitotic spindle and septum assembly (transmitted light). Left: field of cells 3h15 after temperature shift. Right: single cell in which septation occurs before mitotic spindle disassembly. Note the assembly of double septa. t=0: mitotic entry. Bars: 5 μ m. **B.** Left: Ppc89-GFP staining in typical wild type or *cdc10-V50* cells shifted for 5 hours at 36°C and in typical wild type cell treated or not for 5h with 11 mM hydroxyurea at 25°C. Bar: 5 μ m. Enlarged views of SPBs are shown below. Bar: 500 nm. Right: Percentage of similar cells with duplicated SPBs labeled with Ppc89-GFP depending on cell length. n values, collected from 2 independent experiments, are indicated on bars. **D.** Left: Ppc89-GFP distribution in typical long G2 wild type or *sfi1-1*, cells (10 to 12 μ m in length) showing interphase microtubules. Right: Percentage of wild type or *sfi1-1* long G2 cells with interphase microtubules exhibiting 2 Ppc89-GFP stained SPBs. n=155 and 196 respectively from 2 experiments. **E:** Sid2-GFP distribution in *sfi1-1* cells grown at 25°C or for 3 or 4 hours at 36°C. Bar: 5 μ m. **F.** Experimental scheme (top) and example of a typical *cdc31-8 wee1-as8* cell expressing GFP-Atb2 (bottom) treated for 6 hours with 3BrPP1 to produce very short cells at birth that undergo S phase after septation and shifted to 36°C to inactivate Cdc31. **G.** Percentage of monopolar spindles in similar cells shifted from 25°C to 36°C at various cell cycle stages. Sept: septation.

Figure S3: MS spectra of Cdc31 phosphopeptide

Representative MS/MS spectra (MSA enable) of Cdc31 signature peptide for Cdc31 phosphorylation on serine 15 co-purified from mitotic extracts with GFP-tagged Sfi1 N-terminus. The fragmentation spectra shown correspond to phospho-peptide RSRAp(SS)PTPARL at charged state ($460.23^{(3+)m/z}$) and ($689.85^{(2+)m/z}$), the *inset* shows the peptide sequence and the observed ions obtained. Tandem mass spectrum labeled to show singly and doubly charged *b* and *y* ions, as well as ion corresponding to neutral losses of water (*circles*), NH_3 (*asterisks*) and H_3PO_4 (98 Da) groups; *M*, parent ion mass. Fragment ions observed indicate a phosphorylation of one of the two consecutive serines, however none can distinguish localization of the phosphorylation between the two.

Figure S4: Phosphorylation of Cdc31 S15 controls SPB segregation.

A-B. Time-lapse analysis of wild type or *cdc31-S15A* cells expressing Plo1-GFP and Sid4-mCherry. $t=0$: mitotic entry defined by appearance of Plo1-GFP at the SPBs. End of anaphase is determined by maximum extension of mitotic spindle visualized with Sid4-mCherry. Time in minutes. Bar: 5 μm . **C.** Percentage of cells entering mitosis and remaining blocked at the end of 60 min time-lapse (not resolved) or cells resolving their monopolar spindles into a bipolar spindle (resolved). **D.** Time lapse analysis of wild type and *cdc31-S15A* cells expressing Sid2-GFP and mCherry-Atb2. Time after mitosis entry in minutes. Bar: 5 μm . **E:** Number of *cdc31-S15A* post-mitotic cells, identified when Sid2-GFP associates with the septum region, with one or two SPBs.

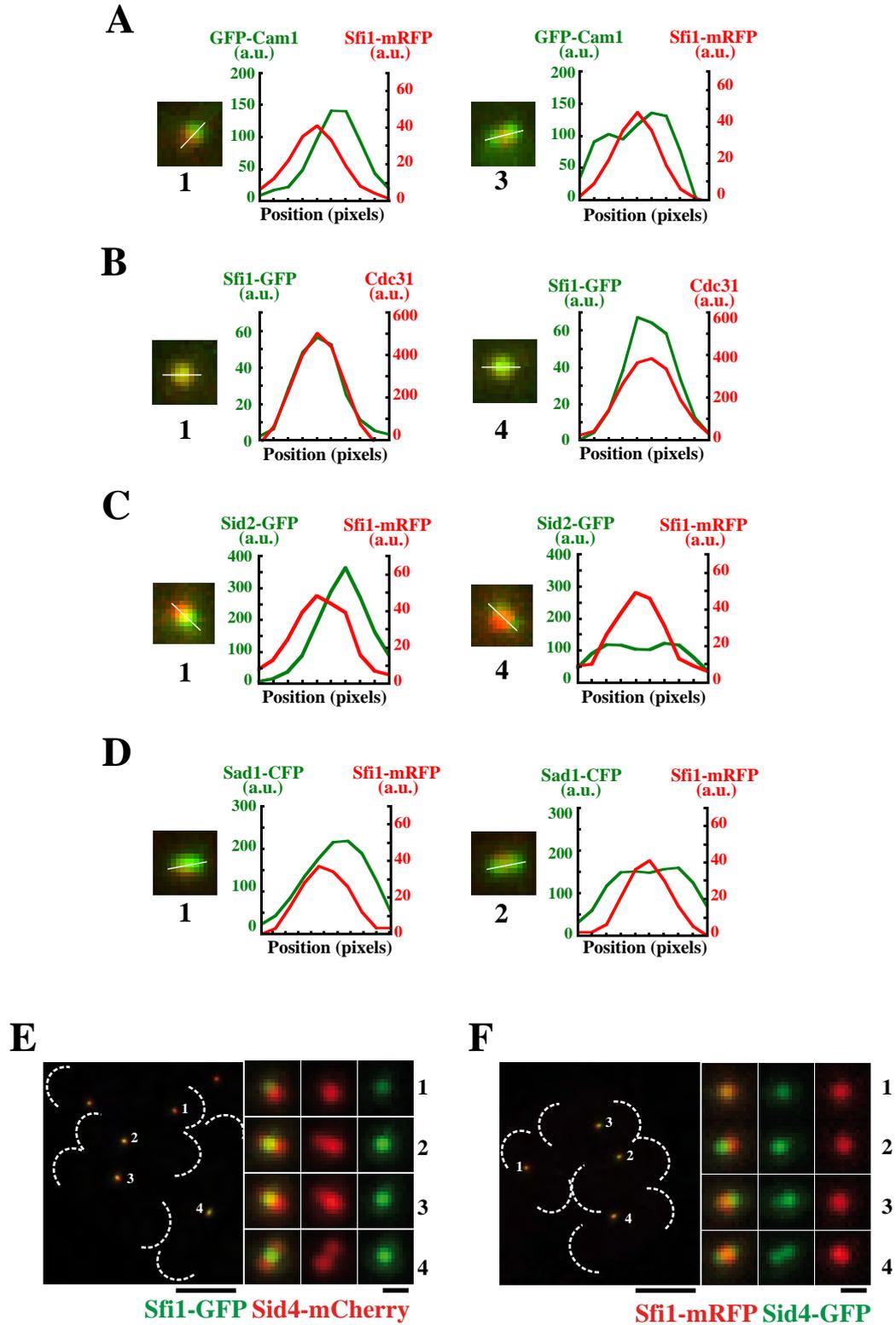
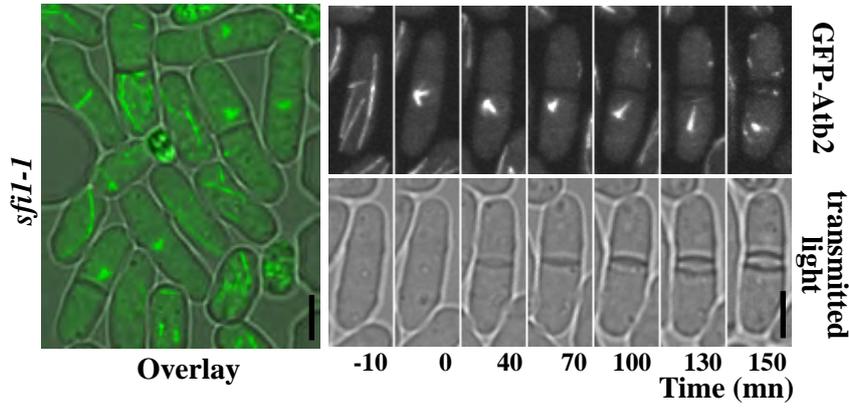
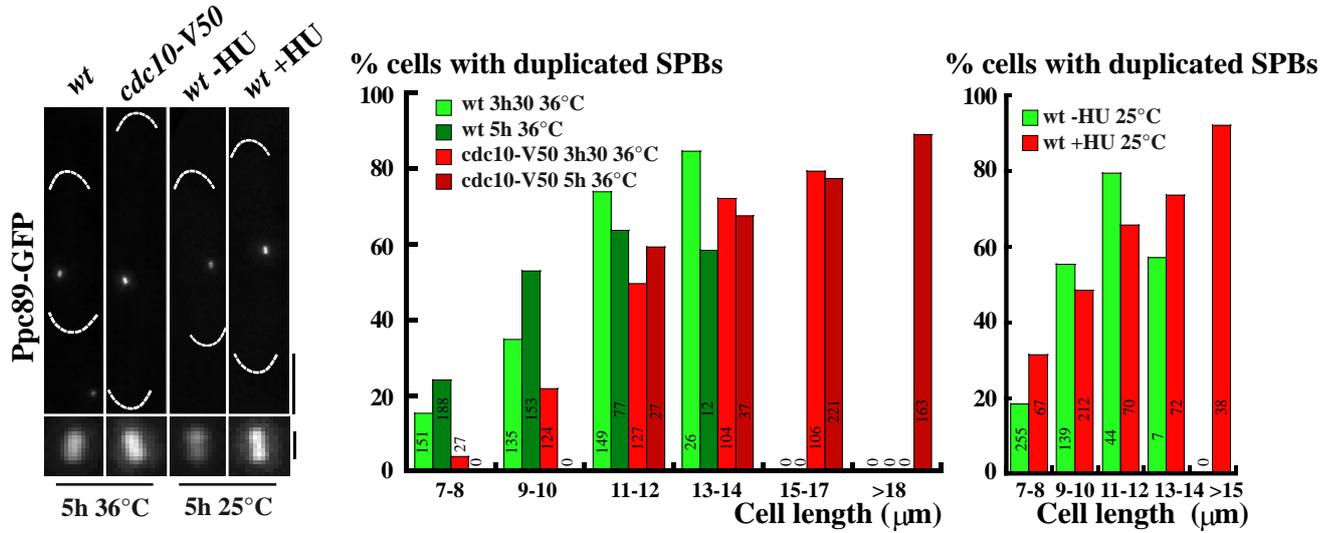


Figure S1

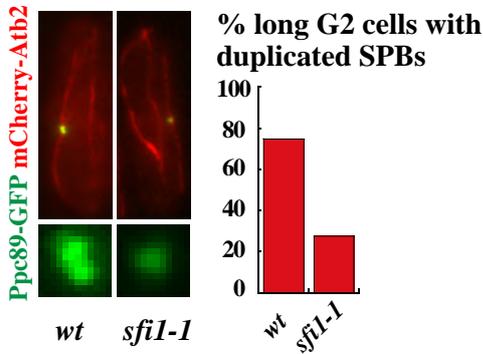
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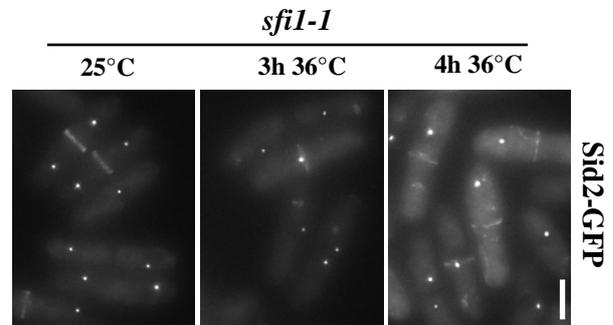
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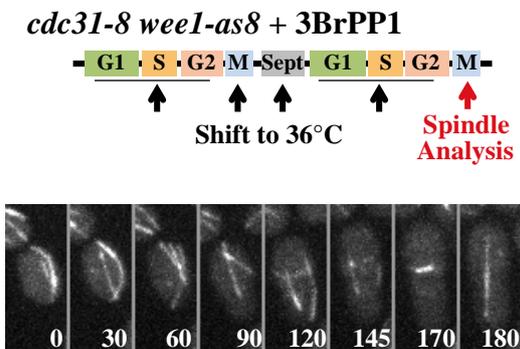
C



D



E



F

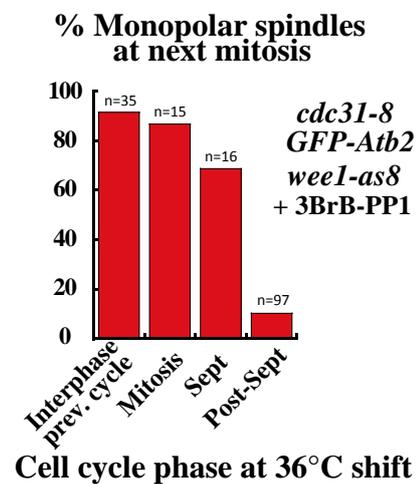


Figure S2

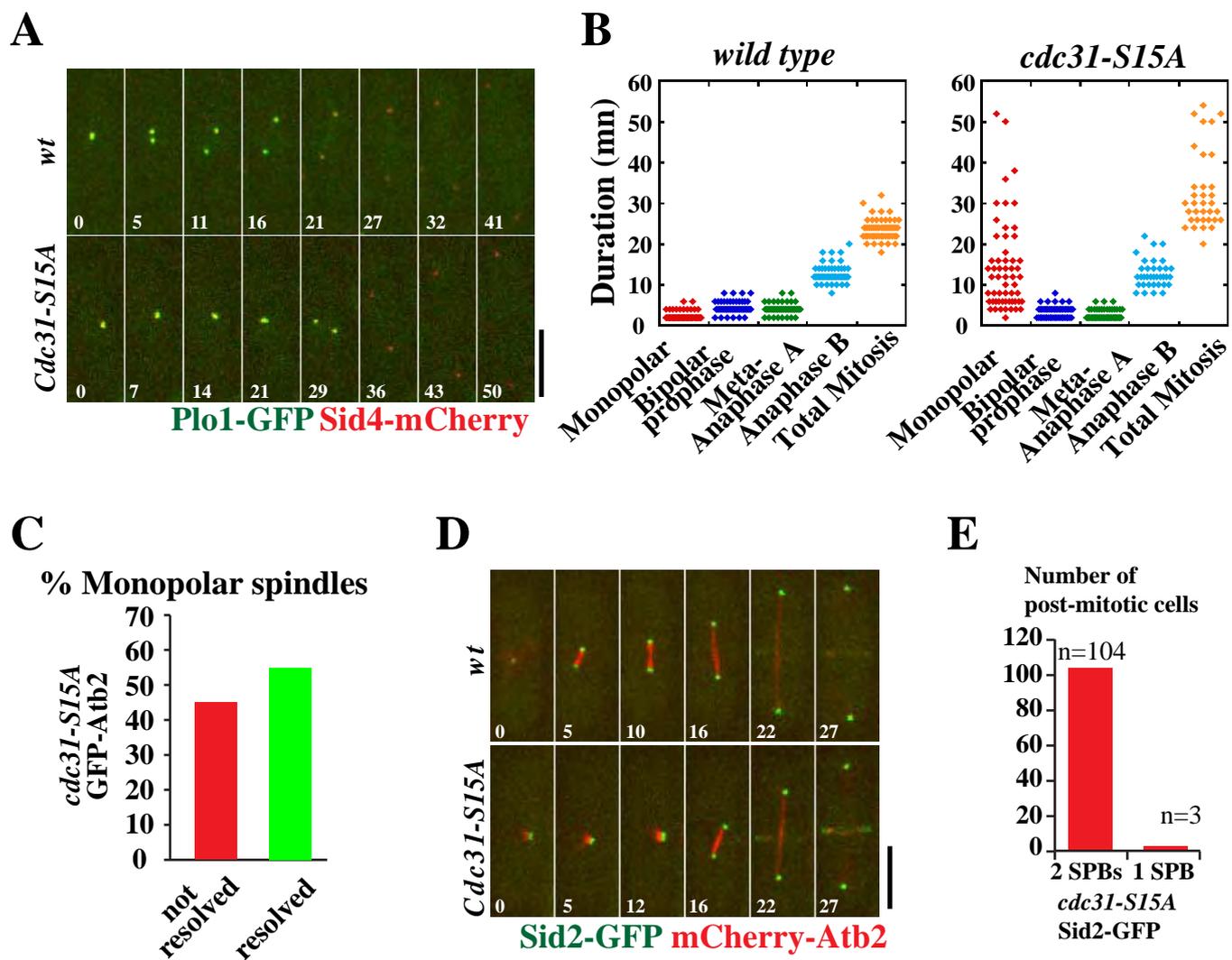


Figure S4

Table S1: List of strain used

Strain	Genotype	Source
AP 770	<i>h- ade6-M210 ura4-D18 leu1-32 + pREP41X</i>	This work
AP 1439	<i>h- sfi1-GFP::KanMX6 ade6-M210 ura4-D18 leu1-32</i>	This work
AP 1542	<i>h- sfi1-GFP::KanMX6 cdc31Δ::KanMX6 leu1+:: pnmt**E147K-cdc31 ade6-M216 ura4D18</i>	This work
AP 1576	<i>h- sfi1-mRFP::KanMX6 sid2-GFP::ura4+ ade6-M210 ura4-D18 leu1-32</i>	This work
AP 1815	<i>h- leu1+::psf1-sfi1-1 sfi1Δ::KanMX6 ade6-M210 ura4-D18</i>	This work
AP 1870	<i>h+ sad1-1 sid2-GFP::ura4+ sfi1-mRFP::KanMX6 leu1-32 ura4-D18 ade6-M216</i>	This work
AP 1872	<i>h- leu1+::psf1-sfi1-GFP ade6-M210 ura4-D18</i>	This work
AP 1876	<i>h- leu1+::psf1-sfi1(1-765)-GFP ade6-M210 ura4-D18</i>	This work
AP 1879	<i>h- leu1+::psf1-sfi1(1-188)-GFP ade6-M210 ura4-D18</i>	This work
AP 1881	<i>h- leu1+::psf1-sfi1(766-840)-GFP ade6-M210 ura4-D18</i>	This work
AP 1884	<i>h- leu1+::psf1-sfi1(189-765)-GFP ade6-M210 ura4-D18</i>	This work
AP 1903	<i>h- leu1+::psf1-sfi1-1 sfi1Δ::KanMX6 sid2-GFP::ura4+ ade6-M210 ura4-D18</i>	This work
AP 1913	<i>h- leu1+::psf1-sfi1(189-840)-GFP ade6-M210 ura4-D18</i>	This work
AP 1973	<i>h+ S65T-GFP::cam1 sfi1-mRFP::KanMX6 ade1? ade6-M210 leu1-32 ura4D18</i>	This work
AP 1982	<i>h- sfi1-mRFP::KanMX6 sad1-mCFP-KanMX6 ade6-M210 ura4-D18 leu1-32</i>	This work
AP 1995	<i>h- leu1+::psf1-sfi1-1 sfi1Δ::KanMX6 ade6-M210 ura4-D18 + pAP257(pnmt-GFP-atb2, ura4+)</i>	This work
AP 3312	<i>h+ leu1+::psf1-sfi1-1 sfi1Δ::KanMX6 GFP-atb2::KanMX6 ade6-M216 leu1-32 ura4-D18</i>	This work
AP 3698	<i>h- cdc31-S15A-KanMX6 + GFP-atb2::ura4+ ade6-M216 leu1-32</i>	This work
AP 3887	<i>h- cdc31-S15A::Nat^R + sid2-GFP::ura4+ mcherry-atb2::Hph^R leu1-32 ade6-M210</i>	This work
AP 3930	<i>h- sid2-GFP::ura4+ mCherry-atb2::Hph^R leu1-32 ade6-M216</i>	This work
AP 3931	<i>h- cdc31-S15A-Nat^R + sfi1-GFP::KanMX6 + mCherry-atb2::Hph^R leu1-32 ura4-D18</i>	This work
AP 3932	<i>h- sfi1-GFP::KanMX6 + mCherry-atb2::Hph^R leu1-32 ura4-D18</i>	This work
AP 3933	<i>h+ cdc31-S15A-Nat^R + plo1-GFP::KanMX6 + sid4-mCherry::Hph^R ura+ leu1-32 ade6M216</i>	This work
AP 3934	<i>h+ plo1-GFP::KanMX6 + sid4-mCherry::Hph^R leu1-32 ade6-M216</i>	This work
AP 4100	<i>h- nda3-KM311 leu1+::psf1-sfi1(1-188)-GFP ade6-M210 ura4-D18</i>	This work
AP 4110	<i>h- nda3-KM311 cdc31-S15A-Nat^R leu1+::psf1-sfi1(1-188)-GFP ade6-M210 ura4-D18</i>	This work
AP 4672	<i>h- cdc25-22 leu1-32 sfi1::GFPKanMX6 ade6-M216 ura4-D18 leu1-32</i>	This work
AP4690	<i>h- ppc89-GFP::KanMX6 mcherry-atb2:hph ura4-D18 leu1-32 ade-M216</i>	This work
AP 4711	<i>h- sfi1::KanMX6 leu1+::psf1-sfi1-1 ppc89-GFP::KanMX6 mCherry-atb2::Hph ura4-D18 ade-M216</i>	This work
AP 4713	<i>h+ sad1-1 ppc89-GFP::KanMX6 mCherry-atb2::hph ura4-D18 leu1-32 ade6-M216</i>	This work
AP 4831	<i>h+ wee1-as8::KanMX6 cdc25-22 cdc31-8::Bsd^R GFP-atb2::KanMX6</i>	This work

AP 4833	<i>h+ wee1-as8::KanMX6 cdc31-8:Bsd^R GFPatb2::KanMX6 ura4-D18 leu1-32</i>	This work
AP4908	<i>h+ sfi1GFP::KanMX6 pnmt-cdc18 ura4-D18</i>	This work
AP4913	<i>h+ cdc10-V50 ppc89-GFP::KanMX6 ade6-M210 leu1-32</i>	This work
AP4918	<i>h+ sfi1GFP::KanMX6 sid4mCherry::Hph leu1-32 ura4-D18 ade6-M210</i>	This work
AP4923	<i>h+ sfi1-mRFP::ura4+ sid4-GFP::KanMX6 leu1-32 ade6-M210</i>	This work
MO 504	<i>h90 cdc31.ts8::Bsd^R GFPatb2::KanMX6 ade6-M210 ura4-D18 leu1-32</i>	This work

2. Article 2: Human Sfil controls Centrin association with centrioles and regulates ciliogenesis and cell cycle progression

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Human Sfil controls Centrin association with centrioles and regulates ciliogenesis and cell cycle progression

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Running Title: Role of Sfil in human cells.

Number of characters:

Abstract

The centrosome is the main microtubule-organizing centre. In interphase, a single centrosome nucleates and organizes microtubules. It is duplicated prior to mitosis to form the two poles of the mitotic spindle segregates chromosomes between the daughter cells. Sfil is a component of the Spindle Pole Body (SPB), the yeast centrosome, and is essential for its duplication. It possesses multiple Cdc31-binding domains distributed along an extended alpha-helix. Sfil and Cdc31 assemble into parallel arrays and form an SPB appendage called the Half-bridge that promotes the assembly of the daughter SPB. Sfil and Cdc31 are conserved in human cells. Cdc31 homologs, the centrins, are concentrated in the distal end of centrioles. Human Sfil fused to GFP was detected to centrioles, but its function remains unknown. Here, we show that human Sfil depletion reduces the pool of centriolar centrins, which becomes asymmetrically distributed. In contrast, it does not alter other centriolar components like CPl10 or CEP135 and does not block centrosome duplication. Nevertheless, centrosome functions are affected. First, Sfil depletion leads to strong cilium assembly defects under serum starvation. Second, Sfil depletion generates a p53-dependent cell cycle arrest in G1, an event previously observed when centriole biogenesis or mitosis progression are affected. Third, depletion of Sfil in p53-deficient cells leads to a prolongation of mitosis indicating that the mitotic spindle is also affected. To conclude, our experiments support a key role for Sfil in centrosome biogenesis and function.

Introduction

The centrosome represents the main microtubule-organizing centre of animal cells. In interphase, a single centrosome is composed of two centrioles surrounded by pericentriolar material that nucleates microtubules. Centrioles are duplicated one and only once per cell cycle by conservative duplication of pre-existing centrioles. The duplication process is under tight control to prevent deleterious effects of supernumerary centrosomes, a hallmark of cancer cells (for reviews see Gonczy, 2015; Nano and Basto, 2017; Rhys and Godinho, 2017).

During duplication, two procentrioles form on the side of the two pre-existing centrioles in a perpendicular orientation (For reviews see Arquint and Nigg, 2016; Conduit et al., 2015).

This multi-step process starts with Plk1 activation in late mitosis. This leads to the disengagement of centrioles which licenses centriole duplication. This initiates Plk4 recruitment to centrioles by CEP152 and CEP192 in G1 (Kim et al., 2013; Park et al., 2014), followed by focusing of Plk4 at the site of procentriole assembly on the proximal side of centrioles. This focusing relies on the degradation of Plk4 except at the site of procentriole assembly where Plk4 is protected by STIL. STIL oligomerization is thought to restrict its distribution to one single site on the centriole proximal end. Plk4/STIL complexes then recruit Sas6, which forms the cartwheel with Cep135. Cep135 also participates in the recruitment MT triplets to the cartwheel in conjunction with CPAP (Arquint et al., 2015; Klebba et al., 2015; Kleylein-Sohn et al., 2007; Kratz et al., 2015; Moyer et al., 2015; Ohta et al., 2014; Sillibourne et al., 2010; Stevens et al., 2010).

New centrioles start elongating in S phase, followed by elongation of the distal region during G2 phase. Cep120 and Spic1 positively regulate centriole elongation (Comartin et al., 2013; Lin et al., 2013). Another protein contributing to this process is centrobilin, which is recruited to the centrosome early during centriole duplication, where it interacts with CPAP and α/β -tubulin dimers and promotes the elongation and stability of centrioles (Gudi et al., 2015; Gudi et al., 2014). The centrin-binding protein, Poc5, and OFD1 localize to the distal portion of centrioles and are required for distal elongation

(Azimzadeh et al., 2009; Singla et al., 2010). Moreover, Pocl play a role in the early steps of centriole duplication and the later step of elongation (Keller et al., 2009).

Finally, CPI10 and its interacting proteins Cep97 and Kif24 act as capping structures that determine the final length of centrioles. Indeed, CPI10 localizes to the distal end of the centrioles and its depletion impairs the regulation of centriole length, resulting in long centrioles (Schmidt et al., 2009; Spektor et al., 2007).

Duplicated centrosomes then remain associated thanks to a centrosomal linker. This linker is dissolved at mitotic entry to free duplicated centrosomes (Nigg and Stearns, 2011; Panic et al., 2015) which move apart to assemble a bipolar spindle of microtubules. Although spindle assembly can proceed in complete absence of centrosomes (Basto et al., 2006; Khodjakov and Rieder, 2001), centrosomes clearly contribute to spindle assembly efficiency and robustness. Centrosomes are for instance essential during fast cycles of early development (Stevens et al., 2007).

Budding yeast spindle pole bodies (SPBs) share all major properties of centrosomes. They are composed of a main core and a side-appendage the half-bridge overlaying the nuclear envelope. Like centrosomes, they duplicate in a conservative manner before nucleating an intranuclear mitotic spindle at mitosis entry (See Cavanaugh and Jaspersen, 2017; Ruthnick et al., 2017 for reviews). The molecular mechanism of SPB duplication has been solved and involves two major proteins, Sfil and the calmodulin-like protein Cdc31 (Baum et al., 1986; Kilmartin, 2003; Li et al., 2006; Paoletti et al., 2003; Spang et al., 1995; Vallen et al., 1994).

In budding yeast, Sfil forms an extended α -helix that possesses multiple Cdc31-binding domains (Li et al., 2006). Sfil molecules assemble together with Cdc31 into a parallel array to form the SPB half-bridge which is attached the SPB main core by Sfil N-terminus and anchored to the nuclear envelope through interactions between Sfil C-terminus and Karl (Li et al., 2006; Seybold et al., 2015). SPB duplication relies on the assembly of an anti-parallel array of Sfil/Cdc31 molecules linked to the first array at the level of Sfil C-termini (Avena et al., 2014; Elserafy et al., 2014; Li et al., 2006; Seybold et al., 2015). Free Sfil N-termini generated by half-bridge duplication serve as an assembly site for the daughter SPB (Li et al., 2006). Likewise, Sfil and Cdc31 are involved in SPB duplication

and bipolar spindle assembly in fission yeast (Bestul et al., 2017; Bouhleb et al., 2015; Lee et al., 2014). Moreover, Cdk1-dependent phosphorylation of Cdc31 destabilizes Sfil arrays at mitotic entry to promote timely assembly of a bipolar mitotic spindle (Bouhleb et al., 2015).

Strikingly, although the core molecular mechanism controlling centrosome and SPB duplication described above differ, Sfil and Cdc31 are among the few SPB components conserved in human cells. Indeed, the ubiquitous centrins Cen2 and Cen3, homologous to Cdc31, are recruited early to procentrioles and concentrate in the distal lumen of centrioles (Laoukili et al., 2000; Middendorp et al., 2000; Paoletti et al., 1996). Nevertheless, they do not seem to be involved in centrosome duplication (Dantas et al., 2011; Strnad et al., 2007). Studies in zebrafish nevertheless show that they are required for proper cilia function and suggest that they could regulate cell division as well (Delaval et al., 2011). It has also been shown that centrin function with various non centrosomal partners, such as XPC, implicated in DNA repair (Araki et al., 2001; Dantas et al., 2011), or nucleoporins to modulate nuclear export (Fischer et al., 2004; Resendes et al., 2008), which may complicate the analysis of their centrosomal function.

On the other hand, Sfil in fusion with GFP, was reported to colocalize with centrin at the centrioles in human cells (Kilmartin, 2003). In addition, Sfil was identified in a centriole biogenesis screen using GFP-centrin as a centriolar marker, suggesting that it may alter centrin recruitment to centrioles or affect centriole biogenesis in human cells (Balestra et al., 2013). Finally Sfil-like proteins were also shown to play a role in basal body organization and duplication in Tetrahymena and paramecium (Gogendeau et al., 2007; Heydeck et al., 2016; Stemm-Wolf et al., 2013).

These data suggest that Sfil may play important functions at centrosomes. Our study confirms that human Sfil is a centriolar component involved in centrin recruitment to the centriole distal lumen. Sfil depletion did not block centriole duplication per se but mother centriole function as a basal body was strongly impaired in these conditions indicating that human Sfil is essential for ciliogenesis like centrin in zebrafish (Delaval et al., 2011). Furthermore, Sfil depletion triggered a p53-dependent cell cycle arrest in G1 in hTERT RPE-1 cells and a prolongation of mitosis in HeLa cells or p53- hTERT RPE-1

cells. We conclude that human Sfil may be required for the biogenesis of fully functional centrioles with impact on ciliogenesis as well as cell cycle progression.

Results

Human Sfil is a centriolar protein in human cells

Human Sfil localization to centrioles has been proposed by Kilmartin based on the transient expression of a fusion of Sfil with GFP in HeLa cells (Kilmartin, 2003). To confirm this localization, we generated an antibody against the Sfil C-terminal region.

Using this antibody in cycling immortalized hTERT RPE-1 cells (hereafter referred to as RPE-1), human Sfil was detected throughout the cell cycle at centrosomes labelled with an anti γ -Tubulin antibody (Figure 1A). Furthermore, Sfil colocalized with centrin stained with mAb 20H5 which recognizes the 4 human centrin (Sanders and Salisbury, 1994), (Paoletti et al., 1996), indicating that Sfil is a centriolar component in human cells (Figure 1B).

To assess whether this was a genuine localization, we generated a stable cell line expressing GFP-Sfil transgene. GFP-Sfil colocalized with the anti-Sfil staining (Figure 1C). Although the anti-Sfil Ab has been affinity purified, we could also detect the Golgi apparatus in interphase cells (our unpublished results). Since this staining does not exist in the GFP-Sfil cell line, we consider that this staining is likely irrelevant to human Sfil.

Finally, Sfil localization to centrioles was observed upon serum starvation when RPE-1 cells assemble a primary cilium with the mother centriole serving as a basal body (Figure 1D).

Overall, our results confirm that Sfil is a centriolar protein at all stages of the cell cycle.

Sfil is recruited gradually to centrioles during S phase

Centrin were shown to be recruited to procentrioles during early phases of centrosome duplication (Middendorp et al., 1997; Paoletti et al., 1996). To test if this was also the case for Sfil, we analysed the timing of Sfil recruitment to centrosomes in RPE-1 cells using the nuclear marker PCNA which starts accumulating in the nucleus in late G1, displays an homogenous nuclear localization in early S and appears in foci in late S (Celis

and Madsen, 1986). In G1 cells, Sfil was localized at the two centrioles. In early S, in addition to its localization at centrioles, we detected additional dots with the anti-Sfil Ab, suggesting the presence of Sfil in centriole satellites (see Hori and Toda, 2017 for a review). As cells progressed through S, Sfil signal increased in the newly assembled centrioles while it decreased in satellites. Localization to satellites was further reduced in G2 cells and disappeared completely in mitotic cells (Figure 2 and Figure 1A and B for G2 and mitotic cells). This data suggest that Sfil is gradually recruited to the centrioles during S phase while procentrioles grow in length.

Sfil is necessary for proper distribution of centrin in centrioles

Next, we investigated the function of Sfil in centriole biogenesis. To do so Sfil was depleted using two previously described SiRNAs (Balestra et al., 2013), Sfil SiRNA#3 and #4). Cells were fixed after three days of SiRNA treatment and the integrated intensities of Sfil and γ -Tubulin were measured at centrosomes using a linescan function. Sfil was partially depleted from centrioles (60% reduction in intensity for SiSfil#4 and 50% for SiSfil#3 whereas γ -Tubulin staining was not modified) (Fig.3A), suggesting a significant but incomplete efficiency of SiRNAs. This could possibly be due to the presence of a static pool of Sfil at centrioles or to a very slow turnover.

Using a co-labelling of centrin and acetylated tubulin, which is enriched in the stable microtubules triplets forming the centriolar barrel (AcTub; (Piperno et al., 1987), we observed that centrin staining was strongly reduced at centrioles. Some centrioles identified by AcTub staining did not show any centrin signal (Figure 3B). This shows that Sfil is essential for centrin recruitment or stabilization at centrioles.

Since Sfil has been proposed to play a role in centriole duplication (Balestra et al., 2013) we further monitored centrioles by double labelling of centrin and CPI10, a capping protein that localizes at the distal end of centrioles (Schmidt et al., 2009). This distal localization allows a very clear distinction of two centrioles, before duplication and four centrioles after duplication (Fig. 3C). Upon Sfil depletion, CPI10 staining showed two centriole pairs at each pole in mitotic cells, while most of these pairs revealed a single centrin-stained centriole (Figure 3C).

To quantify this phenotype in a larger cell population, we counted the number of centrin-stained centrioles in S/G2 cells displaying two pairs of CPl10-stained centrioles (Figure 3D). We observed that the percentage of two centrin-labelled centrioles dropped to ~53% and ~21% of the cases after SiSfl#3 and SiSfl#4 treatment respectively.

We also quantified centrin and CPl10 integrated intensities at centrioles using a linescan function. The total integrated intensity of centrin at centrioles was decreased to respectively 42% and 30% of control intensities measured in cells treated with SiSfl#3 and SiSfl#4 whereas CPl10 levels remained unaffected (Figure 3E).

In addition, cells harbouring two centrin-labelled centrioles per pair showed a higher asymmetry of distribution between centrioles: in cells treated with the control SiRNA (labelled Si NoT) the ratio of intensity between the two centrioles was close to 1.5 for both centrin and CPl10. This ratio increased to 2.4 and 2.9 in cells treated with SiSfl#3 and SiSfl#4 respectively while CPl10 ratios remained identical to the control situation (Figure 3F).

Altogether, this data confirms that Sfl depletion strongly impairs centrin association with centrioles, without affecting other components of centrioles such as CPl10 or impairing centriole duplication.

Sfl is essential for ciliogenesis

Since our results show that centrin levels are decreased in Sfl-depleted cells and centrin is essential for ciliogenesis in zebrafish (Delaval et al., 2011), we next monitored primary cilium formation in Sfl-depleted cells. 48h after an initial SiRNA treatment, we re-transfected the cells with the same SiRNAs and serum-starved them to allow the assembly of a primary cilium (Figure 4A). In the control condition, 75% of the cells displayed a primary cilium stained as detected by acetylated tubulin staining, while this rate dropped to 25% and 8% respectively in cells treated with SiSfl#3 and SiSfl#4 (Figure 4B-C). In order to rule out a defective entry in G0, we stained the cells with proliferative marker Ki67. Our analysis showed that SiSfl#3 and SiSfl#4 treatment did not impair entry into G0 state. We conclude that Sfl is essential for the assembly of a primary cilium.

Sfil depletion affects cell cycle progression

We noticed that mitotic cells were very rare after Sfil depletion in RPE-1 cells suggesting a cell cycle arrest. Since depletion of centrosomal proteins in normal cells p53-positive cells often lead to a cell cycle arrest in G1, we used the RPE1-FUCCI cell line stably expressing the fluorescent markers mKO2-hCdt1 and mAG-hGem to quantify the number of cells in G1 after Sfil depletion (Sakaue-Sawano et al., 2011). We found that in control cells, G1 cells represented 65% of the total population while this percentage increased to $\geq 85\%$ in cells treated with SiSfil#3 and SiSfil#4 Sfil, suggesting a cell cycle arrest in G1 (Figure 5A).

To test if this cell cycle arrest was due to the activation of p53, we measured the mitotic index in HeLa cells, which are p53-negative after DAPI staining. No drop in the mitotic index was observed upon treatment with Sfil siRNA#3 or siRNA#4 in this cell line (Figure 5B). Moreover, although centrin staining was reduced and asymmetrical as seen previously in RPE-1 cells, two CPl10 stained centrioles were clearly detected in mitosis in HeLa cells confirming that centriole duplication is not altered upon Sfil depletion (Figure 5B). Similar observations were made in an RPE-1 cell line stably expressing a shRNA against p53 (hereafter-called RPE1-p53⁻; our unpublished results).

We conclude that Sfil depletion causes a cell cycle arrest in G1 in a p53-dependent manner without blocking centriole duplication altogether.

Sfil depletion leads to a mitotic delay

The alleviation of the cell cycle arrest in G1 observed in HeLa and in the p53⁻ RPE-1 cell lines allowed us to monitor mitosis progression after Sfil depletion. We performed live imaging on these cells for 72h after siRNA treatment. Dividing cells were observed during the entire imaging sequence and mitosis duration was measured. Beginning of mitosis was defined when cells rounded up, inducing a displacement of the medial focal plane. The last point of mitosis was defined when the midbody was formed (Figure 5C). In HeLa cells, the average duration of mitosis was of 41 minutes in control conditions, and increased to 50 or 70 minutes respectively in Sfil-depleted cells using Sfil siRNA#3 and siRNA #4 respectively (Figure 5D). Similar results were obtained in RPE1-p53⁻ cells, where the average duration of mitosis was of 30.9 min in the control

siRNA and increased to 40 min in siRNA#3 and 38.5 in siRNA#4 treated cells. We conclude that Sfil depletion induces a delay in mitosis progression when p53 checkpoint is inactivated.

Discussion

Sfil is a centriolar protein in human cells

Like its yeast counterpart, Sfil is a centrosomal protein. Based on our colocalization studies with centrin, acetylated tubulin and CP110, Sfil seems to be a genuine component of centrioles. In contrast, we did not observe it in the pericentriolar material or in the centrosomal linker which plays the same function as the SPB bridge after centrosome duplication. Furthermore, we did not find evidence for a role of Sfil in promoting centriole duplication although the observed cell cycle arrest in G1 suggests that centriole biogenesis may be affected. Sfil role during centriole biogenesis could be restricted to the recruitment or stability of centrin association with the distal end of centrioles (Paoletti et al., 1996).

Human Sfil is recruited to centrosomes during S phase

Centriole assembly starts at the G1/S transition when a new procentriole is assembled at the proximal end of the mother centriole. Procentrioles elongate into centrioles during S and G2 phase which undergo maturation until they are fully competent in mitosis. However, the order of recruitment of centriolar components is independent of their position (distally or proximally). For example, centrin and CP110 are distal components but are recruited very early (Middendorp et al., 1997; Paoletti and Bornens, 1997; Paoletti et al., 1996; Schmidt et al., 2009). In the case of Sfil, we found that Sfil accumulates first in the centrosome area in several foci in early S phase. This complex pattern is reminiscent of centriole satellites (see (Hori and Toda, 2017) for a review). The functional relevance of these transient foci is unknown. One hypothesis is that they could represent assembly sites for Sfil array, before their incorporation into centrioles. Indeed, Sfil localization to centrioles is gradually reinforced as S phase progresses while extra foci disappear. Live cell imaging at high resolution will be necessary to demonstrate if Sfil is recruited to procentrioles as early as centrin.

Sfil is necessary for centrin association with centrioles and ciliogenesis

We found that in Sfil-depleted cells, centrin levels dropped drastically. Moreover, centrin distribution between centrioles was more asymmetric than in the control condition. This is reminiscent of fission yeast where Sfil is necessary for Cdc31 localization to the half bridge (Bouhleb et al., 2015).

A similar result was already reported for GFP-centrin1 in a screen for centriole biogenesis factors (Balestra et al., 2013). This screen suggested a possible role for Sfil in centriole duplication in human cells. Our experiments using the same SiRNAs show that this is not the case since centriole pairs strongly depleted in centrin retained a normal CPl10 staining in G2 and M. Since most RPE-1 cells arrest in G1, before centrosome duplication, this could prevent the visualization of a centriole duplication defect at later cell cycle stages. Analysis of HeLa cells that are not blocked in G1 demonstrate that this is not the case either since two CPl10 stained centrioles were detected in mitotic cells depleted for Sfil (Figure 5B). This data strongly supports the hypothesis that Sfil is not necessary for centrosome duplication in human cells.

Although it is not necessary for centrosome duplication, Sfil plays an important role at centrosomes by ensuring proper centrin distribution at the distal end of centrioles and promoting ciliogenesis upon serum starvation. Likewise, Centrin2 has been shown to be essential for ciliogenesis in zebrafish (Delaval et al., 2011). Why ciliogenesis is affected upon depletion of human Sfil is unclear. One hypothesis is that the structure of the distal end of centrioles is altered preventing mother centriole docking at the plasma membrane or function as a basal body for cilium assembly.

Sfil depletion leads to a cell cycle arrest and to mitotic delay

RPE-1 cells are non-transformed cells. Many studies have shown that centrosome defects in these cells lead to a cell cycle arrest due to the activation of the so-called p53-dependent centrosome surveillance pathway (Fong et al., 2016; Lambrus et al., 2016; Meitinger et al., 2016; Wong et al., 2015). Cell cycle analysis using RPE1-FUCCI cell line showed an accumulation of G1 cells consistent with this hypothesis. However, this surveillance pathway also monitors mitosis duration and we observed prolonged mitosis

in HeLa cells and p53- RPE-1 cells. Thus, the G1 block in RPE-1 cells could also result from mitosis prolongation during the previous cell cycle.

The exact function of Sfil at the centriole remains an open question. Our results point towards a structural function of Sfil in centrioles with impact on centriole function as a basal body for cilium formation upon serum starvation and on mitotic spindle proper function in chromosome segregation. This identification of additional Sfil partners besides centrin could help understand how Sfil can participate in these functions.

Analysis of Sfil distribution by electron microscopy or super resolution microscopy would also be necessary to determine how Sfil molecules are organized within centrioles. In yeast, Sfil forms anti-parallel arrays at the bridge connected by Sfil C-termini (Li et al., 2006). If these C-terminal interactions were conserved in spite of strong sequence divergence between yeast and human Sfil outside of centrin-binding domains, we could imagine that Sfil C-termini could interact in the centriole lumen, while N-termini domains would interact with centriole walls. Solving these questions would help fully understand Sfil function in human cells.

Material and Methods

Cell Culture:

RPE1 cells, RPE1-FUCCI (MTA agreement with Don Cleveland, San Diego) and RPE1-p53 negative cells were cultured in DMEM-F12 medium supplemented with 10% foetal bovine serum and 1% Penicillin-Streptomycin at 37°C and 5% CO₂. To induce ciliogenesis, cells were starved from serum during 48h. The depletion of p53 in RPE1-p53 cell line was induced by the stable expression of a shRNA against p53. GFP-Sfil cell line was obtained by stable transfection of GFP-Sfil plasmid and maintained under G418 selection.

HeLa cells were cultured in DMEM supplemented with 10% foetal bovine serum at 37°C and 5% CO₂. HeLa cells were selected due to the absence of p53 expression.

SiRNAs and antibodies

The ON-TARGET plus Non-targeting Pool siRNA was used as control and purchased from Dharmacon (Catalogue #D-001810-10-20). It is composed of a pool of four siRNAs designed and microarray tested for minimal targeting of human. The siRNAs used to deplete Sfil were designed as described in ((Balestra et al., 2013)) and purchased from Eurogentec. The sequences are as follows: siSfil#3 (AAGCAAGTACTCATTACAGAA-dTdT) and siSfil#4 (AAGGTTGTCTCTGCAGTGAAA-dTdT). The siRNAs were transfected into cells using RNAi MAX reagents (Invitrogen) according the manufacturer protocol.

The Sfil antibody was raised in rabbit against the GST-fused C-terminal domain of Sfil (aa1021_aa1240) and affinity-purified on AminoLink® Coupling Resin (20381 Thermo Fisher) coupled to the MBP-fused C-terminus (same aa sequence).

The antibodies against γ -tubulin (sc-7396, Santa Cruz Biotechnology, Inc.), centrin clone 20H5 (04-1624, Millipore), SAS-6 (sc-81431, sc-98506 Santa Cruz Biotechnology, Inc.), CPI10 (EPPI1816, Elabscience), and mouse anti acetylated tubulin (T6793, Sigma) were purchased. Cen3 is a homemade antibody described in (Paoletti, Bordes et al. 2003). GT335 (anti polyglutamylated tubulin), human anti Acetylated tubulin and GFP antibodies were purchased from the Institut Curie Recombinant antibodies Platform.

Secondary fluorescent antibodies were purchased from Jackson antibodies and used at 1:500 dilutions.

Immunofluorescence microscopy

For indirect immunofluorescence, cells were grown on 12-mm coverslips and fixed at -20°C with cold methanol for 3 min. The fixed cells were then incubated with the primary antibodies for 1h at room temperature. The cells were washed with PBS and subsequently incubated with the secondary antibodies conjugated with Alexa Fluor-488, 594 or 647. DNA was counterstained with DAPI solution. The samples were mounted in Mowiol and observed with a fluorescence microscope (Upright Leica DMI-5000B) equipped with a CCD Camera 1392x1040 (CoolSnap HQ2 pixel: 6.45 µm from Photometrics). The images were acquired and processed using Metamorph software (Molecular Devices).

Live cell imaging:

Cells grown on 25-mm coverslips for 24 h and were treated with siRNA. 7h after the beginning of siRNA treatment the coverslips were transferred to Chemlode chambers (www.chamlode.com) and the medium was changed to a fresh medium. Image acquisition were performed on workstations of the Nikon Imaging Centre at Institut Curie-CNRS. Live imaging was performed for 72h using an inverted Nikon Ti-E microscope equipped with a CCD camera (1392x1040 CoolSnap HQ2 pixel:6.45 µm) from Photometrics microscope and with a 20× objective and a heated and motorized stage. Cells were imaged by phase-contrast microscopy at the frame rate of one image every 10 min using MetaMorph software. Cells were maintained at 37°C in the presence of 5% CO₂ during the time of the experiment.

Figures Legends:

Figure 1: Sfil is a centriolar protein. A.B. Cycling RPE1 cells were fixed and stained for Sfil (Green), γ Tubulin (Red) and DNA (DAPI, blue) (A).and Sfil (Green), Centrin (Red) and DNA (DAPI, blue) (B). Magnified images, show Sfil localization throughout the cell cycle at two (G1) or four centrioles (G2/S and M cells). C. RPE1 cells stably expressing GFP-Sfil were fixed and stained for GFP (green) and Sfil. D. RPE1 cells were serum starved for 48hours, fixed and stained for polyglutamylated tubulin (Green) and Sfil (Red). Scale bars 5 μ m.

Figure 2: Sfil is recruited gradually to centrioles during S phase. A. RPE1 cycling cells were stained with antibodies against Sfil, AcTub and PCNA to determine the timing of Sfil recruitment to the centrioles. 5 μ m.

Figure 3: Sfil depletion leads to centrin recruitment defects at the centrioles, but does not induce duplication defects. A. RPE1 cells were depleted for Sfil using two different SiRNA. Sfil and γ -Tubulin levels were measured using the linescan tool at the centrioles. B. RPE1 cells were labelled for acetylated Tubulin and Centrin after Sfil depletion. C-D. Mitotic cells (C) and mitotic and G2 cells (D) harbouring four CPl10 foci were analysed for the number of centrin-labelled foci after Sfil depletion. E. Centrin and CPl10 integrated intensities at the centriole were measured. F. Ratios of centrin and CPl10. The total intensity of the strongly-labelled centriole was divided by the weakly labelled one. $p < 0.001$. Scale bars 5 μ m.

Figure4: Sfil is essential for ciliogenesis. A. Experimental protocol for ciliogenesis initiation in RPE1 cells. B. cells induced for ciliogenesis were stained for Ac-Tub, γ -Tub and DAPI. Scale bar 15 μ m. C. Quantifications of B. Mean of three experiments. D. Cells were labelled with the proliferation marker Ki67 to check the presence of G0 cells by monitoring the percentage of Ki67 negative cells.

Figure 5: Sfi1 depletion leads to a cell cycle arrest in RPE1 cells and to a delay in mitosis in HeLa and RPE1-p53⁻ negative cells. **A.** RPE1-FUCCI cells were treated with Sfi1 or Control siRNAs. After 72hours, the cells were imaged and the number of G1 (red) cells was counted. The mean of three experiments is shown. Scale bar 20 μ m. **B.** HeLa cells were treated with control or Sfi1 siRNAs and mitotic cells were stained for CPl10 or centrin. Scale bar 5 μ m. The histograms show that Sfi1 depletion does not change the proportions of mitotic and interphasic cells, suggesting that HeLa cells do not arrest in the cell cycle. **C.** Movies of mitosis duration in HeLa cells treated for control or Sfi1 siRNAs. Beginning of Mitosis corresponds to time zero the elapsed time is indicated in each frame. Scale bar 20 μ m. **D.** HeLa and RPE1-p53⁻ cells were imaged for 72h and mitosis duration was measured. In both cell lines, Sfi1 depletion induced a prolonged mitosis. Mean duration is indicated on the graphs.

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References

- Araki, M., C. Masutani, M. Takemura, A. Uchida, K. Sugawara, J. Kondoh, Y. Ohkuma, and F. Hanaoka. 2001. Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J Biol Chem.* 276:18665-18672.
- Arquint, C., A.M. Gabryjonczyk, S. Imseng, R. Bohm, E. Sauer, S. Hiller, E.A. Nigg, and T. Maier. 2015. STIL binding to Polo-box 3 of PLK4 regulates centriole duplication. *Elife.* 4.
- Arquint, C., and E.A. Nigg. 2016. The PLK4-STIL-SAS-6 module at the core of centriole duplication. *Biochem Soc Trans.* 44:1253-1263.
- Avena, J.S., S. Burns, Z. Yu, C.C. Ebmeier, W.M. Old, S.L. Jaspersen, and M. Winey. 2014. Licensing of yeast centrosome duplication requires phosphoregulation of sfi1. *PLoS Genet.* 10:e1004666.
- Azimzadeh, J., P. Hergert, A. Delouvee, U. Euteneuer, E. Formstecher, A. Khodjakov, and M. Bornens. 2009. hPOC5 is a centrin-binding protein required for assembly of full-length centrioles. *J Cell Biol.* 185:101-114.
- Balestra, F.R., P. Strnad, I. Fluckiger, and P. Gonczy. 2013. Discovering regulators of centriole biogenesis through siRNA-based functional genomics in human cells. *Dev Cell.* 25:555-571.
- Basto, R., J. Lau, T. Vinogradova, A. Gardiol, C.G. Woods, A. Khodjakov, and J.W. Raff. 2006. Flies without centrioles. *Cell.* 125:1375-1386.
- Baum, P., C. Furlong, and B. Byers. 1986. Yeast gene required for spindle pole body duplication: homology of its product with Ca²⁺-binding proteins. *Proc Natl Acad Sci U S A.* 83:5512-5516.
- Bestul, A.J., Z. Yu, J.R. Unruh, and S.L. Jaspersen. 2017. Molecular model of fission yeast centrosome assembly determined by superresolution imaging. *J Cell Biol.* 216:2409-2424.
- Bouhleb, I.B., M. Ohta, A. Mayeux, N. Bordes, F. Dingli, J. Boulanger, G. Velve Casquillas, D. Loew, P.T. Tran, M. Sato, and A. Paoletti. 2015. Cell cycle control of spindle pole body duplication and splitting by Sfi1 and Cdc31 in fission yeast. *J Cell Sci.* 128:1481-1493.
- Cavanaugh, A.M., and S.L. Jaspersen. 2017. Big Lessons from Little Yeast: Budding and Fission Yeast Centrosome Structure, Duplication, and Function. *Annu Rev Genet.*
- Celis, J.E., and P. Madsen. 1986. Increased nuclear cyclin/PCNA antigen staining of non S-phase transformed human amnion cells engaged in nucleotide excision DNA repair. *FEBS Lett.* 209:277-283.
- Comartin, D., G.D. Gupta, E. Fussner, E. Coyaud, M. Hasegan, M. Archinti, S.W. Cheung, D. Pinchev, S. Lawo, B. Raught, D.P. Bazett-Jones, J. Luders, and L.

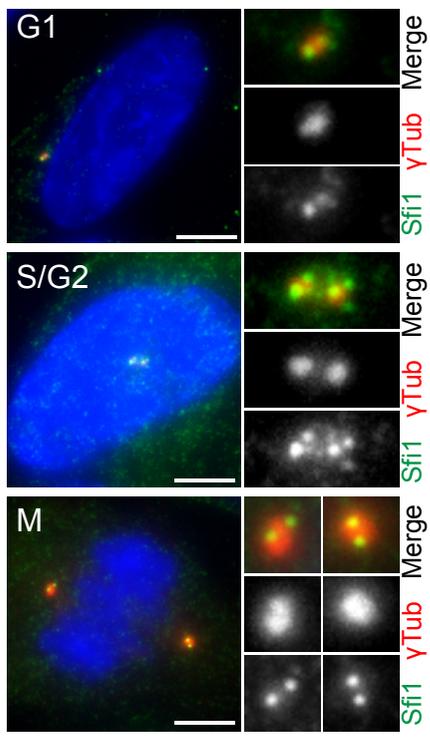
- Pelletier. 2013. CEP120 and SPICE1 cooperate with CPAP in centriole elongation. *Curr Biol.* 23:1360-1366.
- Conduit, P.T., A. Wainman, and J.W. Raff. 2015. Centrosome function and assembly in animal cells. *Nat Rev Mol Cell Biol.* 16:611-624.
- Dantas, T.J., Y. Wang, P. Lalor, P. Dockery, and C.G. Morrison. 2011. Defective nucleotide excision repair with normal centrosome structures and functions in the absence of all vertebrate centrin. *J Cell Biol.* 193:307-318.
- Delaval, B., L. Covassin, N.D. Lawson, and S. Doxsey. 2011. Centrin depletion causes cyst formation and other ciliopathy-related phenotypes in zebrafish. *Cell Cycle.* 10:3964-3972.
- Elserafy, M., M. Saric, A. Neuner, T.C. Lin, W. Zhang, C. Seybold, L. Sivashanmugam, and E. Schiebel. 2014. Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Curr Biol.* 24:1456-1466.
- Fischer, T., S. Rodriguez-Navarro, G. Pereira, A. Racz, E. Schiebel, and E. Hurt. 2004. Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. *Nat Cell Biol.* 6:840-848.
- Fong, C.S., G. Mazo, T. Das, J. Goodman, M. Kim, B.P. O'Rourke, D. Izquierdo, and M.F. Tsou. 2016. 53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis. *Elife.* 5.
- Gogondeau, D., J. Beisson, N.G. de Loubresse, J.P. Le Caer, F. Ruiz, J. Cohen, L. Sperling, F. Koll, and C. Klotz. 2007. An Sfi1p-like centrin-binding protein mediates centrin-based Ca²⁺-dependent contractility in *Paramecium tetraurelia*. *Eukaryot Cell.* 6:1992-2000.
- Gonczy, P. 2015. Centrosomes and cancer: revisiting a long-standing relationship. *Nat Rev Cancer.* 15:639-652.
- Gudi, R., C.J. Haycraft, P.D. Bell, Z. Li, and C. Vasu. 2015. Centrobin-mediated regulation of the centrosomal protein 4.1-associated protein (CPAP) level limits centriole length during elongation stage. *J Biol Chem.* 290:6890-6902.
- Gudi, R., C. Zou, J. Dhar, Q. Gao, and C. Vasu. 2014. Centrobin-centrosomal protein 4.1-associated protein (CPAP) interaction promotes CPAP localization to the centrioles during centriole duplication. *J Biol Chem.* 289:15166-15178.
- Heydeck, W., A.J. Stemm-Wolf, J. Knop, C.C. Poh, and M. Winey. 2016. Sfr1, a *Tetrahymena thermophila* Sfi1 Repeat Protein, Modulates the Production of Cortical Row Basal Bodies. *mSphere.* 1.
- Hori, A., and T. Toda. 2017. Regulation of centriolar satellite integrity and its physiology. *Cell Mol Life Sci.* 74:213-229.
- Keller, L.C., S. Geimer, E. Romijn, J. Yates, 3rd, I. Zamora, and W.F. Marshall. 2009. Molecular architecture of the centriole proteome: the conserved WD40 domain protein POC1 is required for centriole duplication and length control. *Mol Biol Cell.* 20:1150-1166.

- Khodjakov, A., and C.L. Rieder. 2001. Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J Cell Biol.* 153:237-242.
- Kilmartin, J.V. 2003. Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. *J Cell Biol.* 162:1211-1221.
- Kim, T.S., J.E. Park, A. Shukla, S. Choi, R.N. Murugan, J.H. Lee, M. Ahn, K. Rhee, J.K. Bang, B.Y. Kim, J. Loncarek, R.L. Erikson, and K.S. Lee. 2013. Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc Natl Acad Sci U S A.* 110:E4849-4857.
- Klebba, J.E., D.W. Buster, T.A. McLamarrah, N.M. Rusan, and G.C. Rogers. 2015. Autoinhibition and relief mechanism for Polo-like kinase 4. *Proc Natl Acad Sci U S A.* 112:E657-666.
- Kleylein-Sohn, J., J. Westendorf, M. Le Clech, R. Habedanck, Y.D. Stierhof, and E.A. Nigg. 2007. Plk4-induced centriole biogenesis in human cells. *Dev Cell.* 13:190-202.
- Kratz, A.S., F. Barenz, K.T. Richter, and I. Hoffmann. 2015. Plk4-dependent phosphorylation of STIL is required for centriole duplication. *Biol Open.* 4:370-377.
- Lambrus, B.G., V. Daggubati, Y. Uetake, P.M. Scott, K.M. Clutario, G. Sluder, and A.J. Holland. 2016. A USP28-53BP1-p53-p21 signaling axis arrests growth after centrosome loss or prolonged mitosis. *J Cell Biol.* 214:143-153.
- Laoukili, J., E. Perret, S. Middendorp, O. Houcine, C. Guennou, F. Marano, M. Bornens, and F. Tournier. 2000. Differential expression and cellular distribution of centrin isoforms during human ciliated cell differentiation in vitro. *J Cell Sci.* 113 (Pt 8):1355-1364.
- Lee, I.J., N. Wang, W. Hu, K. Schott, J. Bahler, T.H. Giddings, Jr., J.R. Pringle, L.L. Du, and J.Q. Wu. 2014. Regulation of spindle pole body assembly and cytokinesis by the centrin-binding protein Sfi1 in fission yeast. *Mol Biol Cell.* 25:2735-2749.
- Li, S., A.M. Sandercock, P. Conduit, C.V. Robinson, R.L. Williams, and J.V. Kilmartin. 2006. Structural role of Sfi1p-centrin filaments in budding yeast spindle pole body duplication. *J Cell Biol.* 173:867-877.
- Lin, Y.N., C.T. Wu, Y.C. Lin, W.B. Hsu, C.J. Tang, C.W. Chang, and T.K. Tang. 2013. CEP120 interacts with CPAP and positively regulates centriole elongation. *J Cell Biol.* 202:211-219.
- Meitinger, F., J.V. Anzola, M. Kaulich, A. Richardson, J.D. Stender, C. Benner, C.K. Glass, S.F. Dowdy, A. Desai, A.K. Shiau, and K. Oegema. 2016. 53BP1 and USP28 mediate p53 activation and G₁ arrest after centrosome loss or extended mitotic duration. *J Cell Biol.* 214:155-166.
- Middendorp, S., T. Kuntziger, Y. Abraham, S. Holmes, N. Bordes, M. Paintrand, A. Paoletti, and M. Bornens. 2000. A role for centrin 3 in centrosome reproduction. *J Cell Biol.* 148:405-416.

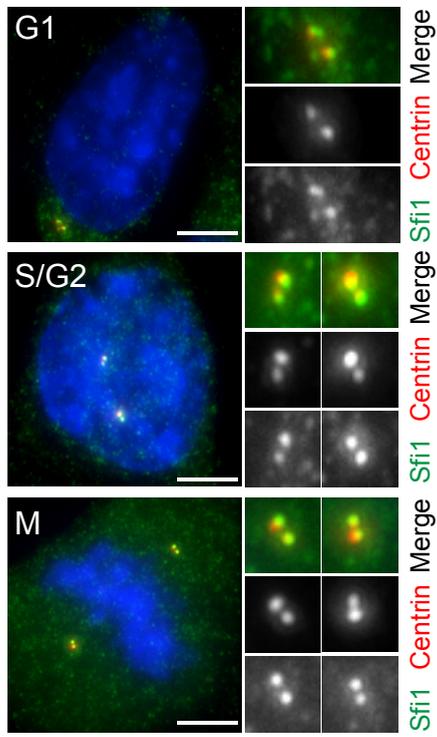
- Middendorp, S., A. Paoletti, E. Schiebel, and M. Bornens. 1997. Identification of a new mammalian centrin gene, more closely related to *Saccharomyces cerevisiae* CDC31 gene. *Proc Natl Acad Sci U S A.* 94:9141-9146.
- Moyer, T.C., K.M. Clutario, B.G. Lambrus, V. Daggubati, and A.J. Holland. 2015. Binding of STIL to Plk4 activates kinase activity to promote centriole assembly. *J Cell Biol.* 209:863-878.
- Nano, M., and R. Basto. 2017. Consequences of Centrosome Dysfunction During Brain Development. *Adv Exp Med Biol.* 1002:19-45.
- Nigg, E.A., and T. Stearns. 2011. The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. *Nat Cell Biol.* 13:1154-1160.
- Ohta, M., T. Ashikawa, Y. Nozaki, H. Kozuka-Hata, H. Goto, M. Inagaki, M. Oyama, and D. Kitagawa. 2014. Direct interaction of Plk4 with STIL ensures formation of a single procentriole per parental centriole. *Nat Commun.* 5:5267.
- Panic, M., S. Hata, A. Neuner, and E. Schiebel. 2015. The centrosomal linker and microtubules provide dual levels of spatial coordination of centrosomes. *PLoS Genet.* 11:e1005243.
- Paoletti, A., N. Bordes, R. Haddad, C.L. Schwartz, F. Chang, and M. Bornens. 2003. Fission yeast cdc31p is a component of the half-bridge and controls SPB duplication. *Mol Biol Cell.* 14:2793-2808.
- Paoletti, A., and M. Bornens. 1997. Organisation and functional regulation of the centrosome in animal cells. *Prog Cell Cycle Res.* 3:285-299.
- Paoletti, A., M. Moudjou, M. Paintrand, J.L. Salisbury, and M. Bornens. 1996. Most of centrin in animal cells is not centrosome-associated and centrosomal centrin is confined to the distal lumen of centrioles. *J Cell Sci.* 109 (Pt 13):3089-3102.
- Park, S.Y., J.E. Park, T.S. Kim, J.H. Kim, M.J. Kwak, B. Ku, L. Tian, R.N. Murugan, M. Ahn, S. Komiyama, H. Hojo, N.H. Kim, B.Y. Kim, J.K. Bang, R.L. Erikson, K.W. Lee, S.J. Kim, B.H. Oh, W. Yang, and K.S. Lee. 2014. Molecular basis for unidirectional scaffold switching of human Plk4 in centriole biogenesis. *Nat Struct Mol Biol.* 21:696-703.
- Piperno, G., M. LeDizet, and X.J. Chang. 1987. Microtubules containing acetylated alpha-tubulin in mammalian cells in culture. *J Cell Biol.* 104:289-302.
- Resendes, K.K., B.A. Rasala, and D.J. Forbes. 2008. Centrin 2 localizes to the vertebrate nuclear pore and plays a role in mRNA and protein export. *Mol Cell Biol.* 28:1755-1769.
- Rhys, A.D., and S.A. Godinho. 2017. Dividing with Extra Centrosomes: A Double Edged Sword for Cancer Cells. *Adv Exp Med Biol.* 1002:47-67.
- Ruthnick, D., A. Neuner, F. Dietrich, D. Kirrmaier, U. Engel, M. Knop, and E. Schiebel. 2017. Characterization of spindle pole body duplication reveals a regulatory role for nuclear pore complexes. *J Cell Biol.* 216:2425-2442.

- Sakaue-Sawano, A., T. Kobayashi, K. Ohtawa, and A. Miyawaki. 2011. Drug-induced cell cycle modulation leading to cell-cycle arrest, nuclear mis-segregation, or endoreplication. *BMC Cell Biol.* 12:2.
- Sanders, M.A., and J.L. Salisbury. 1994. Centrin plays an essential role in microtubule severing during flagellar excision in *Chlamydomonas reinhardtii*. *J Cell Biol.* 124:795-805.
- Schmidt, T.I., J. Kleylein-Sohn, J. Westendorf, M. Le Clech, S.B. Lavoie, Y.D. Stierhof, and E.A. Nigg. 2009. Control of centriole length by CPAP and CP110. *Curr Biol.* 19:1005-1011.
- Seybold, C., M. Elserafy, D. Ruthnick, M. Ozboyaci, A. Neuner, B. Flottmann, M. Heilemann, R.C. Wade, and E. Schiebel. 2015. Kar1 binding to Sfi1 C-terminal regions anchors the SPB bridge to the nuclear envelope. *J Cell Biol.* 209:843-861.
- Sillibourne, J.E., F. Tack, N. Vloemans, A. Boeckx, S. Thambirajah, P. Bonnet, F.C. Ramaekers, M. Bornens, and T. Grand-Perret. 2010. Autophosphorylation of polo-like kinase 4 and its role in centriole duplication. *Mol Biol Cell.* 21:547-561.
- Singla, V., M. Romaguera-Ros, J.M. Garcia-Verdugo, and J.F. Reiter. 2010. Odf1, a human disease gene, regulates the length and distal structure of centrioles. *Dev Cell.* 18:410-424.
- Spang, A., I. Courtney, K. Grein, M. Matzner, and E. Schiebel. 1995. The Cdc31p-binding protein Kar1p is a component of the half bridge of the yeast spindle pole body. *J Cell Biol.* 128:863-877.
- Spektor, A., W.Y. Tsang, D. Khoo, and B.D. Dynlacht. 2007. Cep97 and CP110 suppress a cilia assembly program. *Cell.* 130:678-690.
- Stemm-Wolf, A.J., J.B. Meehl, and M. Winey. 2013. Sfr13, a member of a large family of asymmetrically localized Sfi1-repeat proteins, is important for basal body separation and stability in *Tetrahymena thermophila*. *J Cell Sci.* 126:1659-1671.
- Stevens, N.R., A.A. Raposo, R. Basto, D. St Johnston, and J.W. Raff. 2007. From stem cell to embryo without centrioles. *Curr Biol.* 17:1498-1503.
- Stevens, N.R., H. Roque, and J.W. Raff. 2010. DSas-6 and Anaz coassemble into tubules to promote centriole duplication and engagement. *Dev Cell.* 19:913-919.
- Strnad, P., S. Leidel, T. Vinogradova, U. Euteneuer, A. Khodjakov, and P. Gonczy. 2007. Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. *Dev Cell.* 13:203-213.
- Vallen, E.A., W. Ho, M. Winey, and M.D. Rose. 1994. Genetic interactions between CDC31 and KAR1, two genes required for duplication of the microtubule organizing center in *Saccharomyces cerevisiae*. *Genetics.* 137:407-422.
- Wong, Y.L., J.V. Anzola, R.L. Davis, M. Yoon, A. Motamedi, A. Kroll, C.P. Seo, J.E. Hsia, S.K. Kim, J.W. Mitchell, B.J. Mitchell, A. Desai, T.C. Gahman, A.K. Shiau, and K. Oegema. 2015. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. *Science.* 348:1155-1160.

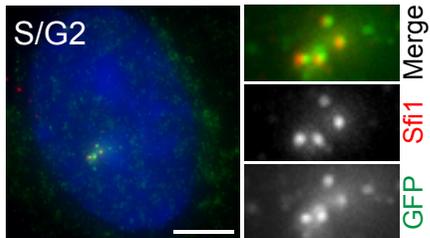
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B RPE-1



C RPE-1 GFP-Sfi1



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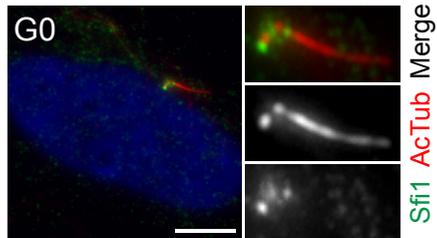


Figure 1

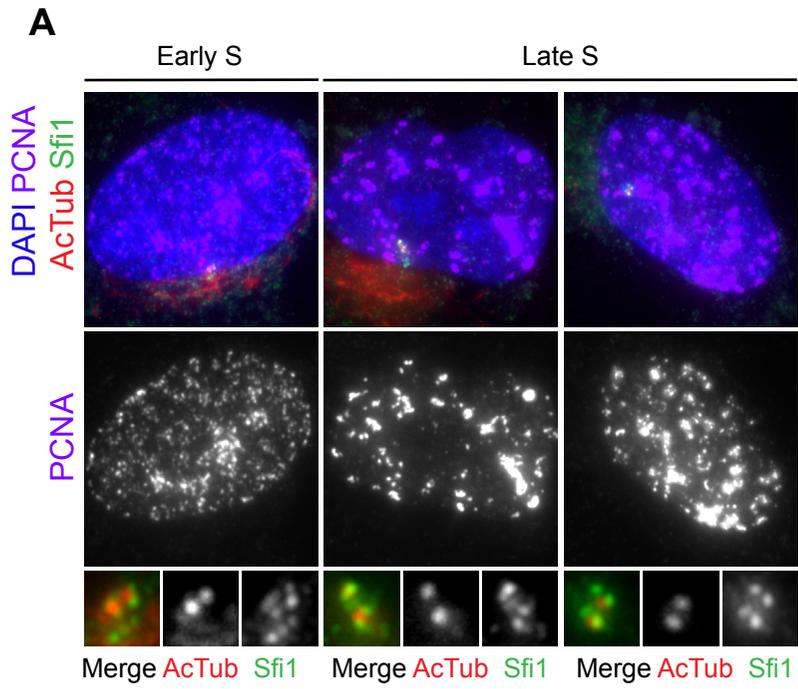


Figure 2

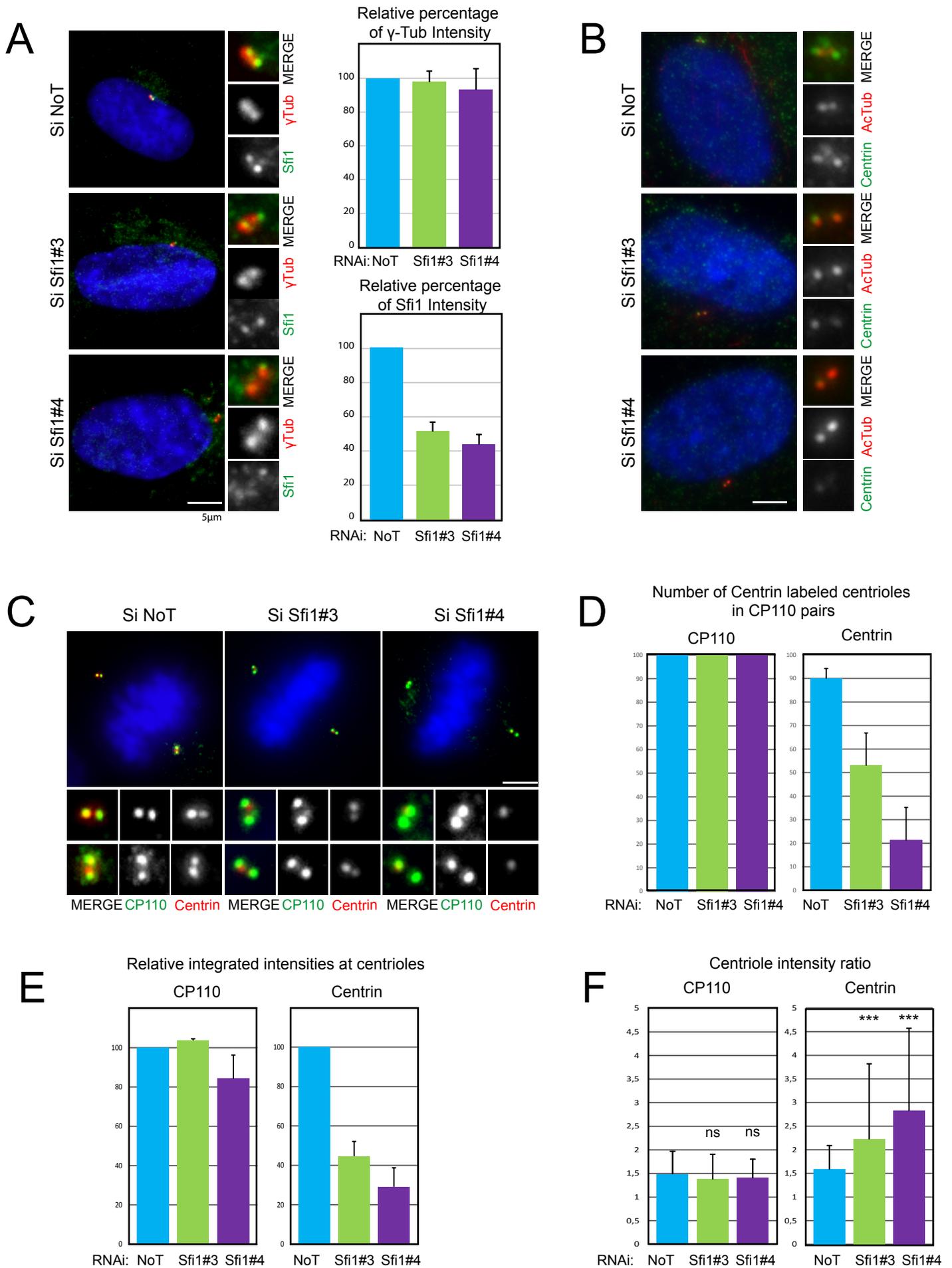


Figure 3

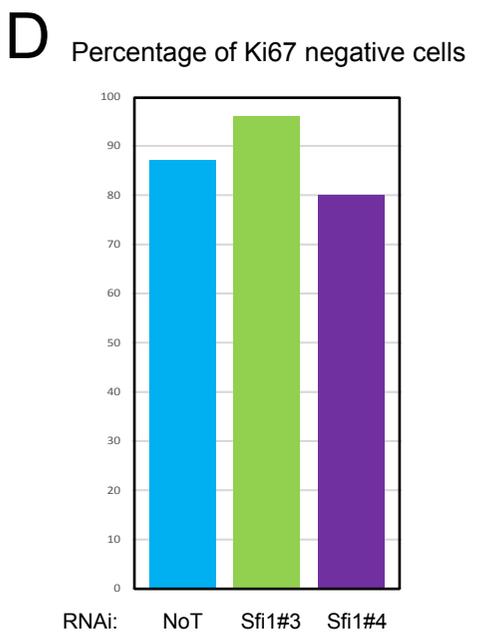
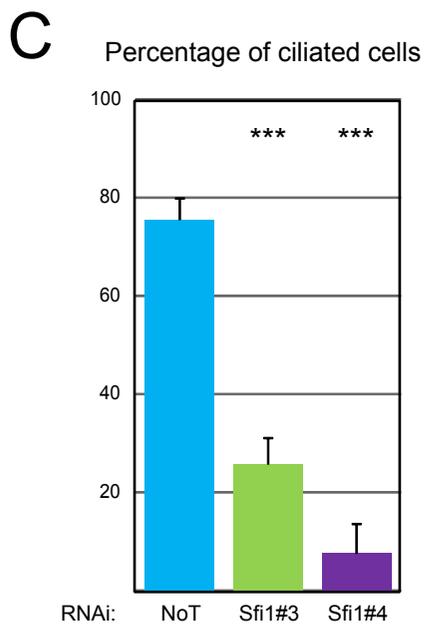
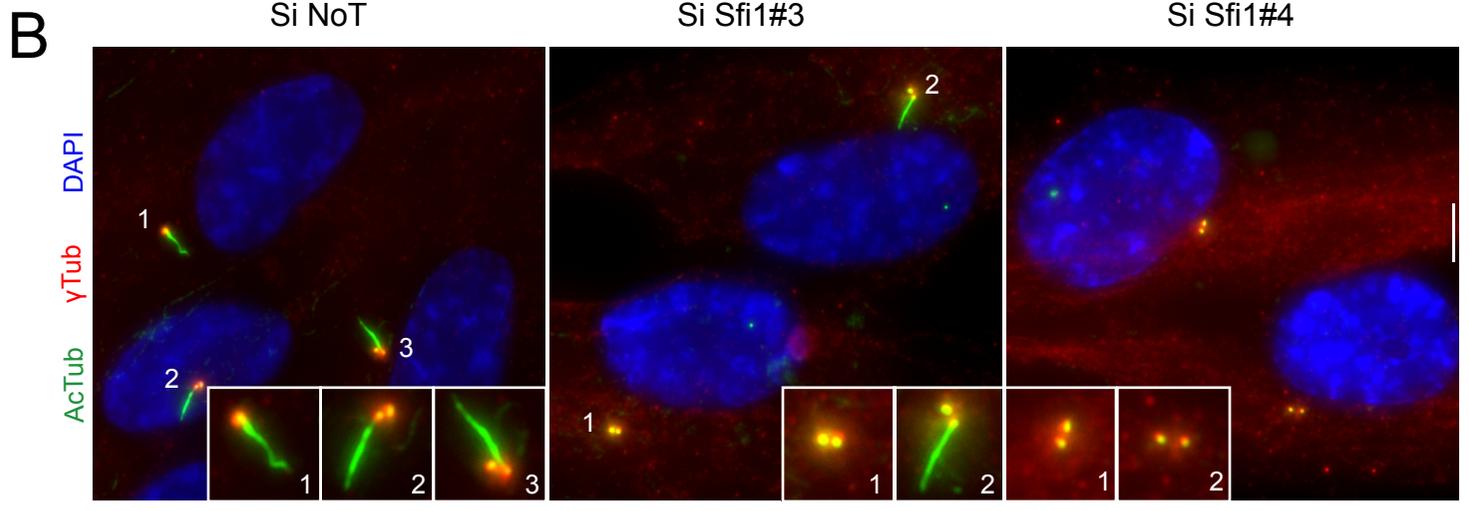
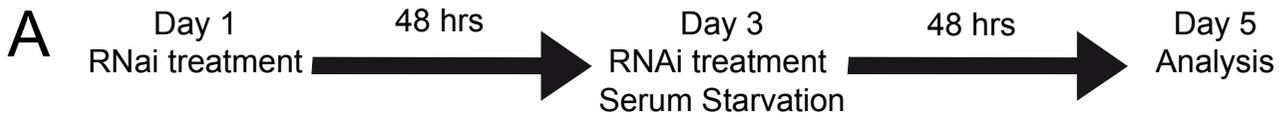


Figure 4

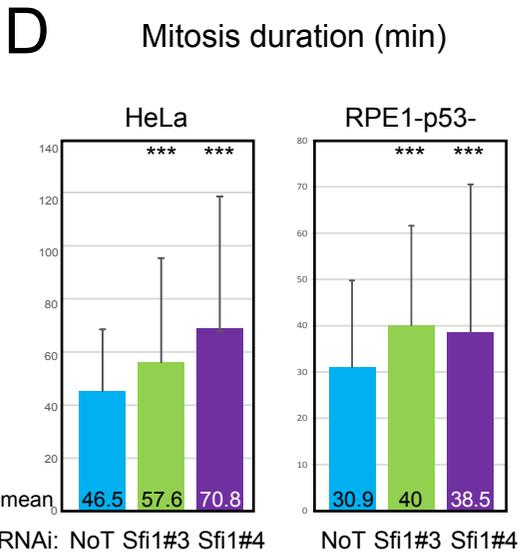
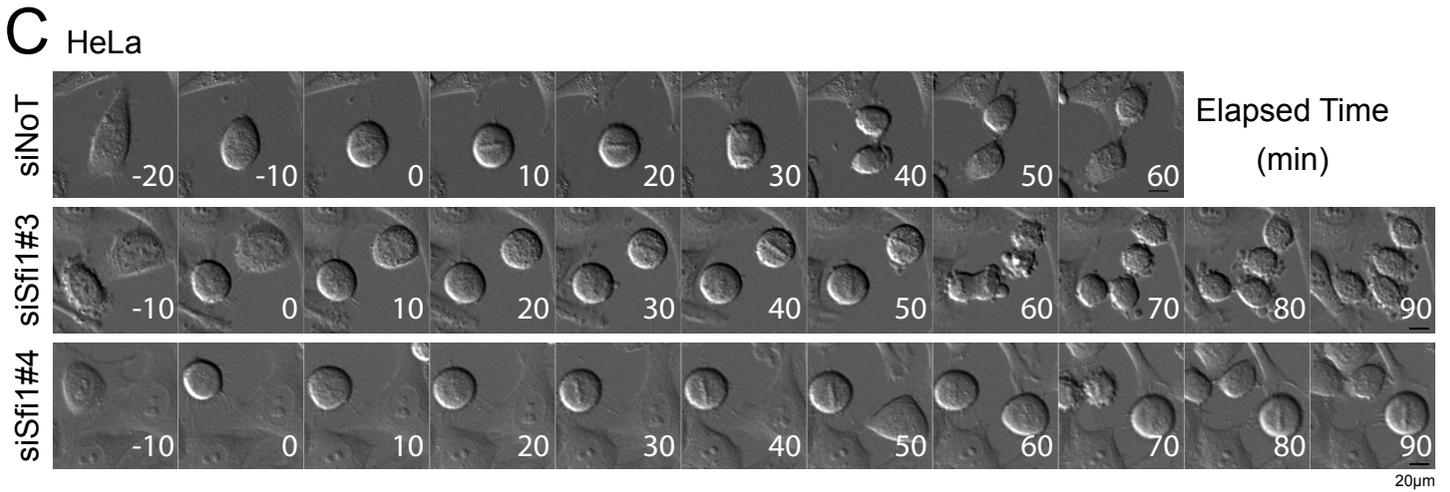
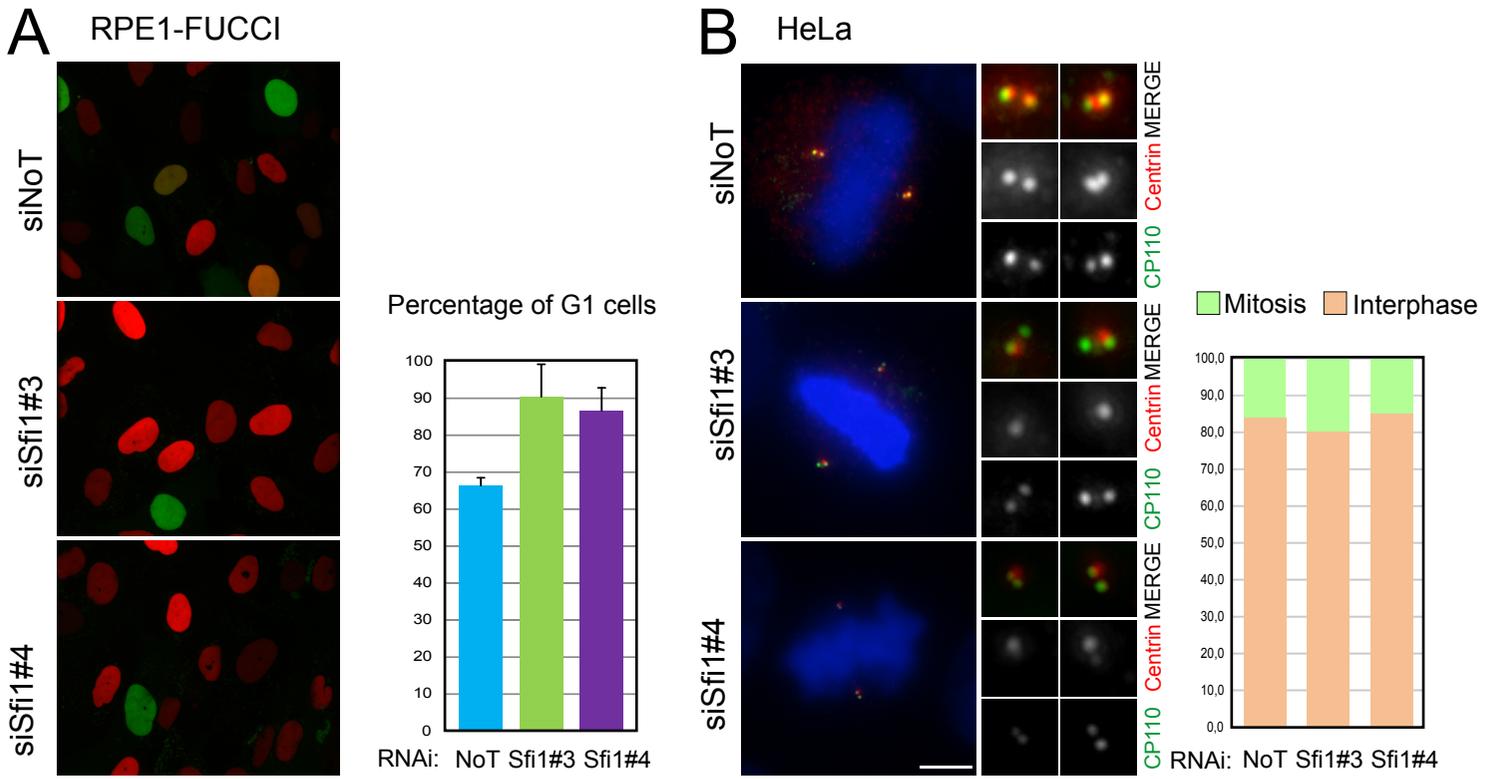


Figure 5

Chapter III: DISCUSSION

1. Cell Cycle Control of Spindle Pole Body Duplication and Splitting by Sfil and Cdc31

1.1 **Sfil is a component of the fission yeast HB and is essential for SPB duplication**

In the first article, I have shown that Sfil is a component of the HB in fission yeast and that it is essential for SPB duplication. Cdc31 has also been shown to be a component of the HB (Paoletti et al., 2003) and our localization studies show that Sfil co-localizes perfectly with Cdc31 but not with Cam1, a core component of the HB. Trying to determine which domain of Sfil was required for Sfil localization at the SPB, we expressed different constructs of Sfil fused to GFP in *S. pombe*. These constructs were expressed in the presence of the endogenous Sfil. Three different domains exist in Sfil: the N-terminal domain, the Central region containing the Sfil repeats and the C-terminal domain. Our experiments show that the C-terminal domain is dispensable for Sfil localization at the SPB, but the N-terminal and the central region are both required for its recruitment to the SPB. The requirement for these domains is not surprising giving the fact that Sfil N-terminal domain is hypothesized to interact with the core SPB like in budding yeast, (Kilmartin, 2003; Li et al., 2006) and the central region is required for Cdc31 localization.

We next established a *thermo-sensitive* (*ts*) mutant of Sfil the *sfil-1* allele. When cultured at 36°C, mitotic cells exhibited monopolar spindles, indicating a SPB duplication or separation defect. Importantly, in this mutant, Cdc31 was unable to localize at the HB as in WT cells. Similarly, depletion of Cdc31 using a *ts* mutant also affected Sfil localization at the SPB. This indicates that Sfil and Cdc31 are interdependent in their recruitment to the HB. Sfil has been shown to be mainly composed of an elongated alpha helix (Li et al., 2006). This likely makes Sfil highly unstable when not interacting with Cdc31 *in vivo*. This may explain why we have failed to express Sfil in bacteria and

why Li and colleagues had to use a bicistronic construct and expressed Cdc31 and Sfil at the same time.

Live imaging of the *sfil-1* mutant has shown that cells assemble a monopolar spindle and exit mitosis after a certain time. Failure of bipolar spindle assembly was due to a defect in SPB duplication in Sfil mutant cells. This indicates that Sfil is essential for SPB duplication in fission yeast, as it is in budding yeast (Kilmartin, 2003; Li et al., 2006).

Another study (Lee et al., 2014) also examined the functions of Sfil in fission yeast and found similar results to ours. This study aimed at characterizing a cytokinesis mutant that assembled multiple septa in short cells. Genome sequencing allowed identification of one mutation in the ninth repeat of Sfil. This *sfil-M46* mutant showed mislocalization of the SIN component Cdc7. Indeed, instead of being restricted to the daughter SPB in late mitosis, both SPBs were now able to recruit Cdc7, which was also observed in other cytoplasmic foci. According to the authors, the mislocalization could explain the cytokinesis defect. However, they didn't investigate further the relationship between Sfil and Cdc7, since they also observed that these mutant could not assemble bipolar mitotic spindles, which could also explain the multiple septa formation as observed in *sfil-1* mutant (Bouhleb et al., 2015). Moreover, EM analysis clearly showed that SPB duplication was impaired in this mutant.

Interestingly, Sfil was asymmetrically localised in the *sfil-M46*, as the old SPB seemed to retain a higher number of Sfil molecules compared to the daughter SPB. This is reminiscent of the situation observed in the *cdc31-S15A* mutant, where a consensus site for Cdk1 phosphorylation has been mutated. SPB separation lead to an asymmetric redistribution of Sfil molecules between the two SPBs (Bouhleb et al., 2015).

Importantly, in some cases, cells inheriting non detectable levels of *sfil-M46* at the SPB nevertheless managed to form a bipolar spindle in the next mitosis. In *cdc31S15A* mutant, we could similarly observe the formation of a bipolar spindle in cells where Sfil-GFP was absent from one SPB at the moment of SPB separation. This indicates that either an initial very low number of Sfil molecules at the bridge is sufficient to template

the recruitment of more molecules or that Sfil arrays can be assembled *de novo* from the SPB during interphase to form a functional HB.

1.2 Timing of HB and SPB duplication in fission yeast

In our study, we were able to time HB duplication using Sfil-GFP signal accumulation and the *cdc31-8 ts* mutant. The execution point experiment with *cdc31-8* inactivation allowed us to show that Cdc31/Sfil arrays function to promote SPB duplication at mitosis before the end of anaphase. Additionally, Sfil-GFP intensity measurement have shown that Sfil recruitment is biphasic; a first phase starts minutes after SPB separation and consists of a rapid accumulation of Sfil molecules while the second phase is slower but lasts throughout G2. This strongly suggests that the first Sfil recruitment event corresponds to HB elongation while the second phase constitutes a maturation phase of the bridge.

Additionally, we used the SPB component Ppc89 fused to GFP to investigate the presence of two adjacent SPB in WT and *cdc10-V50* mutant. The *cdc10-V50* mutant is blocked in G1 prior to S phase entry without blocking cell growth. In WT cells, we could detect two side-by-side duplicated in approximately 20% of short cells (7-8 μm) corresponding to G1/S phase. This frequency increased with cell size and cell cycle progression. Interestingly, in the *cdc10-V50* mutant, very few duplicated SPBs could be found in short cells. However, as in WT, the percentage of unseparated but duplicated SPB increased with cell size. Overall, these results suggest that SPB duplication can occur prior to S phase transition. However, the resolution of light microscopy as well as orientation or size of the nascent SPB could prevent its observation in S phase. This finding is in agreement with the results published by Uzawa and colleagues (Uzawa et al., 2004) where duplicated SPBs could be detected by EM in *cdc10-V50* cells. It is moreover in line with another study where duplicated SPBs could only be observed in a fraction of short cells, confirming the difficulty of assessing SPB duplication in non-synchronized cells even using EM (Ding et al., 1997; Hoog et al., 2013).

Another recent study used super resolution microscopy (3D SIM) to time the SPB duplication (Bestul et al., 2017). Using 3D SIM the authors could resolve the mother SPB and an SPB precursor in short interphasic cells and confirmed that HB duplication is observed in 80% of G1/S cells. Importantly, they confirmed that the initial accumulation in mitosis of Sfil is due to accumulation of Sfil molecules distal to the mother SPB to form the extended HB. Moreover, as the cell cycle continues, Sfil is added to both proximal and distal regions, possibly forming the HB stacked layers as observed by EM (Hoog et al., 2013; Paoletti et al., 2003). The authors hypothesized that this stacking ensures the old and new SPB to associate with approximately the same number of Sfil molecules. This could be of importance for SPB separation and future rounds of SPB duplication. This study fully confirms the hypothesis that we made, based on quantitative analysis of Sfil signal at the SPB.

Besides, the authors were able to observe early recruitment of Ppc89 at the bridge in G1/S cells. This indicates that Ppc89 is the first component of the SPB and its recruitment is concomitant with HB elongation. Although no biochemical interaction with Sfil was tested, this result suggests that Ppc89 could interact with the N-terminal domain of Sfil. Further investigations are required to better understand the mechanisms and regulation of Ppc89 recruitment to the assembly site formed by the N-termini of Sfil (Bestul et al., 2017). This study also brought important novelties about the timing of the recruitment of other SPB components. For instance, it was observed that incorporation of other components occurred in waves, beginning with Sid4 and Pcp1, followed by Cut12 and Cam1. Finally Cdc11 arrived together with Cut12 and Cam1, but did not accumulate to high levels at the new SPB until mitosis, pointing to a maturation event that occurs later in the cell cycle.

1.3 Sfil and Cdc31-dependant regulation of SPB separation

We have shown that Cdc31 is phosphorylated at Serine 15, found within a Cdk1 consensus sequence. *In vitro* phosphorylation assays revealed that Cdk1/CyclinB is able

to phosphorylate Cdc31. Moreover, we could examine that this phosphorylation is enriched in mitotic cells. Additionally, mutation of this Serine to an Alanine abolished all phosphorylated forms in Cdc31. Although this is probably not the only phosphorylation site of Cdc31, it could act as an activation site that triggers further phosphorylation of other sites. This could be achieved by Cdk1 or other mitotic kinases like the polo-like kinase Plol.

Concomitant with our study on Sfil functions in fission yeast, two other studies have shown that Sfil is cell cycle regulated in budding yeast (Avena et al., 2014; Elserafy et al., 2014). These two studies aimed at understanding the cell cycle regulations that apply to the C-terminal domain of Sfil.

The authors have shown that the C-terminal domain of Sfil is heavily phosphorylated. They identified six phospho-sites (S801, T816, S855, S882, S892 and S923) that correspond to the Cdk1 consensus S/TPxK/R and have shown that they are phosphorylated in vivo (Avena et al., 2014; Elserafy et al., 2014).

To study the effect of Sfil phosphorylation on SPB duplication and separation, Elserafy and colleagues have mutated the six phospho-sites to either Alanines (*sfilCdk1-6A* phospho-inhibit mutant) or to an aspartic acid (*sfilCdk1-6D* phospho-mimetic mutant). Following, they have used Spc42-mCherry intensity as a marker for centriole duplication combined to the two Sfil mutants, *sfilCdk1-6A* and *sfilCdk1-6D*. Analysis of *sfilCdk1-6A* cells showed that the Spc42-mCherry signal was 1.5- fold of the corresponding *sfilCdk1-6D* cells or of Sfil WT cells with a single SPB. This suggests that *sfilCdk1-6A* cells have duplicated but unseparated SPB, whereas *sfilCdk1-6D* cells probably have an SPB duplication defect. Interestingly, the mutation of four of the six phospho-sites identified (*sfil-4A*) as Cdk1 sites by Avena and colleagues lead to similar observation that a high percentage of cells where two Spc42 foci could be detected. However, approximately 20% of cells grown at 37°C displayed three or more Spc42 foci, suggesting an overduplication of the SPB. EM analysis confirmed the existence of cells with three or four SPBs, in which two SPBs are adjacent, and the other SPB (or two adjacent SPBs when there are four total) is connected by a short spindle and lies on the

opposite side of the nucleus. Importantly, pairs of SPBs were always found to be connected by a WT-looking bridge suggesting that an aberrant reduplication occurred in these cells. This data not only shows that Sfil C-terminal domain is essential for SPB separation but that the loss of Cdk1 phosphorylation leads to SPB reduplication (Avena et al., 2014; Elserafy et al., 2014). Moreover, Elserafy and colleagues indicated that four of the six phospho-site could also be phosphorylated by the budding yeast polo-like kinase Cdc5 (Elserafy et al., 2014). Finally, these two studies found that Sfil is dephosphorylated by the phosphatase Cdc14 at the end of mitosis and this signals HB extension and SPB duplication. These two studies have also shown that dephosphorylation of Sfil by the phosphatase Cdc14 triggers centrosome duplication for the next cell cycle (Avena et al., 2014; Elserafy et al., 2014).

In fission yeast, we also found a Cdk1 consensus site at the C-terminal domain of Sfil, but the mutation of this site to alanine lead to SPB duplication defects rather than an overduplication (Adeline Mayeux and Anne Paoletti, unpublished data). This observation suggests that this mutation could impair the C-C interactions of Sfil in fission yeast. One possible explanation of this phenotype is that the mutation may affect Sfil C-ter folding globally rather than simply blocking the C-ter phosphorylation, if such a phosphorylation occurs in fission yeast.

Although additional experiments should be performed to completely rule out a role in SPB separation of the C-terminal domain of Sfil in fission yeast, our data points instead towards a role of Cdc31 in SPB separation. It is still however unclear how phosphorylation of Cdc31 could induce SPB separation at mitosis onset. One hypothesis is that phosphorylation of Cdc31 decreases the affinity of Cdc31 and Sfil molecules or destabilizes lateral interactions of adjacent Cdc31 molecules, leading to a less stable bridge. Importantly, this seems to be an initial step for SPB separation since this process also critically requires the molecular motor Cut7 (Hagan and Yanagida, 1992). Accordingly, approximately half of the mitotic cells are able to separate their SPBs (although with a delay) in the *cdc31-S15A* mutant. The observed delay could be due to requirement of additional mechanical forces applied by Cut7 to separate the two SPBs.

Interestingly, SPB separation in this mutant generates a high Sfil asymmetry at the newly separated SPB, suggesting a “forced” separation of the two SPBs.

The mechanisms of SPB separation at mitosis onset seem therefore to be differently regulated in fission and budding yeast. While Sfil C-terminus phosphorylation drives SPB separation in budding yeast, Cdc31 N-terminus phosphorylation initiates SPB separation in fission yeast. This difference could stem from two different factors: first: the C-terminal domain of the fission yeast Sfil is shorter than the C-terminus of budding yeast Sfil, suggesting that additional functions could exist in budding yeast. Second, Sfil and Cdc31 are so far the only identified components of the fission yeast HB, while two additional components have been identified in budding yeast, the transmembrane proteins Mps3 and Karl. Importantly, a recent study has shown that deletion of the C-terminal domain of Sfil does not impair Karl localization at the bridge, although SPB separation takes longer in these cells (Seybold et al., 2015). This observation has led the authors to hypothesize that the C-terminal domain of Sfil could be folded on itself in budding yeast. This would explain why the C-terminal domain is dispensable for assembly of the anti-parallel array of Sfil and SPBs duplication but is required for SPB separation. This conformation would also constitute a perfect platform for additional functions like SPBs separation. However, this region being absent in fission yeast, regulation of SPB separation would rely on Cdc31. Remarkably, the Cdc31 N-terminus might not participate in the Sfil–Cdc31 interaction in budding yeast, as judged by the crystal structure obtained for a fragment of budding yeast Sfil in complex with budding yeast Cdc31, where the Cdc31 N-terminus was not visible and hence not engaged in interactions with the Sfil helix (Li et al., 2006). In addition, a Cdc31 mutant lacking N-terminal residues can replace wild-type Cdc31 in *S. cerevisiae* (Li et al., 2006), similar to the *cdc31S15A* mutant in *S. pombe*. Hopefully, future research on Sfil/Cdc31 regulation in both organisms will bring additional clues about their cell cycle regulation.

2. Human Sfil controls Centrin association with the centriole and regulates ciliogenesis and cell cycle progression

2.1 Sfil in human cells is a centriolar protein required for Centrin localization at the centrioles

I dedicated the second part of my PhD to characterize Sfil in human cells. We confirmed that it is a genuine centriolar protein as previously described (Kilmartin, 2003). We could detect Sfil at the centrioles colocalizing with Centrin and acetylated tubulin. In budding and fission yeast (Kilmartin, 2003), Bouhleb 2015), Sfil is a component of the bridge that links the two SPBs together. In contrast, in human cells, we could not see any Sfil localizing to the PCM or at the centrosomal linker, which plays the same function as the SPB bridge after centrosome duplication. These results indicate that like Centrins (Gavet et al., 2003; Paoletti et al., 1996) Sfil is a centriolar protein that might localize at the distal end of the centrioles like Centrins. So, a first difference between the yeast and human proteins is established.

The second difference stems from Sfil function. In yeast Sfil has been shown in several studies to be essential for SPB duplication (Anderson et al., 2007; Avena et al., 2014; Bouhleb et al., 2015; Elserafy et al., 2014; Kilmartin, 2003; Lee et al., 2014; Li et al., 2006). Our experiments indicate that in human cells, Sfil is largely dispensable for centriole duplication. Indeed, this is not surprising since Cdc31 is also essential for SPB duplication (Baum et al., 1986; Paoletti et al., 2003) but not for centriole duplication (Dantas et al., 2011; Kleylein-Sohn et al., 2007; Strnad et al., 2007). Therefore, the role of Sfil during centriole biogenesis could be restricted to the recruitment or stability of centrin association with the distal end of centrioles (Paoletti et al., 1996).

Accordingly, our Sfil depletion experiments by RNAi, induced a significant decrease of Centrin at the centrioles. This indicates that Sfil could be required either for centrin recruitment or stability at the centriole. In fission yeast, we have shown that Sfil and Cdc31 localization at the HB is interdependent (Bouhleb et al., 2015). It is thus tempting to hypothesize that this co-requirement is conserved in human cells. Investigations of

the consequences of Centrin depletion on Sfil levels at the centriole would provide more insights. Two possibilities exist: first, Sfil recruitment could be affected by Centrin depletion, mimicking their co-requirement in yeast and indicating that both proteins need to be recruited at the centriole simultaneously. Second, Centrin depletion does not perturb Sfil localization at the centriole. Therefore, Sfil would be recruited to centrioles upstream and independently of Centrin. This situation would imply that Sfil could be interacting with other early components of the centriole like CEP135, CPAP or Sas6. Both possibilities are compatible with our observation that Sfil is recruited around the centrioles (and at structures resembling the centrosomal satellites) in early S phase, centriole assembly is initiated.

2.2 Sfil and Centrin are required for ciliogenesis

Although it is not necessary for centriole duplication, Sfil plays an important role at centrosomes by ensuring proper centrin distribution at the distal end of the centriole and promoting ciliogenesis upon serum starvation. Likewise, Centrin2 has been shown to be essential for ciliogenesis in zebrafish (Delaval et al., 2011). Moreover, besides Sfil, hPOC5 and CPII0 also bind to Centrin (Azimzadeh et al., 2009; Kilmartin, 2003; Tsang et al., 2006). Several studies have revealed the importance of hPOC5 and CPII0 for correct centriole maturation (Azimzadeh et al., 2009; Schmidt et al., 2009). A recent study has also shown that Centrins are essential for primary cilia formation in human cells by controlling CPII0 levels at basal bodies (Prosser et al., 2015). Centrins seem therefore to act in centriole biogenesis as structural proteins by recruiting other centriolar components and to regulate ciliogenesis.

Why ciliogenesis is affected upon depletion of human Sfil is unclear. One explanation could be that the structure of the distal end of the centriole is altered preventing mother centriole docking at the plasma membrane or function as a basal body for cilium assembly. Electron microscopy analysis will be required to assess if the distal region of the centriole is affected and confirm this hypothesis.

Moreover, analysis of Sfil organization within centriole could bring insights into its structural function in centriole. In yeast, the C-termini of Sfil interact with each other and this is an essential step for bridge and SPB duplication. If this interaction is conserved in human cells, one could imagine that they interact in the lumen of the centriole while the N-termini point towards the walls of the centrioles. This organization may stabilize the barrel shape of the centriole. Additionally, the distal end of the centriole is decorated with distal (DA) and subdistal (SDA) appendages that are required for ciliogenesis (Anderson, 1972; Bornens, 2002; Ishikawa et al., 2005; Piel et al., 2001). The DA are required for membrane docking of the basal body (BB) while the SDA are essential for MTs anchorage. Investigating the integrity of these appendages in Sfil depleted cells using super resolution microscopy or electron microscopy would bring more information about the role of Sfil in ciliogenesis.

2.3 Sfil depletion leads to a cell cycle arrest and to mitotic delay

RPE-1 cells are non-transformed cells. Many studies have shown that centrosome defects in these cells lead to a cell cycle arrest due to the activation of the so-called p53-dependent centrosome surveillance pathway (Fong et al., 2016; Lambrus et al., 2016; Meitinger et al., 2016; Wong et al., 2015). However, this surveillance pathway also monitors mitosis duration and we observed prolonged mitosis in HeLa cells and p53-RPE-1 cells. Thus, the G1 block in RPE-1 cells could also result from mitosis prolongation during the previous cell cycle.

As mentioned above, Sfil depletion induced a prolonged mitosis in HeLa and RPE1 p53-cells. How could Sfil regulate mitosis duration? Our preliminary observations have shown that upon Sfil depletion, MTs and centrioles acetylation is largely decreased. The effect of a centriolar protein on MTs acetylation have been reported before (Patel et al., 2016; Wang et al., 2016). Kindlin1 (Kin1) is a component of the focal adhesions that is required for integrin activation (Harburger et al., 2009). However, it has been shown recently that it also localizes to the centrioles where it is a substrate of Plk1 (Patel et al., 2013). This centrosomal and integrin-independent localization promotes bipolar spindle assembly (Patel et al., 2013). A follow-up study of the same group has shown

that Kin1 contributed to MTs stability by inhibiting their deacetylation (Patel et al., 2016). This was assessed by the quantification of nocodazole or cold resistant MTs in Kin1 depleted cells. Accordingly, MTs acetylation was decreased in these cells. To determine if acetylation decrease was a direct consequence of Kin1 depletion, the major cellular tubulin deacetylase HDAC6 was inhibited using the specific inhibitor Tubacin. Interestingly, HDAC6 inhibition rescued MTs acetylation and mitotic spindle defects. Moreover, HDAC6 inhibition also prevented the loss of MT stability seen in the Kin1 depleted cells, indicating that the reduction in acetylated tubulin levels seen in Kin1 depleted cells was not just a marker of reduced MT stability but also a direct consequence of Kin1 ability to regulate HDAC6 and contribute to MT stability. Importantly, Kin1, Plk1 and HDAC6 were present in the same complexes on MTs and at the centrosome. As mentioned above, Plk1 phosphorylation of Kin1 is essential for its localization at the centrosome and this study has shown that is required for Kin1 interaction with HDAC6. All these results strongly suggest that Plk1-phosphorylated Kin1 is able to interact with HDAC6 therefore preventing it from deacetylating MTs. In absence of Kin1, HDAC6 activity is not inhibited and MTs are deacetylated leading to more MTs that are unstable and to mitotic spindle defects (Patel et al., 2013).

Based on our preliminary results Sfil might regulate HDAC6 in the same manner. Indeed, the loss of MTs acetylation in Sfil depleted cells is not a consequence of an acetylation defect since HDAC6 inhibition with Tubacin restores the acetylation of MTs in these cells (our preliminary results). This result suggests that the main tubulin acetylase TAT1 is functional. Thus, Sfil depletion could lead to over-activation of HDAC6. This would explain the delay observed in mitosis upon Sfil depletion. Although these are preliminary results, it would be interesting to follow up and test the interaction *in vivo* between Sfil and HDAC6 by performing immunoprecipitations and mass-spectrometry analysis. If this interaction is confirmed, the next step would be to identify potential regulators of Sfil/HDAC6 interaction and if Centrin also play a role in MTs acetylation. This would further unveil Sfil functions in the cell.

2.4 Evolutionary conservation of Sfil/centrin function?

During my PhD, I studied the functions of Sfil and Centrins in fission yeast and in human cells. The results obtained in both organisms raises an important question concerning the evolutionary conservation of Sfil and Centrins: “Why does such a conserved protein seem to have such a variety of functions?” Indeed, as described in different sections of this thesis, Centrins and Sfil have been shown to play a variety of centrosomal function. They seem therefore to form an adaptive module for centriolar/BB functions and maintenance (Azimzadeh and Bornens, 2007; Marshall, 2009).

Centrosomes and BB are conserved structures among all ciliated organisms. Interestingly, the nine fold symmetry is also conserved. The evolutionary conservation of the structure as well as conserved mechanisms of regulation like duplication and separation suggest that centrioles and BB were present among the last common ancestor. Interestingly, their ancestral function was probably to nucleate cilia as evidenced by the distribution of centrioles and BB across the eukaryotic tree (Azimzadeh et al., 2012; Bornens and Azimzadeh, 2007; Marshall, 2009; Mitchell, 2007). Moreover, centrioles have been suggested to have evolved from the internalization of the BB complex, but when and how this happened remains unclear.

Moreover, ultrastructure analysis in a wide range of organisms shows that the distal appendages (DA) are conserved features of the BB and centrioles, and they are often referred to as “transition zones” when associated to a BB. DAs are essential for BB docking at the plasma membrane when ciliogenesis is initiated. Accordingly, the DA protein Cep164 has been shown to be widely conserved (Azimzadeh et al., 2012; Graser et al., 2007).

Considering that genes coding for Centrins appeared very early in evolution, and that they are found to interact with Sfil like protein in ciliates, yeast and human, suggests that the Sfil/complex may have evolved to fulfil different functions according to the organism. Importantly, these functions have been so far always linked to BB

organization or duplication (Heydeck et al., 2016; Stemm-Wolf et al., 2013) or the organization of contractile structures like the ICL in *Paramecium* (Aubusson-Fleury et al., 2017; Gogondeau et al., 2007). It is therefore not surprising that Sfil and Centrin seem to play a role in ciliogenesis in human cells instead of centriole duplication. Indeed, cells have developed a new mechanism of centriole duplication implicating the Plk4-STIL-SAS-6 module while centrin/Sfil module remained required for cilia assembly.

The nine symmetry of centrioles and BB has been lost in yeast but Sfil and Centrin have been conserved. Remarkably, the Centrin/Sfil complex conserved its centrosomal function in these organisms. SPB duplication requires the separation of the two existing SPBs and this is achieved by the rupture of the bridge, where Sfil and Centrin are found. The linear structure of Sfil, its multiple centrin-binding domains and its ability to form anti-parallel arrays appear therefore to make this complex perfectly adapted to regulate the separation and duplication of the SPB in yeast.

However, Sfil is not conserved in *Drosophila* and the worm *C. elegans*, although these organisms do assemble cilia (Riparbelli et al., 2010). This suggests that other mechanisms to assemble cilia must exist. Noteworthy, the centrioles in the fruit fly and *C. elegans* differ from vertebrate's centrioles. *Drosophila* and *C. elegans* centrioles are composed of doublets and singlets of MTs instead of triplets in vertebrates. Moreover, the distal part of centriole, where Centrin has been localized in vertebrates is not present in these two organisms. This could explain why Sfil has been lost in *Drosophila* and *C. elegans*.

RÉSUMÉ

Durant la division cellulaire, l'établissement d'un fuseau mitotique bipolaire est crucial pour la bonne ségrégation des chromosomes dans les deux cellules filles. Le fuseau mitotique est composé de microtubules nucléés à partir des centres organisateurs des microtubules : les centrosomes. Le centrosome est lui-même composé de deux centrioles entourés de la matrice pericentriolaire ou PCM.

Bien que des études aient montré que les centrosomes n'étaient pas essentiels à la formation du fuseau mitotique (Basto et al., 2006; Khodjakov et al., 2002), ces derniers jouent un rôle important dans son maintien, sa stabilité et son orientation. Les centrosomes sont produits par duplication, mécanisme rigoureusement régulé au cours du cycle cellulaire. En effet, le centrosome se duplique une fois par cycle cellulaire par un mécanisme rigoureusement régulé par la cellule. Des erreurs de duplication conduisant à plus de deux centrosomes induisent la formation de fuseaux multipolaires et provoquent des défauts de ségrégation des chromosomes (Fig. 1 et 2).

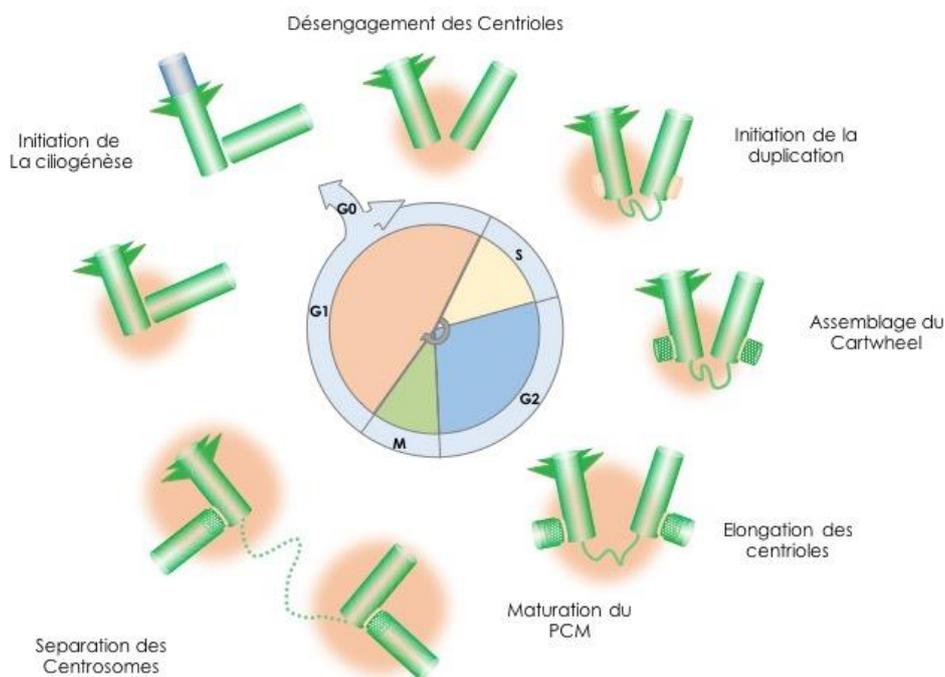


Figure 27- Cycle de duplication du Centrosome

La duplication du centrosome est initiée en G1 lors du désengagement des deux centrioles. Cette étape est suivie par l'initiation de la duplication, qui consiste à définir le site d'assemblage du centriole fils sur le centriole père. En début de la phase G2, le « cartwheel », structure de base du centriole est assemblé, cette étape est suivie par l'élongation des nouveaux centrioles et la maturation de la matrice pericentriolaire (PCM). En mitose, les deux paires de centrioles se séparent afin d'établir le fuseau mitotique. Ainsi, à la fin de la division cellulaire, chaque cellule fille hérite d'une paire de centrioles entourée par le PCM. En G0, le centrosome est aussi à la base de la formation du cil, puisque le centriole père est à la base du cil.

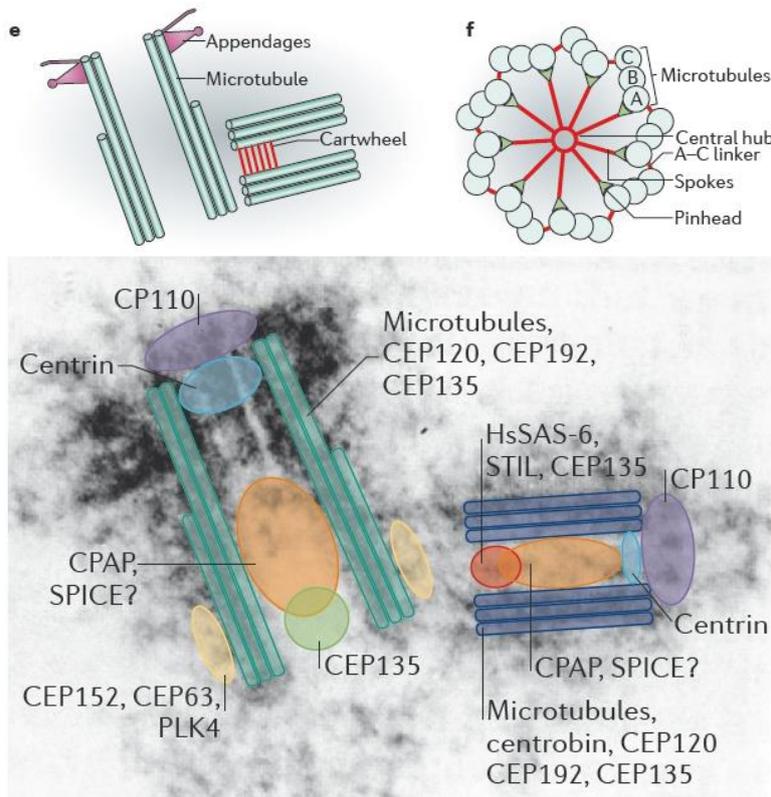


Figure 2: composition et architecture des centrioles

Les centrioles sont formés par neuf triplets de microtubules ce qui leur confère une symétrie en neuf. Les deux centrioles possèdent différentes caractéristiques. Le centriole père possède des appendices distaux. De plus, la région la plus distale du centriole père est composée de neuf paires de microtubules au lieu de triplets.

Le centriole fils, dupliqué à partir du centriole père est caractérisé par la présence d'une structure appelée « la roue » ou « cartwheel » en anglais. Cette structure se trouve sur le bout proximal du centriole et est perdue lors des étapes de maturation dans les centrioles chez les cellules mammifères.

Les composants moléculaires des deux centrioles varient aussi selon leur position : certains composants comme la centrine ou CP110 sont distaux alors que d'autres comme

1. Rôle de Sfil dans la duplication et séparation des SPBs

Chez la levure *Schizosaccharomyces pombe*, un organisme modèle pour l'étude de la division cellulaire, les homologues des centrosomes sont les SPBs (pour Spindle Pole Body). Bien qu'ils possèdent une structure en plaques attachées à l'enveloppe nucléaire très différente des centrosomes, ils sont essentiels à l'établissement d'un fuseau mitotique intra-nucléaire. Ainsi, les défauts de duplication du SPB aboutissent systématiquement à la formation de fuseaux monopolaires incapables de ségréger les chromosomes. De plus, Les SPBs sont liés à une structure annexe spécifique appelée demi-pont, quand ils ne sont pas dupliqués, puis pont, quand elle relie les deux SPBs dupliqués. Les deux principaux composants du pont sont Cdc31 et Sfil (Kilmartin, 2003;

Li et al., 2006; Paoletti et al., 2003). Sfil est une protéine linéaire formée de répétitions d'hélice α (20 répétitions chez *S.pombe* et 17 dans la protéine humaine) formant des sites de liaison pour la protéine globulaire Cdc31. Sfil s'assemble en réseau de molécules parallèles interagissant avec le SPB via leur domaine N-terminal. Au cours de la duplication, un second réseau antiparallèle s'assemble, connecté au premier via le domaine C-terminal de Sfil (Anderson et al., 2007; Li et al., 2006 ; Fig. 3). Ceci crée un site d'assemblage pour un nouvel SPB. Cependant, on ignore complètement quand le second réseau de Sfil s'assemble, comment son assemblage est régulé afin de limiter le nombre de cycles de duplication à un par cycle cellulaire et enfin comment le pont se rompt à l'entrée en mitose pour permettre l'assemblage du fuseau mitotique.

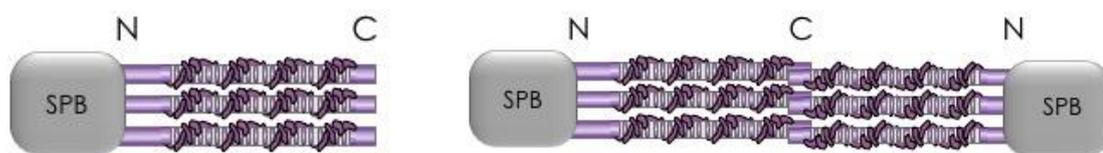


Figure 3 - Composition et duplication du demi-pont chez la levure *S.cerevisiae*

Chez la levure *S.cerevisiae*, le demi-pont est composé d'un réseau parallèle de molécules de Sfil et Cdc31. La duplication du SPB est précédée par la duplication du demi-pont qui consiste en l'assemblage d'un réseau anti-parallèle de Sfil et Cdc31. Adapté de Rüttnik et al. 2017

Au cours de ma thèse, je me suis intéressée dans un premier temps au rôle du demi-pont dans la duplication et la séparation des SPBs conduisant à l'établissement d'un fuseau bipolaire chez la levure *S.pombe*. Cette étude a permis d'établir la fonction de Sfil chez la levure et les résultats ont été publiés dans la revue « Journal of Cell Science ».

Dans un premier temps, nous avons confirmé que Sfil est aussi un composant du demi-pont chez la levure *S.pombe*. Pour cela, nous avons colocalisé Sfil avec des composants du SPB et avec Cdc31. Nous avons observé que la localisation de Sfil avec les composants de demi-pont n'était pas parfaite, et que lorsqu'on pouvait définir deux SPBs dupliqués, le signal de Sfil était localisé entre les deux. En revanche la colocalisation avec Cdc31 était parfaite, et ce, tout au long du cycle cellulaire. Cette observation permet de conclure que Sfil est bien un composant du demi-pont (Bouhleb et al., 2015).

Une étude quantitative de l'intensité de Sfil au SPB au cours du cycle cellulaire réalisée au laboratoire a révélé deux phases d'augmentation du signal au cours du cycle cellulaire ; une première phase rapide et de courte durée a lieu, dès l'anaphase jusqu'à la septation; une seconde s'étend sur toute la phase G2. Puis, quand le fuseau s'assemble à l'entrée en mitose, les deux SPBs se séparent. Le signal de chaque SPB correspond alors à 1/3 du signal observé avant séparation. Ceci montre que les demi-ponts sont déstabilisés à l'entrée en mitose et perdent 1/3 des molécules Sfil.

Par ailleurs, l'utilisation d'un mutant thermosensible de Cdc31 (Ohta et al., 2012) combinée à un système ultra-rapide de changement de température basé sur la microfluidique (Velve Casquillas et al., 2011), montre que la duplication des SPBs a lieu très rapidement en sortie de mitose, coïncidant avec la première phase d'augmentation de Sfil. Ainsi la première phase correspondrait à la duplication du SPB et aurait lieu en G1.

D'autre part, j'ai étudié les effets de la mutation d'un site potentiel de phosphorylation de Cdc31 par la kinase mitotique Cdk1. Ce mutant (*cdc31-S15A*) présente un délai de séparation des SPBs à l'entrée en mitose et assemble un fuseau monopolaire transitoire. De plus j'ai démontré que la déstabilisation des demi-ponts, lors de l'entrée en mitose, observée dans une souche sauvage est abolie dans ce mutant. J'ai aussi montré que le complexe Cdk1/CyclineB1 phosphoryle Cdc31 *in vitro*. Enfin, des expériences d'immunoprécipitation sur celles synchronisées ont permis d'observer que la forme phosphorylée de Cdc31 était enrichie en mitose et que la mutation de la Sérine 15 en Alanine, conduit à la disparition de cette forme phosphorylée.

A partir de ces résultats, nous avons proposé un modèle dans lequel Sfil et Cdc31 sont régulées par Cdk1 : la phosphorylation de Cdc31 par Cdk1 à l'entrée en mitose déstabiliserait le réseau Sfil/Cdc31 pour favoriser la séparation des SPBs, alors que l'inactivation de Cdk1 en sortie de mitose provoquerait la duplication du demi-pont (Fig. 4).

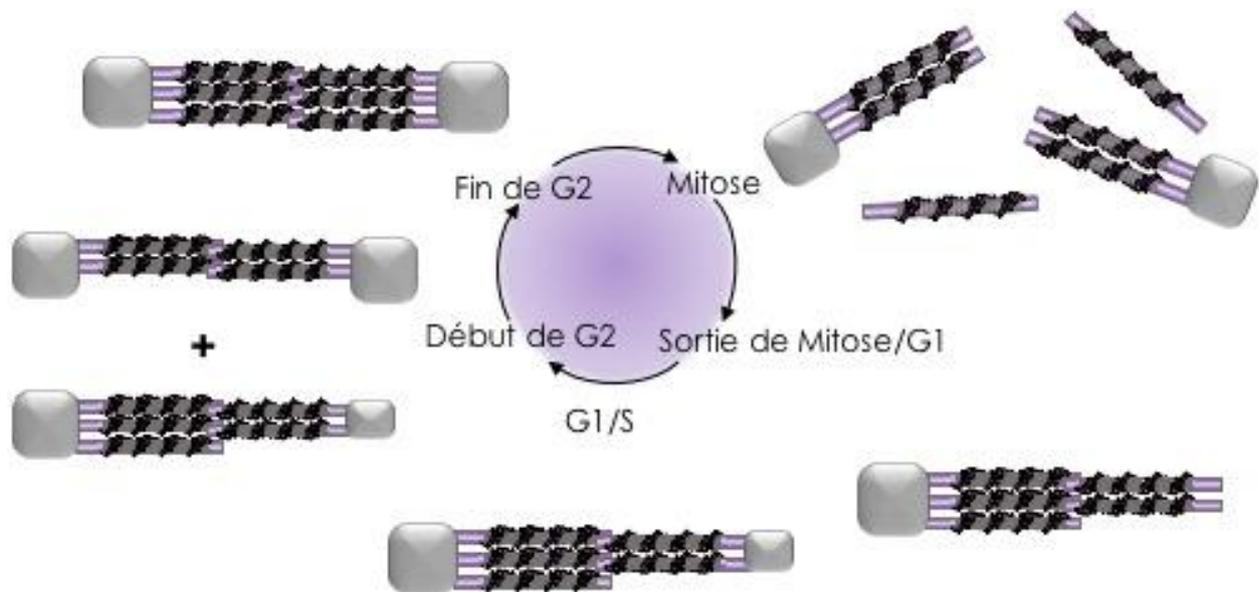


Figure 4 – Modèle de la fonction de Sfi1 et Cdc31 dans le contrôle de la duplication et la séparation des SPBs

Lors de l'entrée en mitose, la phosphorylation de Cdc31 par Cdk1 induit la déstabilisation du pont, et facilite la bonne séparation des SPBs. Dès la séparation des deux SPBs, la duplication du demi-pont est induite, par l'assemblage de nouvelles molécules de Sfi1 et Cdc31. Nos expériences suggèrent que, comme chez *S.cerevisiae*, un précurseur du SPB, le satellite, est assemblé en phase S. En G2, le pont continue d'être assemblé par addition de molécules de Sfi1 et Cdc31 et le SPB est assemblé.

2. Etude de la fonction de Sfi1 chez l'homme

Sfi1 est conservé dans les cellules humaines. La protéine Sfi1 possède une structure similaire à la protéine de *S. pombe* et a été localisée aux centrosomes (Kilmartin, 2003; Li et al., 2006). Cependant, sa fonction reste indéterminée. De la même façon, la fonction centrosomale des Centrinines 2 et 3, homologues de Cdc31, n'est pas clairement définie. En effet, les Centrinines interviennent aussi dans de nombreux processus non liés au centrosome comme la réparation de l'ADN ou l'export d'ARNm (Araki et al. 2001; Fisher et al. 2014). Ainsi, l'étude de Sfi1 et de son interaction avec les Centrinines pourrait aboutir à une meilleure compréhension de leur fonctions centrosomales.

J'ai d'abord confirmé la localisation aux centrioles de Sfil à l'aide de l'anticorps anti-hSfil (produit dans le laboratoire de M. Bornens, par J. Azimzadeh) et en fusionnant la protéine avec la GFP. Ces expériences de localisation ont montré que Sfil colocalise avec la Centrine (Fig. 5).

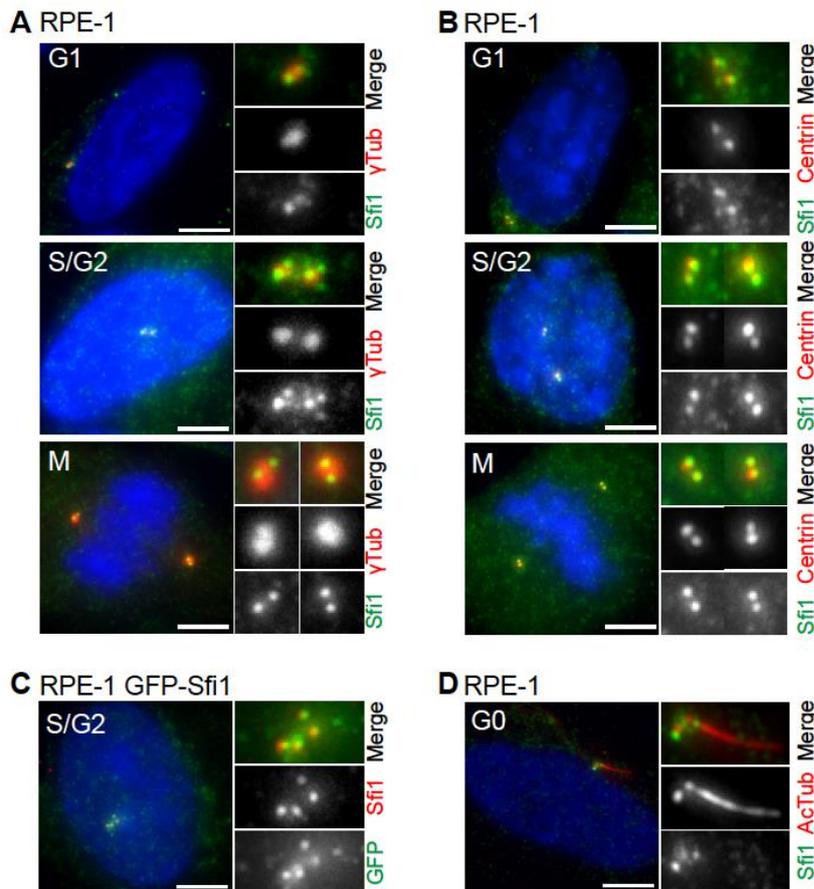


Figure 5 localisation de Sfil dans les cellules humaines RPE-1:

Colocalisation de Sfil au cours du cycle cellulaire avec en **A** la γ -Tubuline et en **B** avec la centrine. Les cellules possèdent deux centrioles en G1 et quatre en S/G2 et en mitose.

C. Une lignée RPE-1 exprimant stablement la protéine GFP-Sfil a été établie. Dans cette lignée, le signal GFP colocalise avec le signal obtenu par l'anticorps anti-Sfil, dans une cellule en G2.

D. Localisation de Sfil dans des cellules en G0 ciliées.

Afin de comprendre les fonctions de Sfil dans les cellules humaines, j'ai utilisé deux siARN (pour short interfering RNA) afin de dépléter Sfil dans les cellules. J'ai observé que la déplétion de Sfil dans les cellules RPE-1, induisait une réduction des niveaux de la Centrine aux centrioles, alors que des composants comme la γ -Tubuline ou CPlI0 (bout distal du centrioles) ne sont pas affectés. De plus, la déplétion de Sfil affecte la localisation de la Centrine aux centrioles de façon asymétrique. En effet, les deux centrioles possèdent des intensités différentes conduisant à une augmentation du ratio de l'intensité du centriole le plus brillant sur le plus faible (Fig. 6). Cette observation

nous conduit à conclure que Sfil régule les niveaux de Centrine aux centrioles soit en la recrutant ou en la stabilisant.

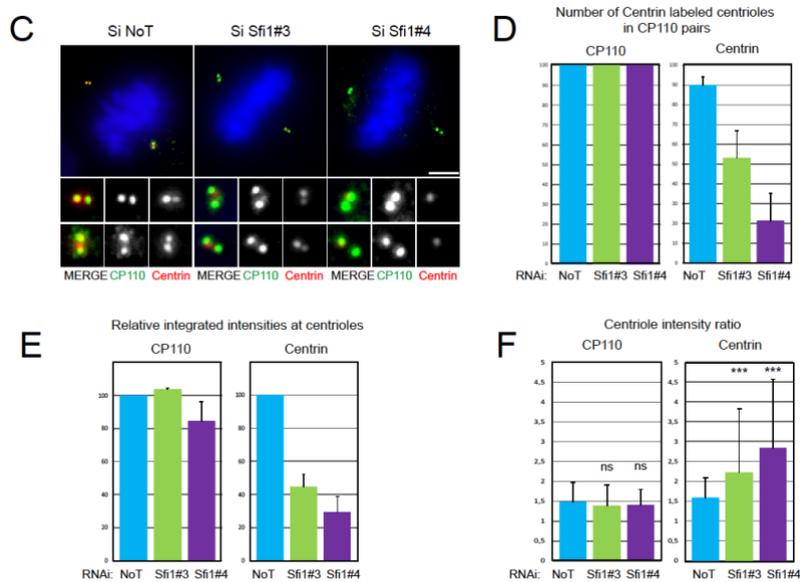


Figure 6: La depletion de Sfil affecte la localisation de la centrine

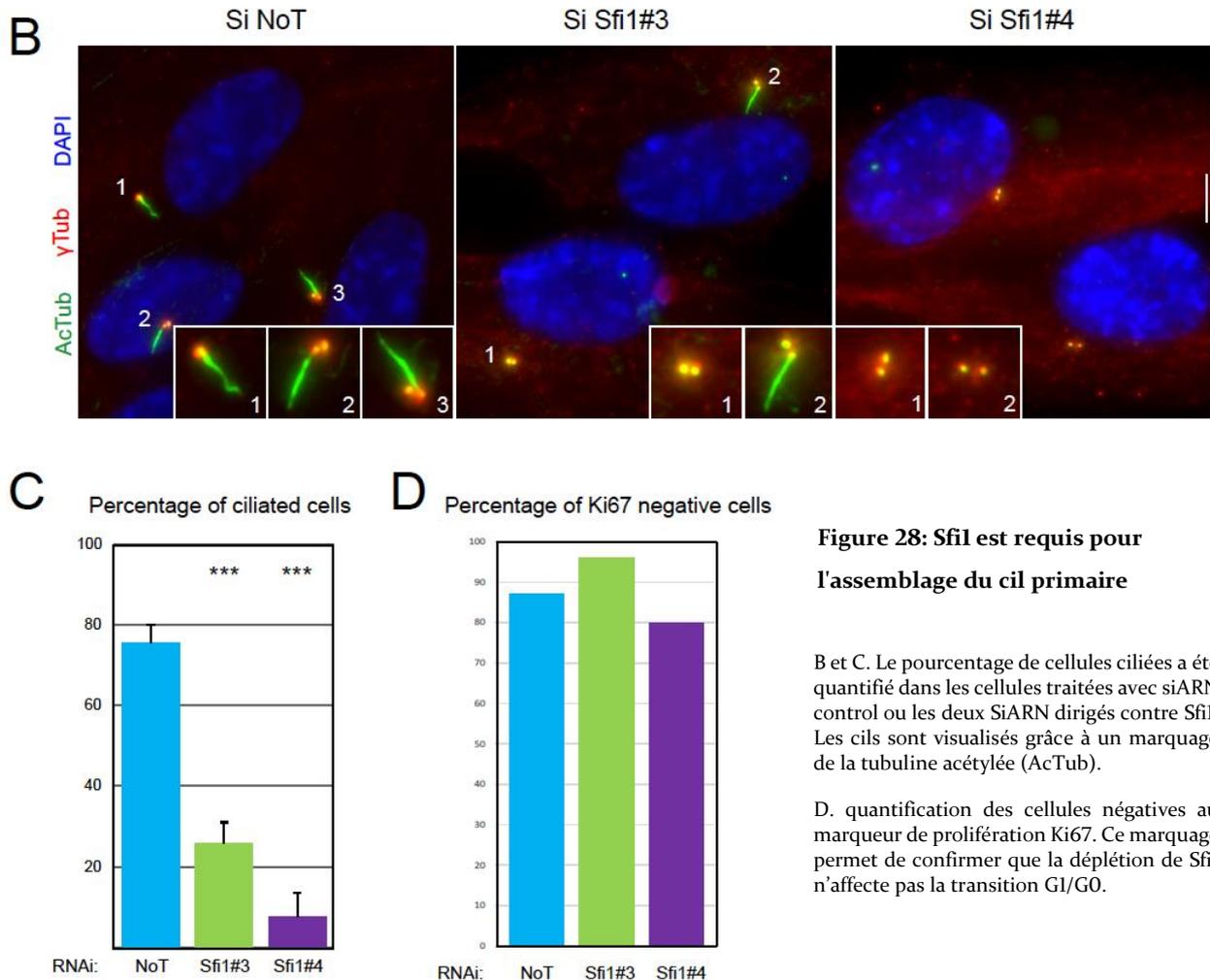
Colocalisation de Sfil au cours du cycle cellulaire avec en **A** la γ -Tubuline et en **B** avec la centrine. Les cellules possèdent deux centrioles en G1 et quatre en S/G2 et en mitose.

Dans cette étude, j'ai utilisé les cellules hTERT-RPE-1 (RPE-1), qui sont des cellules non cancéreuses immortalisées. Ces cellules sont largement utilisées dans l'étude des protéines centrosomales. Les cellules RPE-1 sont capable d'assembler un cil primaire en G0, le centrosome étant à la base de la formation du cil, ces cellules constituent un excellent modèle pour l'étude des fonctions du centrosome. De plus, il a été montré que des défauts d'assemblage du centrosome produisent un arrêt de prolifération dans ces cellules et un arrêt du cycle cellulaire en phase G1.

Lorsque les cellules RPE-1 sont privées de sérum, elles arrêtent de proliférer et sortent du cycle, à ce moment-là, elles sont capables de former un cycle primaire. Il a été démontré que la Centrine était essentielle à l'assemblage du cil en G0 (Delaval et al. 2007). J'ai donc testé l'effet de la déplétion de Sfil sur la ciliogénèse. Quarante-huit heures après le traitement des cellules par siARN, les cellules sont privées de sérum. Dans

ces conditions, 75% des cellules traitées par le siARN contrôle assemblent un cil. Les cellules ciliées ne représentent que 25% et 8% des populations traitées par les siARN anti Sfil#3 et Sfil#4 respectivement (Fig. 7 B et C). Afin de vérifier que la déplétion de Sfil n'altère pas la transition en G0, requise pour l'assemblage d'un cil primaire, j'ai utilisé le marqueur Ki67 qui est uniquement exprimé dans le noyau des cellules qui prolifèrent. Dans les trois conditions, le pourcentage des cellules Ki67 négatives est similaire, indiquant que ces cellules sont bien en phase G0, et que l'absence de cil est liée à l'absence de Sfil aux centrioles (Fig. 7).

Ce résultat montre que comme la Centrine, Sfil est aussi essentiel à la ciliogénèse. Sfil étant requis pour le recrutement ou la stabilisation de la Centrine, sa déplétion pourrait affecter la ciliogénèse à cause de la déstabilisation de cette dernière aux centrioles. La fonction précise de Sfil et de la Centrine dans l'assemblage des cils reste encore à définir. La déplétion de Sfil pourrait conduire à des défauts structuraux du bout distal du centriole, ce qui causerait l'absence de cils dans ces cellules.



D'autre part, j'ai aussi vérifié si la progression du cycle cellulaire était affectée après la déplétion de Sfi1. Pour cela, j'ai utilisé des cellules RPE1-FUCCI. Ces cellules expriment les deux marqueurs nucléaires : mKO-hCdt1 et mAG-hGeminin. Ces deux marqueurs sont exprimés à différentes phases du cycle ; mKO-hCdt1 (rouge) est exprimé en phase G1 et S alors que mAG-hGeminin (vert) est exprimé en phases S et G2 (Fig. 8). Après déplétion de Sfi1, j'ai quantifié le pourcentage de cellules en G1 (rouges). Dans la condition contrôle, le pourcentage de cellule en G1 est d'environ 65% alors que cette proportion est de 90% et 85% après déplétion de Sfi1 (Fig. 8). Ces résultats indiquent que Sfi1 est essentiel à la progression du cycle cellulaire.

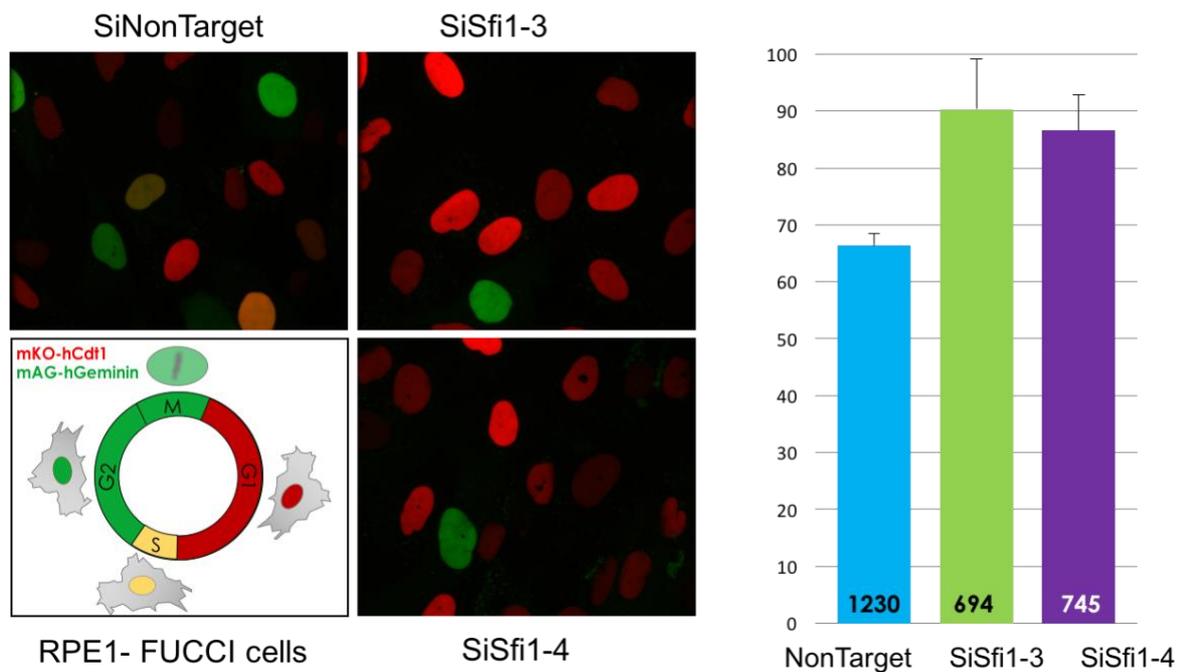


Figure 8 - La déplétion de Sfi1 affecte la progression du cycle

A. Les cellules REP-1-FUCCI ont été utilisées afin de déterminer si Sfi1 est essentiel à la progression du cycle cellulaire.
 B. quantifications de A.

Etant donné que des défauts au centrosome peuvent induire un arrêt du cycle cellulaire dans les cellules non cancéreuses comme les RPE-1, nos résultats suggèrent que l'arrêt des cellules en phase G1 pourrait être dû à des défauts aux centrosomes.

Afin de vérifier cette hypothèse, j'ai déplété Sfi1 dans des cellules HeLa. Ces cellules sont des cellules cancéreuses et il a été montré qu'elles pouvaient continuer de proliférer en présence de défauts ou même d'absence totale du centrosome (Wong et al. 2015). Après déplétion de Sfi1 les cellules continuent de proliférer, la progression du cycle cellulaire n'est donc pas affectée dans ces cellules. J'ai pu aussi confirmer ce résultat dans des cellules RPE-1 p53⁻. Ces cellules n'expriment pas la protéine p53, qui contrôle, entre autres, la progression du cycle cellulaire. Ce résultat suggère que l'arrêt du cycle observé dans les RPE-1 est dû à une activation de p53, probablement via la voie de surveillance centrosomale (Lambrus et al. 2016 ; Fong et al. 2016 ; Meitingner et al. 2016).

Etant donné que les cellules HeLa et RPE-1 p53⁻ continuent de proliférer après la déplétion de Sfi1, j'ai pu mesurer la durée de la mitose dans ces cellules. En effet, des

défauts centrosomaux peuvent induire une prolongation du temps de divisions souvent causée par des défauts d'assemblage du fuseau mitotique et/ou une augmentation de défauts de ségrégation de l'ADN.

Dans la condition contrôle, la durée de mitose des cellules HeLa est en moyenne de 46,5 minutes. Cette durée augmente après déplétion de Sfi1 à 57,6 et 70,8 minutes dans les cellules traitées aux siARN Sfi1#3 et Sfi1#4 respectivement. Cette prolongation du temps de division est aussi observée dans les cellules RPE1-p53- (Figure 9).

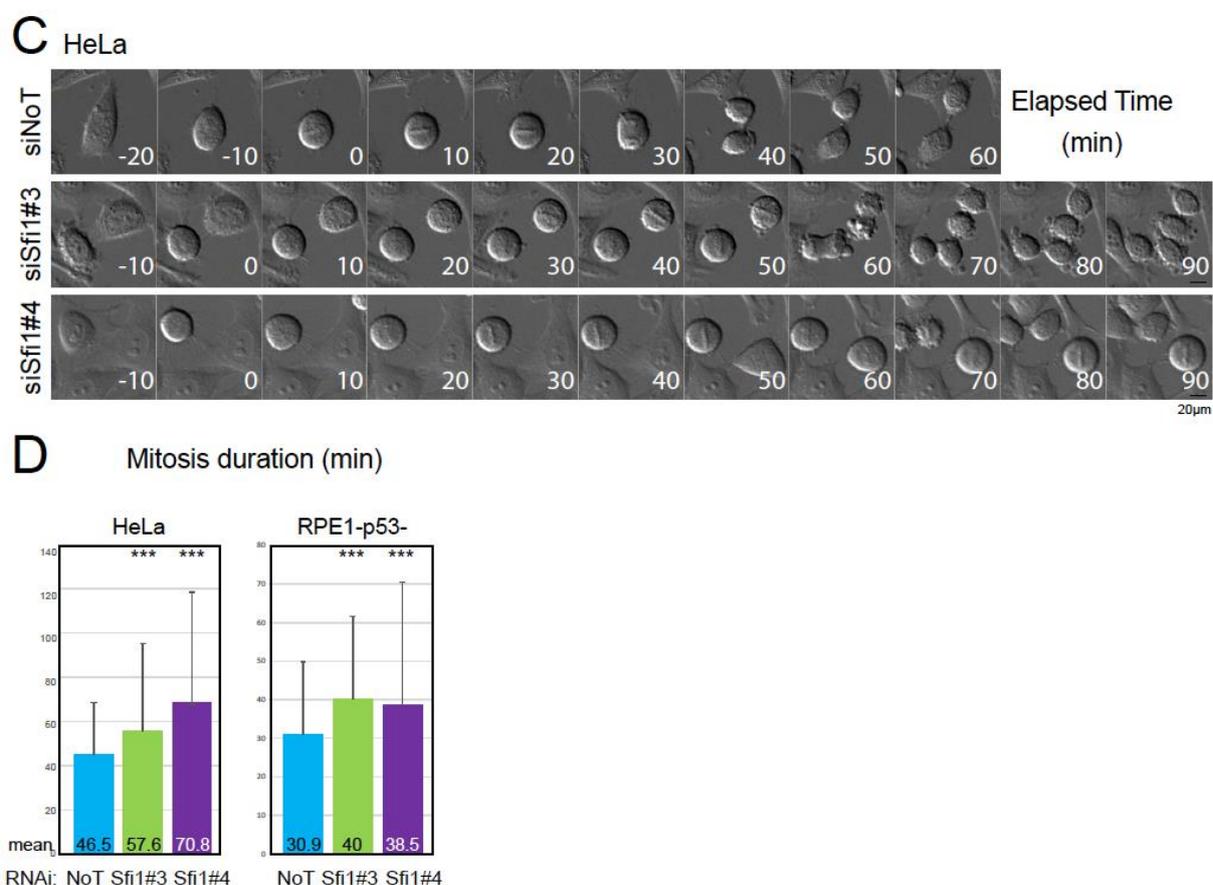


Figure 9: La déplétion de Sfi1 induit une prolongation du temps de mitose

C. Des cellules HeLa ont été filmées pendant 72h et la durée de la mitose a été mesurée. D. Quantification de la durée de mitose dans les cellules HeLa et RPE1-p53-

En

conclusion, notre étude de Sfi1 dans les cellules humaines a confirmé que Sfi1 est bien une protéine centrosomale et est requise pour la stabilisation ou le recrutement de la

Centrine aux centrioles. De plus, nos résultats montrent que Sfil est essentiel à la ciliogénèse, à la progression du cycle cellulaire ainsi qu'au bon déroulement de la mitose. En revanche, la fonction exacte de Sfil aux centrioles reste inconnue, notre hypothèse est que Sfil avec la Centrine seraient des protéines structurales de centriole et leur perte induirait des défauts d'assemblage de ces structures ce qui affecterait les fonctions du centrosome.

BIBLIOGRAPHY

- Adams, I.R., and J.V. Kilmartin. 1999. Localization of core spindle pole body (SPB) components during SPB duplication in *Saccharomyces cerevisiae*. *J Cell Biol.* 145:809-823.
- Akhmanova, A., and M.O. Steinmetz. 2015. Control of microtubule organization and dynamics: two ends in the limelight. *Nat Rev Mol Cell Biol.* 16:711-726.
- Al Jord, A., A.I. Lemaitre, N. Delgehyr, M. Faucourt, N. Spassky, and A. Meunier. 2014. Centriole amplification by mother and daughter centrioles differs in multiciliated cells. *Nature.* 516:104-107.
- Alfaro-Aco, R., and S. Petry. 2015. Building the Microtubule Cytoskeleton Piece by Piece. *J Biol Chem.* 290:17154-17162.
- Alvarez-Fernandez, M., R. Sanchez-Martinez, B. Sanz-Castillo, P.P. Gan, M. Sanz-Flores, M. Trakala, M. Ruiz-Torres, T. Lorca, A. Castro, and M. Malumbres. 2013. Greatwall is essential to prevent mitotic collapse after nuclear envelope breakdown in mammals. *Proc Natl Acad Sci U S A.* 110:17374-17379.
- Anders, A., and K.E. Sawin. 2011. Microtubule stabilization in vivo by nucleation-incompetent gamma-tubulin complex. *J Cell Sci.* 124:1207-1213.
- Andersen, J.S., C.J. Wilkinson, T. Mayor, P. Mortensen, E.A. Nigg, and M. Mann. 2003. Proteomic characterization of the human centrosome by protein correlation profiling. *Nature.* 426:570-574.
- Anderson, R.G. 1972. The three-dimensional structure of the basal body from the rhesus monkey oviduct. *J Cell Biol.* 54:246-265.
- Anderson, V.E., J. Prudden, S. Prochnik, T.H. Giddings, Jr., and K.G. Hardwick. 2007. Novel sfil alleles uncover additional functions for Sfilp in bipolar spindle assembly and function. *Mol Biol Cell.* 18:2047-2056.
- Araki, M., C. Masutani, M. Takemura, A. Uchida, K. Sugasawa, J. Kondoh, Y. Ohkuma, and F. Hanaoka. 2001. Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J Biol Chem.* 276:18665-18672.
- Araki, Y., C.K. Lau, H. Maekawa, S.L. Jaspersen, T.H. Giddings, Jr., E. Schiebel, and M. Winey. 2006. The *Saccharomyces cerevisiae* spindle pole body (SPB) component Nbp1p is

- required for SPB membrane insertion and interacts with the integral membrane proteins Ndc1p and Mps2p. *Mol Biol Cell*. 17:1959-1970.
- Arquint, C., A.M. Gabryjonczyk, S. Imseng, R. Bohm, E. Sauer, S. Hiller, E.A. Nigg, and T. Maier. 2015. STIL binding to Polo-box 3 of PLK4 regulates centriole duplication. *Elife*. 4.
- Aubusson-Fleury, A., G. Balavoine, M. Lemullois, K. Bouhouche, J. Beisson, and F. Koll. 2017. Centrin diversity and basal body patterning across evolution: new insights from Paramecium. *Biol Open*. 6:765-776.
- Aumeier, C., L. Schaedel, J. Gaillard, K. John, L. Blanchoin, and M. They. 2016. Self-repair promotes microtubule rescue. *Nat Cell Biol*. 18:1054-1064.
- Avasthi, P., J.F. Scheel, G. Ying, J.M. Frederick, W. Baehr, and U. Wolfrum. 2013. Germline deletion of Cetn1 causes infertility in male mice. *J Cell Sci*. 126:3204-3213.
- Avena, J.S., S. Burns, Z. Yu, C.C. Ebmeier, W.M. Old, S.L. Jaspersen, and M. Winey. 2014. Licensing of yeast centrosome duplication requires phosphoregulation of sfil. *PLoS Genet*. 10:e1004666.
- Azimzadeh, J., and M. Bornens. 2007. Structure and duplication of the centrosome. *J Cell Sci*. 120:2139-2142.
- Azimzadeh, J., P. Hergert, A. Delouvee, U. Euteneuer, E. Formstecher, A. Khodjakov, and M. Bornens. 2009. hPOC5 is a centrin-binding protein required for assembly of full-length centrioles. *J Cell Biol*. 185:101-114.
- Azimzadeh, J., M.L. Wong, D.M. Downhour, A. Sanchez Alvarado, and W.F. Marshall. 2012. Centrosome loss in the evolution of planarians. *Science*. 335:461-463.
- Bailly, E., M. Doree, P. Nurse, and M. Bornens. 1989. p34cdc2 is located in both nucleus and cytoplasm; part is centrosomally associated at G2/M and enters vesicles at anaphase. *EMBO J*. 8:3985-3995.
- Balasubramanian, M.K., E. Bi, and M. Glotzer. 2004. Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells. *Curr Biol*. 14:R806-818.
- Barros, T.P., K. Kinoshita, A.A. Hyman, and J.W. Raff. 2005. Aurora A activates D-TACC-Msps complexes exclusively at centrosomes to stabilize centrosomal microtubules. *J Cell Biol*. 170:1039-1046.
- Bartek, J., and J. Lukas. 2001. Mammalian G1- and S-phase checkpoints in response to DNA damage. *Curr Opin Cell Biol*. 13:738-747.

- Bartolini, F., and G.G. Gundersen. 2006. Generation of noncentrosomal microtubule arrays. *J Cell Sci.* 119:4155-4163.
- Basto, R., J. Lau, T. Vinogradova, A. Gardiol, C.G. Woods, A. Khodjakov, and J.W. Raff. 2006. Flies without centrioles. *Cell.* 125:1375-1386.
- Bauer, M., F. Cubizolles, A. Schmidt, and E.A. Nigg. 2016. Quantitative analysis of human centrosome architecture by targeted proteomics and fluorescence imaging. *EMBO J.* 35:2152-2166.
- Baum, P., C. Furlong, and B. Byers. 1986. Yeast gene required for spindle pole body duplication: homology of its product with Ca²⁺-binding proteins. *Proc Natl Acad Sci U S A.* 83:5512-5516.
- Bernhard, W., and E. De Harven. 1956. Electron microscopic study of the ultrastructure of centrioles in vertebra. *Z Zellforsch Mikrosk Anat.* 45:378-398.
- Berriman, M., E. Ghedin, C. Hertz-Fowler, G. Blandin, H. Renault, D.C. Bartholomeu, N.J. Lennard, E. Caler, N.E. Hamlin, B. Haas, U. Bohme, L. Hannick, M.A. Aslett, J. Shallom, L. Marcello, L. Hou, B. Wickstead, U.C. Alsmark, C. Arrowsmith, R.J. Atkin, A.J. Barron, F. Bringaud, K. Brooks, M. Carrington, I. Cherevach, T.J. Chillingworth, C. Churcher, L.N. Clark, C.H. Corton, A. Cronin, R.M. Davies, J. Doggett, A. Djikeng, T. Feldblyum, M.C. Field, A. Fraser, I. Goodhead, Z. Hance, D. Harper, B.R. Harris, H. Hauser, J. Hostetler, A. Ivens, K. Jagels, D. Johnson, J. Johnson, K. Jones, A.X. Kerhornou, H. Koo, N. Larke, S. Landfear, C. Larkin, V. Leech, A. Line, A. Lord, A. Macleod, P.J. Mooney, S. Moule, D.M. Martin, G.W. Morgan, K. Mungall, H. Norbertczak, D. Ormond, G. Pai, C.S. Peacock, J. Peterson, M.A. Quail, E. Rabbinowitsch, M.A. Rajandream, C. Reitter, S.L. Salzberg, M. Sanders, S. Schobel, S. Sharp, M. Simmonds, A.J. Simpson, L. Tallon, C.M. Turner, A. Tait, A.R. Tivey, S. Van Aken, D. Walker, D. Wanless, S. Wang, B. White, O. White, S. Whitehead, J. Woodward, J. Wortman, M.D. Adams, T.M. Embley, K. Gull, E. Ullu, J.D. Barry, A.H. Fairlamb, F. Opperdoes, B.G. Barrell, J.E. Donelson, N. Hall, C.M. Fraser, et al. 2005. The genome of the African trypanosome *Trypanosoma brucei*. *Science.* 309:416-422.
- Bestul, A.J., Z. Yu, J.R. Unruh, and S.L. Jaspersen. 2017. Molecular model of fission yeast centrosome assembly determined by superresolution imaging. *J Cell Biol.* 216:2409-2424.

- Bettencourt-Dias, M., A. Rodrigues-Martins, L. Carpenter, M. Riparbelli, L. Lehmann, M.K. Gatt, N. Carmo, F. Balloux, G. Callaini, and D.M. Glover. 2005. SAK/PLK4 is required for centriole duplication and flagella development. *Curr Biol.* 15:2199-2207.
- Biggins, S., and M.D. Rose. 1994. Direct interaction between yeast spindle pole body components: Karlp is required for Cdc3lp localization to the spindle pole body. *J Cell Biol.* 125:843-852.
- Bobinnec, Y., A. Khodjakov, L.M. Mir, C.L. Rieder, B. Edde, and M. Bornens. 1998. Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. *J Cell Biol.* 143:1575-1589.
- Bornens, M. 2002. Centrosome composition and microtubule anchoring mechanisms. *Curr Opin Cell Biol.* 14:25-34.
- Bornens, M., and J. Azimzadeh. 2007. Origin and evolution of the centrosome. *Adv Exp Med Biol.* 607:119-129.
- Bouhrel, I.B., M. Ohta, A. Mayeux, N. Bordes, F. Dingli, J. Boulanger, G. Velve Casquillas, D. Loew, P.T. Tran, M. Sato, and A. Paoletti. 2015. Cell cycle control of spindle pole body duplication and splitting by Sfil and Cdc3l in fission yeast. *J Cell Sci.* 128:1481-1493.
- Bouissou, A., C. Verollet, A. Sousa, P. Sampaio, M. Wright, C.E. Sunkel, A. Merdes, and B. Raynaud-Messina. 2009. γ -Tubulin ring complexes regulate microtubule plus end dynamics. *J Cell Biol.* 187:327-334.
- Boveri, T. 1887. Ueber den Antheil des Spermatozoon an der Teilung des Eies. *Sitzungsber Ges. Morph. Physio. München.* 3:151-164.
- Boveri, T. 2008. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci.* 121 Suppl 1:1-84.
- Brouhard, G.J., J.H. Stear, T.L. Noetzel, J. Al-Bassam, K. Kinoshita, S.C. Harrison, J. Howard, and A.A. Hyman. 2008. XMAP215 is a processive microtubule polymerase. *Cell.* 132:79-88.
- Bullitt, E., M.P. Rout, J.V. Kilmartin, and C.W. Akey. 1997. The yeast spindle pole body is assembled around a central crystal of Spc42p. *Cell.* 89:1077-1086.
- Burns, R.G. 1991. Alpha-, beta-, and gamma-tubulins: sequence comparisons and structural constraints. *Cell Motil Cytoskeleton.* 20:181-189.

- Byers, B., and L. Goetsch. 1975. Electron microscopic observations on the meiotic karyotype of diploid and tetraploid *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 72:5056-5060.
- Cabral, G., S.S. Sans, C.R. Cowan, and A. Dammermann. 2013. Multiple mechanisms contribute to centriole separation in *C. elegans*. *Curr Biol*. 23:1380-1387.
- Cajane, L., and E.A. Nigg. 2014. Cep164 triggers ciliogenesis by recruiting Tau tubulin kinase 2 to the mother centriole. *Proc Natl Acad Sci U S A*. 111:E2841-2850.
- Cavalier-Smith, T. 1974. Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardtii*. *J Cell Sci*. 16:529-556.
- Cavanaugh, A.M., and S.L. Jaspersen. 2017. Big Lessons from Little Yeast: Budding and Fission Yeast Centrosome Structure, Duplication, and Function. *Annu Rev Genet*.
- Chang-Jie, J., and S. Sonobe. 1993. Identification and preliminary characterization of a 65 kDa higher-plant microtubule-associated protein. *J Cell Sci*. 105 (Pt 4):891-901.
- Chang, J., O. Cizmecioglu, I. Hoffmann, and K. Rhee. 2010. PLK2 phosphorylation is critical for CPAP function in procentriole formation during the centrosome cycle. *EMBO J*. 29:2395-2406.
- Chen, D., A. Purohit, E. Halilovic, S.J. Doxsey, and A.C. Newton. 2004. Centrosomal anchoring of protein kinase C betaII by pericentrin controls microtubule organization, spindle function, and cytokinesis. *J Biol Chem*. 279:4829-4839.
- Chen, J., C.J. Smoyer, B.D. Slaughter, J.R. Unruh, and S.L. Jaspersen. 2014. The SUN protein Mps3 controls Ndc1 distribution and function on the nuclear membrane. *J Cell Biol*. 204:523-539.
- Chen, X.P., H. Yin, and T.C. Huffaker. 1998. The yeast spindle pole body component Spc72p interacts with Stu2p and is required for proper microtubule assembly. *J Cell Biol*. 141:1169-1179.
- Chial, H.J., M.P. Rout, T.H. Giddings, and M. Winey. 1998. *Saccharomyces cerevisiae* Ndc1p is a shared component of nuclear pore complexes and spindle pole bodies. *J Cell Biol*. 143:1789-1800.
- Comartin, D., G.D. Gupta, E. Fussner, E. Coyaud, M. Hasegan, M. Archinti, S.W. Cheung, D. Pinchev, S. Lawo, B. Raught, D.P. Bazett-Jones, J. Luders, and L. Pelletier. 2013. CEP120 and SPICE1 cooperate with CPAP in centriole elongation. *Curr Biol*. 23:1360-1366.

- Conduit, P.T., K. Brunk, J. Dobbelaere, C.I. Dix, E.P. Lucas, and J.W. Raff. 2010. Centrioles regulate centrosome size by controlling the rate of Cnn incorporation into the PCM. *Curr Biol.* 20:2178-2186.
- Conduit, P.T., J.H. Richens, A. Wainman, J. Holder, C.C. Vicente, M.B. Pratt, C.I. Dix, Z.A. Novak, I.M. Dobbie, L. Schermelleh, and J.W. Raff. 2014. A molecular mechanism of mitotic centrosome assembly in *Drosophila*. *Elife.* 3:e03399.
- Cottee, M.A., J.W. Raff, S.M. Lea, and H. Roque. 2011. SAS-6 oligomerization: the key to the centriole? *Nat Chem Biol.* 7:650-653.
- Courtheoux, T., G. Gay, Y. Gachet, and S. Tournier. 2009. Asel/Prcl-dependent spindle elongation corrects merotelically during anaphase in fission yeast. *J Cell Biol.* 187:399-412.
- Couwenbergs, C., J.C. Labbe, M. Goulding, T. Marty, B. Bowerman, and M. Gotta. 2007. Heterotrimeric G protein signaling functions with dynein to promote spindle positioning in *C. elegans*. *J Cell Biol.* 179:15-22.
- Creanor, J., and J.M. Mitchison. 1990. Continued DNA synthesis after a mitotic block in the double mutant *cut1 cdcl1* of the fission yeast *Schizosaccharomyces pombe*. *J Cell Sci.* 96 (Pt 3):435-438.
- Cuella-Martin, R., C. Oliveira, H.E. Lockstone, S. Snellenberg, N. Grolmusova, and J.R. Chapman. 2016. 53BP1 Integrates DNA Repair and p53-Dependent Cell Fate Decisions via Distinct Mechanisms. *Mol Cell.* 64:51-64.
- Dammermann, A., T. Muller-Reichert, L. Pelletier, B. Habermann, A. Desai, and K. Oegema. 2004. Centriole assembly requires both centriolar and pericentriolar material proteins. *Dev Cell.* 7:815-829.
- Dantas, T.J., O.M. Daly, and C.G. Morrison. 2012. Such small hands: the roles of centrins/caltractins in the centriole and in genome maintenance. *Cell Mol Life Sci.* 69:2979-2997.
- Dantas, T.J., Y. Wang, P. Lalor, P. Dockery, and C.G. Morrison. 2011. Defective nucleotide excision repair with normal centrosome structures and functions in the absence of all vertebrate centrins. *J Cell Biol.* 193:307-318.
- Davie, E., and J. Petersen. 2012. Environmental control of cell size at division. *Curr Opin Cell Biol.* 24:838-844.

- de Forges, H., A. Bouissou, and F. Perez. 2012. Interplay between microtubule dynamics and intracellular organization. *Int J Biochem Cell Biol.* 44:266-274.
- Debec, A., and C. Montmory. 1992. Cyclin B is associated with centrosomes in *Drosophila* mitotic cells. *Biol Cell.* 75:121-126.
- Delattre, M., C. Canard, and P. Gonczy. 2006. Sequential protein recruitment in *C. elegans* centriole formation. *Curr Biol.* 16:1844-1849.
- Delaval, B., L. Covassin, N.D. Lawson, and S. Doxsey. 2011. Centrin depletion causes cyst formation and other ciliopathy-related phenotypes in zebrafish. *Cell Cycle.* 10:3964-3972.
- Delgehyr, N., J. Sillibourne, and M. Bornens. 2005. Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. *J Cell Sci.* 118:1565-1575.
- Desai, A., and T.J. Mitchison. 1997. Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol.* 13:83-117.
- Dimitrov, A., M. Quesnoit, S. Moutel, I. Cantaloube, C. Pous, and F. Perez. 2008. Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. *Science.* 322:1353-1356.
- Ding, R., R.R. West, D.M. Morphew, B.R. Oakley, and J.R. McIntosh. 1997. The spindle pole body of *Schizosaccharomyces pombe* enters and leaves the nuclear envelope as the cell cycle proceeds. *Mol Biol Cell.* 8:1461-1479.
- Dippell, R.V. 1968. The development of basal bodies in paramecium. *Proc Natl Acad Sci U S A.* 61:461-468.
- Dompiere, J.P., J.D. Godin, B.C. Charrin, F.P. Cordelieres, S.J. King, S. Humbert, and F. Saudou. 2007. Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. *J Neurosci.* 27:3571-3583.
- Doxsey, S.J., P. Stein, L. Evans, P.D. Calarco, and M. Kirschner. 1994. Pericentrin, a highly conserved centrosome protein involved in microtubule organization. *Cell.* 76:639-650.
- Drechsel, D.N., and M.W. Kirschner. 1994. The minimum GTP cap required to stabilize microtubules. *Curr Biol.* 4:1053-1061.
- Drummond, D.R., and R.A. Cross. 2000. Dynamics of interphase microtubules in *Schizosaccharomyces pombe*. *Curr Biol.* 10:766-775.

- Dutertre, S., S. Descamps, and C. Prigent. 2002. On the role of aurora-A in centrosome function. *Oncogene*. 21:6175-6183.
- Dzhindzhev, N.S., Q.D. Yu, K. Weiskopf, G. Tzolovsky, I. Cunha-Ferreira, M. Riparbelli, A. Rodrigues-Martins, M. Bettencourt-Dias, G. Callaini, and D.M. Glover. 2010. Asterless is a scaffold for the onset of centriole assembly. *Nature*. 467:714-718.
- Elliott, S., M. Knop, G. Schlenstedt, and E. Schiebel. 1999. Spc29p is a component of the Spc110p subcomplex and is essential for spindle pole body duplication. *Proc Natl Acad Sci U S A*. 96:6205-6210.
- Elserafy, M., M. Saric, A. Neuner, T.C. Lin, W. Zhang, C. Seybold, L. Sivashanmugam, and E. Schiebel. 2014. Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Curr Biol*. 24:1456-1466.
- Errabolu, R., M.A. Sanders, and J.L. Salisbury. 1994. Cloning of a cDNA encoding human centrin, an EF-hand protein of centrosomes and mitotic spindle poles. *J Cell Sci*. 107 (Pt 1):9-16.
- Evans, L., T. Mitchison, and M. Kirschner. 1985. Influence of the centrosome on the structure of nucleated microtubules. *J Cell Biol*. 100:1185-1191.
- Fankhauser, C., J. Marks, A. Reymond, and V. Simanis. 1993. The *S. pombe* *cdcl6* gene is required both for maintenance of p34cdc2 kinase activity and regulation of septum formation: a link between mitosis and cytokinesis? *EMBO J*. 12:2697-2704.
- Fankhauser, C., and V. Simanis. 1994. The *cdc7* protein kinase is a dosage dependent regulator of septum formation in fission yeast. *EMBO J*. 13:3011-3019.
- Fischer, T., S. Rodriguez-Navarro, G. Pereira, A. Racz, E. Schiebel, and E. Hurt. 2004. Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. *Nat Cell Biol*. 6:840-848.
- Flory, M.R., M. Morphew, J.D. Joseph, A.R. Means, and T.N. Davis. 2002. Pcplp, an Spc110p-related calmodulin target at the centrosome of the fission yeast *Schizosaccharomyces pombe*. *Cell Growth Differ*. 13:47-58.
- Fong, C.S., G. Mazo, T. Das, J. Goodman, M. Kim, B.P. O'Rourke, D. Izquierdo, and M.F. Tsou. 2016. 53BP1 and USP28 mediate p53-dependent cell cycle arrest in response to centrosome loss and prolonged mitosis. *Elife*. 5.
- Frankel, J. 2000. Cell biology of *Tetrahymena thermophila*. *Methods Cell Biol*. 62:27-125.
- Friederichs, J.M., S. Ghosh, C.J. Smoyer, S. McCroskey, B.D. Miller, K.J. Weaver, K.M. Delventhal, J. Unruh, B.D. Slaughter, and S.L. Jaspersen. 2011. The SUN protein Mps3 is

- required for spindle pole body insertion into the nuclear membrane and nuclear envelope homeostasis. *PLoS Genet.* 7:e1002365.
- Fry, A.M., M.J. Leaper, and R. Bayliss. 2014. The primary cilium: guardian of organ development and homeostasis. *Organogenesis.* 10:62-68.
- Fu, J., and D.M. Glover. 2012. Structured illumination of the interface between centriole and peri-centriolar material. *Open Biol.* 2:120104.
- Funaya, C., S. Samarasinghe, S. Pruggnaller, M. Ohta, Y. Connolly, J. Muller, H. Murakami, A. Grallert, M. Yamamoto, D. Smith, C. Antony, and K. Tanaka. 2012. Transient structure associated with the spindle pole body directs meiotic microtubule reorganization in *S. pombe*. *Curr Biol.* 22:562-574.
- Gadadhar, S., S. Bodakuntla, K. Natarajan, and C. Janke. 2017. The tubulin code at a glance. *J Cell Sci.* 130:1347-1353.
- Gavet, O., C. Alvarez, P. Gaspar, and M. Bornens. 2003. Centrin4p, a novel mammalian centrin specifically expressed in ciliated cells. *Mol Biol Cell.* 14:1818-1834.
- Gavet, O., and J. Pines. 2010. Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Dev Cell.* 18:533-543.
- Geiser, J.R., H.A. Sundberg, B.H. Chang, E.G. Muller, and T.N. Davis. 1993. The essential mitotic target of calmodulin is the 110-kilodalton component of the spindle pole body in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 13:7913-7924.
- Geiser, J.R., D. van Tuinen, S.E. Brockerhoff, M.M. Neff, and T.N. Davis. 1991. Can calmodulin function without binding calcium? *Cell.* 65:949-959.
- Gerdes, J.M., E.E. Davis, and N. Katsanis. 2009. The vertebrate primary cilium in development, homeostasis, and disease. *Cell.* 137:32-45.
- Gharbi-Ayachi, A., J.C. Labbe, A. Burgess, S. Vigneron, J.M. Strub, E. Brioude, A. Van-Dorselaer, A. Castro, and T. Lorca. 2010. The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. *Science.* 330:1673-1677.
- Gheghiani, L., and O. Gavet. 2014. Deciphering the spatio-temporal regulation of entry and progression through mitosis. *Biotechnol J.* 9:213-223.
- Gheghiani, L., D. Loew, B. Lombard, J. Mansfeld, and O. Gavet. 2017. PLK1 Activation in Late G2 Sets Up Commitment to Mitosis. *Cell Rep.* 19:2060-2073.

- Giddings, T.H., Jr., E.T. O'Toole, M. Morphew, D.N. Mastronarde, J.R. McIntosh, and M. Winey. 2001. Using rapid freeze and freeze-substitution for the preparation of yeast cells for electron microscopy and three-dimensional analysis. *Methods Cell Biol.* 67:27-42.
- Glotzer, M. 2017. Cytokinesis in Metazoa and Fungi. *Cold Spring Harb Perspect Biol.* 9.
- Glotzer, M., A.W. Murray, and M.W. Kirschner. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature.* 349:132-138.
- Goetz, S.C., K.F. Liem, Jr., and K.V. Anderson. 2012. The spinocerebellar ataxia-associated gene Tau tubulin kinase 2 controls the initiation of ciliogenesis. *Cell.* 151:847-858.
- Gogendeau, D., J. Beisson, N.G. de Loubresse, J.P. Le Caer, F. Ruiz, J. Cohen, L. Sperling, F. Koll, and C. Klotz. 2007. An Sfilp-like centrin-binding protein mediates centrin-based Ca²⁺-dependent contractility in *Paramecium tetraurelia*. *Eukaryot Cell.* 6:1992-2000.
- Gogendeau, D., C. Klotz, O. Arnaiz, A. Malinowska, M. Dadlez, N.G. de Loubresse, F. Ruiz, F. Koll, and J. Beisson. 2008. Functional diversification of centrins and cell morphological complexity. *J Cell Sci.* 121:65-74.
- Golsteyn, R.M., K.E. Mundt, A.M. Fry, and E.A. Nigg. 1995. Cell cycle regulation of the activity and subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle function. *J Cell Biol.* 129:1617-1628.
- Gomez-Ferreria, M.A., U. Rath, D.W. Buster, S.K. Chanda, J.S. Caldwell, D.R. Rines, and D.J. Sharp. 2007. Human Cep192 is required for mitotic centrosome and spindle assembly. *Curr Biol.* 17:1960-1966.
- Gopalan, G., C.S. Chan, and P.J. Donovan. 1997. A novel mammalian, mitotic spindle-associated kinase is related to yeast and fly chromosome segregation regulators. *J Cell Biol.* 138:643-656.
- Goshima, G., M. Mayer, N. Zhang, N. Stuurman, and R.D. Vale. 2008. Augmin: a protein complex required for centrosome-independent microtubule generation within the spindle. *J Cell Biol.* 181:421-429.
- Gould, K.L., and P. Nurse. 1989. Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. *Nature.* 342:39-45.
- Gould, R.R., and G.G. Borisy. 1977. The pericentriolar material in Chinese hamster ovary cells nucleates microtubule formation. *J Cell Biol.* 73:601-615.

- Grallert, A., E. Boke, A. Hagting, B. Hodgson, Y. Connolly, J.R. Griffiths, D.L. Smith, J. Pines, and I.M. Hagan. 2015. A PPI-PP2A phosphatase relay controls mitotic progression. *Nature*. 517:94-98.
- Grallert, A., K.Y. Chan, M.L. Alonso-Nunez, M. Madrid, A. Biswas, I. Alvarez-Tabares, Y. Connolly, K. Tanaka, A. Robertson, J.M. Ortiz, D.L. Smith, and I.M. Hagan. 2013. Removal of centrosomal PPI by NIMA kinase unlocks the MPF feedback loop to promote mitotic commitment in *S. pombe*. *Curr Biol*. 23:213-222.
- Grallert, A., Y. Connolly, D.L. Smith, V. Simanis, and I.M. Hagan. 2012. The *S. pombe* cytokinesis NDR kinase Sid2 activates Fin1 NIMA kinase to control mitotic commitment through Pom1/Wee1. *Nat Cell Biol*. 14:738-745.
- Graser, S., Y.D. Stierhof, S.B. Lavoie, O.S. Gassner, S. Lamla, M. Le Clech, and E.A. Nigg. 2007. Cep164, a novel centriole appendage protein required for primary cilium formation. *J Cell Biol*. 179:321-330.
- Gruneberg, U., K. Campbell, C. Simpson, J. Grindlay, and E. Schiebel. 2000. Nud1p links astral microtubule organization and the control of exit from mitosis. *EMBO J*. 19:6475-6488.
- Gudi, R., C.J. Haycraft, P.D. Bell, Z. Li, and C. Vasu. 2015. Centrobin-mediated regulation of the centrosomal protein 4.1-associated protein (CPAP) level limits centriole length during elongation stage. *J Biol Chem*. 290:6890-6902.
- Gudi, R., C. Zou, J. Dhar, Q. Gao, and C. Vasu. 2014. Centrobin-centrosomal protein 4.1-associated protein (CPAP) interaction promotes CPAP localization to the centrioles during centriole duplication. *J Biol Chem*. 289:15166-15178.
- Guerra, C., Y. Wada, V. Leick, A. Bell, and P. Satir. 2003. Cloning, localization, and axonemal function of *Tetrahymena* centrin. *Mol Biol Cell*. 14:251-261.
- Guertin, D.A., L. Chang, F. Irshad, K.L. Gould, and D. McCollum. 2000. The role of the sid1p kinase and cdcl4p in regulating the onset of cytokinesis in fission yeast. *EMBO J*. 19:1803-1815.
- Guichard, P., A. Desfosses, A. Maheshwari, V. Hachet, C. Dietrich, A. Brune, T. Ishikawa, C. Sachse, and P. Gonczy. 2012. Cartwheel architecture of *Trichonympha* basal body. *Science*. 337:553.
- Guichard, P., V. Hachet, N. Majubu, A. Neves, D. Demurtas, N. Olieric, I. Fluckiger, A. Yamada, K. Kihara, Y. Nishida, S. Moriya, M.O. Steinmetz, Y. Hongoh, and P. Gonczy. 2013. Native

- architecture of the centriole proximal region reveals features underlying its 9-fold radial symmetry. *Curr Biol.* 23:1620-1628.
- Habedanck, R., Y.D. Stierhof, C.J. Wilkinson, and E.A. Nigg. 2005. The Polo kinase Plk4 functions in centriole duplication. *Nat Cell Biol.* 7:1140-1146.
- Hagan, I., and M. Yanagida. 1992. Kinesin-related cut7 protein associates with mitotic and meiotic spindles in fission yeast. *Nature.* 356:74-76.
- Hagan, I.M. 1998. The fission yeast microtubule cytoskeleton. *J Cell Sci.* 111 (Pt 12):1603-1612.
- Hagan, I.M., and J.S. Hyams. 1988. The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. *J Cell Sci.* 89 (Pt 3):343-357.
- Hamill, D.R., A.F. Severson, J.C. Carter, and B. Bowerman. 2002. Centrosome maturation and mitotic spindle assembly in *C. elegans* require SPD-5, a protein with multiple coiled-coil domains. *Dev Cell.* 3:673-684.
- Hannak, E., M. Kirkham, A.A. Hyman, and K. Oegema. 2001. Aurora-A kinase is required for centrosome maturation in *Caenorhabditis elegans*. *J Cell Biol.* 155:1109-1116.
- Harburger, D.S., M. Bouaouina, and D.A. Calderwood. 2009. Kindlin-1 and -2 directly bind the C-terminal region of beta integrin cytoplasmic tails and exert integrin-specific activation effects. *J Biol Chem.* 284:11485-11497.
- Harper, J.V., and G. Brooks. 2005. The mammalian cell cycle: an overview. *Methods Mol Biol.* 296:113-153.
- Hart, P.E., J.N. Glantz, J.D. Orth, G.M. Poynter, and J.L. Salisbury. 1999. Testis-specific murine centrin, Cetn1: genomic characterization and evidence for retroposition of a gene encoding a centrosome protein. *Genomics.* 60:111-120.
- Hartwell, L.H., J. Culotti, J.R. Pringle, and B.J. Reid. 1974. Genetic control of the cell division cycle in yeast. *Science.* 183:46-51.
- Hatano, T., and G. Sluder. 2012. The interrelationship between APC/C and Plk1 activities in centriole disengagement. *Biol Open.* 1:1153-1160.
- Hayward, D., J. Metz, C. Pellacani, and J.G. Wakefield. 2014. Synergy between multiple microtubule-generating pathways confers robustness to centrosome-driven mitotic spindle formation. *Dev Cell.* 28:81-93.

- Hergovich, A., and B.A. Hemmings. 2012. Hippo signalling in the G2/M cell cycle phase: lessons learned from the yeast MEN and SIN pathways. *Semin Cell Dev Biol.* 23:794-802.
- Heydeck, W., A.J. Stemm-Wolf, J. Knop, C.C. Poh, and M. Winey. 2016. Sfr1, a *Tetrahymena thermophila* Sfil Repeat Protein, Modulates the Production of Cortical Row Basal Bodies. *mSphere.* 1.
- Hinchcliffe, E.H., F.J. Miller, M. Cham, A. Khodjakov, and G. Sluder. 2001. Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. *Science.* 291:1547-1550.
- Hirono, M. 2014. Cartwheel assembly. *Philos Trans R Soc Lond B Biol Sci.* 369.
- Hollenbeck, P.J., and W.M. Saxton. 2005. The axonal transport of mitochondria. *J Cell Sci.* 118:5411-5419.
- Hoog, J.L., S.M. Huisman, D. Brunner, and C. Antony. 2013. Electron tomography reveals novel microtubule lattice and microtubule organizing centre defects in +TIP mutants. *PLoS One.* 8:e61698.
- Hotta, T., Z. Kong, C.M. Ho, C.J. Zeng, T. Horio, S. Fong, T. Vuong, Y.R. Lee, and B. Liu. 2012. Characterization of the Arabidopsis augmin complex uncovers its critical function in the assembly of the acentrosomal spindle and phragmoplast microtubule arrays. *Plant Cell.* 24:1494-1509.
- Howard, J., and A.A. Hyman. 2007. Microtubule polymerases and depolymerases. *Curr Opin Cell Biol.* 19:31-35.
- Hu, H., and W.J. Chazin. 2003. Unique features in the C-terminal domain provide caltractin with target specificity. *J Mol Biol.* 330:473-484.
- Huang, B., A. Mengersen, and V.D. Lee. 1988. Molecular cloning of cDNA for caltractin, a basal body-associated Ca²⁺-binding protein: homology in its protein sequence with calmodulin and the yeast CDC31 gene product. *J Cell Biol.* 107:133-140.
- Humphrey, T., and A. Pearce. 2005. Cell cycle molecules and mechanisms of the budding and fission yeasts. *Methods Mol Biol.* 296:3-29.
- Hutchins, J.R., Y. Toyoda, B. Hegemann, I. Poser, J.K. Heriche, M.M. Sykora, M. Augsburg, O. Hudecz, B.A. Buschhorn, J. Bulkescher, C. Conrad, D. Comartin, A. Schleiffer, M. Sarov, A. Pozniakovsky, M.M. Slabicki, S. Schloissnig, I. Steinmacher, M. Leuschner, A. Szykor, S. Lawo, L. Pelletier, H. Stark, K. Nasmyth, J. Ellenberg, R. Durbin, F. Buchholz, K.

- Mechtler, A.A. Hyman, and J.M. Peters. 2010. Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science*. 328:593-599.
- Ishikawa, H., A. Kubo, and S. Tsukita. 2005. Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nat Cell Biol*. 7:517-524.
- Ivanovska, I., and M.D. Rose. 2001. Fine structure analysis of the yeast centrin, Cdc31p, identifies residues specific for cell morphology and spindle pole body duplication. *Genetics*. 157:503-518.
- Izquierdo, D., W.J. Wang, K. Uryu, and M.F. Tsou. 2014. Stabilization of cartwheel-less centrioles for duplication requires CEP295-mediated centriole-to-centrosome conversion. *Cell Rep*. 8:957-965.
- Jackman, M., C. Lindon, E.A. Nigg, and J. Pines. 2003. Active cyclin B1-Cdk1 first appears on centrosomes in prophase. *Nat Cell Biol*. 5:143-148.
- Jakobsen, L., K. Vanselow, M. Skogs, Y. Toyoda, E. Lundberg, I. Poser, L.G. Falkenby, M. Bennetzen, J. Westendorf, E.A. Nigg, M. Uhlen, A.A. Hyman, and J.S. Andersen. 2011. Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods. *EMBO J*. 30:1520-1535.
- Janke, C. 2014. The tubulin code: molecular components, readout mechanisms, and functions. *J Cell Biol*. 206:461-472.
- Janson, M.E., T.G. Setty, A. Paoletti, and P.T. Tran. 2005. Efficient formation of bipolar microtubule bundles requires microtubule-bound gamma-tubulin complexes. *J Cell Biol*. 169:297-308.
- Jaspersen, S.L., and S. Ghosh. 2012. Nuclear envelope insertion of spindle pole bodies and nuclear pore complexes. *Nucleus*. 3:226-236.
- Jaspersen, S.L., T.H. Giddings, Jr., and M. Winey. 2002. Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. *J Cell Biol*. 159:945-956.
- Jimenez, J., and J. Oballe. 1994. Ethanol-hypersensitive and ethanol-dependent cdc- mutants in *Schizosaccharomyces pombe*. *Mol Gen Genet*. 245:86-95.

- Johnson, A.E., S.E. Collier, M.D. Ohi, and K.L. Gould. 2012. Fission yeast Dmal requires RING domain dimerization for its ubiquitin ligase activity and mitotic checkpoint function. *J Biol Chem.* 287:25741-25748.
- Joukov, V., J.C. Walter, and A. De Nicolo. 2014. The Cep192-organized aurora A-Plkl cascade is essential for centrosome cycle and bipolar spindle assembly. *Mol Cell.* 55:578-591.
- Katta, S.S., J. Chen, J.M. Gardner, J.M. Friederichs, S.E. Smith, M. Gogol, J.R. Unruh, B.D. Slaughter, and S.L. Jaspersen. 2015. Sec66-Dependent Regulation of Yeast Spindle-Pole Body Duplication Through Pom152. *Genetics.* 201:1479-1495.
- Keller, D., M. Orpinell, N. Olivier, M. Wachsmuth, R. Mahen, R. Wyss, V. Hachet, J. Ellenberg, S. Manley, and P. Gonczy. 2014. Mechanisms of HsSAS-6 assembly promoting centriole formation in human cells. *J Cell Biol.* 204:697-712.
- Keller, L.C., S. Geimer, E. Romijn, J. Yates, 3rd, I. Zamora, and W.F. Marshall. 2009. Molecular architecture of the centriole proteome: the conserved WD40 domain protein POCl is required for centriole duplication and length control. *Mol Biol Cell.* 20:1150-1166.
- Keller, L.C., E.P. Romijn, I. Zamora, J.R. Yates, 3rd, and W.F. Marshall. 2005. Proteomic analysis of isolated chlamydomonas centrioles reveals orthologs of ciliary-disease genes. *Curr Biol.* 15:1090-1098.
- Kemp, C.A., K.R. Kopish, P. Zipperlen, J. Ahringer, and K.F. O'Connell. 2004. Centrosome maturation and duplication in *C. elegans* require the coiled-coil protein SPD-2. *Dev Cell.* 6:511-523.
- Khodjakov, A., and C.L. Rieder. 1999. The sudden recruitment of gamma-tubulin to the centrosome at the onset of mitosis and its dynamic exchange throughout the cell cycle, do not require microtubules. *J Cell Biol.* 146:585-596.
- Khodjakov, A., and C.L. Rieder. 2001. Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J Cell Biol.* 153:237-242.
- Khodjakov, A., C.L. Rieder, G. Sluder, G. Cassels, O. Sibon, and C.L. Wang. 2002. De novo formation of centrosomes in vertebrate cells arrested during S phase. *J Cell Biol.* 158:1171-1181.
- Kilmartin, J.V. 2003. Sfilp has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. *J Cell Biol.* 162:1211-1221.

- Kilmartin, J.V., S.L. Dyos, D. Kershaw, and J.T. Finch. 1993. A spacer protein in the *Saccharomyces cerevisiae* spindle pole body whose transcript is cell cycle-regulated. *J Cell Biol.* 123:1175-1184.
- Kilmartin, J.V., and P.Y. Goh. 1996. Spc110p: assembly properties and role in the connection of nuclear microtubules to the yeast spindle pole body. *EMBO J.* 15:4592-4602.
- Kim, S., and K. Rhee. 2014. Importance of the CEP215-pericentrin interaction for centrosome maturation during mitosis. *PLoS One.* 9:e87016.
- Kim, T.S., J.E. Park, A. Shukla, S. Choi, R.N. Murugan, J.H. Lee, M. Ahn, K. Rhee, J.K. Bang, B.Y. Kim, J. Loncarek, R.L. Erikson, and K.S. Lee. 2013. Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc Natl Acad Sci U S A.* 110:E4849-4857.
- Kimura, M., S. Kotani, T. Hattori, N. Sumi, T. Yoshioka, K. Todokoro, and Y. Okano. 1997. Cell cycle-dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of *Drosophila* and yeast Ipl1. *J Biol Chem.* 272:13766-13771.
- Kirkham, M., T. Muller-Reichert, K. Oegema, S. Grill, and A.A. Hyman. 2003. SAS-4 is a *C. elegans* centriolar protein that controls centrosome size. *Cell.* 112:575-587.
- Kitagawa, D., I. Vakonakis, N. Olieric, M. Hilbert, D. Keller, V. Olieric, M. Bortfeld, M.C. Erat, I. Fluckiger, P. Gonczy, and M.O. Steinmetz. 2011. Structural basis of the 9-fold symmetry of centrioles. *Cell.* 144:364-375.
- Kiyomitsu, T. 2016. Analyzing Spindle Positioning Dynamics in Cultured Cells. *Methods Mol Biol.* 1413:239-252.
- Klebba, J.E., D.W. Buster, T.A. McLamarrah, N.M. Rusan, and G.C. Rogers. 2015. Autoinhibition and relief mechanism for Polo-like kinase 4. *Proc Natl Acad Sci U S A.* 112:E657-666.
- Klein, H.C., P. Guichard, V. Hamel, P. Gonczy, and U.S. Schwarz. 2016. Computational support for a scaffolding mechanism of centriole assembly. *Sci Rep.* 6:27075.
- Kleylein-Sohn, J., J. Westendorf, M. Le Clech, R. Habedanck, Y.D. Stierhof, and E.A. Nigg. 2007. Plk4-induced centriole biogenesis in human cells. *Dev Cell.* 13:190-202.
- Kline-Smith, S.L., and C.E. Walczak. 2002. The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells. *Mol Biol Cell.* 13:2718-2731.
- Klotz, C., M.C. Dabauvalle, M. Paintrand, T. Weber, M. Bornens, and E. Karsenti. 1990. Parthenogenesis in *Xenopus* eggs requires centrosomal integrity. *J Cell Biol.* 110:405-415.

- Knoblich, J.A. 2010. Asymmetric cell division: recent developments and their implications for tumour biology. *Nat Rev Mol Cell Biol.* 11:849-860.
- Knop, M., and E. Schiebel. 1997. Spc98p and Spc97p of the yeast gamma-tubulin complex mediate binding to the spindle pole body via their interaction with Spc110p. *EMBO J.* 16:6985-6995.
- Kobayashi, T., W.Y. Tsang, J. Li, W. Lane, and B.D. Dynlacht. 2011. Centriolar kinesin Kif24 interacts with CPL10 to remodel microtubules and regulate ciliogenesis. *Cell.* 145:914-925.
- Koblenz, B., J. Schoppmeier, A. Grunow, and K.F. Lehtreck. 2003. Centrin deficiency in *Chlamydomonas* causes defects in basal body replication, segregation and maturation. *J Cell Sci.* 116:2635-2646.
- Kohlmaier, G., J. Loncarek, X. Meng, B.F. McEwen, M.M. Mogensen, A. Spektor, B.D. Dynlacht, A. Khodjakov, and P. Gonczy. 2009. Overly long centrioles and defective cell division upon excess of the SAS-4-related protein CPAP. *Curr Biol.* 19:1012-1018.
- Kollman, J.M., A. Merdes, L. Mourey, and D.A. Agard. 2011. Microtubule nucleation by gamma-tubulin complexes. *Nat Rev Mol Cell Biol.* 12:709-721.
- Kollman, J.M., J.K. Polka, A. Zelter, T.N. Davis, and D.A. Agard. 2010. Microtubule nucleating gamma-TuSC assembles structures with 13-fold microtubule-like symmetry. *Nature.* 466:879-882.
- Kollman, J.M., A. Zelter, E.G. Muller, B. Fox, L.M. Rice, T.N. Davis, and D.A. Agard. 2008. The structure of the gamma-tubulin small complex: implications of its architecture and flexibility for microtubule nucleation. *Mol Biol Cell.* 19:207-215.
- Kong, D., V. Farmer, A. Shukla, J. James, R. Gruskin, S. Kiriyama, and J. Loncarek. 2014. Centriole maturation requires regulated Plkl activity during two consecutive cell cycles. *J Cell Biol.* 206:855-865.
- Kotwaliwale, C.V., S.B. Frei, B.M. Stern, and S. Biggins. 2007. A pathway containing the Ipl1/aurora protein kinase and the spindle midzone protein Asel regulates yeast spindle assembly. *Dev Cell.* 13:433-445.
- Krapp, A., M.P. Gulli, and V. Simanis. 2004. SIN and the art of splitting the fission yeast cell. *Curr Biol.* 14:R722-730.

- Krapp, A., and V. Simanis. 2008. An overview of the fission yeast septation initiation network (SIN). *Biochem Soc Trans.* 36:411-415.
- Kratz, A.S., F. Barenz, K.T. Richter, and I. Hoffmann. 2015. Plk4-dependent phosphorylation of STIL is required for centriole duplication. *Biol Open.* 4:370-377.
- Kupke, T., L. Di Cecco, H.M. Muller, A. Neuner, F. Adolf, F. Wieland, W. Nickel, and E. Schiebel. 2011. Targeting of Nbp1 to the inner nuclear membrane is essential for spindle pole body duplication. *EMBO J.* 30:3337-3352.
- Kupke, T., J. Malsam, and E. Schiebel. 2017. A ternary membrane protein complex anchors the spindle pole body in the nuclear envelope in budding yeast. *J Biol Chem.* 292:8447-8458.
- Kuriyama, R., and G.G. Borisy. 1981. Centriole cycle in Chinese hamster ovary cells as determined by whole-mount electron microscopy. *J Cell Biol.* 91:814-821.
- Lambrus, B.G., V. Daggubati, Y. Uetake, P.M. Scott, K.M. Clutario, G. Sluder, and A.J. Holland. 2016. A USP28-53BP1-p53-p21 signaling axis arrests growth after centrosome loss or prolonged mitosis. *J Cell Biol.* 214:143-153.
- Lattao, R., L. Kovacs, and D.M. Glover. 2017. The Centrioles, Centrosomes, Basal Bodies, and Cilia of *Drosophila melanogaster*. *Genetics.* 206:33-53.
- Lawo, S., M. Hasegan, G.D. Gupta, and L. Pelletier. 2012. Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material. *Nat Cell Biol.* 14:1148-1158.
- LeDizet, M., J.C. Beck, and W.E. Finkbeiner. 1998. Differential regulation of centrin genes during ciliogenesis in human tracheal epithelial cells. *Am J Physiol.* 275:L1145-1156.
- Lee, I.J., N. Wang, W. Hu, K. Schott, J. Bahler, T.H. Giddings, Jr., J.R. Pringle, L.L. Du, and J.Q. Wu. 2014. Regulation of spindle pole body assembly and cytokinesis by the centrin-binding protein Sfil in fission yeast. *Mol Biol Cell.* 25:2735-2749.
- Lee, K., and K. Rhee. 2011. PLK1 phosphorylation of pericentrin initiates centrosome maturation at the onset of mitosis. *J Cell Biol.* 195:1093-1101.
- Lee, V.D., and B. Huang. 1993. Molecular cloning and centrosomal localization of human caltractin. *Proc Natl Acad Sci U S A.* 90:11039-11043.
- Leidel, S., M. Delattre, L. Cerutti, K. Baumer, and P. Gonczy. 2005. SAS-6 defines a protein family required for centrosome duplication in *C. elegans* and in human cells. *Nat Cell Biol.* 7:115-125.

- Lemullois, M., G. Fryd-Versavel, and A. Fleury-Aubusson. 2004. Localization of centrins in the hypotrich ciliate *Paraurostyla weissei*. *Protist*. 155:331-346.
- Li, S., A.M. Sandercock, P. Conduit, C.V. Robinson, R.L. Williams, and J.V. Kilmartin. 2006. Structural role of Sfilp-centrin filaments in budding yeast spindle pole body duplication. *J Cell Biol*. 173:867-877.
- Lin, Y.N., C.T. Wu, Y.C. Lin, W.B. Hsu, C.J. Tang, C.W. Chang, and T.K. Tang. 2013. CEP120 interacts with CPAP and positively regulates centriole elongation. *J Cell Biol*. 202:211-219.
- Lindqvist, A., H. Kallstrom, A. Lundgren, E. Barsoum, and C.K. Rosenthal. 2005. Cdc25B cooperates with Cdc25A to induce mitosis but has a unique role in activating cyclin B1-Cdk1 at the centrosome. *J Cell Biol*. 171:35-45.
- Lobjois, V., C. Froment, E. Braud, F. Grimal, O. Burlet-Schiltz, B. Ducommun, and J.P. Bouche. 2011. Study of the docking-dependent PLK1 phosphorylation of the CDC25B phosphatase. *Biochem Biophys Res Commun*. 410:87-90.
- Loiodice, I., J. Staub, T.G. Setty, N.P. Nguyen, A. Paoletti, and P.T. Tran. 2005. Aselp organizes antiparallel microtubule arrays during interphase and mitosis in fission yeast. *Mol Biol Cell*. 16:1756-1768.
- Loncarek, J., P. Hergert, and A. Khodjakov. 2010. Centriole reduplication during prolonged interphase requires procentriole maturation governed by Plk1. *Curr Biol*. 20:1277-1282.
- Loncarek, J., P. Hergert, V. Magidson, and A. Khodjakov. 2008. Control of daughter centriole formation by the pericentriolar material. *Nat Cell Biol*. 10:322-328.
- Lopes, C.A., S.C. Jana, I. Cunha-Ferreira, S. Zitouni, I. Bento, P. Duarte, S. Gilberto, F. Freixo, A. Guerrero, M. Francia, M. Lince-Faria, J. Carneiro, and M. Bettencourt-Dias. 2015. PLK4 trans-Autoactivation Controls Centriole Biogenesis in Space. *Dev Cell*. 35:222-235.
- Lukasiewicz, K.B., T.M. Greenwood, V.C. Negron, A.K. Bruzek, J.L. Salisbury, and W.L. Lingle. 2011. Control of centrin stability by Aurora A. *PLoS One*. 6:e21291.
- Ma, P., J. Winderickx, D. Nauwelaers, F. Dumortier, A. De Doncker, J.M. Thevelein, and P. Van Dijck. 1999. Deletion of SF11, a novel suppressor of partial Ras-cAMP pathway deficiency in the yeast *Saccharomyces cerevisiae*, causes G(2) arrest. *Yeast*. 15:1097-1109.

- Ma, S., S. Vigneron, P. Robert, J.M. Strub, S. Cianferani, A. Castro, and T. Lorca. 2016. Greatwall dephosphorylation and inactivation upon mitotic exit is triggered by PPl. *J Cell Sci.* 129:1329-1339.
- Magidson, V., F. Chang, and A. Khodjakov. 2006. Regulation of cytokinesis by spindle-pole bodies. *Nat Cell Biol.* 8:891-893.
- Mahjoub, M.R., Z. Xie, and T. Stearns. 2010. Cep120 is asymmetrically localized to the daughter centriole and is essential for centriole assembly. *J Cell Biol.* 191:331-346.
- Maniotis, A., and M. Schliwa. 1991. Microsurgical removal of centrosomes blocks cell reproduction and centriole generation in BSC-1 cells. *Cell.* 67:495-504.
- Mardin, B.R., and E. Schiebel. 2012. Breaking the ties that bind: new advances in centrosome biology. *J Cell Biol.* 197:11-18.
- Marshall, W.F. 2009. Centriole evolution. *Curr Opin Cell Biol.* 21:14-19.
- Matei, E., S. Miron, Y. Blouquit, P. Duchambon, I. Durussel, J.A. Cox, and C.T. Craescu. 2003. C-terminal half of human centrin 2 behaves like a regulatory EF-hand domain. *Biochemistry.* 42:1439-1450.
- Matsuo, K., K. Ohsumi, M. Iwabuchi, T. Kawamata, Y. Ono, and M. Takahashi. 2012. Kendrin is a novel substrate for separase involved in the licensing of centriole duplication. *Curr Biol.* 22:915-921.
- McKenney, R.J., W. Huynh, R.D. Vale, and M. Sirajuddin. 2016. Tyrosination of alpha-tubulin controls the initiation of processive dynein-dynactin motility. *EMBO J.* 35:1175-1185.
- McKinley, K.L., and I.M. Cheeseman. 2017. Large-Scale Analysis of CRISPR/Cas9 Cell-Cycle Knockouts Reveals the Diversity of p53-Dependent Responses to Cell-Cycle Defects. *Dev Cell.* 40:405-420 e402.
- Meadows, J.C., and J. Millar. 2008. Latrunculin A delays anaphase onset in fission yeast by disrupting an Ase1-independent pathway controlling mitotic spindle stability. *Mol Biol Cell.* 19:3713-3723.
- Meitinger, F., J.V. Anzola, M. Kaulich, A. Richardson, J.D. Stender, C. Benner, C.K. Glass, S.F. Dowdy, A. Desai, A.K. Shiau, and K. Oegema. 2016. 53BP1 and USP28 mediate p53 activation and G1 arrest after centrosome loss or extended mitotic duration. *J Cell Biol.* 214:155-166.

- Mennella, V., B. Keszthelyi, K.L. McDonald, B. Chhun, F. Kan, G.C. Rogers, B. Huang, and D.A. Agard. 2012. Subdiffraction-resolution fluorescence microscopy reveals a domain of the centrosome critical for pericentriolar material organization. *Nat Cell Biol.* 14:1159-1168.
- Merdes, A., K. Ramyar, J.D. Vechio, and D.W. Cleveland. 1996. A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. *Cell.* 87:447-458.
- Middendorp, S., T. Kuntziger, Y. Abraham, S. Holmes, N. Bordes, M. Paintrand, A. Paoletti, and M. Bornens. 2000. A role for centrin 3 in centrosome reproduction. *J Cell Biol.* 148:405-416.
- Middendorp, S., A. Paoletti, E. Schiebel, and M. Bornens. 1997. Identification of a new mammalian centrin gene, more closely related to *Saccharomyces cerevisiae* CDC31 gene. *Proc Natl Acad Sci U S A.* 94:9141-9146.
- Mikule, K., B. Delaval, P. Kaldis, A. Jurczyk, P. Hergert, and S. Doxsey. 2007. Loss of centrosome integrity induces p38-p53-p21-dependent G1-S arrest. *Nat Cell Biol.* 9:160-170.
- Minet, M., P. Nurse, P. Thuriaux, and J.M. Mitchison. 1979. Uncontrolled septation in a cell division cycle mutant of the fission yeast *Schizosaccharomyces pombe*. *J Bacteriol.* 137:440-446.
- Mitchell, D.R. 2007. The evolution of eukaryotic cilia and flagella as motile and sensory organelles. *Adv Exp Med Biol.* 607:130-140.
- Mochida, S., S.L. Maslen, M. Skehel, and T. Hunt. 2010. Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. *Science.* 330:1670-1673.
- Mogensen, M.M., A. Malik, M. Piel, V. Bouckson-Castaing, and M. Bornens. 2000. Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. *J Cell Sci.* 113 (Pt 17):3013-3023.
- Morin, X., and Y. Bellaiche. 2011. Mitotic spindle orientation in asymmetric and symmetric cell divisions during animal development. *Dev Cell.* 21:102-119.
- Moritz, M., M.B. Braunfeld, J.W. Sedat, B. Alberts, and D.A. Agard. 1995. Microtubule nucleation by gamma-tubulin-containing rings in the centrosome. *Nature.* 378:638-640.
- Morrell, J.L., G.C. Tomlin, S. Rajagopalan, S. Venkatram, A.S. Feoktistova, J.J. Tasto, S. Mehta, J.L. Jennings, A. Link, M.K. Balasubramanian, and K.L. Gould. 2004. Sid4p-Cdc11p assembles the septation initiation network and its regulators at the *S. pombe* SPB. *Curr Biol.* 14:579-584.

- Moudjou, M., N. Bordes, M. Paintrand, and M. Bornens. 1996. gamma-Tubulin in mammalian cells: the centrosomal and the cytosolic forms. *J Cell Sci.* 109 (Pt 4):875-887.
- Moyer, T.C., K.M. Clutario, B.G. Lambrus, V. Daggubati, and A.J. Holland. 2015. Binding of STIL to Plk4 activates kinase activity to promote centriole assembly. *J Cell Biol.* 209:863-878.
- Murphy, S.M., A.M. Preble, U.K. Patel, K.L. O'Connell, D.P. Dias, M. Moritz, D. Agard, J.T. Stults, and T. Stearns. 2001. GCP5 and GCP6: two new members of the human gamma-tubulin complex. *Mol Biol Cell.* 12:3340-3352.
- Murray, A.W. 1989. Cyclin synthesis and degradation and the embryonic cell cycle. *J Cell Sci Suppl.* 12:65-76.
- Nakajima, H., F. Toyoshima-Morimoto, E. Taniguchi, and E. Nishida. 2003. Identification of a consensus motif for Plk (Polo-like kinase) phosphorylation reveals Myt1 as a Plk1 substrate. *J Biol Chem.* 278:25277-25280.
- Nemoto, N., T. Udagawa, T. Ohira, L. Jiang, K. Hirota, C.R. Wilkinson, J. Bahler, N. Jones, K. Ohta, R.C. Wek, and K. Asano. 2010. The roles of stress-activated Styl and Gcn2 kinases and of the protooncogene homologue Int6/eIF3e in responses to endogenous oxidative stress during histidine starvation. *J Mol Biol.* 404:183-201.
- Nguyen-Ngoc, T., K. Afshar, and P. Gonczy. 2007. Coupling of cortical dynein and G alpha proteins mediates spindle positioning in *Caenorhabditis elegans*. *Nat Cell Biol.* 9:1294-1302.
- Nguyen, T., D.B. Vinh, D.K. Crawford, and T.N. Davis. 1998. A genetic analysis of interactions with Spc110p reveals distinct functions of Spc97p and Spc98p, components of the yeast gamma-tubulin complex. *Mol Biol Cell.* 9:2201-2216.
- Nigg, E.A., and J.W. Raff. 2009. Centrioles, centrosomes, and cilia in health and disease. *Cell.* 139:663-678.
- Nigg, E.A., and T. Stearns. 2011. The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. *Nat Cell Biol.* 13:1154-1160.
- Nishita, M., T. Satake, Y. Minami, and A. Suzuki. 2017. Regulatory mechanisms and cellular functions of non-centrosomal microtubules. *J Biochem.* 162:1-10.
- Nogales, E., S.G. Wolf, and K.H. Downing. 1998. Structure of the alpha beta tubulin dimer by electron crystallography. *Nature.* 391:199-203.

- Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. *Nature*. 344:503-508.
- Nurse, P., P. Thuriaux, and K. Nasmyth. 1976. Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. *Mol Gen Genet*. 146:167-178.
- O'Connell, K.F., C. Caron, K.R. Kopish, D.D. Hurd, K.J. Kempfues, Y. Li, and J.G. White. 2001. The *C. elegans* *zyg-1* gene encodes a regulator of centrosome duplication with distinct maternal and paternal roles in the embryo. *Cell*. 105:547-558.
- O'Toole, E.T., M. Winey, and J.R. McIntosh. 1999. High-voltage electron tomography of spindle pole bodies and early mitotic spindles in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell*. 10:2017-2031.
- Oliferenko, S., T.G. Chew, and M.K. Balasubramanian. 2009. Positioning cytokinesis. *Genes Dev*. 23:660-674.
- Oliveira, R.A., and K. Nasmyth. 2013. Cohesin cleavage is insufficient for centriole disengagement in *Drosophila*. *Curr Biol*. 23:R601-603.
- Panier, S., and S.J. Boulton. 2014. Double-strand break repair: 53BP1 comes into focus. *Nat Rev Mol Cell Biol*. 15:7-18.
- Paoletti, A., N. Bordes, R. Haddad, C.L. Schwartz, F. Chang, and M. Bornens. 2003. Fission yeast *cdc31p* is a component of the half-bridge and controls SPB duplication. *Mol Biol Cell*. 14:2793-2808.
- Paoletti, A., M. Moudjou, M. Paintrand, J.L. Salisbury, and M. Bornens. 1996. Most of centrin in animal cells is not centrosome-associated and centrosomal centrin is confined to the distal lumen of centrioles. *J Cell Sci*. 109 (Pt 13):3089-3102.
- Park, S.Y., J.E. Park, T.S. Kim, J.H. Kim, M.J. Kwak, B. Ku, L. Tian, R.N. Murugan, M. Ahn, S. Komiya, H. Hojo, N.H. Kim, B.Y. Kim, J.K. Bang, R.L. Erikson, K.W. Lee, S.J. Kim, B.H. Oh, W. Yang, and K.S. Lee. 2014. Molecular basis for unidirectional scaffold switching of human Plk4 in centriole biogenesis. *Nat Struct Mol Biol*. 21:696-703.
- Parker, L.L., and H. Piwnica-Worms. 1992. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. *Science*. 257:1955-1957.
- Patel, H., I. Stavrou, R.L. Shrestha, V. Draviam, M.C. Frame, and V.G. Brunton. 2016. Kindlin1 regulates microtubule function to ensure normal mitosis. *J Mol Cell Biol*. 8:338-348.

- Patel, H., J. Zich, B. Serrels, C. Rickman, K.G. Hardwick, M.C. Frame, and V.G. Brunton. 2013. Kindlin-1 regulates mitotic spindle formation by interacting with integrins and Plk-1. *Nat Commun.* 4:2056.
- Patten, S.A., P. Margaritte-Jeannin, J.C. Bernard, E. Alix, A. Labalme, A. Besson, S.L. Girard, K. Fendri, N. Fraisse, B. Biot, C. Poizat, A. Campan-Fournier, K. Abelin-Genevois, V. Cunin, C. Zaouter, M. Liao, R. Lamy, G. Lesca, R. Menassa, C. Marcaillou, M. Letexier, D. Sanlaville, J. Berard, G.A. Rouleau, F. Clerget-Darpoux, P. Drapeau, F. Moldovan, and P. Ederly. 2015. Functional variants of POC5 identified in patients with idiopathic scoliosis. *J Clin Invest.* 125:1124-1128.
- Pelletier, L., N. Ozlu, E. Hannak, C. Cowan, B. Habermann, M. Ruer, T. Muller-Reichert, and A.A. Hyman. 2004. The *Caenorhabditis elegans* centrosomal protein SPD-2 is required for both pericentriolar material recruitment and centriole duplication. *Curr Biol.* 14:863-873.
- Peris, L., M. Wagenbach, L. Lafanechere, J. Brocard, A.T. Moore, F. Kozielski, D. Job, L. Wordeman, and A. Andrieux. 2009. Motor-dependent microtubule disassembly driven by tubulin tyrosination. *J Cell Biol.* 185:1159-1166.
- Petersen, J. 2009. TOR signalling regulates mitotic commitment through stress-activated MAPK and Polo kinase in response to nutrient stress. *Biochem Soc Trans.* 37:273-277.
- Petersen, J., and I.M. Hagan. 2005. Polo kinase links the stress pathway to cell cycle control and tip growth in fission yeast. *Nature.* 435:507-512.
- Petersen, J., and P. Nurse. 2007. TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. *Nat Cell Biol.* 9:1263-1272.
- Picard, A., E. Karsenti, M.C. Dabauvalle, and M. Doree. 1987. Release of mature starfish oocytes from interphase arrest by microinjection of human centrosomes. *Nature.* 327:170-172.
- Piel, M., P. Meyer, A. Khodjakov, C.L. Rieder, and M. Bornens. 2000. The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. *J Cell Biol.* 149:317-330.
- Piel, M., J. Nordberg, U. Euteneuer, and M. Bornens. 2001. Centrosome-dependent exit of cytokinesis in animal cells. *Science.* 291:1550-1553.
- Pitaval, A., F. Senger, G. Letort, X. Gidrol, L. Guyon, J. Sillibourne, and M. Thery. 2017. Microtubule stabilization drives 3D centrosome migration to initiate primary ciliogenesis. *J Cell Biol.*

- Pollard, T.D. 2017. Nine unanswered questions about cytokinesis. *J Cell Biol.* 216:3007-3016.
- Portran, D., L. Schaedel, Z. Xu, M. Thery, and M.V. Nachury. 2017. Tubulin acetylation protects long-lived microtubules against mechanical ageing. *Nat Cell Biol.* 19:391-398.
- Prosser, S.L., and C.G. Morrison. 2015. Centrin2 regulates CPII0 removal in primary cilium formation. *J Cell Biol.* 208:693-701.
- Prosser, S.L., M.D. Samant, J.E. Baxter, C.G. Morrison, and A.M. Fry. 2012. Oscillation of APC/C activity during cell cycle arrest promotes centrosome amplification. *J Cell Sci.* 125:5353-5368.
- Pugacheva, E.N., S.A. Jablonski, T.R. Hartman, E.P. Henske, and E.A. Golemis. 2007. HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell.* 129:1351-1363.
- Purohit, A., S.H. Tynan, R. Vallee, and S.J. Doxsey. 1999. Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *J Cell Biol.* 147:481-492.
- Reber, S., and A.A. Hyman. 2015. Emergent Properties of the Metaphase Spindle. *Cold Spring Harb Perspect Biol.* 7:a015784.
- Reed, N.A., D. Cai, T.L. Blasius, G.T. Jih, E. Meyhofer, J. Gaertig, and K.J. Verhey. 2006. Microtubule acetylation promotes kinesin-1 binding and transport. *Curr Biol.* 16:2166-2172.
- Rhind, N., and P. Russell. 2012. Signaling pathways that regulate cell division. *Cold Spring Harb Perspect Biol.* 4.
- Rhys, A.D., and S.A. Godinho. 2017. Dividing with Extra Centrosomes: A Double Edged Sword for Cancer Cells. *Adv Exp Med Biol.* 1002:47-67.
- Rincon, S.A., M. Estravis, F. Dingli, D. Loew, P.T. Tran, and A. Paoletti. 2017a. SIN-Dependent Dissociation of the SAD Kinase Cdr2 from the Cell Cortex Resets the Division Plane. *Curr Biol.* 27:534-542.
- Rincon, S.A., A. Lamson, R. Blackwell, V. Syrovatkina, V. Fraiser, A. Paoletti, M.D. Betterton, and P.T. Tran. 2017b. Kinesin-5-independent mitotic spindle assembly requires the antiparallel microtubule crosslinker Ase1 in fission yeast. *Nat Commun.* 8:15286.
- Riparbelli, M.G., R. Dallai, and G. Callaini. 2010. The insect centriole: A land of discovery. *Tissue Cell.* 42:69-80.

- Robbins, E., G. Jentsch, and A. Micali. 1968. The centriole cycle in synchronized HeLa cells. *J Cell Biol.* 36:329-339.
- Roberts-Galbraith, R.H., and K.L. Gould. 2008. Stepping into the ring: the SIN takes on contractile ring assembly. *Genes Dev.* 22:3082-3088.
- Rodrigues-Martins, A., M. Riparbelli, G. Callaini, D.M. Glover, and M. Bettencourt-Dias. 2007. Revisiting the role of the mother centriole in centriole biogenesis. *Science.* 316:1046-1050.
- Roghi, C., R. Giet, R. Uzbekov, N. Morin, I. Chartrain, R. Le Guellec, A. Couturier, M. Doree, M. Philippe, and C. Prigent. 1998. The *Xenopus* protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. *J Cell Sci.* 111 (Pt 5):557-572.
- Rose, M.D., and G.R. Fink. 1987. KAR1, a gene required for function of both intranuclear and extranuclear microtubules in yeast. *Cell.* 48:1047-1060.
- Rosenberg, J.A., G.C. Tomlin, W.H. McDonald, B.E. Snyderman, E.G. Muller, J.R. Yates, 3rd, and K.L. Gould. 2006. Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. *Mol Biol Cell.* 17:3793-3805.
- Roshak, A.K., E.A. Capper, C. Imburgia, J. Fornwald, G. Scott, and L.A. Marshall. 2000. The human polo-like kinase, PLK, regulates cdc2/cyclin B through phosphorylation and activation of the cdc25C phosphatase. *Cell Signal.* 12:405-411.
- Ruiz, F., N. Garreau de Loubresse, C. Klotz, J. Beisson, and F. Koll. 2005. Centrin deficiency in *Paramecium* affects the geometry of basal-body duplication. *Curr Biol.* 15:2097-2106.
- Ruthnick, D., A. Neuner, F. Dietrich, D. Kirrmaier, U. Engel, M. Knop, and E. Schiebel. 2017. Characterization of spindle pole body duplication reveals a regulatory role for nuclear pore complexes. *J Cell Biol.* 216:2425-2442.
- Salimova, E., M. Sohrmann, N. Fournier, and V. Simanis. 2000. The *S. pombe* orthologue of the *S. cerevisiae* *mob1* gene is essential and functions in signalling the onset of septum formation. *J Cell Sci.* 113 (Pt 10):1695-1704.
- Salisbury, J.L., A. Baron, B. Surek, and M. Melkonian. 1984. Striated flagellar roots: isolation and partial characterization of a calcium-modulated contractile organelle. *J Cell Biol.* 99:962-970.

- Salisbury, J.L., A.T. Baron, and M.A. Sanders. 1988. The centrin-based cytoskeleton of *Chlamydomonas reinhardtii*: distribution in interphase and mitotic cells. *J Cell Biol.* 107:635-641.
- Salisbury, J.L., K.M. Suino, R. Busby, and M. Springett. 2002. Centrin-2 is required for centriole duplication in mammalian cells. *Curr Biol.* 12:1287-1292.
- Samejima, I., V.J. Miller, L.M. Grocock, and K.E. Sawin. 2008. Two distinct regions of Mtol are required for normal microtubule nucleation and efficient association with the gamma-tubulin complex in vivo. *J Cell Sci.* 121:3971-3980.
- Samejima, I., V.J. Miller, S.A. Rincon, and K.E. Sawin. 2010. Fission yeast Mtol regulates diversity of cytoplasmic microtubule organizing centers. *Curr Biol.* 20:1959-1965.
- Sanchez, A.D., and J.L. Feldman. 2017. Microtubule-organizing centers: from the centrosome to non-centrosomal sites. *Curr Opin Cell Biol.* 44:93-101.
- Sanders, M.A., and J.L. Salisbury. 1989. Centrin-mediated microtubule severing during flagellar excision in *Chlamydomonas reinhardtii*. *J Cell Biol.* 108:1751-1760.
- Sanders, M.A., and J.L. Salisbury. 1994. Centrin plays an essential role in microtubule severing during flagellar excision in *Chlamydomonas reinhardtii*. *J Cell Biol.* 124:795-805.
- Sawin, K.E., and P.T. Tran. 2006. Cytoplasmic microtubule organization in fission yeast. *Yeast.* 23:1001-1014.
- Schaerer, F., G. Morgan, M. Winey, and P. Philippsen. 2001. Cnm67p is a spacer protein of the *Saccharomyces cerevisiae* spindle pole body outer plaque. *Mol Biol Cell.* 12:2519-2533.
- Schmidt, K.N., S. Kuhns, A. Neuner, B. Hub, H. Zentgraf, and G. Pereira. 2012. Cep164 mediates vesicular docking to the mother centriole during early steps of ciliogenesis. *J Cell Biol.* 199:1083-1101.
- Schmidt, S., M. Sohrmann, K. Hofmann, A. Woollard, and V. Simanis. 1997. The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. *Genes Dev.* 11:1519-1534.
- Schmidt, T.I., J. Kleylein-Sohn, J. Westendorf, M. Le Clech, S.B. Lavoie, Y.D. Stierhof, and E.A. Nigg. 2009. Control of centriole length by CPAP and CPl10. *Curr Biol.* 19:1005-1011.
- Schockel, L., M. Mockel, B. Mayer, D. Boos, and O. Stemmann. 2011. Cleavage of cohesin rings coordinates the separation of centrioles and chromatids. *Nat Cell Biol.* 13:966-972.

- Schramm, C., S. Elliott, A. Shevchenko, and E. Schiebel. 2000. The Bbplp-Mps2p complex connects the SPB to the nuclear envelope and is essential for SPB duplication. *EMBO J.* 19:421-433.
- Schuyler, S.C., J.Y. Liu, and D. Pellman. 2003. The molecular function of Aselp: evidence for a MAP-dependent midzone-specific spindle matrix. Microtubule-associated proteins. *J Cell Biol.* 160:517-528.
- Schuyler, S.C., and D. Pellman. 2001. Microtubule "plus-end-tracking proteins": The end is just the beginning. *Cell.* 105:421-424.
- Seybold, C., M. Elserafy, D. Ruthnick, M. Ozboyaci, A. Neuner, B. Flottmann, M. Heilemann, R.C. Wade, and E. Schiebel. 2015. Karl binding to Sfil C-terminal regions anchors the SPB bridge to the nuclear envelope. *J Cell Biol.* 209:843-861.
- Shi, J., Y. Zhao, T. Vonderfecht, M. Winey, and M.W. Klymkowsky. 2015. Centrin-2 (Cetn2) mediated regulation of FGF/FGFR gene expression in *Xenopus*. *Sci Rep.* 5:10283.
- Sillibourne, J.E., F. Tack, N. Vloemans, A. Boeckx, S. Thambirajah, P. Bonnet, F.C. Ramaekers, M. Bornens, and T. Grand-Perret. 2010. Autophosphorylation of polo-like kinase 4 and its role in centriole duplication. *Mol Biol Cell.* 21:547-561.
- Simanis, V. 2015. Pombe's thirteen - control of fission yeast cell division by the septation initiation network. *J Cell Sci.* 128:1465-1474.
- Singla, V., M. Romaguera-Ros, J.M. Garcia-Verdugo, and J.F. Reiter. 2010. Ofd1, a human disease gene, regulates the length and distal structure of centrioles. *Dev Cell.* 18:410-424.
- Sir, J.H., M. Putz, O. Daly, C.G. Morrison, M. Dunning, J.V. Kilmartin, and F. Gergely. 2013. Loss of centrioles causes chromosomal instability in vertebrate somatic cells. *J Cell Biol.* 203:747-756.
- Sohrman, M., S. Schmidt, I. Hagan, and V. Simanis. 1998. Asymmetric segregation on spindle poles of the *Schizosaccharomyces pombe* septum-inducing protein kinase Cdc7p. *Genes Dev.* 12:84-94.
- Song, K., K.E. Mach, C.Y. Chen, T. Reynolds, and C.F. Albright. 1996. A novel suppressor of ras1 in fission yeast, *byr4*, is a dosage-dependent inhibitor of cytokinesis. *J Cell Biol.* 133:1307-1319.

- Sonnen, K.F., A.M. Gabryjonczyk, E. Anselm, Y.D. Stierhof, and E.A. Nigg. 2013. Human Cep192 and Cep152 cooperate in Plk4 recruitment and centriole duplication. *J Cell Sci.* 126:3223-3233.
- Sorokin, S.P. 1968. Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J Cell Sci.* 3:207-230.
- Sossin, W.S., and L. DesGroseillers. 2006. Intracellular trafficking of RNA in neurons. *Traffic.* 7:1581-1589.
- Spang, A., I. Courtney, U. Fackler, M. Matzner, and E. Schiebel. 1993. The calcium-binding protein cell division cycle 31 of *Saccharomyces cerevisiae* is a component of the half bridge of the spindle pole body. *J Cell Biol.* 123:405-416.
- Spang, A., I. Courtney, K. Grein, M. Matzner, and E. Schiebel. 1995. The Cdc31p-binding protein Kar1p is a component of the half bridge of the yeast spindle pole body. *J Cell Biol.* 128:863-877.
- Spang, A., S. Geissler, K. Grein, and E. Schiebel. 1996. gamma-Tubulin-like Tub4p of *Saccharomyces cerevisiae* is associated with the spindle pole body substructures that organize microtubules and is required for mitotic spindle formation. *J Cell Biol.* 134:429-441.
- Sparks, C.A., M. Morphew, and D. McCollum. 1999. Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. *J Cell Biol.* 146:777-790.
- Spektor, A., W.Y. Tsang, D. Khoo, and B.D. Dynlacht. 2007. Cep97 and CP110 suppress a cilia assembly program. *Cell.* 130:678-690.
- Stearns, T., and M. Kirschner. 1994. In vitro reconstitution of centrosome assembly and function: the central role of gamma-tubulin. *Cell.* 76:623-637.
- Stemm-Wolf, A.J., J.B. Meehl, and M. Winey. 2013. Sfr13, a member of a large family of asymmetrically localized Sfil-repeat proteins, is important for basal body separation and stability in *Tetrahymena thermophila*. *J Cell Sci.* 126:1659-1671.
- Stemm-Wolf, A.J., G. Morgan, T.H. Giddings, Jr., E.A. White, R. Marchione, H.B. McDonald, and M. Winey. 2005. Basal body duplication and maintenance require one member of the *Tetrahymena thermophila* centrin gene family. *Mol Biol Cell.* 16:3606-3619.
- Stevens, N.R., A.A. Raposo, R. Basto, D. St Johnston, and J.W. Raff. 2007. From stem cell to embryo without centrioles. *Curr Biol.* 17:1498-1503.

- Stevens, N.R., H. Roque, and J.W. Raff. 2010. DSas-6 and Ana2 coassemble into tubules to promote centriole duplication and engagement. *Dev Cell*. 19:913-919.
- Stirling, D.A., K.A. Welch, and M.J. Stark. 1994. Interaction with calmodulin is required for the function of Spc110p, an essential component of the yeast spindle pole body. *EMBO J*. 13:4329-4342.
- Strnad, P., S. Leidel, T. Vinogradova, U. Euteneuer, A. Khodjakov, and P. Gonczy. 2007. Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. *Dev Cell*. 13:203-213.
- Sulimenko, V., Z. Hajkova, A. Klebanovych, and P. Draber. 2017. Regulation of microtubule nucleation mediated by gamma-tubulin complexes. *Protoplasma*. 254:1187-1199.
- Sundberg, H.A., and T.N. Davis. 1997. A mutational analysis identifies three functional regions of the spindle pole component Spc110p in *Saccharomyces cerevisiae*. *Mol Biol Cell*. 8:2575-2590.
- Sundberg, H.A., L. Goetsch, B. Byers, and T.N. Davis. 1996. Role of calmodulin and Spc110p interaction in the proper assembly of spindle pole body components. *J Cell Biol*. 133:111-124.
- Takahashi, M., A. Yamagiwa, T. Nishimura, H. Mukai, and Y. Ono. 2002. Centrosomal proteins CG-NAP and kendrin provide microtubule nucleation sites by anchoring gamma-tubulin ring complex. *Mol Biol Cell*. 13:3235-3245.
- Tamm, T., A. Grallert, E.P. Grossman, I. Alvarez-Tabares, F.E. Stevens, and I.M. Hagan. 2011. Brr6 drives the *Schizosaccharomyces pombe* spindle pole body nuclear envelope insertion/extrusion cycle. *J Cell Biol*. 195:467-484.
- Tanaka, K., and T. Kanbe. 1986. Mitosis in the fission yeast *Schizosaccharomyces pombe* as revealed by freeze-substitution electron microscopy. *J Cell Sci*. 80:253-268.
- Tanaka, K., J. Petersen, F. MacIver, D.P. Mulvihill, D.M. Glover, and I.M. Hagan. 2001. The role of Plol kinase in mitotic commitment and septation in *Schizosaccharomyces pombe*. *EMBO J*. 20:1259-1270.
- Tang, C.J., R.H. Fu, K.S. Wu, W.B. Hsu, and T.K. Tang. 2009. CPAP is a cell-cycle regulated protein that controls centriole length. *Nat Cell Biol*. 11:825-831.
- Terada, Y., Y. Uetake, and R. Kuriyama. 2003. Interaction of Aurora-A and centrosomin at the microtubule-nucleating site in *Drosophila* and mammalian cells. *J Cell Biol*. 162:757-763.

- Thompson, J.R., Z.C. Ryan, J.L. Salisbury, and R. Kumar. 2006. The structure of the human centrin 2-xeroderma pigmentosum group C protein complex. *J Biol Chem.* 281:18746-18752.
- Tomlin, G.C., J.L. Morrell, and K.L. Gould. 2002. The spindle pole body protein Cdc11p links Sid4p to the fission yeast septation initiation network. *Mol Biol Cell.* 13:1203-1214.
- Tsang, W.Y., A. Spektor, D.J. Luciano, V.B. Indjeian, Z. Chen, J.L. Salisbury, I. Sanchez, and B.D. Dynlacht. 2006. CPl10 cooperates with two calcium-binding proteins to regulate cytokinesis and genome stability. *Mol Biol Cell.* 17:3423-3434.
- Tsou, M.F., and T. Stearns. 2006. Mechanism limiting centrosome duplication to once per cell cycle. *Nature.* 442:947-951.
- Tsou, M.F., W.J. Wang, K.A. George, K. Uryu, T. Stearns, and P.V. Jallepalli. 2009. Polo kinase and separase regulate the mitotic licensing of centriole duplication in human cells. *Dev Cell.* 17:344-354.
- Uzawa, S., F. Li, Y. Jin, K.L. McDonald, M.B. Braunfeld, D.A. Agard, and W.Z. Cande. 2004. Spindle pole body duplication in fission yeast occurs at the G1/S boundary but maturation is blocked until exit from S by an event downstream of cdc10+. *Mol Biol Cell.* 15:5219-5230.
- Vallen, E.A., W. Ho, M. Winey, and M.D. Rose. 1994. Genetic interactions between CDC31 and KARI, two genes required for duplication of the microtubule organizing center in *Saccharomyces cerevisiae*. *Genetics.* 137:407-422.
- van Beneden, É. 1887. Nouvelles recherches sur la fécondation et la division mitotique chez l'Ascaride mégalocéphale. W. Engelmann.
- van Breugel, M., M. Hirono, A. Andreeva, H.A. Yanagisawa, S. Yamaguchi, Y. Nakazawa, N. Morgner, M. Petrovich, I.O. Ebong, C.V. Robinson, C.M. Johnson, D. Veprintsev, and B. Zuber. 2011. Structures of SAS-6 suggest its organization in centrioles. *Science.* 331:1196-1199.
- Veeraraghavan, S., P.A. Fagan, H. Hu, V. Lee, J.F. Harper, B. Huang, and W.J. Chazin. 2002. Structural independence of the two EF-hand domains of caltractin. *J Biol Chem.* 277:28564-28571.
- Vigneron, S., E. Brioudes, A. Burgess, J.C. Labbe, T. Lorca, and A. Castro. 2009. Greatwall maintains mitosis through regulation of PP2A. *EMBO J.* 28:2786-2793.

- Vinh, D.B., J.W. Kern, W.O. Hancock, J. Howard, and T.N. Davis. 2002. Reconstitution and characterization of budding yeast gamma-tubulin complex. *Mol Biol Cell*. 13:1144-1157.
- Walczak, C.E., and S.L. Shaw. 2010. A MAP for bundling microtubules. *Cell*. 142:364-367.
- Walker, R.A., E.T. O'Brien, N.K. Pryer, M.F. Soboeiro, W.A. Voter, H.P. Erickson, and E.D. Salmon. 1988. Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J Cell Biol*. 107:1437-1448.
- Wang, G., Q. Chen, X. Zhang, B. Zhang, X. Zhuo, J. Liu, Q. Jiang, and C. Zhang. 2013. PCMI recruits Plk1 to the pericentriolar matrix to promote primary cilia disassembly before mitotic entry. *J Cell Sci*. 126:1355-1365.
- Wang, L., and A. Brown. 2002. Rapid movement of microtubules in axons. *Curr Biol*. 12:1496-1501.
- Wang, S., and Y. Zheng. 2011. Identification of a novel dynein binding domain in nudel essential for spindle pole organization in *Xenopus* egg extract. *J Biol Chem*. 286:587-593.
- Wang, X., Y. Yang, Q. Duan, N. Jiang, Y. Huang, Z. Darzynkiewicz, and W. Dai. 2008. sSgo1, a major splice variant of Sgo1, functions in centriole cohesion where it is regulated by Plk1. *Dev Cell*. 14:331-341.
- Wang, Y.L., H. Chen, Y.Q. Zhan, R.H. Yin, C.Y. Li, C.H. Ge, M. Yu, and X.M. Yang. 2016. EWSR1 regulates mitosis by dynamically influencing microtubule acetylation. *Cell Cycle*. 15:2202-2215.
- Watanabe, N., H. Arai, J. Iwasaki, M. Shiina, K. Ogata, T. Hunter, and H. Osada. 2005. Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways. *Proc Natl Acad Sci U S A*. 102:11663-11668.
- West, R.R., E.V. Vaisberg, R. Ding, P. Nurse, and J.R. McIntosh. 1998. cut11(+): A gene required for cell cycle-dependent spindle pole body anchoring in the nuclear envelope and bipolar spindle formation in *Schizosaccharomyces pombe*. *Mol Biol Cell*. 9:2839-2855.
- Westermann, S., and K. Weber. 2003. Post-translational modifications regulate microtubule function. *Nat Rev Mol Cell Biol*. 4:938-947.
- White, R.A., Z. Pan, and J.L. Salisbury. 2000. GFP-centrin as a marker for centriole dynamics in living cells. *Microsc Res Tech*. 49:451-457.
- Wiese, C., and Y. Zheng. 2000. A new function for the gamma-tubulin ring complex as a microtubule minus-end cap. *Nat Cell Biol*. 2:358-364.

- Wigge, P.A., O.N. Jensen, S. Holmes, S. Soues, M. Mann, and J.V. Kilmartin. 1998. Analysis of the *Saccharomyces* spindle pole by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. *J Cell Biol.* 141:967-977.
- Winey, M., L. Goetsch, P. Baum, and B. Byers. 1991. MPS1 and MPS2: novel yeast genes defining distinct steps of spindle pole body duplication. *J Cell Biol.* 114:745-754.
- Wittmann, T., A. Hyman, and A. Desai. 2001. The spindle: a dynamic assembly of microtubules and motors. *Nat Cell Biol.* 3:E28-34.
- Wloga, D., and J. Frankel. 2012. From molecules to morphology: cellular organization of *Tetrahymena thermophila*. *Methods Cell Biol.* 109:83-140.
- Wolfe, B.A., and K.L. Gould. 2005. Split decisions: coordinating cytokinesis in yeast. *Trends Cell Biol.* 15:10-18.
- Wong, Y.L., J.V. Anzola, R.L. Davis, M. Yoon, A. Motamedi, A. Kroll, C.P. Seo, J.E. Hsia, S.K. Kim, J.W. Mitchell, B.J. Mitchell, A. Desai, T.C. Gahman, A.K. Shiau, and K. Oegema. 2015. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. *Science.* 348:1155-1160.
- Wright, R.L., J. Salisbury, and J.W. Jarvik. 1985. A nucleus-basal body connector in *Chlamydomonas reinhardtii* that may function in basal body localization or segregation. *J Cell Biol.* 101:1903-1912.
- Xu, Z., L. Schaedel, D. Portran, A. Aguilar, J. Gaillard, M.P. Marinkovich, M. Thery, and M.V. Nachury. 2017. Microtubules acquire resistance from mechanical breakage through intraluminal acetylation. *Science.* 356:328-332.
- Yamashita, A., M. Sato, A. Fujita, M. Yamamoto, and T. Toda. 2005. The roles of fission yeast *ase1* in mitotic cell division, meiotic nuclear oscillation, and cytokinesis checkpoint signaling. *Mol Biol Cell.* 16:1378-1395.
- Yim, H., S.B. Shin, S.U. Woo, P.C. Lee, and R.L. Erikson. 2017. Plk1-mediated stabilization of 53BP1 through USP7 regulates centrosome positioning to maintain bipolarity. *Oncogene.* 36:966-978.
- Zhang, Y., and C.Y. He. 2012. Centrioles in unicellular organisms: functional diversity and specialization. *Protoplasma.* 249:459-467.

- Zhao, H., L. Zhu, Y. Zhu, J. Cao, S. Li, Q. Huang, T. Xu, X. Huang, X. Yan, and X. Zhu. 2013. The Cep63 paralogue Deupl enables massive de novo centriole biogenesis for vertebrate multiciliogenesis. *Nat Cell Biol.* 15:1434-1444.
- Zheng, Y., M.L. Wong, B. Alberts, and T. Mitchison. 1995. Nucleation of microtubule assembly by a gamma-tubulin-containing ring complex. *Nature.* 378:578-583.
- Zimmerman, W.C., J. Sillibourne, J. Rosa, and S.J. Doxsey. 2004. Mitosis-specific anchoring of gamma tubulin complexes by pericentrin controls spindle organization and mitotic entry. *Mol Biol Cell.* 15:3642-3657.

Appendix: Protocol Chapter

Article 1-B: Monitoring SPB biogenesis in fission yeast with high resolution and quantitative fluorescent microscopy

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Monitoring SPB biogenesis in fission yeast with high resolution and quantitative fluorescent microscopy

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Abstract

Like centrosomes, yeast spindle pole bodies (SPBs) undergo a tightly controlled duplication cycle in order to restrict their number to one or two per cell and promote the assembly of a bipolar spindle at mitotic entry. This conservative duplication cycle is tightly coordinated with cell cycle progression although the mechanisms that ensure this coordination remain largely unknown. In this chapter, we describe simple high resolution microscopy- and quantitative light microscopy-based methods that allow to monitor SPB biogenesis in fission yeast and may be useful to study the molecular pathways controlling the successive phases of the duplication cycle.

INTRODUCTION

Spindle pole bodies or SPBs are the yeast equivalent of centrosomes and have an essential function in bipolar spindle assembly. They share key functional features with centrosomes, including microtubule nucleation and anchoring, attachment to the nucleus as well as a strict regulation of their copy number, controlled by a conservative duplication mechanism that restricts them to one copy per cell before duplication, and two copies afterward. Nevertheless, the composition and structure of SPBs is quite different from that of centrosomes. Indeed, SPB components generally share limited homology with centrosomal proteins outside of the γ -TurC involved in microtubule nucleation. Moreover, these organelles are lacking centrioles, and instead are formed of stacked layers referred to as SPB plaques, tightly associated with one another and with the nuclear envelope (Adams & Kilmartin, 2000; Ding, West, Morphew, Oakley, & McIntosh, 1997; Jaspersen & Ghosh, 2012; Jaspersen & Winey, 2004; Lim, Zhang, & Surana, 2009; Uzawa et al., 2004). (Figure 1(A)).

In fission yeast, electron microscopy analysis performed in the late-1990s revealed the ultrastructure of SPBs at different cell cycle stages (Ding et al., 1997). It showed that during interphase, the SPB is sitting on top of the nuclear envelope rather than being inserted in it like in *Saccharomyces cerevisiae*. It also identified, like in budding yeast, an appendage called the half-bridge, on the side of the SPB plaques, which duplicates first to create at its tip an assembly site for the new SPB (Bouhleb et al., in press; Elserafy et al., 2014; Kilmartin, 2003; Lee et al., 2014; Li et al., 2006; Paoletti et al., 2003). Side-by-side duplicated SPBs eventually insert in the nuclear envelope at mitotic entry and separate after cleavage of the bridge and nucleation of intranuclear microtubules to generate a bipolar spindle that can segregate chromosomes.

Determining the duplication status of SPBs by electron microscopy remains very challenging and tedious in fission yeast, and only allows the analysis of a handful of samples. New progress will be aided by faster methods to track SPB duplication. In addition, the availability of methods to monitor SPB biogenesis in live cells is important to establish how the different steps of the duplication cycle are coordinated with cell cycle progression.

In this chapter, we describe two methods to monitor SPB duplication cycle based on high resolution microscopy of fixed cells and quantitative light microscopy of live cells. These analyses can be performed on fission yeast strains expressing red and green fluorescent SPB components from their endogenous locus.

1. MONITORING SPB DUPLICATION IN FIXED SPB-LABELED STRAINS

SPB duplication can be monitored by imaging some of the key structural components of the SPB plaques such as Ppc89 (Rosenberg et al., 2006) and Sid4 that

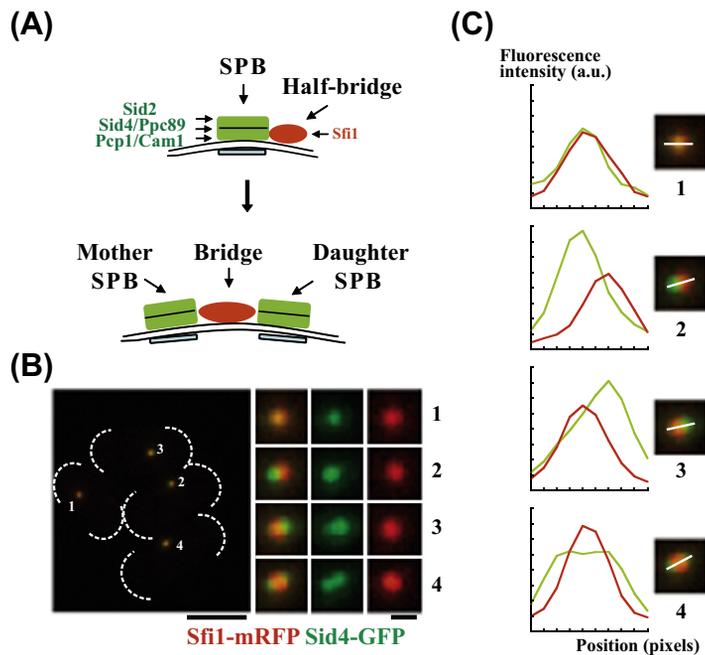


FIGURE 1

Monitoring SPB duplication in fixed SPB-labeled strains. (A) Scheme of the SPB with SPB plaques in green and half-bridge in red before duplication (top) or the duplicated SPBs linked by the bridge that maintain them side by side (bottom). The localization of the plaque components Sid2, Sid4, Ppc89, Pcp1, and Cam1 and of the half-bridge component Sfi1 is indicated. (B) Localization at SPBs of Sid4-GFP and Sfi1-mRFP. Bar: 5 μ m. Insets: magnification of SPBs from cells numbered according to cell cycle progression in the left panel. Bars: 500 nm. (*Reproduced from Bouhleb et al., in press.*) (C) Fluorescence intensity profiles of Sfi1-mRFP and Sid4-GFP across SPBs shown in insets measured on a 4-pixels-wide line oriented as shown in white. These SPBs are the same as those shown in (B). Note that Sfi1 maximum intensity does not coincide with Sid4 maximum intensity (SPB 2,3), except for SPB 1 where the SPB-half-bridge axis might be oriented perpendicular to the field of view. After duplication (SPB 4), Sfi1 localizes between the two maxima of intensity of Sid4-GFP. Note the asymmetric peak of Sid4-GFP in SPB 3 suggesting that the assembly of the daughter SPB has started. (See color plate)

are C-terminally tagged with GFP (Chang & Gould, 2000) in combination with the half-bridge/bridge component Sfi1 (Bouhleb et al., in press; Lee et al., 2014; Ohta, Sato, & Yamamoto, 2012) C-terminally tagged with mRFP (Figure 1(B)). This analysis can be completed with strains expressing Sid2-GFP associated with the SPB cytoplasmic surface (Sparks, Morphew, & McCollum, 1999), and GFP-tagged calmodulin Cam1 or Pcp1 that associate with the nuclear-facing surface of the SPB (Flory, Morphew, Joseph, Means, & Davis, 2002; Fong, Sato, & Toda,

2010). Imaging is performed on methanol-fixed cells, which preserves the fluorescence of SPB components best, in order to block microtubule-dependent SPB oscillations that would blur the fluorescent signal and lower the resolution. Imaging can be performed on any classical epifluorescence microscope or on a spinning disc confocal microscope equipped with a high aperture 100 \times objective, an automated z control and a high resolution CCD camera. We typically use a DM 5000 B upright microscope (Leica Microsystems), equipped with a 100 \times /1.4NA oil immersion PlanApo objective, a Pifoc objective stepper, and a Coolsnap HQ CCD camera (Photometrics).

1.1 CELL GROWTH AND FIXATION

1. Grow cells at 25 °C in YE5S medium until exponential phase (OD at 595 nm 0.2–0.8).
2. Filter 20–30 mL of culture on 0.45 μ m Durapore membrane filter (Millipore, HVLP4700).
3. Place the filter into a 50 mL tube half-filled with cold methanol (–20 °C) and vortex.
4. Quickly remove the membrane from the tube and centrifuge cells at 4000 rpm for 2 min.
5. Remove methanol and rehydrate the cell pellet with 1 mL PEM buffer (100 mM Pipes, 1 mM EGTA, 1 mM MgSO₄).
6. Transfer cells to a 1.5 mL microcentrifuge tube and wash three times with 1 mL PEM.

At this stage, the cells resuspended in PEM buffer can be stored in the dark and at 4 °C for several days before imaging.

1.2 IMAGING SPBs IN FIXED CELLS

1. Mount 2 μ L of cells between coverslip and slide.
2. Select a field with numerous cells, focus on a medial focal plane, and acquire an image of cells in transmitted light or DIC mode to define cell contours.
3. Acquire two successive series of green and red z-stacks of nine planes spaced by 0.5 μ m (i.e., –2 μ m below to +2 μ m above the medial focal plane).
4. Image multichannel fluorescent beads (100 nm diameter) in the same conditions.

1.3 ANALYSIS OF SPB STATUS

1. Make maximum intensity projections of the green and red z-stacks. Check the perfect superimposition of signals between the two stacks acquired successively with the same color channel to ensure that the cells did not move during the imaging process. Check also the proper registration of red and green channels with the fluorescent beads.

2. Determine the SPB status based on the signal obtained for the SPB plaque component on high magnification views. A duplicated SPB appears as two juxtaposed points separated by the completely isotropic bridge signal. It can also appear as an elongated anisotropic signal when the two duplicated SPBs are not fully resolved. It should be noted that a minority of duplicated SPBs may be overlooked if the elongation axis is oriented perpendicular to the field of view. This is however expected to happen at low frequency only since duplicated SPBs orient along the cell long axis due to the spatial constraints imposed by cell geometry on microtubule bundles that are anchored to SPBs, and run parallel to their duplication axis (Vogel et al., 2007).
3. Trace the mean fluorescence intensity profile along a 4-pixels-wide line covering all the signal and parallel to the signal elongation axis using the linescan tool of Metamorph software, or equivalent, to confirm the SPB status. A broad signal with two maxima is observed upon duplication compared to the central single peak obtained for the half-bridge or bridge throughout the duplication cycle (Figure 1(C)).
4. Correlate the SPB duplication status with cell cycle progression as judged by cell length during G2 phase that can be measured from the recorded DIC or bright field image or presence of two separated SPBs during mitosis, or of two separated SPBs as well as a septum in postmitotic cells.

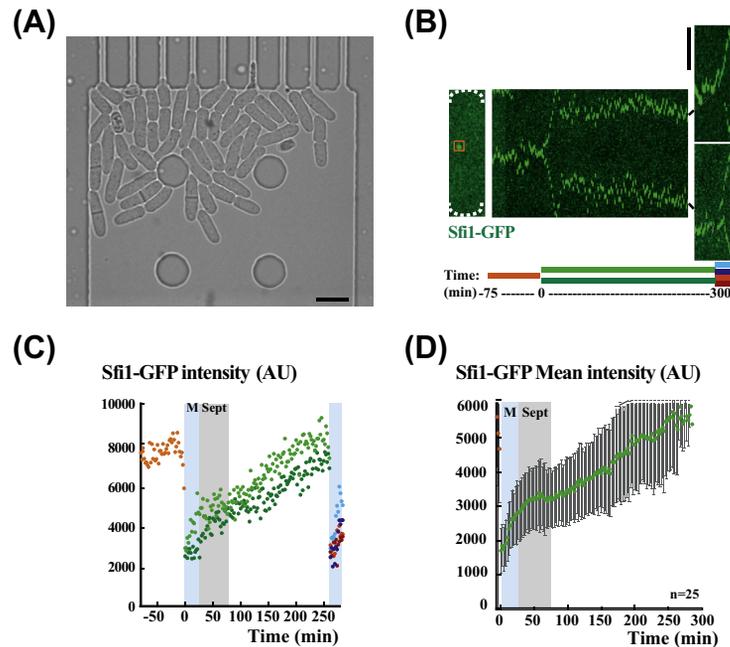
2. QUANTITATIVE ANALYSIS OF SPB BIOGENESIS IN LIVE CELLS

The SPB duplication cycle is accompanied by modifications of the content of individual components of SPB plaques and bridge, which can be tracked by quantitative live cell imaging. This method can complement the qualitative analysis of SPB duplication status proposed in the first part of the chapter.

2.1 PDMS CHAMBERS FOR LIVE IMAGING

Long-term imaging for over a cell cycle can be performed on agar pads as described earlier (Tran, Paoletti, & Chang, 2004) or in dedicated 4–5 μm thick poly-dimethylsiloxane (PDMS) microfluidic chambers stucked on glass coverslips (Figure 2(A)). In our hands, such chambers are better suited than agar pads for time-lapse movies with red fluorescent proteins. Production methods for such chambers have been described previously (Terenna et al., 2008; Velve-Casquillas, Le Berre, Piel, & Tran, 2010). Briefly:

1. Spin-coat Su-8 negative photoresist onto a silicon wafer (Su-8 2005 Microchem).
2. Transfer the features of a photomask (Microtronics Engineering GmbH) produced according to chamber design in L-edit software (Tanner EDA) by

**FIGURE 2**

Quantitative analysis of SPB biogenesis by live cell imaging. (A) Transmitted light image of fission yeast cells growing in a PDMS chamber for live imaging. Bar: 10 μm . (B–C) Kymograph of SPBs in a wild-type cell expressing Sfi1-GFP and quantitative analysis of Sfi1-GFP intensity at SPB during cell cycle progression. Bars in (B) (bottom) represent SPBs tracked in (C) with a similar color code. Bar: 5 μm . $t = 0$: SPB segregation. M: Mitosis (blue (very light gray in print versions) zone). Sept: septation (gray zone). *Reproduced from Bouhlej et al., in press.* (D) Mean Sfi1-GFP intensity at SPB before (orange (light gray in print versions)) and after segregation (green (dark gray in print versions)) at $t = 0$. $n = 25$. Bars: SD. *Reproduced from Bouhlej et al., in press.*

laser-etching into a thin layer of chromium on a quartz plate onto the photoresist layer, by exposure and cross-linking with UV light (365 nm).

3. Develop the photoresist with the developer (Su-8 developer, Microchem) and clean it with isopropyl alcohol and nitrogen gas.
4. Use the wafer as a master mold to repeatedly cast PDMS chambers (Sylgard 184, Dow Corning).
5. Assemble chambers by peeling off a PDMS replica from a mold, introducing inlet and outlet holes, and bonding the replica to a glass coverslip after surface activation with a plasma cleaner (Harrick Scientific).
6. Insert metallic connectors in inlet and outlet holes and connect them to appropriate Tygon tubing to allow cell injection into the chamber with a syringe and needle after filling the chamber with YE5S medium.

2.2 LIVE CELL IMAGING

Time-lapse imaging can be performed on an automated spinning disc confocal microscope equipped with a temperature control box to maintain cells at a constant temperature of 25 °C, a high aperture 60× or 100× objective, and a sensitive CCD camera or an EMCCD camera. We typically use Nikon Eclipse Ti-E microscope equipped with the Perfect Focus System, a 100×/1.45 NA PlanApo oil immersion objective, a Mad City Lab piezo stage, a Yokogawa CSU×1 confocal unit, a Photometrics HQ2 CCD camera, and a laser bench (Errol) with 491 and 561 nm diode lasers of 100 mW each (Cobolt). Lasers should be set at a very low power to limit fluorescence bleaching during time-lapse movies. With a 100× objective, camera binning can be set to 2 to increase signals. Acquisition is made in nine-plane z-stacks (+2 μm to −2 μm every 0.5 μm) to always capture SPBs even if their z position changes over time. Acquisitions are performed every 2–5 min depending on the brightness of the signal, to allow proper capture of intensity variations at different phases of the duplication cycle while limiting bleaching that would alter intensity measurements.

2.3 QUANTITATIVE ANALYSIS OF SPB BIOGENESIS

Simple image analysis allows extracting quantitative data from time-lapse movies.

1. Perform quantitative analysis of SPB intensity over time manually on sum or maximum projections of z-stacks in Metamorph (Molecular devices) or ImageJ software by measuring the integrated fluorescence intensity within a small rectangle of a few pixels wide (5–10) centered on the SPB at each time point (Figure 2(B)). Of note, maximum projections are well suited for SPBs that do not span over several planes due to their limited size. Alternatively, automatic tracking of SPBs can be implemented to speedup the analysis using dedicated softwares such as MIA (Racine et al., 2006).
2. Measure background intensity in a square of similar size placed in the cell cytoplasm and deduce it from the SPB intensities measured in 1.
3. Plot SPB intensity over time to follow the SPB duplication cycle. Mitotic entry can be recognized by the sudden drop in SPB intensity when the two SPBs segregate from one another. Intensity increase during the subsequent cell cycle reflects the behavior of specific SPB components during SPB biogenesis (Figure 2(C)).
4. Generate mean curves for several SPBs after time registration between cells selecting an appropriate time reference such as mitotic entry or anaphase completion as evidenced by SPB separation and maximum distance (Figure 2(D)).
5. Estimate overall bleaching rate during the movie by measuring the mean intensity of all SPBs at the first and last time points. Bleaching corrections can be introduced if necessary assuming an exponential decay of fluorescence over time.
6. Complete the analysis by ratio measurement between different time points along the cell cycle, for instance, between mitotic entry and end of anaphase.

CONCLUSION

The simple linear geometry of side-to-side duplicated SPBs allows for their direct visualization by classical high resolution light microscopy in strains expressing SPB plaque components tagged with fluorescent proteins. This property permits an easy monitoring of SPB duplication process that can be coupled to quantitative analysis of fluorescent signals to describe the dynamics of their accumulation on SPBs as cells progress along the cell cycle. Such methods were recently used to describe the behavior of the SPB component Sfi1 (Bouhlel et al., *in press*; Lee et al., 2014). In this particular case, based on fluorescence intensity profiles, we managed to distinguish Sfi1 localization on the half-bridge, from that of components of SPB plaques, before or after SPB duplication. Quantitative analysis of fluorescent signals allowed us to identify two phases of Sfi1 accumulation. The first one, at mitotic exit, could be attributed to half-bridge duplication, an event that precedes the assembly of the new SPB. It also revealed that the bridge is destabilized at mitotic entry and loses about a third of its Sfi1 molecules when it splits into two half-bridges. We found that this event is under the control of Cdc31 phosphorylation on a Cdk1 consensus site and controls the timely separation of the two SPBs necessary for bipolar spindle assembly. Quantitative analysis of fluorescence was also used recently to study the nuclear side SPB component Pcp1. This revealed a late phase of accumulation, shortly before mitosis entry (Walde & King, 2014). These examples illustrate how the systematic use of quantitative microscopy can reveal the differential behavior of various SPB components during SPB biogenesis and help deciphering how this complex process is controlled molecularly.

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REFERENCES

- Adams, I. R., & Kilmartin, J. V. (2000). Spindle pole body duplication: a model for centrosome duplication? *Trends in Cell Biology*, *10*, 329–335.
- Bouhlel, I. B., Ohta, M., Mayeux, A., Bordes, N., Dingli, F., Boulanger, J., et al. Cell cycle control of spindle poles bodies duplication and splitting by Sfi1 and Cdc31 in fission yeast. *Journal of Cell Science*, *in press*.
- Chang, L., & Gould, K. L. (2000). Sid4p is required to localize components of the septation initiation pathway to the spindle pole body in fission yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 5249–5254.

- Ding, R., West, R. R., Morpew, D. M., Oakley, B. R., & McIntosh, J. R. (1997). The spindle pole body of *Schizosaccharomyces pombe* enters and leaves the nuclear envelope as the cell cycle proceeds. *Molecular Biology of the Cell*, *8*, 1461–1479.
- Elserafy, M., Saric, M., Neuner, A., Lin, T. C., Zhang, W., Seybold, C., et al. (2014). Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Current Biology*, *24*, 1456–1466.
- Flory, M. R., Morpew, M., Joseph, J. D., Means, A. R., & Davis, T. N. (2002). Pcp1p, an Spc110p-related calmodulin target at the centrosome of the fission yeast *Schizosaccharomyces pombe*. *Cell Growth & Differentiation*, *13*, 47–58.
- Fong, C. S., Sato, M., & Toda, T. (2010). Fission yeast Pcp1 links polo kinase-mediated mitotic entry to gamma-tubulin-dependent spindle formation. *EMBO Journal*, *29*, 120–130.
- Jaspersen, S. L., & Ghosh, S. (2012). Nuclear envelope insertion of spindle pole bodies and nuclear pore complexes. *Nucleus*, *3*, 226–236.
- Jaspersen, S. L., & Winey, M. (2004). The budding yeast spindle pole body: structure, duplication, and function. *Annual Review of Cell and Developmental Biology*, *20*, 1–28.
- Kilmartin, J. V. (2003). Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. *Journal of Cell Biology*, *162*, 1211–1221.
- Lee, I. J., Wang, N., Hu, W., Schott, K., Bahler, J., Giddings, T. H., Jr., et al. (2014). Regulation of spindle pole body assembly and cytokinesis by the centrin-binding protein Sfi1 in fission yeast. *Molecular Biology of the Cell*, *25*, 2735–2749.
- Lim, H. H., Zhang, T., & Surana, U. (2009). Regulation of centrosome separation in yeast and vertebrates: common threads. *Trends in Cell Biology*, *19*, 325–333.
- Li, S., Sandercock, A. M., Conduit, P., Robinson, C. V., Williams, R. L., & Kilmartin, J. V. (2006). Structural role of Sfi1p-centrin filaments in budding yeast spindle pole body duplication. *Journal of Cell Biology*, *173*, 867–877.
- Ohta, M., Sato, M., & Yamamoto, M. (2012). Spindle pole body components are reorganized during fission yeast meiosis. *Molecular Biology of the Cell*, *23*, 1799–1811.
- Paoletti, A., Bordes, N., Haddad, R., Schwartz, C. L., Chang, F., & Bornens, M. (2003). Fission yeast cdc31p is a component of the half-bridge and controls SPB duplication. *Molecular Biology of the Cell*, *14*, 2793–2808.
- Racine, V., Hertzog, A., Jouanneau, J., Salamero, J., Kervrann, C., & Sibarita, J.-B. (2006). Multiple-target tracking of 3D fluorescent objects based on simulated annealing. In *ISBI* (pp. 1020–1023).
- Rosenberg, J. A., Tomlin, G. C., McDonald, W. H., Snyderman, B. E., Muller, E. G., Yates, J. R., III, et al. (2006). Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. *Molecular Biology of the Cell*, *17*, 3793–3805.
- Sparks, C. A., Morpew, M., & McCollum, D. (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. *Journal of Cell Biology*, *146*, 777–790.
- Terenna, C. R., Makushok, T., Velve-Casquillas, G., Baigl, D., Chen, Y., Bornens, M., et al. (2008). Physical mechanisms redirecting cell polarity and cell shape in fission yeast. *Current Biology*, *18*, 1748–1753.
- Tran, P. T., Paoletti, A., & Chang, F. (2004). Imaging green fluorescent protein fusions in living fission yeast cells. *Methods*, *33*, 220–225.
- Uzawa, S., Li, F., Jin, Y., McDonald, K. L., Braunfeld, M. B., Agard, D. A., et al. (2004). Spindle pole body duplication in fission yeast occurs at the G1/S boundary but maturation is

blocked until exit from S by an event downstream of *cdc10+*. *Molecular Biology of the Cell*, 15, 5219–5230.

Velve-Casquillas, G., Le Berre, M., Piel, M., & Tran, P. T. (2010). Microfluidic tools for cell biological research. *Nano Today*, 5, 28–47.

Vogel, S. K., Raabe, I., Dereli, A., Maghelli, N., & Tolić-Nørrelykke, I. (2007). Interphase microtubules determine the initial alignment of the mitotic spindle. *Current Biology*, 17, 438–444.

Walde, S., & King, M. C. (2014). The KASH protein Kms2 coordinates mitotic remodeling of the spindle pole body. *Journal of Cell Science*, 127, 3625–3640.

Titre : Fonctions de la protéine centrosomale Sfil chez la levure et l'homme

Mots clés : Centrosome, Centriole, SPB, mitose, division cellulaire

Le centrosome est le centre organisateur des microtubules dans les cellules animales, il nucléé les microtubules interphasiques ainsi que le fuseau mitotique. Les centrosomes sont produits par duplication, mécanisme rigoureusement régulé au cours du cycle cellulaire. En effet, un centrosome comporte deux centrioles qui se dupliquent une seule fois par cycle cellulaire. Des erreurs de duplication conduisant à plus de deux centrosomes induisent la formation de fuseaux multipolaires et provoquent des défauts de ségrégation des chromosomes.

Chez la levure *Schizosaccharomyces pombe*, un organisme modèle pour l'étude de la division cellulaire, les homologues des centrosomes sont les SPBs (pour Spindle Pole Body). Une structure annexe spécifique liée aux SPBs est appelée demi-pont (quand les SPBs ne sont pas dupliqués) puis pont (quand elle relie les deux SPBs dupliqués). Les deux principaux composants du pont chez la levure *S. pombe* sont Cdc31 et Sfil. Sfil est une protéine linéaire formée de répétitions en hélice α formant des sites de liaison pour la Centrine/Cdc31. Sfil s'assemble en réseau de molécules parallèles interagissant avec le SPB via leur domaine N-terminal. Dans la première partie de ma thèse, je me suis intéressée aux fonctions de Sfil chez la levure. Cette étude a permis de démontrer que Sfil

est un composant du demi-pont et qu'il est essentiel pour la duplication des SPBs et l'assemblage d'un fuseau bipolaire. De plus, nous avons déterminé que le pont est dupliqué en fin de mitose. Enfin, nous avons aussi montré que la déstabilisation du pont menant à sa rupture en mitose, dépend de la phosphorylation de Cdc31 par la kinase mitotique Cdkl.

Lors de la seconde partie de ma thèse, je me suis intéressée au complexe Sfil/Centrine dans les cellules humaines. J'ai confirmé que Sfil est localisée aux centrioles. De plus, j'ai montré que la déplétion de Sfil dans les cellules RPE1, conduit à une perte de localisation de la Centrine, suggérant soit un défaut de recrutement, soit une déstabilisation. De plus, en absence de Sfil, les cellules RPE1 ne sont plus capables de former de cil primaire. Ce résultat suggère que Sfil et la Centrine sont requis pour la ciliogénèse. Enfin, j'ai aussi démontré que la déplétion de Sfil induit un arrêt de cycle cellulaire dans les cellules non tumorales RPE1. Dans les cellules cancéreuses, HeLa, le cycle n'est pas arrêté mais j'ai pu observer une prolongation du temps de mitose.

En conclusion mes travaux montrent que bien que la fonction de Sfil/Centrine ne soit pas conservée, le complexe reste essentiel pour l'intégrité structurale et fonctionnelle du centrosome.

Title: The Centrin-Binding Protein Sfil: Functions in Fission Yeast and Human

Keywords: Centrosome, Centriole, SPB, mitosis, cell division

The centrosome is the main microtubule organizing center. It nucleates and organizes interphase microtubule and contributes to the assembly of the bipolar mitotic spindle. To do so, the centrosome, present in one copy at the beginning of the cell cycle, duplicates to produce a second copy. The duplication process is tightly controlled and regulated since centrosome over-duplication can lead to multipolar mitotic spindles and promote genome instability and tumorigenesis.

The duplication of the yeast centrosome, the SPB (Spindle pole body), begins with the duplication of the half bridge. This appendage is composed of Sfil/Cdc31 complex organized in a parallel array attached to the core SPB. SPB duplication relies on the assembly of a second array of Sfil/Cdc31, anti-parallel to the first one, creating thereby an assembly site for the new SPB.

Therefore, Sfil is essential for SPB duplication and our work defined the timing of half-bridge duplication and

some of the regulatory mechanisms that favor bridge splitting to release duplicated centrosomes and allow spindle assembly at mitotic onset.

Sfil and Cdc31/Centrins are conserved in human cells where the centrosome is composed of two centrioles surrounded by the pericentriolar material. Centrins are concentrated in the distal end of centrioles. Sfil has also been localized to centrioles, but its function remained unknown. Thus, we started investigating Sfil function in human cells. We found that Sfil depletion leads to a decrease in Centrin recruitment to the centrioles. It also leads to a cell cycle arrest in G1 in RPE1 cells, an event previously observed in presence of defects in centriole biogenesis. In HeLa cells where the cell cycle is not affected, Sfil depletion leads to a mitotic delay. Moreover, Sfil depletion leads to cilium assembly. To conclude, these results altogether point towards a role of human Sfil in centriole biogenesis.

