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MICROTUBULE AND CHROMOSOME DYNAMICS DURING MITOSIS IN BUDDING YEAST

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Microtubule and chromosome dynamics during mitosis in budding yeast
Thesis for Doctoral Degree (Ph.D.)

By

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Popular science summary of the thesis

Cell division is a process where a mother cell duplicates, generating a daughter cell. When a cell divides it must first copy its DNA, the blueprint for cellular functions, and then carefully distribute it evenly between the mother cell and the newly created daughter cell. The DNA resides inside the nucleus of the cell in the form of several chromosomes. Chromosomes are long threads of DNA, wrapped around proteins which compacts the DNA and enables the cell to regulate how and when DNA is used. When a chromosome has been copied, the two copies are held together, preventing them from separating prematurely. Cells have evolved an elegant system to ensure that the mother and daughter cells each receive an equal set of chromosomes when they divide. This is important, as receiving an incomplete set, or too many chromosomes, can lead to cell death or disease (such as cancer).

So how does it work? The cell creates long cylindrical filaments called microtubules that grow from two poles in the cell. Each chromosome of the copied pairs become attached to opposing poles through the microtubules. A single microtubule will bind to each of the chromosomes through a large protein complex called the kinetochore. As the cell divides, the mother and daughter cell will each inherit one of the poles and the chromosomes attached to it (Figure 3 in chapter 1.2.1 shows an outline of what this looks like). This way, the chromosomes are segregated equally between mother and daughter cells.

All the experiments in this thesis have been performed using baker's yeast as a model organism to understand fundamental aspect of cell biology. Baker's yeast (*Saccharomyces cerevisiae*, also known as budding yeast) makes it easier and cheaper to design many experiments compared to other cell model systems. It's also a great system to study cell division, as many core mechanisms are very similar to those in human cells.

In this thesis, I present two papers that have investigated different aspects of microtubule regulation, both at the poles that create the microtubules, and at the ends of microtubules where they connect to the kinetochores and chromosomes. I also present a study of how loops of chromosomal DNA affect the distance between specific locations on the of the chromosome.

In **paper I**, we have studied the function of a protein called Bik1 that has previously been found to bind to ends of microtubules. We did this by changing the protein so that it is forced out of the nucleus and can no longer bind to the microtubules that connect to the kinetochores. We then looked at how cells without Bik1 in the nucleus divide. This revealed that cells without nuclear Bik1 divide slower than normal cells and lose the ability to neatly cluster the kinetochores before separating the chromosomes. The same

defect has been observed in yeast cells lacking motor proteins that walk along the microtubules. This led us to explore if Bik1 works together with such motor proteins, and could show that Bik1 indeed is in close proximity to some of those motor proteins and partially depends on one such motor to associate with the kinetochores. Although we still don't understand the exact molecular mechanisms, the study has identified a previously unknown function of the Bik1 protein and new clues how chromosomes are faithfully segregated during cell division.

In **paper II** we investigated how the poles that create microtubules are modified inside the cell. As a cell prepares to divide, it not only copies its DNA, but also the pole that creates microtubules. In yeast, these are known as spindle pole bodies, or SPBs for short. These SPBs are chemically modified by the cell in order to control their function. Hundreds of these of modifications have been identified, but the function of most modifications is still not well understood.

A newly created cell has a single SPB that was inherited from the mother cell, and when it's time for the cell to divide, the SPB must be duplicated. Interestingly, previous research has shown that the old SPB and the newly created one will be modified differently from each other, and that this is important for cells to divide efficiently.

To better understand how old and new SPBs are modified by the cell, we adapted an existing technique to separate old and new components of the SPB and analyzed their chemical composition. This revealed modifications that were only present in old SPB components. To understand what the function of those modifications are in the cell, we genetically modified yeast cells in a way that prevents those specific modifications from occurring. This resulted in cells dividing slightly slower than normal, and with brighter microtubules, suggesting that the SPB modifications we identified are important for the cell to regulate microtubules. We believe these modifications to be important for cells to divide efficiently, and possibly helps the cell when it is disassembling the microtubules after dividing.

In **paper III** we describe a system to study how chromosomes behave in live yeast cells using microscopy. As mentioned above, chromosomes are large, bundled thread structures that consist of a single, long molecule of DNA and proteins. The organization of the DNA fiber is not random. Instead, specific regions of the chromosome are more likely to be in close contact and many such areas are actively shaped into loops. The organization of DNA into these structures has been shown to be important for regulating how the DNA is utilized by the cell, and also helps in compacting the long DNA molecules to fit inside the nucleus. Much of the work that has helped us understand how DNA is organized in cells comes from studies that report the average organization from millions of cells. Such studies give detailed information about all regions of every chromosome; however, they do not tell us how the DNA of individual cells are organized. This has

proven to be an important distinction, as some recent studies suggest that fully formed chromosomal loops are not the norm. Instead, regions that can form loops were found to be non-looped or in partially looped states. To better understand how baker's yeast chromosomes are organized, we have generated a system that allows us to follow two specific positions of a single chromosome in living cells using microscopy. We then used this system in cells where the machinery that creates the loops is unable to be loaded onto the chromosomes. By measuring the distance between the two regions in cells with and without chromosomal loops, we observed little difference between the two scenarios. This suggests that chromosomal looping does not have a major influence on overall distances between these two regions. In contrast, when we followed how this distance changed over time in live cells, we observed that the cells without loops were more dynamic and had a greater change in distance over time. This suggests that chromosomal looping restricts the dynamic movement of chromosomal regions. In the future, this technique could be used to better understand how specific chromosomal regions behave and can complement the information gained from other techniques.

In summary, this thesis presents new data on how microtubules, kinetochores and chromosomes are regulated in yeast cells. It provides novel insight into fundamental mechanisms that enable cells to divide.

Abstract

As a cell divides, DNA must be replicated and faithfully segregated between the mother and daughter cells. This segregation is facilitated by the mitotic spindle, assembled to pull sister chromatids apart as the cell divides. In budding yeast, spindle pole bodies nucleate microtubules that make up the mitotic spindle, position it at the site of division, and physically link chromosomes to opposing poles via the kinetochores. The chromosomes are held together by cohesin, which is also involved in the architecture of chromatin.

In this thesis, I have explored mechanisms controlling microtubule dynamics, kinetochore positioning and chromosome dynamics during mitotic cell division in budding yeast.

Bik1 is a microtubule-associated protein shown to play a role in the cytosol to position the spindle before anaphase. In **paper I**, we have characterized the nuclear function of Bik1 and identified a novel role in clustering kinetochores prior to spindle elongation. Cells lacking nuclear Bik1 have a delayed cell cycle progression, with prolonged metaphase, and fail to cluster kinetochores. We also connect this function to the nuclear kinesin Cin8, which has previously been described to regulate kinetochore microtubule dynamics in metaphase.

The spindle pole body anchors microtubule nucleating γ-tubulin complexes using two different receptors, Spc72 in the cytosol and Spc110 in the nucleus. In **paper II**, we have isolated 'old' Spc110, originating from the previous cell cycle, and mapped its phosphorylation sites. These analyses revealed that old Spc110 is phosphorylated at serine 36 and at a novel site, serine 11. Non-phosphorylatable mutant strains revealed that these sites influence microtubule dynamics and cell cycle progression. The Spc110S11A mutant strain frequently had brighter spindle microtubules with asymmetric distribution of α-tubulin. Furthermore, Spc110S11A S36A cells had slightly delayed cell cycle progression and spindle disassembly.

The cohesin complex has been shown to shape the chromosomes into loops in budding yeast through a mechanism known as loop extrusion. This phenomenon has primarily been studied using genome-wide sequencing techniques, which report detailed population averages of contact frequencies throughout the genome. How chromosomes of individual cells are affected, and whether this looping affects physical compaction remains poorly understood. In **paper III** we have generated a microscopy-based system to study chromosome dynamics in single yeast cells by fluorescently tagging specific chromosomal loci. We then used this system to investigate how physical distances between the fluorescently marked loci change after inhibiting loop extruding cohesin.

This study revealed that loop extrusion does not significantly affect physical distances but may limit the dynamic movement of chromosomes.

In conclusion, these studies reveal novel mechanisms controlling spindle and chromosome dynamics during mitotic cell division: 1) We have uncovered a new role of Bik1 at the spindle. 2) We have mapped phosphorylation sites in old Spc110 and characterized a novel site. 3) We have created a system to study chromosome dynamics in single cells and found that loop extrusion does not significantly compact mitotic yeast chromosomes.

List of scientific papers

I. **Alexander Julner**, Marjan Abbasi, Victoria Menéndez-Benito; The microtubule plus-end tracking protein Bik1 is required for chromosome congression. Molecular biology of the cell, 2022, Vol.33 (5), br7. doi: 10.1091/mbc.E21-10-0500

II. Marjan Abbasi, **Alexander Julner**, Yan Ting Lim, Tianyun Zhao, Radoslaw Mikolaj Sobota, Victoria Menéndez-Benito; Phosphosites of the yeast centrosome component Spc110 contribute to cell cycle progression and mitotic exit. Biol Open 1 November 2022; 11 (11): bio059565. doi: 10.1242/bio.059565

III. **Alexander Julner,** Kristian Jeppsson, Camilla Björkegren; The effect of DNA loop extrusion on chromosome dynamics in budding yeast. Manuscript

Contents

List of abbreviations

+TIP	Microtubule plus-end tracking protein
AID	Auxin-inducible degron
aMT	Astral microtubule
APC/C	Anaphase promoting complex/cyclosome
BiFC	Bi-molecular fluorescence complimentation
Bik1	Bilateral karyogami 1
Bim1	Binding to microtubule 1
Bub3	Budding inhibited by benzimidazole 3
Cdc20	Cell division cycle 20
CDE	Centromere-determining element
Cdh1	Cdc20 homolog 1
CDK	Cyclin-dependent kinase
CEN	Centromere
Cfi1	Cdc14 inhibitor 1
CG	Cap-glycine
CH	Calponin homology
CP	Central plaque
Cryo-EM	Cryo-electron microscopy
CTCF	CCTC-binding factor
FEAR	Cdc14 early anaphase release
FISH	Fluorescence *in situ* hybridization
FRET	Förster resonance energy transfer
IL	Intermediate layer
KT	Kinetochore
KT MT	Kinetochore microtubule
MAD	Mitotic arrest-deficient
MAP	Microtubule-associated protein
MCC	Mitotic checkpoint complex
MEN	Mitotic exit network

MSCD	Mean squared change in distance
MSD	Mean squared displacement
MT	Microtubule
MTOC	Microtubule organizing center
NES	Nuclear export signal
Net1.	Nucleolar silencing establishing factor and telophase regulator 1
NIPBL	Nipped-B-Like
PTM	Post-translational modification
RITE	Recombination-induced tag exchange
SAC	Spindle assembly checkpoint
SMC	Structural maintenance of chromosomes
SPB	Spindle pole body
SPOC	Spindle position checkpoint
STAG	Stromal antigen
γ-TuRC	γ-Tubulin ring complex
γ-TuSC	γ-Tubulin small complex

1 Literature review

1.1 Yeast mitotic spindle

The work presented in this thesis revolves around the machinery that segregates the chromosomes during cell division. These structures are together known as the mitotic spindle and is made up of several components: 1) The spindle pole body (SPB) that organizes the spindle. 2) Long protein filaments called microtubules (MTs). 3) A protein interphase that physically connects the chromosomes to the MTs called kinetochores (KTs). Not only does the mitotic spindle separate the genomic material between mother and daughter cells, but it also plays regulatory roles to ensure that each cell ends up with a single copy of each chromosome. In this section, I will present the components of the mitotic spindle and associated proteins.

1.1.1 Spindle pole bodies

Centrosomes are the main microtubule organizing centers (MTOCs) of metazoan cells and serve to organize the mitotic spindle during mitosis and meiosis. At the start of the cell cycle each cell has a single centrosome, made up of two cylindrical centrioles surrounded by amorphous pericentriolar material. The centrioles are made up of nine groups of triplet MTs rather than the doublets that make up microtubule filaments (Winey & O'Toole, 2014). For a cell to divide, the centrosome must be duplicated to function as opposing poles that segregate a complete set of the replicated chromosomes to the mother and daughter cells. Spindle pole bodies (SPBs) are the yeast equivalent of centrosomes and, like centrosomes, organize MTs for faithful segregation of chromosomes during cell division. Unlike centrosomes, the budding yeast SPB is a layered structure that resides embedded in the nuclear envelope throughout the cell cycle. At the start of the cell cycle it exists as a single copy and will be duplicated once to be able to form the mitotic spindle. Interestingly, the duplication occurs in a conservative fashion, meaning that a dividing cell will have SPBs that differ in age.

The SPB is made up of at least 5 distinct layers (reviewed in (Jaspersen & Winey, 2004)): The central plaque (CP), intermediate layer 1 and -2 (IL1 and 2) and an inner and an outer plaque (Figure 1). The CP lies embedded in the nuclear envelope, and IL1 and 2 sit between the CP and outer plaques. The inner and outer plaques face towards the nuclear and cytosolic sides, respectively, and anchor the MT nucleating γ-tubulin complexes. Additionally, mature SPBs have an extension protruding parallel to the nuclear envelope, known as a half-bridge. This structure will later be the site of SPB

duplication. In total, there are 18 core proteins that make up the SPB (Table 1). Although budding yeast SPBs are structurally different from centrosomes, several components of the SPB are homologs of centrosomal proteins.

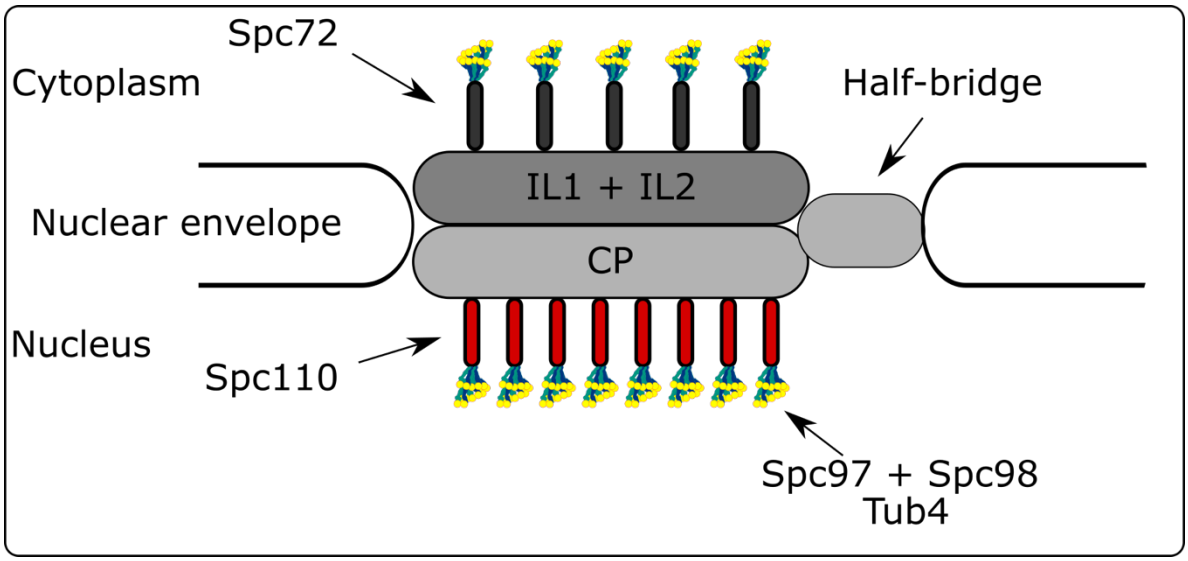

Figure 1 - Structure of the budding yeast spindle pole body. The SPB is a layered structure that lies embedded in the nuclear envelope. The different layers are indicated. γ-tubulin complex receptors Spc72 and Spc110 are located at the outer and inner plaques, respectively. IL1/2 – Intermediate layers 1 and 2, CP – central plaque.

The structure of the SPB core was first explored using cryo-electron microscopy (cryo-EM) of cells overexpressing Spc42. These studies revealed a hexagonal crystal lattice of Spc42 molecules that is embedded in the nuclear envelope to make up the CP (Bullitt et al., 1997; Donaldson & Kilmartin, 1996; O'Toole et al., 1999). This structure was refined through Förster resonance energy transfer (FRET) experiments. CFP- and YFP-tags were introduced at the C- or N-terminus in pairs of SPB CP components followed by measurements of FRET signal intensity. As FRET efficiency is proportional to the distance between the two fluorescent proteins, the orientation and relative distances between CP components could be mapped in live cells (Muller et al., 2005). This revealed that Spc42 spans from IL2 and extends into the CP. Furthermore, the C-terminal end of Spc42 binds Cnm67 in IL2, while the N-terminal end in the CP binds the C-terminal ends of Spc110, Spc29 and Cmd1. Spc110 extends from the CP with its N-terminal end binding the γ-tubulin small complex (γ-TuSC) in the inner plaque. The outer plaque similarly anchors γ-TuSC with the N-terminal end of Spc72. Spc72 connects to IL2 via Cnm67 and the protein Nud1, which has additional roles in mitotic exit network signaling (Stegmeier & Amon, 2004).

Protein	Function/localization	Human homolog
Tub4	γ-Tubulin	TUBG1/2
Spc97	γ-TuSC	GCP2
Spc98	γ-TuSC	GCP3
Spc72	Cytoplasmic γ-TuSC linker	
Spc110	Nuclear γ-TuSC linker	Pericentrin/kendrin*
Cmd1	Core (CP)	Calmodulin
Nud1	Core (IL1)	Centriolin
Cnm67	Core (IL1/2)	
Spc42	Core (CP)	
Spc29	Core (CP)	
Mps2	Membrane	
Ndc1	Membrane	Ndc1
Nbp1	Membrane	
Bbp1	Membrane	
Mps3	Half-bridge/Duplication	
Kar1	Half-bridge/Duplication	
Cdc31	Half-bridge/Duplication	Centrin3
Sfi1	Half-bridge/Duplication	

Table 1. *Core components of the budding yeast SPB. Human homologues obtained from the Saccharomyces genome database (Cherry et al., 2012) homology section (sourced from the Alliance of genome resources database (Agapite et al., 2022)). *Spc110 homology based on (Flory et al., 2000).*

Like centrosomes, the main role of the SPB is to nucleate microtubules and build the mitotic spindle. In budding yeast, it does so via MT nucleating γ-TuSCs anchored to the inner and outer plaques. The budding yeast γ-TuSC consists of two γ-tubulin subunits (Tub4), each bound to one copy of Spc97 and Spc98 (Knop, 1997). Cryo EM studies of *in vitro* reconstituted γ-TuSC shows a y-shaped structure where Spc97 and Spc98 make up the stem and arms, and Tub4 is located at the ends of the arms (Kollman et al., 2008). Flexibility in one of the arms is thought to give rise to an active and an inactive conformation, where opening the arms suppresses the nucleating activity (Kollman et al., 2008, 2015). Furthermore, γ-TuSCs can oligomerize into rings with a structure that matches a 13 protofilament MT (explained in chapter 1.1.6). Spc110 stabilizes oligomerization and γ-TuSC rings are found in in the closed, active conformation when bound to MTs. However, it is not currently known how this conformational shift occurs (Kollman et al., 2008, 2015). Taken together, γ-TuSCs anchor at the SPB to nucleate microtubules through oligomerization, forming a base from which MTs can grow.

1.1.2 SPB phosphorylation

The SPB is heavily phosphorylated throughout the cell cycle. Isolation of intact SPBs followed by mass spectrometry has revealed that virtually all core proteins of the budding yeast SPB are phosphorylated, and the phosphorylation states of individual sites and components vary depending on the cell cycle stage (Fong et al., 2018; Keck et al., 2011). Although hundreds of SPB phosphorylation sites have been identified, few have been functionally characterized. However, SPB duplication has been linked to phosphorylation by specific kinases. The kinase Monopolar spindle 1 (Mps1) is a key regulator of SPB duplication and has been shown to phosphorylate Spc42, Spc110, Spc98 and Spc29 (Castillo et al., 2002; Friedman et al., 2001; Holinger et al., 2009; Lauzé et al., 1995; Pereira et al., 1998; Schutz & Winey, 1998; Weiss & Winey, 1996; Winey et al., 1991). Inactivating Mps1 results in monopolar spindles and failure to elongate the SPB half-bridge during SPB duplication (Winey et al., 1991). Furthermore, different inactivating mutations of Mps1 halt SPB duplication at various stages, suggesting that Mps1 is needed for multiple steps in this process (Castillo et al., 2002; Schutz & Winey, 1998). Furthermore, SPB duplication is tightly regulated to only occur once per cell cycle. This has been shown to depend on phosphoryalation of the half-bridge component Sfi1 via budding yeast Cdk1 and polo kinase Cdc5, which is thought to block SPB duplication (Avena et al., 2014; Elserafy et al., 2014). The phosphatase Cdc14 reverses these phosphorylations during mitotic exit (covered in chapter 1.2.5) to prime the SPB for duplication during the next cell cycle.

Another well-studied function of SPB phosphorylation is regulation of MTs via phosphorylation of the γ-tubulin complex and its receptors. Mutational studies show that phosphorylation of the γ-tubulin complex is important for proper cell cycle progression and spindle morphology (Keck et al., 2011; T. chen Lin et al., 2011; Vogel et al., 2001). Mutating phosphorylation sites in the γ-tubulin complex that potentially modulate interactions with Spc110 affects spindle length, cell cycle progression, tubulin distribution and even compromises viability (Table 1,(Fong et al., 2018; Friedman et al., 2001; Huisman et al., 2007; Keck et al., 2011; T. C. Lin et al., 2014; T. chen Lin et al., 2011; Nazarova et al., 2013; Vogel et al., 2001)). Similarly, phosphomimetic mutations in the γ-tubulin complex cause spindles to elongate slower, hyper elongation of spindles and confer temperature sensitivity (Table 1). Furthermore, other phosphomimetic mutations modulate the recruitment of Tub4 to SPBs, with $Tub4^{S100E}$ decreasing Tub4 recruitment and $Tub4^{S74E}$ increasing recruitment (T. chen Lin et al., 2011). Additionally, the Cdk1 site S360 has been implicated in regulation of spindle formation, as a phosphomimetic mutant was shown to fail to generate interpolar MTs during spindle assembly (Nazarova et al., 2013).

MT dynamics are also affected by phosphorylation of the γ-tubulin complex anchor Spc110 (Table 1). As described in chapter 1.1.1, Spc110 links the central plaque to the inner

plaque, anchoring the γ-tubulin complex on the nuclear side of the SPB via its N-terminal domain (Knop, 1997; Knop & Schiebel, 1998; Sundberg & Davis, 1997). Early studies showed that Spc110 is phosphorylated in a cell cycle-dependent manner, with low levels of phosphorylated protein in G1, and a peak of phosphorylated protein in metaphase cells (Friedman et al., 1996; Stirling & Stark, 1996). These observations were based on the appearance of a 120 KDa band of Spc110 when analyzed by gel electrophoresis. This 120 KDa band was subsequently shown to be dependent on Mps1 and could be reconstituted *in vitro* by incubating Spc110 with Mps1 (Friedman et al., 2001). Mass spectrometry identified the Spc110 phosphorylation sites targeted by Mps1 *in vitro* as S60, T64 and T68. Although not recognized as a target of Mps1, the study found that mutating serine 36 in combination with the three identified sites resulted in a synthetic lethal phenotype when combined with mutations of the γ-TuSC subunit Spc97. Later studies identified serine 36 and serine 91 as cyclin-dependent kinase 28 (Cdk28, lone CDK of budding yeast) phosphorylation sites, and non-phosphorylatable mutants resulted in longer spindles at metaphase (Huisman et al., 2007). Furthermore, phosphomimetic mutations of the N-terminal domain of Spc110 at T18 and S91 was shown to slow down spindle elongation in anaphase (T. C. Lin et al., 2014). This suggests that microtubule dynamics are regulated via their minus-end through the SPB. These studies demonstrate that the γ-tubulin complex and its nuclear anchor Spc110 are regulated by phosphorylation in mitosis, which has strong effects on the metaphase and anaphase spindles.

How these mutations at the γ-tubulin complex or its nuclear anchor, Spc110, affect MT dynamics is not known. As described below, the γ-tubulin complex interacts with the stable minus-end of MTs which do not see much growth or shrinkage compared to the dynamic plus-end. In line with this, phosphorylation of the Spc110 N-terminus facilitates γ-tubulin complex oligomerization *in vivo*, promoting MT nucleation (T. C. Lin et al., 2014). Future studies will hopefully broaden our understanding of how MT dynamics are regulated by the γ-tubulin complex and its receptors.

Protein	Mutation	Effect	Reference
Spc97	S130E	Slow growth at 37°C	Lin 2011
Spc97	S152D	Increased spindle length, cell cycle delay	Fong 2018
Spc97	S208A S209A	Lethal	Fong 2018
Spc97	S40A/E	Slow growth	Lin 2011
Spc97	S471E	Slow growth at 37°C	Lin 2011
Spc97	S84E	Slow growth	Lin 2011
Spc97	T88A/D T84A/D	Increased spindle length, cell cycle delay	Fong 2018
Spc97	T88A/E	Slow growth	Lin 2011
Tub4	S100E	Lethal, delayed metaphase spindle, metaphase arrest, less Tub4 at SPB	Lin 2011
Tub4	S360A	Faster bipolar spindle formation, uniform spindle length	Nazarova 2013
Tub4	S360D S360E	Slow spindle elongation, increased spindle length, no interpolar MTs, spindle instability, lethal at 37°C	Keck 2011, Nazarova 2013
Tub4	Y445D	Lethal at 37°C, metaphase arrest, increased spindle length	Vogel 2001
Tub4	S71A	Cell cycle delay	Fong 2018
Tub4	S71D	Increased spindle length, cell cycle delay	Fong 2018
Tub4	S74E	Lethal, metaphase arrest, misaligned nuclear microtubules, more Tub4 at SPB	Lin 2011
Spc110	S36A S60A T64A T68A	Lethal in combination with spc97-62	Friedman 2001
Spc110	S36A T64A	Lethal in combination with spc97-62	Friedman 2001
Spc110	S36A T64A	Lethal in combination with spc97-62	Friedman 2001
Spc110	S36A	Increased metaphase spindle length	Huisman 2006
Spc110	S91A	Increased metaphase spindle length, continued elongation during metaphase	Huisman 2007
Spc110	S18D S91D	Delayed spindle elongation	Lin 2014

***Table 2** - Summary of phosphomutations in the budding yeast γ-tubulin complex and its nuclear receptor Spc110.*

1.1.3 Structure of mitotic spindle in budding yeast

The budding yeast spindle is assembled from the SPBs to segregate chromosomes in anaphase. The nuclear side of the SPB nucleates two types of MTs: Kinetochore microtubules (KT MTs) and interpolar microtubules. KT MTs connect the centromeres of the chromosomes to the SPB, while interpolar MTs connect the opposing SPBs, bundling together at the midzone. Each centromere is connected by a single KT MT and each SPB nucleates approximately 4 interpolar MTs (Peterson & Ris, 1976; Winey et al., 1995). On the cytoplasmic side, 4-6 astral MTs (aMTs) orient the spindle prior to anaphase (Shaw et al., 1997) (see chapter 1.2.1 for details on spindle positioning).

Electron microscopy has revealed how MTs are organized at different stages of the cell cycle. Nuclear MTs are present throughout the cell cycle. Indeed, chromosomes remain attached via KT MTs throughout most of the cell cycle, except during centromeric replication in S-phase (Kitamura et al., 2007). Spindle formation occurs once SPBs have been duplicated. In metaphase, the spindle consists of two opposed SPBs, each with approximately 20 microtubules, of which 16 appear to be short (~500 nm) KT MTs (matching the 16 chromosomes of haploid budding yeast cells) and a few longer interpolar MTs identified as stretching from pole-to-pole (O'Toole et al., 1999; Winey et al., 1995). Anaphase spindles maintain KT attachments, although the KT MTs shrink to about 30-50 nm, while interpolar MTs elongate and become fewer in number around the time the spindle extends beyond the bud neck.

Fluorescence microscopy has complemented these studies showing that centromeres are kept clustered together and that sister chromatids become attached to opposing poles (bi-oriented) via kinetochore attachments as the spindle assembles (Goshima & Yanagida, 2000; Jin et al., 2000; Pearsona et al., 2001).

1.1.4 Microtubules

MTs are cellular filaments conserved through evolution among eukaryotes, from yeast to animal cells. They play important roles as structural proteins, facilitate intracellular movement of cargo, such as mRNA, signaling molecules and even organelles, enable cell motility, and segregate chromosomes during cell division. In this chapter, I will focus on their role in spindle positioning and chromosome segregation, primarily in budding yeast. Individual MTs are hollow tubes made up of heterodimers of α- and β-tubulin, often referred to as simply tubulin. Budding yeast has a single gene coding for β-tubulin (Tub2) and two genes coding for α-tubulin (Tub1 and Tub3). Tub1 contributes about 70% of the α-tubulin subunits found in cells (Aiken et al., 2019) and is an essential gene, while Tub3 is not (Schatz et al., 1986). Overexpression of Tub3 can rescue the lethal phenotype of Δtub1 and the genes have traditionally been described as functionally

identical. However, recent studies have revealed that the cells expressing only one of the two α-tubulin isotypes favor different spindle positioning pathways depending on the isotype expressed, and the resulting MTs appear to have different affinities for microtubule-associated proteins (MAPs) (Aiken et al., 2019; Nsamba et al., 2021). Most eukaryotic cells have multiple isotypes of tubulin, and specific cell types utilize different isotypes (for a review, see (Nsamba & Gupta Jr, 2022)).

Tubulin subunits are arranged as linear beads (known as protofilaments), with dimers aligned along the filament in a head to tail-configuration. Although the number of protofilaments that make up the MT can vary (Chrétien & Wade, 1991; Dallai et al., 2006; Howes et al., 2018), a typical MT is usually described to be made up of 13 protofilaments in longitudinal alignment. MTs are polar polymers, and the two ends display different properties. The end where β-tubulin is exposed, known as the plus-end, is more dynamic and is the site that sees most growth and shrinking events (Bergen & Borisy, 1980; Mitchison & Kirschner, 1984a; Walker et al., 1988). Conversely, the α-tubulin exposed end, or minus end, is more stable and is often bound to an MTOC. The structure of the microtubule plus-end can take on different. Electron microscopy of MTs have revealed many different structures of the dynamic plus-end. A variety of structures have been observed during both MT growth and shrinking, ranging from tapered, straight ends to curled and flared sheets (see (Gudimchuk & McIntosh, 2021) for a review). Growing budding yeast MTs are thought to become more tapered at the plus-ends as the MT length increases, as shown by fluorescence microscopy and simulations (Coombes et al., 2013).

1.1.5 Microtubule dynamics

MTs are highly dynamic and often in a state of "dynamic instability", where the filament switches between polymerization and rapid depolymerization (Mitchison & Kirschner, 1984a). The switching from growth to rapid depolymerization is known as catastrophe. Early studies described dynamic instability after growing MTs at a steady-state concentration of tubulin and then diluting these to different concentrations of free tubulin (Mitchison & Kirschner, 1984a, 1984b). Rather than growing or shrinking in uniformly as a function of tubulin concentration, MTs either rapidly depolymerized or continued to grow. This dynamic instability serves as a fundamental mechanism for many of the functions of MTs and can be modified by associated proteins, tubulin concentration and post-translational modifications (PTMs) (Gudimchuk & McIntosh, 2021).

MT polymerization requires soluble GTP-bound tubulin (Cote & Borisy, 1981) which is then hydrolyzed to GDP once incorporated into the MT. However, the hydrolysis of GTP to GDP does not promote growth but rather gives rise to dynamicity by promoting depolymerization. Indeed, tubulin in the presence a slow-hydrolyzing analogue of GTP

(GMPCPP), polymerizes microtubules at a rate comparable to that of GTP-tubulin *in vitro*. However, the resulting MTs are two orders of magnitude more stable than MTs generated from GTP-tubulin when free tubulin is removed (Hyman et al., 1992). This suggests that catastrophe is dependent on the hydrolysis of GTP.

Dynamic instability is thought to arise from a thin layer of GTP-bound tubulin at the plus-end. As this GTP-bound tubulin, known as the GTP-cap, is more stable than the GDP-bound lattice, the plus-end is protected from depolymerization. Interestingly, the GTP-cap stabilizes the entire polymer, as exposing the GDP-bound tubulin by severing the MT plus-end causes rapid depolymerization of the whole MT (Walker et al., 1989).

Labeling MTs with nucleotide-specific antibodies reveals a thin layer of GTP-tubulin at the plus-tip, as well as sporadic patches of GTP-tubulin positioned along the MT filament (Dimitrov et al., 2008). These islands of GTP-tubulin within the MT lattice are thought to allow for rescue events during depolymerization (Dimitrov et al., 2008; Tropini et al., 2012). *In vitro* studies further support the GTP-cap model. Increasing the MT polymerization rate increases the area of the MT coated with plus-end binding protein EB1 (Duellberg et al., 2016), which is thought to bind GTP-bound tubulin (Maurer et al., 2012). The thicker cap seems to protect the plus-end, as the thickness of the EB coated cap correlates with the time it takes for MTs to switch to catastrophe when free tubulin is depleted (Duellberg et al., 2016). Furthermore, slowing down GTP hydrolysis also increases the EB1-coated tip thickness and protects from catastrophe (Roostalu et al., 2020). Taken together, MTs are highly dynamic polymers that switch between states of growth and depolymerization. Dynamic instability arises from the unstable nature of GDP-tubulin and the existence of a decaying GTP-tubulin cap at the plus-end.

1.1.6 Microtubule nucleation

New MTs rarely form spontaneously and require high concentrations of α- and β-tubulin to form *in vitro* by themselves. Instead, formation of new MTs *in vivo* is facilitated by nucleation via MTOCs, such as centrosomes or SPBs. MTOCs anchor a specialized form of tubulin, γ-tubulin, and as described in chapter 1.1.1, γ-tubulin in the form of γ-TuSC oligomerizes into a ring (γ-tubulin ring complexes, γ-TuRC). The γ-TuRC has been shown to closely resemble a 13 protofilament MT (Kollman et al., 2010, 2015) and is thought to function as a seed to establish a new MT and protect the minus-end once formed (Wiese & Zheng, 2000). γ-TuRCs are present in all eukaryotic cells and the budding yeast γ-TuRC is perhaps the simplest, only containing 2 γ-tubulin complex proteins, Spc97 and Spc98 (known as GCP2 and GCP3 in humans, respectively) and γ-tubulin. GCP2 and GCP3 are conserved in all γ-TuRCs, however, many organisms possess alternative GCPs and accessory proteins (for a review, see (Tovey & Conduit, 2018)).

1.1.7 Centromeres and kinetochores

During mitosis, replicated chromosomes are separated faithfully between mother and daughter cells. To enable this, the mitotic spindle needs to bind the chromosomes. This is facilitated by a specialized region called centromeres, where a large protein complex called the kinetochore is assembled to serve as an interphase between the spindle MTs and the chromosome.

Budding yeast centromeres are so-called point-centromeres and consist of three distinct centromere-determining elements (CDEs) spanning ~125 basepairs (Bloom & Carbon, 1982; Carbon & Clarke, 1984). This is in stark contrast to higher eukaryotic centromeres, which span hundreds of kilobasepairs to several megabasepairs (Talbert & Henikoff, 2020). The CDEs are present on all 16 budding yeast chromosomes and are required for the kinetochore to assemble. Indeed, the CDE sequences define the centromere, as introducing the sequence into a plasmid results in the plasmid being segregated like chromosomes during mitotic and meiotic division (Clarke & Carbon, 1980). Centromeric chromatin differs from that of other chromosomal regions. First, nucleosomes at the centromere have the core histone protein H3 replaced with CENP-A (Cse4 in budding yeast). Furthermore, cohesin is enriched at and around centromeres and is specifically loaded at centromeres via kinetochores (Fernius & Marston, 2009; Hinshaw et al., 2015, 2017). Additionally, the budding yeast pericentromeric chromatin forms loops via cohesin that aid in bi-orienting sister chromatids in metaphase spindles (Paldi et al., 2020; Stephens et al., 2011; Yeh et al., 2008).

KTs are protein structures that serve as the interphase between chromosomes and the mitotic spindle by connecting the centromeric DNA with dynamic plus-ends of spindle microtubules (Figure 2). They also serve as signaling hubs for cell cycle progression and tension sensors to ensure that sister chromatids are segregated correctly (Biggins et al., 1999; Cheeseman, Anderson, et al., 2002; Dewar et al., 2004; Gillett et al., 2004; T. U. Tanaka et al., 2002). The budding yeast kinetochore consists of more than 60 different proteins, assembled into subcomplexes and associated proteins (Westermann et al., 2007). These are typically described according to their position relative to the chromosome: The inner kinetochore, which is assembled around the Cse4 nucleosome, the outer kinetochore, which connects to the KT MT and the central kinetochore proteins which connect the inner and outer kinetochore complexes (Cheeseman, Drubin, et al., 2002; Westermann et al., 2007).

Kinetochore assembly can occur independently of the cell cycle phase the cell is in, and depends on Cse4, as shown by experiments using a conditional centromere (Collins et al., 2005). The kinetochore has been thought to be static once assembled as almost no turnover was initially observed in any subcomplex in fluorescence recovery after photobleaching (FRAP) experiments of metaphase and anaphase spindles (Joglekar

2006). However, it has since been reported that kinetochores are dynamic in anaphase, with around a 50% increase in fluorescence after the incorporation of new subunits in all subcomplexes except the Dam1 complex (Dhatchinamoorthy et al., 2017).

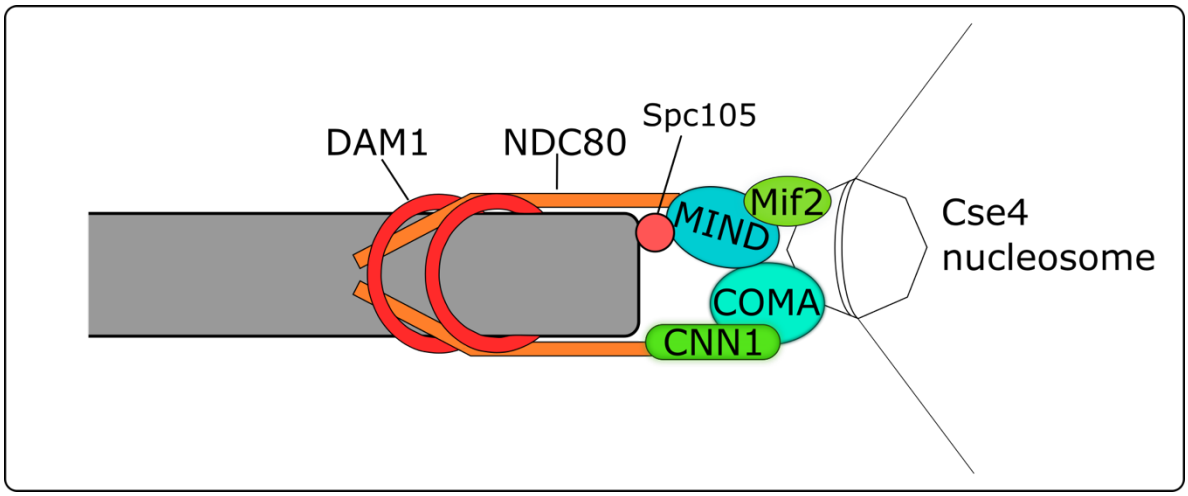

***Figure 2** – Schematic structure of the budding yeast kinetochore. DAM1 ring complex in the outer KT wraps around a KT MT and connects to the central KT complexes via the Ndc80 complex. Ndc80 complexes binds to the inner KT via the MIND complex and the CNN1 complex.*

The inner kinetochore proteins connect to the centromere via Mif2 (the yeast homolog of human CENP-C), which binds directly to the Cse4 nucleosome (Hornung et al., 2014; Westermann et al., 2003; Xiao et al., 2017). Furthermore, the inner kinetochore CBF3 complex is essential for KT assembly and recognizes the CDE III DNA sequence (Lechner & Carbon, 1991; R. Ng & Carbon, 1987). On the opposite side of the kinetochore, the outer kinetochore complexes connect to the KT MTs. The DASH/Dam1 complex forms a 10-subunit ring that encircles the KT MT plus-tip (Jenni & Harrison, 2018; Miranda et al., 2005; C. T. Ng et al., 2019). There are conflicting reports as to whether one or two rings encircle each MT. Quantitative microscopy experiments suggest that a single ring binds each MT (Dhatchinamoorthy et al., 2017; Joglekar et al., 2006), while crosslinking and *in vitro* reconstitution experiments show that the Ndc80 complex can bridge two rings (J. O. Kim et al., 2017). Cryo-EM studies of the Dam1 complex *in vivo* suggests that most KT MTs have a single ring, although two rings were occasionally observed (C. T. Ng et al., 2019). Furthermore, most rings appeared to be incomplete and were suggested to become fully formed rings as tension increased. The rod-shaped Ndc80 complex requires Dam1 to localize to KT MT plus-ends (Lampert et al., 2013; Schleiffer et al., 2012; Tien et al., 2010) and connects the Dam1 complex to the inner kinetochore via the MIND complex (Malvezzi et al., 2013). The interaction between Ndc80 and the Dam1 complex is abolished when Ndc80 is phosphorylated by the aurora B kinase Ipl1 (Cheeseman et al., 2006; Tien et al., 2010) and is part of the spindle assembly checkpoint (SAC) that ensures correct chromosome segregation (see chapter 1.2.2). Additionally, the Ndc80 complex has also been found to bind to the inner kinetochore via the Cnn1 complex, which in turn binds the Ctf19 complex (part of the COMA complex) (Hinshaw & Harrison,

2019; Malvezzi et al., 2013). Cnn1 has been shown to be stably associated with the kinetochore throughout the cell cycle but increases up to 4-fold in anaphase (Bock et al., 2012). This anaphase recruitment of Ndc80 by Cnn1 is further supported by immunoprecipitation experiments that found an increased interaction between Cnn1 and Ndc80 in anaphase cells (Schleiffer et al., 2012).

The small size of the yeast spindle and the fact that kinetochores are kept clustered have made it difficult to study how kinetochores are initially captured by MTs. However, microscopy studies where centromeric replication of one chromosome can be delayed until metaphase have revealed key aspects. Kinetochores were found to be captured by attaching to the sides of microtubules before being transported towards the pole (K. Tanaka et al., 2005). Once at the pole, KTs are instead found at the plus-end of the KT MT rather than the side. Initial capture was found to be dependent on proteins in the Ndc80, Mtw1 and Ctf19 complexes, but not Dam1. Instead, the Dam1 complex is required to establish and maintain the biorientation of sister chromatids (Janke et al., 2002), suggesting that the complex facilitates end-on attachments once captured.

The KTs link MTs to the centromeres via a complex network of proteins that are modulated by tension and phosphorylation. This allows for a tight regulation and serves to ensure that sister chromatids are segregated correctly at anaphase. How this is regulated will be further explained in the chapter 1.2.2.

1.1.8 Microtubule-associated proteins

In vitro studies of isolated tubulin on its own have revealed many of the fundamental mechanisms that give rise to MT dynamics. However, the mechanisms that govern MT dynamics *in vivo* are more complex as many proteins bind and associate with MTs. As *in vitro* studies show, MTs on their own are unstable and spontaneously depolymerize, while *in vivo*, MT stability can be regulated to perform specific functions. Different types of MT binding proteins have been identified, such as stabilizing and destabilizing proteins, bundling proteins that interact with multiple adjacent MTs, and motor proteins that walk along microtubules, to name a few. MT binding proteins often have partly redundant functions or can otherwise be compensated for. Additionally, different conformations of MT binding proteins in heterodimers and complexes can have opposing effects, complicating the interpretation of *in vitro* studies.

Budding yeast has many MT associated proteins (MAPs) that perform a wide range of functions. I will below highlight a few of these, their effect on MT dynamics as well as their biological function.

1.1.9 +TIPs

A subset of MAPs preferentially bind the dynamic plus-end and are known as MT plus-end tracking proteins (+TIPs). In budding yeast, binding to microtubule 1 (Bim1) and

bilateral karyogamy 1 (Bik1) both localize along the microtubule lattice but accumulate at the plus-ends (Bergman et al., 2019; Wolyniak et al., 2006; Zimniak et al., 2009). Bim1 and Bik1 are both homologues of protein families that are well conserved through evolution (EB1 and CLIP-170, respectively). Bim1, like other EB1 proteins, contains an N-terminal calponin homology (CH) domain which can bind tubulin (Hayashi & Ikura, 2003). Clip-170 contains two cap-glycine (CG) domains, which increases its affinity for MTs (K. K. Gupta et al., 2009; Pierre et al., 1992). Bik1 only contains a single CG domain and has not been demonstrated to bind MTs on its own *in vitro*. Both Bik1 and Bim1 bind free tubulin on their own, however, only Bim1 homodimers localize to plus-ends *in vitro* while Bik1 requires Bim1 to localize to plus-ends in the same assay (Blake-Hodek et al., 2010). Further characterization showed that interactions between Bik1 and Bim1 depends on the C-terminal of Bim1 and the CG domain of Bik1 (Stangier et al., 2018). Additionally, phosphoregulation and dimerization of Bim1 greatly affects its ability to bind MTs (Zimniak et al., 2009). Interestingly, while Bik1 does not localize to plus-ends alone *in vitro*, it is still found at both the MT lattice as well as plus-ends in bim1Δ strains (Stangier et al., 2018).

Functionally, both EB1 and CLIP-170 proteins have been shown to promote MT stability, either through promoting MT growth or rescue events (reviewed in (Akhmanova & Steinmetz, 2008)). While *in vitro* studies of Bik1 and Bim1 support this as heterodimers of Bik1-Bim1 and Bim1 homodimers promote MT rescue events (Blake-Hodek et al., 2010), the *in vivo* functions of these proteins are more complex.

1.1.10 Bik1

Bik1 was first identified as a gene required for nuclear fusion during mating (Trueheart et al., 1987). In a follow up report, Bik1 was described as a MAP that is essential for normal spindle morphology (Berlin et al., 1990). The null mutant displayed short or absent cytoplasmic MTs, shorter spindles and genetic instability (Berlin et al., 1990). Overexpression of Bik1 via the *GAL1* promoter, on the other hand, resulted in long aMTs, absent spindle MTs and cell cycle arrest.

Bik1 localizes to both cytoplasmic and nuclear microtubules. Using FRAP, Bik1 at astral MTs was shown to be dynamically associated with the plus-ends of MTs (Carvalho et al., 2004), while the nuclear pool showed no recovery in most cells. These results suggest that Bik1 has distinct dynamics at astral and spindle MTs.

Bik1 and Bim1 have also been implicated in chromosome capture following centromere replication. As described in chapter 1.1.3, budding yeast centromeres remain close to the SPB throughout most of the cell cycle, except for a brief time in S-phase when centromeres are replicated. During this time, the centromere is released from the spindle MTs and moves 1-1.5 µm away from the SPB and is subsequently captured again by dynamic spindle MTs (Kitamura et al., 2007; K. Tanaka et al., 2005). By inactivating

CEN3 and re-activating it in metaphase, centromere capture events can be visualized by microscopy. In this assay, deletion of either Bim1 or Bik1 greatly reduces the number of spindle MTs that elongate to capture the re-activated centromere (K. Tanaka et al., 2005). Although the capture distance during S-phase release is considerably shorter, the nucleation-promoting function of Bik1 is consistent with the observations from the null mutant.

The best studied function of Bik1 is its role in spindle positioning via aMTs and will be explained in detail in chapter 1.2.1.

1.1.11 Cin8 and other kinesins

Kinesins are motor proteins that walk along microtubules in an ATP-dependent manner. The general structure of a kinesin motor is made up of two motor domains linked to a common stalk. The motor domains bind tubulin, have ATPase activity, and are connected to the stalk with a flexible neck-domain. The affinity for tubulin depends on nucleotide binding, with ATP-bound and completely unbound motor domains having a high affinity for tubulin, and ADP-bound motor domains having a low affinity for tubulin (Cross, 2004). By cycling and pausing between states, the variable affinity for microtubules coupled with the flexible neck allows kinesin motors to walk along MTs as ATP is hydrolyzed (Tomishige et al., 2006). Kinesins are diverse motor proteins with some walking towards plus-ends, others towards minus-ends, and some being bi-directional with different directionalities depending on molecular context. This variety allows kinesins to carry out diverse tasks in cells, often dictated by their directionality and affinity for different types of cargo.

Cin8 is a member of the kinesin-5 family of motor proteins. It's a bi-directional motor conserved between yeasts and higher eukaryotes (Mann & Wadsworth, 2019). Together with another kinesin-5 family protein, Kip1, Cin8 plays important roles in kinetochore clustering prior to anaphase onset (Gardner, Bouck, et al., 2008; Tytell & Sorger, 2006) and spindle elongation during anaphase (Gerson-Gurwitz et al., 2009; Saunders et al., 1995; Straight et al., 1998). The interpolar MTs meet at the midzone with their plus-ends where Cin8 and Kip1 are thought to push MTs apart by binding antiparallel MTs and provide plus-end directed force (Pandey et al., 2021). Cin8 also plays a key role in regulating MT dynamics early in mitosis by specifically destabilizing long KT MTs to cluster KTs prior to anaphase, although the mechanism for this is unknown (Gardner, Bouck, et al., 2008).

Cin8 and other kinesin-5 motors were thought to exclusively move towards plus-ends. However, *in vitro* experiments have demonstrated that Kip1, Cin8 and fission yeast Cut7 can be made to move towards both plus- and minus-ends (Edamatsu, 2014; Fridman et al., 2013; Gerson-Gurwitz et al., 2011). Directionality of Cin8 can be modulated by mutating CDK1 phosphosites in the catalytic domain, where phosphomimetic mutants

have a lower affinity for MTs and move towards minus-ends (Shapira & Gheber, 2016). Cells expressing the phosphomimetic mutant variant has overall less Cin8 at the spindle, fails to recruit the protein to the midzone, and display slower rates of spindle elongation (Avunie-Masala et al., 2011). Furthermore, changing the ionic strength and motor concentration also affects directionality, with single motors or motors in high-ionic strength buffer moving towards the minus-end, and clusters of Cin8 moving towards the plus-end (Roostalu et al., 2011; Shapira et al., 2017). Although the exact mechanisms are not established, bidirectionality could explain how Cin8 is positioned at different populations of MTs of the spindle.

1.1.12 Sister chromatid cohesion

When chromosomes are duplicated during S-phase, the resulting sister chromatids need to be held together up until all chromosome pairs are properly attached to opposing spindle poles and are ready to be segregated into mother and daughter cells. This phenomenon is known as sister chromatid cohesion and is mediated by the ring-shaped structural maintenance of chromosomes (SMC) complex cohesin. Sister chromatid cohesion counteracts the pulling forces of KT MTs and allows the spindle to bi-orient chromosomes before segregation (T. Tanaka et al., 2000). When proper biorientation is achieved, cohesin is cleaved, and chromosome segregation is initiated. Chromosome biorientation, error correction, SAC and anaphase initiation will be covered in chapters 1.2.2 and 1.2.4.

The cohesin complex consists of 4 core components: Two elongated SMC proteins, Smc1 and Smc3, that make up most of the ring, the kleisin protein Rad21 (Scc1 in budding yeast) and stromal antigen (STAG1 or STAG2 in humans, Scc3 in budding yeast) at the base of the ring (reviewed in (Nasmyth & Haering, 2009)). Cohesin complexes have been shown to topologically entrap minichromosomes within their ring, thus holding them together (Haering et al., 2008; Murayama & Uhlmann, 2014; Srinivasan et al., 2018). This is thought to give rise to cohesin's ability to hold sister chromatids together. Key molecular details of cohesin loading have been described (reviewed in (Nasmyth & Haering, 2009)). First, ATP binding, but not hydrolysis, of Smc1 is required for Scc1 to interact with Smc1/Smc3 dimers (Arumugam et al., 2003; Weitzer et al., 2003). On the other hand, disruption of the ATPase activity of either Smc1 or Smc3 prevents the cohesin complex from binding to chromosomes (Arumugam et al., 2003; Weitzer et al., 2003). Furthermore, the cohesin loading factor Nipped-B-like (NIPBL, Scc2 in budding yeast) is required to establish stable cohesin binding to chromosomes (Arumugam et al., 2003; Ciosk et al., 2000; Tóth et al., 1999). Once cohesion has been established, NIPBL/Scc2 is no longer required to maintain cohesion (Ciosk et al., 2000). Although cohesin can bind DNA after S-phase, cohesin must be loaded onto chromosomes before DNA replication has finished to establish sister chromatid cohesion (Uhlmann & Nasmyth, 1998). Once cohesin has been loaded onto chromosomes in S-phase, Smc3 is

acetylated by the acetyltransferase Eco1, stabilizing the chromosomal association of cohesin (Ben-Shahar et al., 2008; Ünal et al., 2008). Like NIPBL/Scc2, Eco1 is not needed for maintaining sister chromatid cohesion once established and, in its absence, cohesin can still bind chromosomes cells (Ben-Shahar et al., 2008).

1.1.13 Cohesin regulation

Much is known about cohesin regulation in loading, positioning and removal and has been reviewed in (Choudhary & Kupiec, 2022). In human cells, most chromosome-bound cohesin is removed in prometaphase, with only centromeric cohesin remaining (Waizenegger et al., 2000). This unloading of cohesin is carried out by Wapl (known as Wpl1 or Rad61 in budding yeast) (Gandhi et al., 2006; Kueng et al., 2006). In budding yeast, the lethal phenotype of eco1Δ can be suppressed by deleting Wpl1/Rad61, mutating Pds5, which recruits Wpl1, or introducing acetylation-mimicking mutations in Smc3 (Ben-Shahar et al., 2008; Rowland et al., 2009). Furthermore, inducing Wpl1 expression in G2/M in the absence of Eco1 leads to a loss of cohesion (Chan et al., 2012). Taken together, these data suggest that acetylation of Smc3 blocks Wpl1-dependent removal of cohesin to establish sister chromatid cohesion. In budding yeast, cohesin is instead found along chromosome arms up until anaphase onset, although Wpl1/Rad61 is continuously unloading non-cohesive cohesin (Dauban et al., 2020).

The centromeric cohesin keeps sister chromatid cohesion to counteract the pulling forces of KT microtubules attached at centromeres. When chromosomes have correctly been bi-oriented and SAC is satisfied (see chapter 1.2.2), the anaphase-promoting complex ubiquitinates securin followed by proteolytic degradation. This in turn activates separin, a cysteine protease that will cleave Rad21/Scc1 and initiate chromosome segregation (Hauf et al., 2001; Uhlmann et al., 2000).

1.1.14 Cohesin positioning

Cohesin is found at distinct loci along chromosomes in many organisms. In budding yeast, cohesin was found to be enriched at centromeres and at specific positions along the chromosome arms (Blat & Kleckner, 1999; T. Tanaka et al., 1999). It was later shown that these cohesin binding sites on chromosome arms are situated at sites of convergently transcribed genes (Glynn et al., 2004; Lengronne et al., 2004). The identified cohesin binding sites differ from NIPBL/Scc2 binding sites, suggesting cohesin moves along chromosomes after loading. Altering transcription affects cohesin positioning, further implicating the transcription machinery in cohesin is positioning (Bausch et al., 2007; Lengronne et al., 2004).

In mammalian cells, cohesin is similarly found at centromeres and along chromosome arms (Parelho et al., 2008). However, unlike in budding yeast, the positioning along chromosome arms depends on the transcription insulator CCTC-binding factor (CTCF)

(Parelho et al., 2008; Wendt et al., 2008). CTCF is found throughout the genome and recognizes a consensus motif that is highly conserved among species, although not in budding yeast (T. H. Kim et al., 2007). In the absence of CTCF, cohesin is instead found accumulating at transcription start sites of active genes. Furthermore, by depleting both CTCF and the cohesin unloader Wapl, cohesin localization resembles that of budding yeast, with cohesin instead found between convergently transcribed genes (Busslinger et al., 2017). This suggests that cohesin movement along chromosomes in mammalian cells, like in yeast, is mediated by the transcription machinery.

1.2 Cell cycle of budding yeast

When a cell divides, it is essential that the genomic material is faithfully replicated and, in turn, segregated between daughter cells. To this end, cells have evolved a hierarchical, stepwise program for cell division, the cell cycle, with key checkpoints along the way to pause progression until the checkpoint is satisfied. Here I will focus on some of the key events during the budding yeast cell cycle relevant to the findings presented in papers I, II and III.

Although budding yeast and mammalian cells are separated by billions of years of evolution, basic regulatory mechanisms of cell cycle progression are conserved. The cell cycle is divided into phases, and the standard cell cycle consists of the gap 1 phase (G1), during which cells grow, followed by the synthesis phase (S-phase), where DNA is replicated. Then, a second gap phase (G2), in which the cell grows further and prepares for mitosis (M phase). During mitosis, the replicated DNA is segregated between mother and daughter cells, and cells begin to reset the cell cycle. The central regulators of cell cycle progression are cyclin-dependent kinases (Cdks), protein kinases that when paired with regulatory cyclin subunits will promote cell cycle progression through phosphorylation (Morgan, 1997). Budding yeast has a single Cdk (Cdc28/Cdk1) that together with cyclins progress the cell cycle (Nasmyth, 1996).

Budding yeast divides through closed mitosis, that is, the nuclear envelope remains intact throughout the cell cycle. Furthermore, as the name suggests, it divides through budding, in which the daughter cell grows from the cell cortex of the mother cell. This means that the plane of division is predetermined and can't be positioned according to the spindle. To this end, budding yeast have evolved tightly regulated mechanisms to position the nucleus and spindle before chromosome segregation. The morphology of a dividing budding yeast cell and mitotic spindle is outlined in Figure 3.

1.2.1 Spindle positioning

There are two main pathways for nuclear positioning, the Kar9 pathway and the dynein pathway. Kar9 is an adaptor protein that links the plus-end of aMTs with the actin cytoskeleton to orient the spindle towards the bud (R. K. Miller et al., 1999; R. K. Miller &

Rose, 1998). Kar9 is first found at the SPB and is loaded onto MTs through its interaction with Bim1 (Korinek et al., 2000; L. Lee et al., 2000; R. K. Miller et al., 2000). Once at the plus-end, Kar9 interacts with the myosin motor protein Myo2, which walks along polarized actin cables towards the bud tip (Yin et al., 2000), thus guiding the spindle towards the bud. Once the mitotic spindle is aligned along the mother-daughter cell axis via the actin cytoskeleton, the aMTs are captured at the bud neck and cortex by the actin-binding protein Bud6 and the +TIP Bim1, and aMT are shortened by depolymerization mediated by the kinesin Kip3 (M. L. Gupta et al., 2006; Segal et al., 2000; ten Hoopen et al., 2012).

How then is the spindle aligned perpendicular to the bud neck? In principle, aMTs emanating from either pole could be transported towards the neck, resulting in a misaligned spindle. As described earlier, the old SPB is inherited by the daughter cell, and in line with this, the old SPB is oriented towards the bud prior to spindle elongation (Liakopoulos et al., 2003; Pereira et al., 2001). Although Kar9 initially localizes to both SPBs during spindle assembly (Cepeda-García et al., 2010; Huisman et al., 2004), it is preferentially associated with the bud-bound pole as the spindle aligns (Huisman et al., 2004). This has is a highly regulated process that involves age-specific signals on the bud-directed SPB, intrinsic differences between old and new SPBs, and PTMs of Kar9 itself.

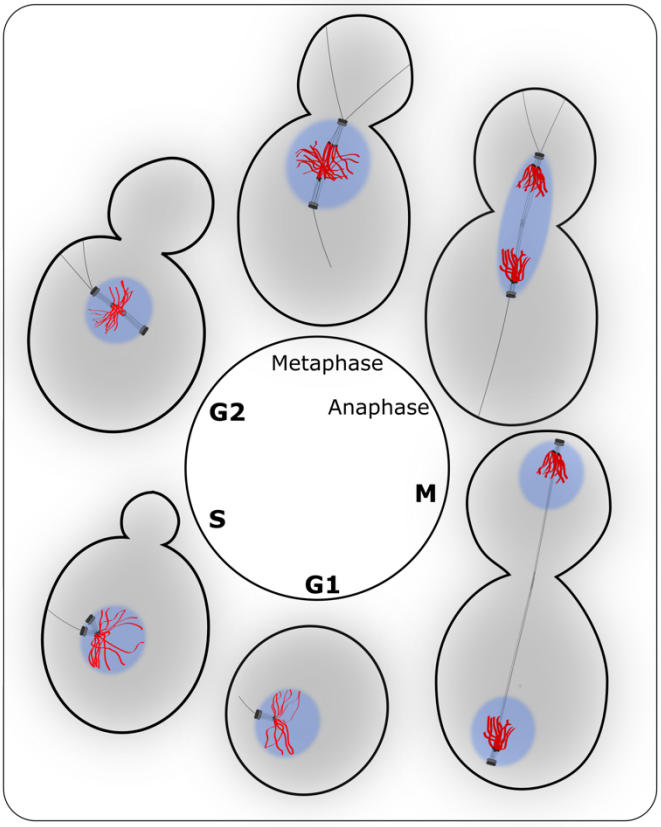

Figure 3 *- Overview of cell cycle progression and spindle assembly in budding yeast. As cells commit to dividing, the bud emerges from the mother cell cortex. The SPB is embedded in the nuclear envelope and is duplicated in G1/S. A bipolar spindle is formed in S-phase and chromosomes become bioriented leading up to metaphase. In anaphase, spindle elongation occurs through shortening of the KT MTs and polymerization of interpolar MTs. Cells then exit from mitosis, disassemble the spindle and divide through cytokinesis.*

Kar9 is phosphorylated by Cdc28 with the cyclins Clb4 and Clb5 at serine 197 and serine 496 (Liakopoulos et al., 2003; Maekawa et al., 2003; Maekawa & Schiebel, 2004; Moore et al., 2006; Moore & Miller, 2007). The exact function of these phosphorylation sites has been debated, but a non-phosphorylatable mutant (S197A S496A) resulted in Kar9 binding to both SPBs (Liakopoulos et al., 2003; Meziane et al., 2021; Moore & Miller, 2007). Furthermore, Bik1 has been shown to be important for Kar9 S496 phosphorylation (Moore et al., 2006). Bik1 binds aMTs emanating from both SPBs. However, deleting Bik1 has been shown to greatly reduce the levels of phosphorylated Kar9, leads to symmetric loading of Kar9, and breaks the interaction between Kar9 and Clb5 as analyzed by two-hybrid analysis (Moore et al., 2006). Furthermore, components of the mitotic exit network (MEN, covered later in chapter 1.2.5) have been shown to influence asymmetric retention of Kar9 at the old SPB. The MEN components Dbf2/20, Tem1 and Cdc15 have been suggested to act via Nud1 on the old SPB to stabilize Kar9 retention by phosphorylating Kar9 (Hotz et al., 2012).

Another key aspect of spindle alignment is the maturity of 'old' and 'new' SPBs. Swe1 phosphorylation of Nud1 during G1 has been suggested to mark the existing SPB for subsequent maturation via the acetyltransferase NuA4 and the kinase Kin3 (Lengefeld et al., 2017) This in turn has been related to MEN activity specifically at the old SPB to retain Kar9 (Lengefeld et al., 2017). Another factor contributing to the spindle alignment is that old and newly forming SPBs differ in their capacity to nucleate MTs. During SPB duplication, the pre-existing SPB nucleates aMTs while the newly assembling SPB does not (Shaw et al., 1997). Moreover, it has been shown that recruitment of the outer plaque γ-TuSC anchor Spc72 is delayed in the new SPB, and tethering Spc72 to both SPBs results in spindle orientation defects and randomized inheritance of the SPBs (Juanes et al., 2013). Recruitment of Spc72 depends on the phosphorylation of Nud1, and a non-phosphorylatable allele of Nud1 results in symmetric recruitment of Spc72 and randomized inheritance of SPBs (Geymonat et al., 2020). Furthermore, Cdc5 activity has been shown to regulate the recruitment of Spc72 to SPBs as cells enter anaphase, and inhibiting the kinase abolishes age-dependent inheritance of SPBs in the following cell cycle (Matellán et al., 2020). Moreover, inhibiting Cdc5 activity increased the proportion of cells with Kar9 loaded on the newly formed SPB instead of the old, possibly by phosphorylating Kar9 directly. This suggests that Cdc5 plays a key role in SPB maturation that is coupled to cell cycle progression to prime the newly formed SPB in the next round of division. Taken together, the alignment of the old SPB towards the bud is dependent on differences in maturation of the two SPBs and is given directionality by Kar9-guided MT capture via Bud6 in the bud.

Once the spindle has been correctly positioned and oriented, the dynein-mediated pathway subsequently facilitates the pull of the spindle into the bud in anaphase (Yeh et al., 2000). Additionally, the dynein pathway contributes to the elongation of the spindle

and mediates the separation of SPBs (Moore et al., 2009). The main effector of this pathway is the ATPase-driven motor protein dynein. Dynein is conserved between a wide range of organisms (Wickstead & Gull, 2007) and is involved in many different processes. However, the only known function of dynein in budding yeast is spindle positioning. Dynein motors walk in a stepwise manner along MTs and are important for intracellular traffic of cargo and positioning of organelles. Budding yeast only has cytoplasmic dynein, encoded by the DYN1 gene. Dyn1 is a homolog of the dynein heavy chain, and deletion mutants result in spindle misalignment while nuclear spindle morphology is unaffected (Eshel et al., 1993; Y. Y. Li et al., 1993). Dynein is a minus-directed motor and exerts a pulling force on MTs while anchored at the cell cortex. Interestingly, although it is a minus-directed motor, dynein is delivered to the cortex via plus-end directed transport along aMTs. This transport is mediated through Dyn1 binding to Ndl1 and Pac1 (homologs of human NdlEL and Lis1, respectively), forming a complex which in turn binds Bik1 for plus-end directed transport (W. L. Lee et al., 2003; Markus et al., 2011; Markus & Lee, 2011). At the cortex, Dyn1 encounters patches of Num1 that function as an anchor and is off-loaded (Markus & Lee, 2011). Cortical dynein will then guide the spindle across the bud neck.

1.2.2 Chromosome biorientation and spindle assembly checkpoint

As explained in chapter 1.1.3, in budding yeast kinetochores remain attached to KT MTs throughout most of the cell cycle. As the centromere is replicated, the kinetochore is rapidly assembled and captured by KT MTs. Early in mitosis, the spindle will assume a bilobed distribution, with MTs emanating from each SPB and KTs distributed as two clusters between them. KTs are initially preferentially attached to the old SPB, and KT capture results in a mix of syntelic (sister chromatids attached to the same SPB) and amphitelic (sisters attached to opposing SPBs) attachments that are gradually converted to amphitelic before anaphase (Marco et al., 2013; Maure et al., 2007; T. U. Tanaka et al., 2002). How does the cell ensure that sister chromatids are attached to opposing SPBs (known as chromosome biorientation)? Amphitelic attachment to dynamic microtubules is hypothesized to generate more tension across the KTs and centromeres, giving rise to the hypothesis that tension sensing is key to stabilizing KT MT interactions. Interestingly, *in vitro* experiments with MTs bound to KTs show that tension itself slows MT disassembly, stabilizes MT-KT interactions and promotes MT elongation (Akiyoshi et al., 2010; Franck et al., 2007; Grishchuk et al., 2005). The tension-mediated stabilization of yeast KT-MT interactions seem to depend on the microtubule polymerase Stu2 (M. P. Miller et al., 2016). However, *in vitro* assays show that the KT bound Stu2 does not impact MT dynamics but is critical for stabilizing KT MT attachments under tension. Disrupting Stu2's ability to bind directly to KTs decreases inter-kinetochore distances in metaphase arrested cells and results in failure to bi-orient centromeres, and chromosome missegregation (M. P. Miller et al., 2019).

Furthermore, the aurora B kinase is a key player in error correction (Figure 4). The budding yeast Aurora kinase Ipl1 is part of the chromosome passenger complex together with Bir1 (Survivin), Nbl1 (Borealin) and Sli15 (INCENP) (Carmena et al., 2012). Ipl1 is crucial for metaphase spindle formation by phosphorylating components of the Ndc80 and Dam1 complexes to destabilize MT-KT interactions (Biggins et al., 1999; Cheeseman, Anderson, et al., 2002; T. U. Tanaka et al., 2002). Ipl1 localizes to the kinetochores and destabilizes syntelic attachments by specifically phosphorylating kinetochores not under tension (Biggins & Murray, 2001; Fischböck-Halwachs et al., 2019; García-Rodríguez et al., 2019; T. U. Tanaka et al., 2002). Tension sensing by aurora kinase relies on its localization to the centromere in mammalian cells (reviewed in (Broad & DeLuca, 2020)). Centromere stretching has been hypothesized to control aurora kinase-mediated phosphorylation; Kinetochores under tension have an increased physical distance between the centromeric kinase and KT target proteins, leading to specific detachments incorrectly attached kinetochores not under tension (Liu et al., 2009; Wang et al., 2011; Welburn et al., 2010). However, budding yeast cells where Bir1-Sli15 binding is disrupted, which should eliminate centromeric localization of the CPC, still segregate chromosomes normally (Campbell 2013). Additionally, Sli15/Ipl1 bound directly to the KT via Ctf19 in the COMA complex is sufficient for correct segregation (Fischböck-Halwachs et al., 2019). Instead, it has been suggested that centromeric localization of the CPC is primarily involved in signaling on unattached kinetochores, while kinetochore-bound CPC is involved in correcting syntelic attachments (Marsoner et al., 2022).

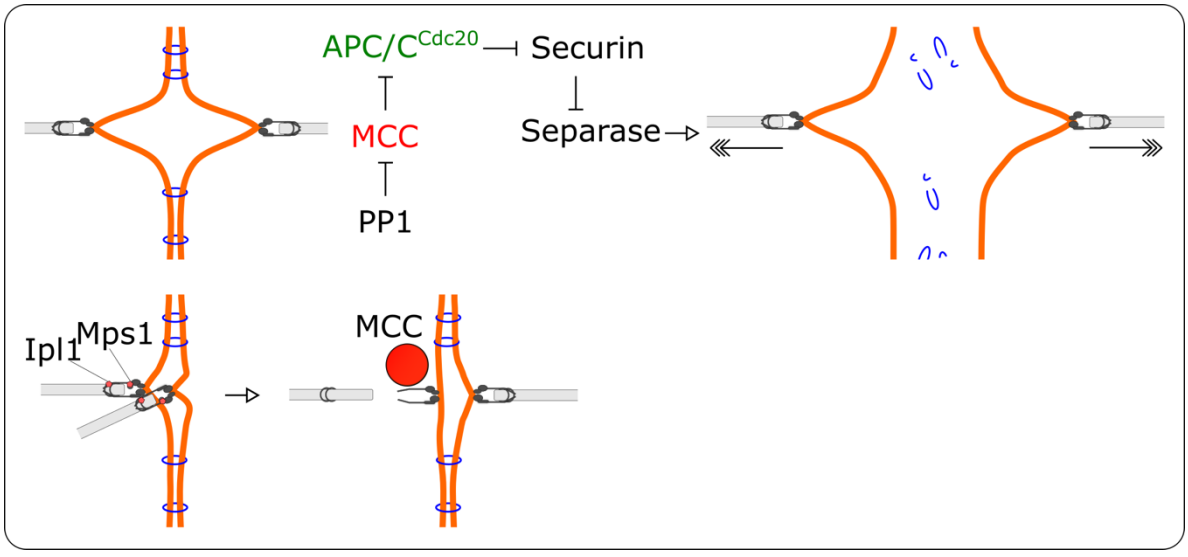

Figure 4 *- Error correction and spindle assembly checkpoint. KT-MT interactions not under tension are eliminated via phosphorylation of Mps1 and Ipl1. This in turn recruits the MCC, halting cell cycle progression until correct KT attachment has been achieved. Once all chromosomes are bioriented, PP1 turns off SAC, activating APC/C^{Cdc20}, leading to cohesin cleavage via separase and anaphase progression.*

Sister chromatids are held together by cohesin, counteracting the outward directed force from attached microtubules. Stretching of the centromeres of bioriented sisters results in tension across the spindle, which stabilizes KT-MT attachments (Figure 4). Unattached kinetochores, such as those destabilized by the lack of tension (Pinsky et al., 2006), activate a signaling cascade known as the spindle assembly checkpoint (SAC), which blocks anaphase initiation. SAC appears to primarily play a checkpoint role, as improper spindle attachments can be rescued by S-phase arrest via hydroxyurea in cells lacking SAC signaling (R. Li & Murray, 1991). SAC functions by inhibiting the anaphase promoting complex/cyclosome (APC/C), an E3 ligase that, when bound to its co-activators Cell Division Cycle 20 (Cdc20) and later Cdc20 Homolog 1 (Cdh1), ubiquitylates mitotic regulators (Barford, 2011). APC/C bound to Cdc20 is activated in metaphase once SAC is satisfied and targets the Securin (Pds1 in budding yeast) for proteasomal degradation (Cohen-Fix et al., 1996; Lim et al., 1998). Securin is bound to and inhibits the protease separase (Esp1), which, when active, will cleave the cohesin complex allowing spindle elongation and chromosome segregation (Ciosk et al., 1998; Uhlmann et al., 1999, 2000). APC/C^{Cdc20} also initiates cyclin degradation (Peters 2006). Cdh1, on the other hand, is blocked from binding the APC/C by Cdk1-mediated phosphorylation during early anaphase (Höckner et al., 2016; Zachariae et al., 1998) As Cdk1 activity drops, the APC/C will bind to Cdh1, leading to cyclin degradation that is maintained through the next G1 phase (Irniger & Nasmyth, 1997; Kramer et al., 2000; Schwab et al., 2001).

Cdc20 is also involved in SAC signaling to block anaphase onset. Cdc20 can only interact with the APC/C when it has been phosphorylated (Rudner & Murray, 2000; Shteinberg et al., 1999), possibly explaining the mechanism enabling the dual functions of Cdc20. During spindle assembly, Cdc20 is part of the mitotic checkpoint complex (MCC), which is recruited to unattached kinetochores and is a potent inhibitor of APC/C (reviewed in (Lara-Gonzalez et al., 2021)). The MCC consists of mitotic arrest-deficient 2 and 3 (Mad2, Mad3), budding inhibited by benzimidazole 3 (Bub3) and Cdc20. Bub3 exists in a complex with Bub1, which is recruited to the KT-MT interphase through phosphorylation of the outer kinetochore protein Spc105 by Mps1 (London et al., 2012; Primorac et al., 2013). Mps1 then phosphorylates Bub1 for further recruitment of Mad1-Mad2 and checkpoint activation (London & Biggins, 2014). Cdc20 is recruited for MCC formation via Ipl1-dependent phosphorylation, meaning that Ipl1 not only activates SAC via destabilizing tensionless KT-MT interactions, but is also involved in SAC signaling (Roy et al., 2022). What triggers Mps1 recruitment to unattached kinetochores in budding yeast is not well understood. However, in human cells, Mps1 recruitment is increased by the activity of Aurora B kinase (Santaguida et al., 2010; Saurin et al., 2011).

Error correction and SAC signaling is further entangled. Mps1 has been implicated in tension sensing in both human cells and budding yeast. Human MPS1 has been shown to

bind directly to Ndc80 and destabilize MT-KT interactions, even after inhibition of Aurora B (Ji et al., 2015; Maciejowski et al., 2017). In budding yeast, Mps1 has been shown to promote the biorientation of chromosomes independently of the CPC (Benzi et al., 2020; Maure et al., 2007; Storchová et al., 2011). Tethering Bub1 to Spc105 rescues SAC signaling and error correction defects of an Mps1 mutant deficient in both, suggesting a common mechanism for Mps1 in SAC and error correction (Benzi et al., 2020). Additionally, Mps1 co-purified with intact budding yeast kinetochores has been shown to phosphorylate Ndc80 independently of Ipl1, weakening KT MT attachments (Sarangapani et al., 2021). In summary, error correction and SAC are tightly linked and promote faithful segregation of chromosomes by halting anaphase onset and promoting correcting kinetochore attachments.

SAC signaling is reversed by protein phosphatase 1 (PP1) which is recruited via Spc105 (London et al., 2012; Pinsky et al., 2009; Rosenberg et al., 2011; Roy et al., 2019). How PP1 is specifically recruited to correctly attached kinetochores is not well understood. However, it has been shown that the PP1 catalytic subunit, Glc7, interacts with the kinesin Cin8, and mutations that abolish their interaction result in loss of tension (Suzuki et al., 2018). Additionally, it has been suggested that phosphorylation of Spc105 blocks the recruitment of Glc7 to prevent premature SAC silencing (Roy et al., 2019).

1.2.3 Motor proteins at the pre-anaphase spindle

As described above, KT capture occurs early after replication, resulting in a mix of syntelic and amphitelic attachments that are gradually corrected before anaphase. During this time, the budding yeast spindle is in a bi-lobed configuration where KTs are localized in two opposing clusters between the SPBs (Goshima & Yanagida, 2000; Pearsona et al., 2001; Xiangwei et al., 2000). This chromosome positioning before anaphase, known as chromosome congression, is a common theme in yeasts and mammalian cells (Kops et al., 2010). However, in mammalian cells the nuclear envelope breaks down as the spindle assembles. Furthermore, budding yeast KTs are attached to a single KT MT and do not possess polar ejection forces from astral MTs or MT flux as in higher eukaryotes, meaning that some mechanisms regulating chromosome positioning before anaphase differ (Maddox et al., 2000). However, kinesin motor proteins remain key players in all eukaryotic cells for proper spindle assembly, tension generation and elongation.

KT positioning in budding yeast depends on kinesin motor proteins Cin8, Kip1 and Kip3 (Gardner, Bouck, et al., 2008; Marco et al., 2013; Tytell & Sorger, 2006; Wargacki et al., 2010). Kinesin-5 motors (Cin8 and Kip1 in budding yeast) have been identified as critical components for spindle assembly in most eukaryotic cells (Xenopus (le Guellec et al., 1991), Pombe (Hagan & Yanagida, 1990), Drosophila (Heck et al., 1993), Human (Slangy et al., 1995)). Deletion mutants in budding yeast show defects in clustering kinetochores,

where instead of a neatly clustered single foci of KTs, mutants have a fuzzy streak of KTs between SPBs. This effect is greatest in cin8Δ cells, although cin8Δ mutants also have defects in spindle assembly (Gardner, Bouck, et al., 2008; Tytell & Sorger, 2006; Wargacki et al., 2010). The spindle defect in cin8Δ and kip1Δ cells manifest as collapsed spindles, where SPBs fail to separate (Saunders & Hoyt, 1992). The motor proteins are thought to generate an outward directed force by sliding antiparallel interpolar microtubule bundles, stabilizing the spindle (Hildebrandt & Hoyt, 2000; Kapitein et al., 2005; Saunders & Hoyt, 1992; Thiede et al., 2012). This idea is supported by the decreased spindle length observed in the subset of cin8Δ cells that do separate SPBs, and increased spindle length in cells overexpressing Cin8 (Gardner, Bouck, et al., 2008; Saunders & Hoyt, 1992). In budding yeast, MT bundles are stabilized by the MT crosslinker Ase1, and are aligned by the minus-directed kinesin Kar3 (Hepperla et al., 2014; Molodtsov et al., 2016). Ase1 is dispensable for pre-anaphase spindle assembly but is required in anaphase, as spindles collapse shortly after starting to elongate in ase1Δ cells (Schuyler et al., 2003). Kar3 on the other hand, aligns antiparallel MTs in the pre-anaphase spindle, allowing Kip1 and Cin8 to direct outward force (Hepperla et al., 2014). When Kar3-mediated bundling is abolished, cells become reliant on Ase1 for pre-anaphase spindle formation (Kornakov et al., 2020). Interestingly, cells lacking Cin8 also require the microtubule bundling protein Ase1 to assemble bipolar spindles (Kotwaliwale et al., 2007), suggesting that Cin8 might be required for bundling of MTs prior to anaphase. Cin8 is a bidirectional motor and can switch between minus-end and plus-end directed motility (Gerson-Gurwitz et al., 2011; Roostalu et al., 2011). So far not much is known about the biological function of this, however, it has been suggested that minus-directed motility of Cin8 is important during spindle assembly to cluster motors close to the SPBs to crosslink MTs and form the bilobed spindle (Shapira et al., 2017). Taken together, spindle assembly relies on MT bundling and kinesins to balance spindle stability and length before anaphase.

Interestingly, Cin8 has also been reported to affect MT dynamics in an MT length-dependent manner in pre-anaphase spindles. Photo-bleaching experiments showed that cells lacking Cin8 had less tubulin turnover at long KT MTs (Gardner, Bouck, et al., 2008). Furthermore, electron microscopy experiments have revealed that cin8Δ cells have more numerous spindle microtubules, and that they extend to the KT MTs from the opposing pole (Gardner 2008). This suggests that Cin8 positions kinetochores by destabilizing long KT MTs. This idea is additionally supported by live-cell imaging in which cin8Δ cells have more dynamic centromeric movements and faster centromeric displacement (Marco et al., 2013).

Recent findings further complicate interpreting the function of Cin8 at the spindle. Cin8 was shown to directly bind to kinetochores *in vitro* and depend on Dam1 and Ndc80 for spindle localization *in vivo* (Suzuki 2018). Furthermore, cells lacking Cin8 showed lower

kinetochore tension, possibly through recruiting PP1 to the kinetochores (Suzuki et al., 2018). Cin8's potential role in generating tension is further supported by the fact that cells require SAC activity for viability when Cin8 is depleted and have an increased frequency of chromosome missegregation (Sherwin et al., 2022).

Other motor proteins have also been implicated in kinetochore positioning. Kip3 is a member of the kinesin-8 family of motor proteins, localizes to plus-ends of astral and spindle MTs *in vivo*, and can bind plus-ends by itself *in vitro* (M. L. Gupta et al., 2006). Cells lacking Kip3 show a weak kinetochore positioning defect in pre-anaphase spindles and lagging chromosomes in anaphase (Tytell & Sorger, 2006). This phenotype is likely explained by Kip3 promoting MT depolymerization, and stems from observations that kip3Δ cells have longer aMTs, longer spindles at late anaphase and delayed spindle disassembly, as well as Kip3 promoting MT disassembly *in vitro* (M. L. Gupta et al., 2006; Straight et al., 1998). Kinesin-8 proteins Klp5 and Klp6 in fission yeast also affect MT length and play a role in positioning KTs before anaphase. Deletion mutants display fluctuating KT distances before anaphase, as well as longer spindles and lagging chromosomes in anaphase (Gergely et al., 2016).

In fission yeast, inward directed force is thought to be generated by the minus-directed kinesin-14 members Pkl1 and Klp2 to balance the outward force from kinesin-8 (Yukawa et al., 2018). Budding yeast kinesin-14 Kar3 has also been suggested to oppose the outward force generated by Cin8 and Kip1 (Saunders & Hoyt, 1992). However, altering centromeric stiffness and the number of kinetochores in a cell also changes the pre-anaphase spindle length (Nannas et al., 2014; Stephens et al., 2011). This shows that KTs, centromeres, and/or KT MTs, coupled to sister chromatid cohesion, opposes the inward force generated from the interpolar microtubules.

Taken together, motor proteins play conserved and varied roles in spindle assembly, MT positioning, error correction and tension generation. This is achieved via modulating MT dynamics and motor function in MT bundling and sliding. The multiple roles and sometimes redundant functions of motor proteins and MAPs complicates the interpretation of deletion mutant phenotypes.

1.2.4 Anaphase

Once biorientation has been achieved and SAC is satisfied, cohesin is cleaved by separase and chromosomes can be segregated. Anaphase is characterized by two stages (although these sometimes occur simultaneously): Poleward movement of KTs (anaphase A) and spindle elongation (anaphase B). Anaphase A is mediated by MT shrinkage while anaphase B is powered by MT sliding via motor proteins. In budding yeast, KT MTs will shrink from around 0.5 µm at the onset of anaphase to about 30-50 nm at the end of mitosis (O'Toole et al., 1999). Unlike in mammalian cells, MT flux, that is, disassembly at the minus-end coupled with growth at the plus-end, does not appear to

occur in budding yeast cells (Maddox et al., 2000). Therefore, the shortening of KT MTs during anaphase most likely stems from depolymerization at the plus-end.

At the onset of anaphase, centromeres move towards the SPBs with chromosome arms lagging (Pearsona et al., 2001; Xiangwei et al., 2000). At the same time, spindle elongation starts and plays a major role in segregating chromosomes, as the spindle elongates from ~2 µm to ~6 µm. The elongation rate depends on Cin8 and Kip1, with Cin8 being important during early anaphase when the spindle elongates rapidly, and Kip1 at later stages when the elongation rate is slower (Straight et al., 1998). Furthermore, cin8Δ elongation defects can be compensated for by mild overexpression of Kip1, suggesting they have overlapping functions. Both Cin8 and Kip1 are found at the spindle midzone, where plus-ends from opposing interpolar MTs meet, during anaphase.

How then is anaphase regulated? In budding yeast, cleavage of Scc1 by an inducible TEV protease is enough to segregate chromosomes (Uhlmann et al., 2000). However, in cells where Scc1 is cleaved by ectopic protease expression, MT dynamics in the elongating spindle remains high, KT MTs fail to shorten and spindle elongation is erratic, and the spindle frequently collapses (Higuchi & Uhlmann, 2005). This suggests that additional regulation is required for anaphase to progress normally. The anaphase spindle midzone is assembled by Ase1 and the minus-directed kinesin Kar3, which are both important to form MT bundles, and contribute to spindle integrity (Braun et al., 2011; Gardner, Haase, et al., 2008; Khmelinskii et al., 2007; Pellman et al., 1995; Schuyler et al., 2003; Thomas et al., 2020). Ase1 is recruited to the midzone after being dephosphorylated by the phosphatase Cdc14 (Khmelinskii et al., 2007). Cdc14 is a key regulator of anaphase, and subsequently mitotic exit, and is localized to the nucleolus for most of the cell cycle. In the nucleolus, Cdc14 is bound by nucleolar silencing establishing factor and telophase regulator 1 (Net1, also known as Cdc14 inhibitor 1, Cfi1) and kept sequestered (Shou et al., 1999; Visintin et al., 1999). Cdc14 is released in two waves: first a limited release upon anaphase onset that is limited to the nucleus, known as the Cdc-14 early anaphase release (FEAR) pathway, and later a full release that reaches the cytoplasm for mitotic exit. Phosphorylation of Net1 via Cdk1 will release Cdc14. However, this phosphorylation is counteracted by the phosphatase $PP2A^{Cdc55}$. $PP2A^{Cdc55}$ is downregulated by Zds1 and separase, coordinating the initial Cdc14 release with the onset of anaphase (Játiva et al., 2019; Queralt et al., 2006; Queralt & Uhlmann, 2008). Furthermore, Cdc5, which is needed for both FEAR and mitotic exit, contributes to Net1 phosphorylation and Cdc14 release (Shou et al., 2002; Visintin et al., 2003).

The FEAR pathway is important for several events in anaphase. Cdc14 and the FEAR components Spo12 are needed for nucleolar segregation and rDNA compaction (D'Amours et al., 2004; Sullivan et al., 2004). The FEAR pathway is also important for the anaphase spindle. Cdc14 dephosphorylates several spindle-associated proteins, such as Ask1, Fin1, and Ipl1-Sli15, and is needed to decrease MT dynamics and stabilize the

anaphase spindle as well as relocalize Sli15-Ipl1 to the spindle (Higuchi & Uhlmann, 2005; Pereira & Schiebel, 2003; Woodbury & Morgan, 2007). In early anaphase, Cdc14 dephosphorylates Ase1, which targets it to the midzone (Khmelinskii et al., 2007). Simultaneous inactivation of Cdc14 and Cdc5 results in cell cycle arrest with large-budded cells with short mitotic spindles (Roccuzzo et al., 2015). Spindle elongation can be restored to these cells by expressing Ase1 or Cin8 with non-phosphorylatable Cdk1 sites, indicating that Cdc14 signaling is required to generate the outward force at the midzone. This is further supported by the observation that Cin8 no longer localizes to the anaphase spindle after Cdc14 inactivation (Higuchi & Uhlmann, 2005). Thus, Cdc14 and Cdc5 regulate spindle dynamics and stability early in anaphase, ensuring correct segregation of genomic material.

1.2.5 Mitotic exit

When the spindle has elongated and chromosomes are segregated, the cells need to divide and reset for the next round of division to start. In budding yeast, this is controlled by the mitotic exit network (MEN), a signaling cascade that triggers the second, full release of Cdc14. MEN coordinates Cdc14 release in late anaphase via the GTPase Tem1 (Figure 5). Tem1 is localized to the daughter bound SPB via Nud1 on the outer plaque (Bardin et al., 2000; Gruneberg et al., 2000). There, Tem1 is kept inactive by a complex consisting of Bfa1 and Bub2 (Pereira et al., 2000). In anaphase, when SPBs have been segregated correctly, Tem1 becomes active and, together with Cdc5, activates a cascade that includes the kinases Cdc15, Dbf2-Dbf20 together with the co-activator Mob1, which leads to Net1 phosphorylation and Cdc14 release (reviewed in (Hotz & Barral, 2014)).

As the plane of division is predetermined in budding yeast, the cell integrates spatial cues to inhibit MEN until the spindle is positioned correctly. This checkpoint, known as the spindle position checkpoint (SPOC), uses the SPBs as a signaling platform and detects mother and bud-specific signals (Figure 5). The kinase Kin4 is an effector of SPOC and is localized to the mother cell cortex and becomes recruited to SPBs in the mother cell compartment when the spindle is mispositioned (D'Aquino et al., 2005; Pereira & Schiebel, 2005). Kin4's role in SPOC is to block MEN by phosphorylating Bfa1-Bub2 (Caydasi & Pereira, 2009; Maekawa et al., 2007). This prevents Cdc5 from phosphorylating Bfa1 (Maekawa et al., 2007; Pereira & Schiebel, 2005), which otherwise inactivates Bfa1-Bub2 and facilitates Tem1 activity for MEN to become active (Hu et al., 2001).

The kinase Kin4 is localized to the mother cell cortex and is found at the daughter bound SPB in anaphase (Pereira & Schiebel, 2005). In cells with mispositioned spindles, Kin4 and Bfa1 is recruited to both SPBs (Caydasi & Pereira, 2009; Molk et al., 2004; Pereira et al., 2001). Kin4 is removed from the daughter bound SPB once it enters the bud (Molk

2004). This is dependent on Lte1, which is localized to the bud cortex and is required for MEN activation (Bardin et al., 2000; Bertazzi et al., 2011; Falk et al., 2011; Pereira et al., 2000). At the same time, Tem1 fluorescence increases at the daughter bound SPB and has been shown to be dependent on bud localization rather than anaphase onset (Molk et al., 2004). Once relieved of Bfa1-Bub2, Tem1 can activate Cdc15 together with Cdc5 (Rock & Amon, 2011) which recruits and activates Dbf2-Mob1 (Rock et al., 2013). Dbf2/Mob1 is then phosphorylated by Cdc5 and will then translocate to the nucleolus and release Cdc14 (Zhou et al., 2021).

This way, SPOC integrates spatial cues to ensure that the genomic material is distributed between mother and daughter cells before initiating mitotic exit. Once Cdc14 is released via MEN, the phosphatase reverts Cdk1-mediated phosphorylations and upregulates the Cdk1 inhibitor Sic1 (Visintin et al., 1998).

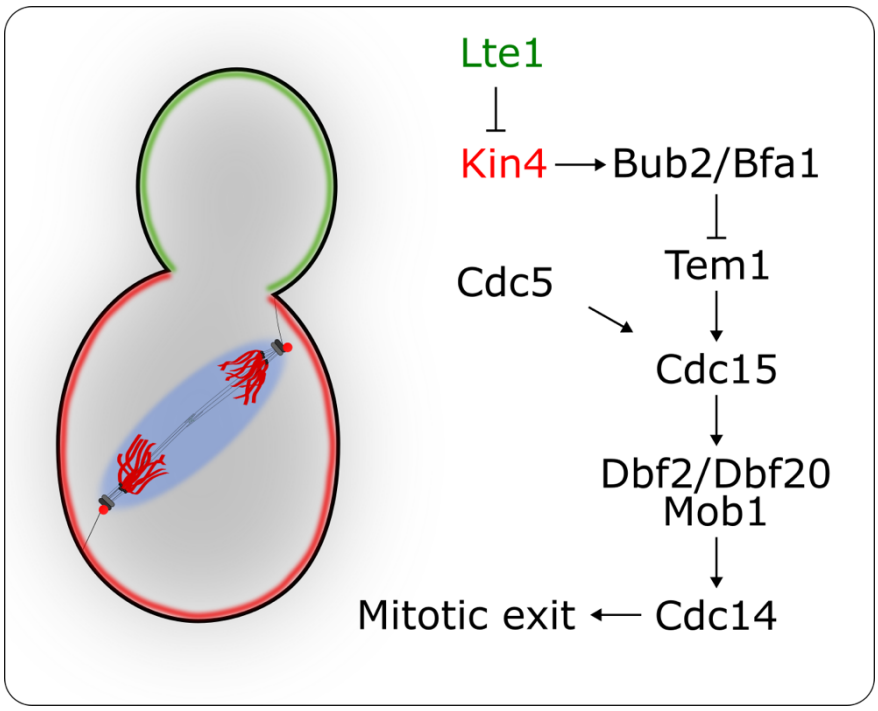

Figure 5 *– Outline of SPOC activation in a cell with a mispositioned spindle. Lte1 and Kin4 localizations are marked in green and red, respectively. MEN signaling is inhibited by Kin4 until a SPB has been transferred into the bud. Lte1 will then remove Kin4, alleviating the inhibitory signal from Bub2/Bfa1.*

1.2.6 Spindle disassembly

After the chromosomes have been segregated and MEN is active, the mitotic spindle needs to be disassembled prior to cytokinesis. How spindle disassembly is regulated is still not fully understood, but has been shown to be mediated by the CPC and APC/C. First, the midzone protein Ase1 is a target of APC/C and is degraded upon mitotic exit (Juang et al., 1997). Expression of an APC/C resistant Ase1 mutant delays spindle disassembly but does not prevent it completely. Secondly, Sli15-Ipl1 is found at the spindle midzone in anaphase and is needed for both spindle integrity and disassembly (Buvelot et al., 2003; Pereira & Schiebel, 2003). The relocalization of the CPC from kinetochores to the spindle has been shown to be regulated by dephosphorylation of Sli15 via Cdc14 (Pereira & Schiebel, 2003). How the CPC regulates spindle disassembly is not fully understood, but in late anaphase, Ipl1 binds to and phosphorylates Bim1 (Zimniak et al., 2009). Phosphomimetic mutants of Bim1 have been shown to have

reduced binding to microtubules, and a non-phosphorylatable variant of Bim1 was shown to remain at the spindle midzone, eventually resulting in spindle breakage (Zimniak et al., 2009). Further evidence for Ipl1's function in spindle disassembly comes from a high-throughput screen which found that cells lacking Mcm21, a component of the COMA complex, have reduced Ipl1 and Sli15 at the kinetochores at metaphase (Vizeacoumar et al., 2010). In anaphase, mcm21Δ cells failed to recruit Ipl1 and Sli15 to the midzone and displayed bent, elongated spindles that stretched longer than the cell. This spindle phenotype was further exacerbated by deletion of the FEAR component Slk19 and was also present in FEAR and MEN mutant strains (Vizeacoumar et al., 2010). Lastly, the MEN components Dbf2-Mob1 are required to maintain Ipl1 at the spindle in late anaphase (Stoepel et al., 2005), possibly explaining how MEN signaling promotes spindle disassembly via the CPC.

1.3 Chromatin organization

The genome at its most basic level is made up of DNA, but to allow for its compaction, regulation, repair and duplication, it is carefully organized at different levels. First, DNA is wrapped around spool-like histone proteins, forming a nucleosome. This compacts the DNA and allows for epigenetic regulation (see (Khorasanizadeh, 2004) for a review). Nucleosome wrapped DNA is the basic building block of the chromatin fiber, which has been described as 'beads on a string'. This description comes from early electron microscopy studies where isolated chromatin spreads presented as a 10 nm wide fiber with regularly spaced nucleosome beads (Olins & Olins, 1974). By varying the salt composition, isolated chromatin will take on different structures. In specific salt conditions and low chromatin concentrations, a regularly folded, 30 nm wide fiber is formed and was long thought to be the general arrangement of chromatin in cells (see (Ricci et al., 2015; Woodcock & Horowitz, 1995) for a review). However, the 30 nm, highly regular chromatin fiber remained elusive in cryo-EM studies of fixed cells. As new techniques have become available, our understanding of chromatin organization *in vivo* has expanded. A recent study employing super-resolution microscopy of core histone proteins in human interphase cells found that nucleosomes are clustered in small clutches containing 2-10 nucleosomes each (Ricci et al., 2015). Average nucleosome clutch sizes varied between cell types, with stem cells typically having smaller clutches than somatic cells. Furthermore, RNA pol II colocalization was associated with smaller clusters, suggesting transcriptionally active areas of the chromatin fiber are arranged into smaller clutches of nucleosomes.

Advances in electron microscopy and DNA-specific dyes has allowed for detailed mapping of chromatin organization in single cells. These techniques enable specific labeling of DNA and have revealed that rather than the regularly folded 30 nm fiber, chromatin in human interphase cells consists of a disordered polymer, 5-24 nm in diameter (Ou et al., 2017). Many different densities and clustering motifs have been

observed, such as linear stacks of clustered nucleosomes and looped regions of chromatin. Furthermore, imaging of mitotic chromatin revealed that these are also made up of 5-24 nm disordered chromatin chains, although more densely packed than that of interphase cells.

Genome-wide analysis of chromatin in human cells shows that chromatin is organized in hierarchical domains. This has been revealed through chromosome capture techniques (such as Hi-C) in which chromatin in close spatial proximity is crosslinked, digested and ligated with neighboring overhangs and then isolated (Lieberman-Aiden et al., 2009). The isolated DNA is then sequenced to map the average chromosomal contact frequencies in a population of cells. Hi-C maps have identified megabasepair-scale interacting domains of chromatin that correlate with specific histone modifications and biological function (Lieberman-Aiden et al., 2009; Schwarzer et al., 2017; Sexton et al., 2012). Initially termed A- and B-compartments, these have higher contact frequencies within their domains than with surrounding chromatin. A-compartments are gene-rich, have higher expression levels and are more accessible to DNAse digestion (Lieberman-Aiden et al., 2009). B compartments in contrast, have overall higher contact frequencies suggesting a more compact chromatin.

Genome-wide contact frequency maps have also revealed the existence of topologically associating domains (TADs). TADs are smaller than compartmental regions (median size of 185 kb (Rao et al., 2014)) but also show higher contact frequencies within them than that of bordering regions. TADs show up as pyramid shaped patterns in contact frequency maps (Dixon et al., 2012). TAD boundaries are associated with CTCF binding, housekeeping genes, tRNAs and retrotransposons (Dixon et al., 2012; Rao et al., 2014). Furthermore, many TADs show signs of being looped chromatin regions, evident by a local peak of high contact frequencies (9500 identified loops, 39% of TADs) (Rao et al., 2014). 90% of loop boundaries overlap with CTCF binding sites, Smc3 and Rad21 binding (Rao 2014), suggesting the involvement of cohesin. Indeed, depletion of Scc1 or NIPBL abolishes loop peaks and decreases the contact frequencies within TADs (Rao et al., 2017; Schwarzer et al., 2017; Wutz et al., 2017). Furthermore, depletion of Wapl or Pds5, which stabilizes cohesin binding on chromosomes, increases the number of observed loops and the loop sizes (Haarhuis et al., 2017; Wutz et al., 2017). Furthermore, transient depletion of Rad21 by auxin-mediated degradation followed by washout of auxin restores chromatin loops (Wutz et al., 2017). In contrast, depletion of CTCF does not affect the distances between interactions. However, TAD boundaries appear weaker, and the number of identified loops decreases, presumably as an effect of the weakened boundaries (Wutz et al., 2017) Taken together, chromatin loops are formed and maintained by the cohesin complex and are dynamic, as interacting frequencies at long ranges increase when cohesin binding is stabilized. Furthermore, loop and TAD boundaries are established by flanking convergently oriented CTCF sites.

Cohesin-dependent chromatin loops have been identified in budding yeast as well (Costantino et al., 2020; Dauban et al., 2020; Jeppsson et al., 2022; Schalbetter et al., 2017). These cohesin-dependent loops are smaller than those found in higher eukaryotes, and typically span 3-15 kilobase pairs (Costantino et al., 2020; Dauban et al., 2020). Furthermore, as with cohesin binding sites, budding yeast chromatin loop anchors are found at sites of convergently oriented genes (Dauban 2020). Unlike in higher eukaryotes, chromatin loops in budding yeast first appear in S-phase, approximately 15 minutes after cohesin can be detected at the same genomic loci (Costantino et al., 2020). However, chromatin loops do not require DNA replication to be formed. Depleting the replication initiating factor Cdc45 results in cells reaching metaphase without replicating the genome (Schalbetter et al., 2017). These cells were shown to still be able to form cohesin-dependent loops that strongly resemble those of wildtype cells (Dauban et al., 2020; Schalbetter et al., 2017). Furthermore, inducing cohesin loading in G1 arrested cells results in chromatin loop formation (Dauban et al., 2020). As in higher eukaryotes, chromatin loop formation in budding yeast is a dynamic process and is regulated by cohesin loading and offloading. Depleting the cohesin loading factor Scc2 in G2/M arrested cells abolishes chromatin looping while leaving sister chromatid cohesion intact (Jeppsson et al., 2022). Stabilizing cohesin on chromatin by depletion of Wpl1 increases the number of loops observed and increases the size of loops (Costantino et al., 2020; Dauban et al., 2020; Jeppsson et al., 2022). As in mammalian cells, Wpl1 depleted cells appear to have intact boundaries, as contact frequency peaks become “crisper” and overlap with cohesin binding sites. What then establishes loop boundaries in budding yeast? It was recently shown that inhibition of transcription, either by the drug thiolutin, or by inactivation of RNA pol II, decreases short range intra-chromosomal interactions, increases long range interactions and decreasing the number of observed loops, similar to what has been observer in CTCF depleted cells (Jeppsson et al., 2022). Furthermore, cells arrested in S-phase by hydroxyurea, which stalls replications forks, showed new loop boundaries at the stalled forks. Taken together, this suggests that transcription and replication forks act as chromosome loop boundaries in budding yeast.

In animal cells, chromosomes are condensed during mitosis into characteristic rod-shaped structures consisting of a central axis from which small chromatin loops emanate (Marsden & Laemmli, 1979; Naumova et al., 2013). Mitotic compaction is mediated by another SMC complex, the condensin complex (Hirano & Mitchison, 1994; Kruitwagen et al., 2015; Ono et al., 2003; Samejima et al., 2012), and depletion of condensin abolishes mitotic chromatin loops (Gibcus et al., 2018)). Isolated condensin complexes can compact naked DNA *in vitro* in an ATP-dependent manner (Strick et al., 2004). In budding yeast, mitotic chromosomes are less compacted than metazoan chromosomes (Guacci et al., 1994), although the condensin complex is important for

condensation of ribosomal DNA and contraction of chromosome arms during anaphase (Kruitwagen et al., 2015; Lazar-Stefanita et al., 2017; Vas et al., 2007).

1.3.1 Loop extrusion as a mechanism for chromatin loops

Cohesin and condensin complexes are thought to generate chromatin loops through a mechanism known as loop extrusion, in which the SMC complex binds to chromatin and functions as a motor to generate a growing loop dynamically (Fudenberg et al., 2017). Although it had long been speculated that SMC complexes could generate loops via loop extrusion (Nasmyth, 2001), it has only recently been directly observed. Condensin complexes isolated from budding yeast were first observed to translocate along naked DNA in an ATP-dependent manner, demonstrating that they can function as a motor (Terakawa et al., 2017). Then, Ganji et al showed that condensin can form DNA loops in an ATP-dependent manner on tethered DNA, and time-lapse imaging revealed gradually growing loops (Ganji et al., 2018). Later, similar experiments showed that the human cohesin complex fused to NIPBL could compact both naked and nucleosomal DNA in an ATP-dependent manner (Y. Kim et al., 2019). Furthermore, this cohesin complex can generate growing loops on tethered DNA, like condensin. In summary, loop extrusion is a plausible and attractive model for chromatin loop generation that seems common between SMC complexes and conserved through evolution.

Genome-wide chromosome capture techniques, such as Hi-C, have provided great insight into how the genome is organized. However, most such studies report average contact frequencies from a population of cells. How chromatin is organized in single cells and the cell-to-cell variability is less well understood. Single-cell Hi-C and super resolution imaging studies can recapitulate population average contact maps and replicate them as distance frequency maps based on spatial separation of specific genomic regions (Bintu et al., 2018; Finn et al., 2019; Flyamer et al., 2017; Gu et al., 2020; Luppino et al., 2020). However, these studies reveal that TAD-like domains are dynamic and show high cell-to-cell variability (Bintu et al., 2018; Finn et al., 2019; Luppino et al., 2020). Single-cell measurements have also revealed that TAD boundaries are not absolute insulators of chromatin contacts. Indeed, neighbouring cohesin-dependent domains flanked by CTCF binding sites often intermingle when imaged by microscopy (Bintu et al., 2018; Luppino et al., 2020; Szabo et al., 2020). Furthermore, depletion of WAPL or CTCF brings neighboring TADs closer together and increases overlap between domains, suggesting that these regions are controlled by cohesin-dependent loops, but that they are not completely insulated (Luppino et al., 2020; Szabo et al., 2020). Conversely, depletion of Rad21 decreases distances between neighboring TADs and the overlap between fluorescent DNA probes within the same TAD (Luppino et al., 2020; Szabo et al., 2020). Whether domain intermingling is affected by cohesin depletion appears to vary between domains, as two studies have reported conflicting results (Luppino et al., 2020; Szabo et al., 2020). Taken together, microscopy studies of

chromosome organization have shown that domain boundaries are dynamic, and that intermingling between domains is a general phenomenon, promoted by cohesin, and counteracted by CTCF and WAPL. Furthermore, TADs are present in single cells and are not an emergent property from population averages, although highly variable between individual cells.

Considering the heterogeneity observed between individual cells, the question of loop dynamics of individual cells arises. It is unclear if loops are stable once formed and how cohesin and CTCF modulate the dynamics *in vivo*. Two recent studies have begun to address these questions by super resolution live cell imaging. Gabriele et al. studied the dynamics of a single 500 kilobasepair TAD in mouse embryonic stem cells by inserting Tet operator repeats and Anchor3 arrays at the boundaries (Gabriele et al., 2022). The boundary loci can be visualized in live cells by expressing fluorescently tagged binding partners. Cells were then imaged using super resolution microscopy at 20 second intervals for 2 hours, and the distance between the two loci was measured for each frame. Depletion of Rad21 increases the mean dot distance while depletion of Wapl or CTCF had the opposite effect, as expected of intra-TAD distances controlled by loop extrusion. By depleting Rad21, they defined an un-looped state of the TAD and used polymer modeling informed by Micro-C data to identify the extent to which the TAD is looped in their experimental measurements. They found that the studied TAD is rarely in a fully looped state in wildtype conditions, spending 92% of the imaged time in a non-looped, or partially looped state. However, once in a fully looped state, the distances between dots remained relatively stable for 10-30 minutes, suggesting that the looped state is stabilized. These results suggest that TADs primarily exist in a dynamic state, as observed in fixed cells, but once a loop reaches two convergently oriented CTCF sites, it can be stabilized. Whether this is a general phenomenon is unclear, especially as the studied TAD contains a single gene that is not expressed in the cell type studied.

A recent study used a similar approach to study the dynamics of loop extrusion by introducing Tet and lac operator repeats 150 kb apart within a region devoid of enhancers or expressed genes where endogenous CTCF sites had been removed (Mach et al., 2022). They introduced new, convergently oriented CTCF sites flanking the operator sequences to establish a new loop barrier. Live cell imaging revealed that the dots transition between a proximal and distal state, consistent with what had been reported previously (Gabriele et al., 2022). The proximal state closely matched perfectly overlapping signals where both fluorescent proteins bind the same operator sequence, showing that dots in the proximal state are in close contact. In wildtype conditions, dots were observed in close contact 78% of the time imaged. Furthermore, the proximal configuration was stable for ~16 minutes and would reform every 5 minutes. Removal of the ectopic CTCF sites reduced the time dots spent in close proximity and the stability of the proximal state (down to 6 minutes). This effect was even greater upon depletion

of Rad21 where the proximal state would only last for 2 minutes. In conclusion, CTCF and cohesin can bring two genomic loci separated by 150 kilobasepairs within the same TAD in close contact within minutes. Furthermore, once in close contact, cohesin and CTCF stabilize the proximal state, consistent with loop extrusion models of cohesin with loop anchoring by convergently oriented CTCF sites. The difference observed in looping frequency between the two studies highlights the heterogeneity observed in single cell studies and could be caused by factors such as other chromosomal features, loop size or boundary strength.

Details of loop extrusion in budding yeast still need to be better understood. While loops and TAD-like structures have been observed in Hi-C studies, cell-to-cell variability and whether looping contributes to chromatin compaction is not known.

2 Research aims

The projects presented in this thesis focus on furthering our understanding of fundamental mechanisms underlying nuclear organization during cell division with two primary aims:

- To understand how extrinsic regulation of microtubules affects cell cycle progression in budding yeast.
- To develop a system to investigate chromosome organization in live yeast cells.

Specifically, the goals of the papers presented herein were:

Paper I

To understand the function of the +TIP Bik1 at nuclear MTs in its role in cell cycle progression.

Paper II

To map the phosphosites of old Spc110, the nuclear receptor for the γ-tubulin complex, from a pool of protein originating from the previous cell cycle, and to characterize their biological function.

Paper III

To generate a system to visualize chromosome dynamics in budding yeast by live cell microscopy and to apply it to explore the role of loop extruding cohesin on metaphase chromosome dynamics.

3 Materials and methods

3.1.1 Saccharomyces cerevisiae as a model organism

The budding yeast *Saccharomyces cerevisiae* has been utilized by humans for thousands of years to bake bread and brew beer. It is a single-cell eukaryotic organism, about 4-5 µm in diameter that divides, as the name suggests, by budding. As the yeast cell begins to divide, the daughter cell grows from the mother cell cortex and is eventually separated through cytokinesis, resulting in a mother and daughter cell. Each cell has a replicative capacity to divide about 25-40 times and this capacity is generally renewed in daughter cells, while the mother cell ages (for a review, see (Steinkraus et al., 2008)). Under correct nutrient and temperature conditions, the cells can divide about once every 90 minutes. Budding yeast has long been used as a model organism to study cell biology. The cerevisiae genome was the first to be fully sequenced in 1996 and revealed that the 16 chromosomes, totaling around 12 megabasepairs, contains around 6000 genes (Goffeau et al., 1996). As a model system, it has several advantages to more complex organisms. First, with the long history of use, many techniques and resources have been developed to create mutant strains and isolate specific mutational combinations. Libraries of strains are readily available, such as deletion, GFP-tagged and overexpression libraries, facilitating large screens or strain creation (Giaever et al., 2002; Huh et al., 2003; Jones et al., 2008). Additionally, the relative ease of genetic manipulation in budding yeast makes it cheaper and faster to study gene function. Although yeasts and animal cells evolutionarily separated around a billion years ago (Douzery et al., 2004), many genes and cellular functions are conserved. Indeed, around 50% of essential budding yeast genes that have a single orthologous gene in humans can be complemented by the corresponding human gene (Kachroo et al., 2015). Together, this makes budding yeast an ideal model organism to understand basic cell biology, which can then be applied to studies in higher eukaryotes and human cells.

Most of my experimental work has centered around microscopy. Microscopy in budding yeast presents great opportunities as well as challenges. As stated above, generating strains where a protein of interest is tagged with a fluorescent protein is relatively simple, making it easy to visualize its localization. However, the signal depends entirely on protein abundance and the stability and function of the fusion protein. Furthermore, the small cell size can provide a barrier to visualizing specific localizations, such as spindles during the early stages of mitosis.

3.1.2 Cell cycle analysis in budding yeast

In papers I and II, we analyzed cell cycle progression by cell and spindle morphology. Cell morphology is determined by transmitted light microscopy, showing the contour of the cell. The bud emerges from the mother cell cortex as cells commit to dividing at the G1/S

transition and will continue to grow through mitosis (Bean et al., 2006; Moffat & Andrews, 2004). Therefore, bud size can be used to track cell cycle progression. Furthermore, the spindle changes with the cell cycle phase. In G1, a single SPB exists that nucleates astral microtubules. In late S-phase, when the SPB has been duplicated, the bipolar mitotic spindle is formed. The spindle is initially short and will grow to around 1.5-2 µm as chromosomes become bioriented in metaphase (Winey et al., 1995; Winey & O'Toole, 2001). Once all chromosomes are bioriented and SAC is fulfilled, cells enter anaphase and elongate the spindle, which quickly reaches 4-6 µm in length and then elongates slower until chromosomes are fully segregated in mother and bud (Kahana et al., 1995; Straight et al., 1998; Winey et al., 1995). Fluorescently tagged alpha tubulin (Tub1) can therefore be used to track cell cycle progression by measuring spindle length and morphology. This has traditionally been done by immunofluorescence using α-tubulin antibodies (Cohen-Fix et al., 1996; Kilmartin & Adams, 1984; Muñoz-Barrera & Monje-Casas, 2017). Practically, cells are synchronized in G1 using a mating pheromone, typically α-factor for MATa cells. Cells are then released by washing away the α factor and samples are taken and fixed at regular intervals. Fixed cells are then imaged, and cell cycle progression is scored by bud appearance (G1/S), short 1.5-2 µm spindles (metaphase) and long 2+ µm spindles (anaphase).

In paper II, we incorporated Cdc14 localization to distinguish later cell cycle events further. Cdc14 is a key regulator of anaphase and mitotic exit (see chapters 1.2.4 and 1.2.5) and is kept sequestered in the nucleolus until anaphase when it is released in waves. First through the FEAR pathway in early anaphase and then during mitotic exit. In early anaphase, Cdc14 release is limited to the nucleus, while during mitotic exit, Cdc14 reaches the cytosol and is found at the daughter bound SPB, then both SPBs, the bud neck and later accumulates in the nucleolus again (Yoshida et al., 2002). This allows for further characterization of cell cycle progression in late anaphase leading up to mitotic exit.

3.1.3 Live cell imaging

While imaging of fixed cells can reveal protein localization and allows for the analysis of many cells, the dynamics and sequences of events can be hard to evaluate from snapshot images. Live cell imaging is a good complement, as time series of the same population of cells can reveal additional information. However, photobleaching and phototoxicity are concerns. Fusions of fluorescently marked proteins expressed from their endogenous promotor can prove difficult to detect and bleach rapidly from repeated imaging. Additionally, high-intensity light will damage cells and slow down or even halt cell division. Both issues must be balanced with the image signal-to-noise ratio, as lowering the excitation light intensity and imaging frequency helps counteract bleaching and toxicity (Carlton et al., 2010).

3.1.4 Visualizing chromosome dynamics

There are a variety of tools and techniques that enable studies of chromosomes in individual yeast cells. Fluorescence in situ hybridization (FISH) can be used to label specific DNA sequences in fixed cells to study different aspects of chromosomes, such as compaction in mitosis, centromere positioning, and chromosome pairing in meiosis (Guacci et al., 1994, 1997; Weiner & Kleckner, 1994). FISH is based on the ability of single-stranded DNA to hybridize with complementary DNA. By denaturing chromosomal DNA and introducing a labelled probe that is complementary to the sequence of interest, the localization of the hybridized probe can be visualized. The technique can also be combined with immunofluorescence to study the localization of DNA relative to other cellular structures (Trelles-Sticken et al., 1999). FISH can, in theory, be used to visualize any DNA sequence within the cell and does not require specific strains to be constructed. However, it requires cells to be fixed. Chromosomes of live cells can be visualized with DNA-binding dyes; however, these are not specific to any chromosomal loci or sequence. rDNA repeats have been visualized using fluorescently tagged Net1 (D'Ambrosio et al., 2008), which not only sequesters Cdc14 to the nucleolus, but also binds to rDNA repeats to silence transcription (Huang & Moazed, 2003; Straight et al., 1999).

To specifically visualize a chromosomal locus, bacterial operator repeats can be integrated at the region of interest. When combined with a fluorescently tagged repressor protein, the operator repeats can be visualized in both fixed and live cells (Straight et al., 1996). This technique has been used to study sister chromatid cohesion, centromere positioning, biorientation and chromosome segregation (Goshima & Yanagida, 2000; Jeppsson et al., 2014; Pearsona et al., 2001; Straight et al., 1996, 1997; T. Tanaka et al., 2000), chromosome condensation (Herbert et al., 2017; Kruitwagen et al., 2015; Neurohr et al., 2011; Vas et al., 2007) as well as chromosomal movement inside the nucleus (Heun et al., 2001; Marshall et al., 1997; Miné-Hattab & Rothstein, 2012). By tracking a single chromosomal locus in live cells, it was shown that chromosome arms follow diffusive movement within the nucleus and become less dynamic in S-phase (Heun et al., 2001; Marshall et al., 1997). The dynamic movement of chromosomal loci is often measured relative to another point of reference, such as the nuclear envelope or a second chromosome in diploid cells, as this helps reduce errors from nuclear movements or drift. Labeling of two loci on the same chromosome has been used to study axial compaction of chromosomes in mitosis by measuring the spatial distance between the two marked regions (Herbert et al., 2017; Kruitwagen et al., 2015; Neurohr et al., 2011; Vas et al., 2007).

Mean squared displacement (MSD) (or mean squared change in distance (MSCD), when two particles are tracked instead (Miné-Hattab & Rothstein, 2012)) has been calculated to understand the mode of diffusive movement and estimate constraints of

chromosomal diffusion (Heun et al., 2001; Marshall et al., 1997). MSD/MSCD analysis measures the displacement of a particle over increasing time steps (Δt). MSD is initially small but will, on average, increase as Δt becomes greater, as the particle has more time to diffuse further away. A freely diffusing particle will have a linear increase in MSD as Δt increases (Qian et al., 1991). A constrained particle, such as a fluorescently tagged chromosomal locus attached to a tethered chromosome, is confined to move within a limited radius and the MSD curve will eventually reach a plateau. The height of the plateau reflects the confinement radius (Heun et al., 2001; Marshall et al., 1997). We used MSCD analysis in paper III to investigate the effect of loop extruding cohesin on chromosome dynamics in G2/M arrested cells.

3.1.5 Recombination-induced tag exchange to track old and new proteins

Recombination-induced tag exchange (RITE) is a technique that allows for the tracking of proteins based on their relative age and is based on a DNA tagging cassette and an inducible Cre recombinase. The RITE cassette consists of a tag flanked by LoxP sequences, followed by a second tag. Activating the inducible Cre recombinase will result in site-specific recombination between the two LoxP sites, replacing the first tag with the second tag, meaning newly synthesized proteins can be distinguished from old ones. The RITE system was initially designed to track histone turnover using chromatin immunoprecipitation (Verzijlbergen et al., 2010) and then further developed to follow organelle inheritance and SPB dynamics by microscopy (Hotz et al., 2012; Menendez-Benito et al., 2013). Other methods to track proteins of different age typically rely on photoconversion or the different maturation times of fluorescent proteins (Hotz et al., 2012; Jakobs et al., 2003; Khmelinskii et al., 2012; Pereira et al., 2001). While techniques like tandem-fluorescent timers (Khmelinskii et al., 2012) or photoactivatable fluorescent proteins (Jakobs et al., 2003) or simply the slow maturation time of RFP or mCherry (Hotz et al., 2012; Pereira et al., 2001) can allow for detection of protein dynamics within a shorter time frame than RITE, they are not suitable for biochemistry.

In paper II, we used RITE to isolate old, pre-existing subunits of Spc110 from maternal origin. Labeling with RITE was followed with enrichment of intact SPBs using a previously published method that relies on affinity purification of Spc97 (Fong et al., 2018). SPBs were then disrupted with trichloroacetic acid and resuspended in a mild denaturing buffer to purify old and new proteins using immunoprecipitation using antibodies against the RITE tags. Purifications appear very clean, as almost no cross-contamination was observed in western blot analysis. However, this method does not distinguish between old and new SPBs, as any subunit with the Flag or V5 fusion tag will be recovered, regardless of which SPB it originated from. However, previous results from fluorescent RITE analysis showed that 90% of 'old' Spc110 was located at the daughter bound SPB, while 'new' Spc110 is incorporated in both SPBs (Menendez-Benito et al., 2013).

4 Results and discussion

4.1.1 Paper I – The microtubule plus-end tracking protein Bik1 is required for chromosome congression

The project presented in paper I was initiated after observations from a small screen of cells expressing different proteins tagged with a fluorescent RITE cassette. We had selected a subset of 96 nuclear localization to evaluate the localization of old and newly synthesized proteins. We found Bik1 to have different localizations in the cell depending on the age of the protein, with a GFP-tagged 'old' pool visible as two dots in the nucleus and along short mitotic spindles. In contrast, newly synthesized Bik1 was primarily at aMTs. These results got us interested in the nuclear pool, as the published literature had mostly focused on the spindle positioning and dynein-related functions of Bik1. In paper I, we have characterized the nuclear pool of Bik1 and identified its role in chromosome congression prior to anaphase.

First, we monitored Bik1 localization relative to SPBs (Spc42), kinetochores (Ndc80), and MTs (Tub1) throughout the cell cycle by microscopy. We found that Bik1 overlaps with kinetochores from G1 until the onset of anaphase. As the spindle elongates, Bik1 is instead located at the interpolar MTs and the midzone. Bik1 then disassociates from the spindle prior to disassembly. In the pre-anaphase spindle, kinetochores are kept clustered while chromosomal attachments are corrected, suggesting that Bik1 could be involved in one or more of those steps.

To separate the nuclear and cytoplasmic functions of Bik1, we generated a strain where Bik1 is fused to a strong nuclear export signal (NES), which depletes the nuclear pool of Bik1 while leaving the cytoplasmic pool intact (referred to as Bik1-NES). We then followed cell cycle progression in fixed cells by spindle morphology (Tub1) and found that the NES mutant has a delay in metaphase prior to spindle elongation. We then monitored cell cycle progression in live cells expressing Ndc80-TagRFP-T and could confirm the delay. The live cell imaging experiment revealed two more aspects of the Bik1-NES mutant: First, the spindle would sometimes briefly move into the bud compartment and then back before elongation (observed in about 10% of NES cells analyzed). These cells often had long, bent cytoplasmic MTs decorated with Bik1-NES. This phenotype suggests a spindle defect that could be caused by dosage increase from cytosolic Bik1, which is normally found in the nucleus. Indeed, previous studies have shown a similar phenotype with long and bent aMTs upon overexpression of Kip2, a budding yeast kinesin-7 shown to promote Bik1 association with astral plus-tips (Carvalho et al., 2004). The Kip2 overexpression phenotype of hyper elongation of aMTs could be rescued by deleting Bik1, however, Kip2 overexpression was still lethal in bik1Δ cells (Carvalho et al., 2004). Although not all cells displayed such a phenotype, we cannot exclude the possibility that the cell cycle delay is influenced by the increased cytoplasmic Bik1 levels. Second,

although the signal-to-noise ratio of the live cell imaging is limited (the long imaging time limits the exposure time to avoid photo toxicity), the kinetochores appeared less clustered than WT cells prior to anaphase. Instead, we often observed a fuzzy, continuous distribution of kinetochores before spindle elongation. To better understand this defect, we opted to arrest cells in metaphase to evaluate fully assembled spindles. The arrest was achieved by controlling the expression of the APC/C co-activator Cdc20 via the Gal1 promoter. We found that 60% of Bik1-NES cells had unclustered kinetochores that appeared as a fuzzy streak between the SPBs. These results suggest that nuclear Bik1 at the KTs influence chromosome congression, as was observed in kinesin deletion strains cin8Δ, kip1Δ and kip3Δ (Tytell & Sorger, 2006; Wargacki et al., 2010). This defect appears strongest in Δcin8 (~60% vs 20% in WT, ~25% kip1Δ and ~35% kip3Δ (Tytell & Sorger, 2006)), which matches what we observed in Bik1-NES cells.

As Bik1 accumulation at aMTs plus-ends partially depends on the kinesin Kip2, we hypothesized that a nuclear kinesin might be needed for Bik1 accumulation at kinetochore MTs. To identify interaction partners of Bik1 at metaphase, we arrested cells by Cdc20 depletion and isolated Bik1-5xFlag under native conditions. We then analyzed interaction partners by mass spectrometry. This experiment revealed known interaction partners, such as Bim1 (Blake-Hodek et al., 2010; Roberts et al., 2014; Stangier et al., 2018; van der Vaart et al., 2017; Wolyniak et al., 2006), Kip2 (Carvalho et al., 2004; Roberts et al., 2014; van der Vaart et al., 2017), Kar9 (Moore et al., 2009; Stangier et al., 2018; van der Vaart et al., 2017) and Stu2 (Stangier et al., 2018; van der Vaart et al., 2017; Wolyniak et al., 2006). Interestingly, among the most highly recovered proteins (both by coverage % and unique peptides) was Cin8. We then tried to recapitulate interactions by co-immunoprecipitation in asynchronous cultures but were unable to co-precipitate Bik1 and Cin8. We could neither detect an interaction between Cin8 and the kinetochore protein Dsn1, which has previously been reported (Suzuki et al., 2018), suggesting an issue in detection. It is possible that the interaction is transient in metaphase, or that only a small subset the proteins interact, making detection difficult.

To better understand the potential interaction between Bik1 and Cin8, we turned to a proximity based biotinylation assay. A biotinylating enzyme, TurboID, was linked to Bik1 in cells expressing Kip2, Cin8, Kip1 or Mre11 fused to 5xFlag. Mre11 was included as a negative control, as it's a nuclear protein that should not interact with Bik1. Biotinylated proteins were then purified by streptavidin pulldown and analyzed by western blot. We could recover all kinesins, while the nuclear protein Mre11 could not be detected. This method has previously been used in high-throughput screens to identify interaction partners by mass spectrometry and does not necessarily indicate a direct interaction. However, it does suggest that these proteins are in close proximity. Taken together, the mass spectrometry and biotinylation assays provide evidence that Bik1 is localized close to the mitotic kinesins Cin8 and Kip1.

To understand where in the cell this interaction occurs, we turned to bi-molecular fluorescence complementation (BiFC). This method utilizes a split variant of the Venus fluorescent protein, one half fused to each protein of interest. If the proteins are in close proximity, the Venus fluorescent protein is reconstituted and detected by microscopy. Expressing Cin8 and Bik1 with fragments of Venus reconstituted a fluorescent signal at spindles that increased as the mitotic spindle formed (20% in G1 cells, 80% in metaphase and anaphase cells). Interestingly, these signals presented as two dots strongly resembling the kinetochore clusters. In anaphase cells, a single dot was visible in the middle of the spindle. However, it has been reported that once formed, the reconstituted fluorescent protein is irreversibly fused, cautioning against overinterpretation of the localized interactions later in the cell cycle (Shyu & Hu, 2008). Nonetheless, the BiFC data supports the biochemical assays and shows that Cin8 and Bik1 interact at the spindle from metaphase.

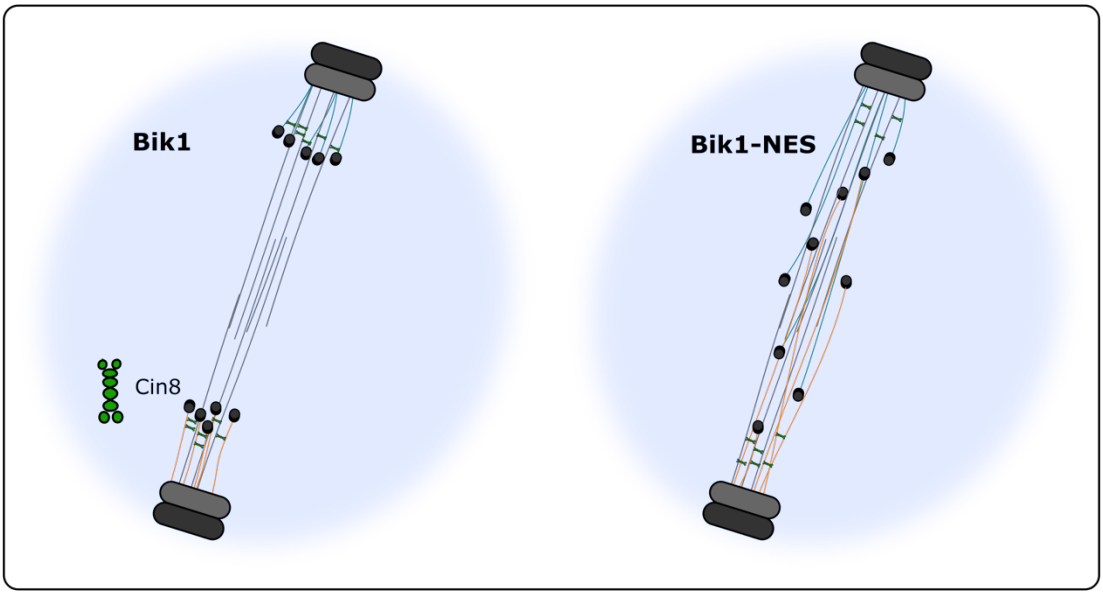

Figure 6 *– Bik1 and Bik1-NES metaphase spindles. In Bik1 spindles (left panel), KTs are kept clustered close to the SPBs with Cin8 localized close to KT clusters. In Bik1-NES spindles (right), KTs appears in a fuzzy streak, presumably due to failure to clusters KTs. Cin8 is still found in foci close to the SPBs.*

Lastly, we investigated the interdependence of Cin8 and Bik1 in their localization to the pre-anaphase spindle. Bik1 levels at pre-anaphase spindles decrease in a cin8Δ strain, suggesting that Cin8 either brings Bik1 to the KT MTs, or that the KT MTs themselves are altered in the absence of Cin8. Conversely, Cin8 was still found at the pre-anaphase spindle in Bik1-NES cells. However, Cin8 did not overlap with the mispositioned Ndc80 (Figure 6). This is intriguing as Cin8 has been shown to bind directly to Dam1 *in vitro* and depends on Dam1 and Ndc80 to localize to the pre-anaphase spindle *in vivo* (Suzuki et al., 2018). At the kinetochores, Cin8 mediates tension, as measured by a FRET sensor inserted in the Ndc80 complex. Deleting the N-terminal domain of Ndc80 produces a loss in tension (Suzuki et al., 2016) and mislocalizes the inner kinetochore protein Mtw1,

resulting in a stretched, fuzzy distribution like the Ndc80 distribution in Bik1-NES cells (Demirel et al., 2012). The role of Bik1 at the KT is to couple Ndc80 to the plus-end, mediating tension generation via Cin8 and the MT plus-end. Another possibility is that Bik1 regulates MTs by increasing Cin8 processivity, as has suggested for Kip2 at astral microtubules (Hibbel et al., 2015; Roberts et al., 2014). This is based on observations that overexpression of Kip2 leads to hyper elongation of aMTs, which is rescued by deletion of Bik1 or Bim1 (Carvalho et al., 2004). Kip2 alone increases MT length *in vitro* (Hibbel et al., 2015) and Bik1-Bim1 increases the length Kip2 travels along MTs *in vitro* (Roberts et al., 2014). This suggests that Bik1 helps bring Kip2 to plus-ends, increasing MT polymerization. Bik1 may play a similar role at the spindle by increasing Cin8 processivity to cluster kinetochores. In this model, Bik1 would help bring Cin8 to long KT MTs to depolymerize them, clustering KTs close to the SPB (Gardner, Bouck, et al., 2008).

4.1.2 Paper II – Phosphosites of the yeast centrosome component Spc110 contribute to cell cycle progression and mitotic exit

As described earlier, SPBs are heavily phosphorylated throughout the cell cycle. Interestingly, previous studies have shown that phosphorylation of SPB components differ between old and new SPBs (Hotz et al., 2012; Lengefeld et al., 2017; Matellán et al., 2020). For example, the cytosolic γ-TuSC anchor, Spc72, has been shown to be recruited to new SPBs at the onset of anaphase via Cdc5-mediated phosphorylation (Matellán et al., 2020). Furthermore, phosphorylation sites of Spc110 have been shown to influence MT dynamics (Huisman et al., 2007; Liang et al., 2013; T. C. Lin et al., 2014). Whether age-specific phosphorylations occur in other SPB components is not known, and our overall understanding of biological functions of specific phosphorylation sites is lacking. With this in mind, we set out to isolate old Spc110, originating in the previous cell cycle, to characterize phosphorylation sites and understand their biological function.

To identify phosphorylation sites specifically in old material, we introduced a RITE cassette consisting of two epitope tags (5xFlag and V5) to the SPC110 gene. To couple Cre-recombination with the cell cycle, we grew cells were grown until they had depleted the glucose in the media, leading to arrest via the diauxic shift where protein expression is minimal (Werner-Washburne et al., 1996). At this point, we induced Cre-recombination by adding β-estradiol to the media. Cells were then released in fresh media and collected for protein extraction when most of the cells were in G2/M. Intact SPBs were isolated by affinity purification of Spc97-TAP, followed by denaturation and isolation of Spc110-5xFlag and Spc110-V5, using immunoprecipitation against Flag and V5 separately. Following immunoprecipitation, western blot analysis revealed no visible cross-contamination between Flag and V5-tagged proteins.

Phosphorylated residues in the purified old (Flag) and new (V5) Spc110 were analyzed by mass spectrometry in two ways: Enrichment of phospho-peptides using titanium

dioxide beads and non-enriched analysis. Phospho-enrichment identified S60 and S11 phosphosites in the Flag-purified sample. In contrast, no phosphorylated peptides were recovered in the V5-purified sample. Because of this, we decided to only analyze the Flag-purified sample without phospho-enrichment. These analyses revealed two phosphosites: S11 and S36. Taken together, 'old' Spc110 was found to be phosphorylated at S11, S36 and S60 at G2/M. Conversely, we did not identify any phosphorylated residues in newly expressed Spc110. Previous reports have revealed more sites to be phosphorylated (Fong et al., 2018; Keck et al., 2011) that were absent in our analysis, although some of those sites are missing in our peptide coverage. Specifically, S91 was not covered, and T64 and T68 had low coverage. However, T18, which has been shown to be phosphorylated in asynchronous cultures as well as G1 and G2/M arrested cells (Fong et al., 2018; Keck et al., 2011), was not detected as being phosphorylated in our analysis. Differences in sample preparation and mass spectrometry could explain our apparent lower sensitivity. Furthermore, V5-tagged proteins represent a transiently expressed pool, and the pre-existing pool could be affected by stationary phase. However, an analysis of the phospho-proteome during the diauxic shift found that Spc110 T18, S60 and S36 (among others) remained phosphorylated 33 hours after inoculation (the last time point analyzed) (Gassaway et al., 2021).

The identified phosphorylated residues reside in the N-terminal domain of Spc110, which is known to interact with γ-TuSC. However, the S11 phosphorylation site has only been identified in proteomic screens, and the function has not been explored (Lanz et al., 2021; Zhou et al., 2021). To this end we generated strains expressing non-phosphorylatable variants of Spc110. Cell cycle analysis revealed that the $Spc110^{S36A}$ and $Spc110^{S11AS36A}$ mutants slightly delayed SPB separation. Furthermore, the S11AS36A showed slower mitotic progress and delayed re-entry into G1. The S11A mutant had an increase in spindle MT intensity in anaphase spindles. This effect was partially suppressed in the S11AS36A mutant, suggesting these sites do not have additive effects on spindle MTs. Furthermore, we observed an increase in asymmetric spindles, where the half of the spindle closest to the bud appeared less bright, in the S11A mutant, and to a lesser extent in the S36A and S11AS36A mutants. These findings suggest that S36 and S11 phosphorylation affects cell cycle progression and modulate spindle dynamics.

Spc110 S36 has been identified as a Cdk1 consensus site (Friedman et al., 2001; Huisman et al., 2007). Spc110 S11, on the other hand has been reported to be phosphorylated in anaphase in a Cdc5-dependent manner in a large phosphoproteome study (Zhou et al., 2021). Since Cdc5 plays a role in regulating exit from mitosis, and our mutant strains had a slower cell cycle progression and delayed re-entry into G1, we next investigated the localization of Cdc14, a key regulator of mitotic exit. Cdc14 is kept sequestered in the nucleolus from G1 until anaphase. Upon release via MEN, Cdc14 is recruited to the SPBs and then to the bud neck before cytokinesis (Bembenek et al., 2005; Yoshida et al.,

2002). We therefore followed synchronized cells released from G1 arrest and categorized them based on Cdc14 localization. These analyses confirmed what we had seen earlier, with fewer cells in late anaphase (full Cdc14 release) in the Spc110^{S11AS36A} mutant than in WT. Furthermore, the mutant took longer to enter G1, where Cdc14 is again found in the nucleolus. This shows that phosphorylation of Spc110 at S11 and S36 influences spindle dynamics, and failure to do so delays the exit from mitosis.

The brighter spindles we observe in the phospho-mutants and the observation that Spc110 S11 phosphorylation depends on Cdc5 suggest that this phosphorylation is involved in spindle disassembly. How phosphorylations of the γ-TuSC receptor influence MT dynamics is not well understood. However, N-terminal phosphorylations have been shown to influence γ-TuSC oligomerization *in vitro* and affect MT intensity *in vivo* (T. C. Lin et al., 2014). Cdk1 sites (S36 and S91 (Huisman et al., 2007)) and Mps1 sites (S60, S64 and S68 (Friedman et al., 2001)) have been shown to promote γ-TuSC oligomerization when phosphorylated (T. C. Lin et al., 2014). Additionally, phosphomimetic mutations of Cdk1 sites, Mps1 sites or both combined promote MT nucleation *in vitro*. Mutating all 5 sites to non-phosphorylatable residues impairs spindle formation and MT nucleation *in vivo* (T. C. Lin et al., 2014). Furthermore, early EM studies found that the number of interpolar MTs decrease as the spindle elongates, with long anaphase spindles typically having 2 from each SPB (Winey et al., 1995). It's possible that S11A delays disassembly of interpolar MTs, explaining the brighter spindles we observed.

Another role of Spc110 S11 phosphorylation may be to regulate the recruitment of MAPs to the MTs. Minus-end regulation of MT dynamics has been demonstrated for aMTs in budding yeast. Bub2 and Bfa1, which bind the SPB and inhibit MEN when spindles are mispositioned, have been shown to affect loading of the kinesin Kip2 at MT minus-ends (Chen et al., 2019). Kip2 will then travel along the MTs towards the plus-end and is involved in dynein transport and regulation of microtubule length (Carvalho et al., 2004; Hibbel et al., 2015; Huyett et al., 1998; Roberts et al., 2014). Interestingly, it has been suggested that dephosphorylation of Spc110 via the FEAR and MEN phosphatases Cdc15 and Spo12 recruits the microtubule polymerase Stu2 to the spindle, increasing the rate of spindle elongation (Liang et al., 2013). Whether Spc110 S11 and S36 regulate γ-TuSC oligomerization or recruitment of MAPs remains to be seen. Taken together, our results show that pre-existing Spc110 is phosphorylated at S36, S60 and S11 at G2/M. Mutating S36 and S11 affects cell cycle progression and spindle dynamics.

4.1.3 Paper III – The effect of DNA loop extrusion on chromosome dynamics in budding yeast

The study presented in paper III was started with the supervision of Professor Camilla Björkegren and aimed to understand the impact of loop extrusion on chromosome dynamics. Cohesin is loaded onto chromosomes in S-phase by the cohesin loading

factor Scc2 and holds sister chromatids together until separase is activated at the onset of anaphase. In addition to sister chromatid cohesion, recent studies have revealed that budding yeast chromosomes are organized into interacting domains and chromosomal loops by the cohesin complex (Costantino et al., 2020; Dauban et al., 2020; Schalbetter et al., 2017). However, most of the studies have used chromatin capture techniques (such as Hi-C) which reveal genome-wide average contact frequencies of a population of cells. How chromosomes of individual cells are organized is still poorly understood. To investigate this, we have generated a microscopy-based system where two loci of chromosome IV can be visualized in live cells. The system is based on fluorescently tagged repressors (GFP-LacI and TetR-tdTomato) and the integration of corresponding operator sequences (LacO and TetO) at specific sites in the budding yeast.

We chose integration sites based on previously published cohesin chromatin immunoprecipitation and Hi-C data (Jeppsson et al., 2022). We chose a region on chromosome IV with loops and interacting domains that were reduced in the absence of dynamic cohesin. Two insertion sites, 240kb apart, were chosen in intergenic regions without cohesin binding sites to not disrupt cohesin binding. We then introduced auxin-inducible degrons (AIDs) at the C-terminus of Scc2 or the cohesin off-loader Rad61 (Wpl1) to modulate cohesin binding to the chromosome. As sister chromatid cohesion is established in S-phase and cohesive cohesin remains stably associated with the chromatin in the absence of Scc2 once established (Ciosk et al., 2000), we performed measurements in cells arrested in G2/M by the drug benomyl. This way, dynamic cohesin is no longer loaded onto chromosomes, while stably bound, cohesive cohesin remains (Jeppsson et al., 2022). Benomyl has been shown to decrease MT dynamics and leads to a loss of tension at the spindle (Chacón et al., 2014; Pearson et al., 2003; Suzuki et al., 2016, 2018).

We first measured the distance between GFP and tdTomato dots in cells arrested in G2/M followed by 1h of auxin treatment before being fixed. We observed a large spread of distances between cells, ranging from completely overlapping dots (0.25 µm apart) to dots separated by up to 1.5 µm. These results are consistent with previous reports from yeast chromosomes where similar genomic distances have been measured (Herbert et al., 2017; Kruitwagen et al., 2015; Neurohr et al., 2011). Neither the Scc2 nor Rad61 depletion had a significant effect on mean distances. However, the Scc2-AID strain had a larger spread of distances. In mammalian cells, depletion of Rad21 (Scc1) in interphase cells has been shown to increase distances and shift the distribution when tracking fluorescent dots placed at the boundaries of a single TAD (Gabriele et al., 2022). In the same study, depletion of Wapl had a much lower effect on distances. These data suggest that dynamic cohesin does not have a major influence on chromosome compaction, at least not in this region of chromosome IV.

Chromosomes move dynamically in the nucleus by diffusion. Two loci on the same chromosome arm move independently but are restricted by the genomic distance in how far they can move apart (Herbert 2017, Gabriele 2022). If a domain forms a loop, the dynamic movement of two dots flanking the domain should be restricted. To test this, we performed live-cell imaging experiments and tracked dot distances over time. To maximize imaging frequency, we took images in a single plane with low exposure times every 1.7 seconds and measured the 2D distance for each frame. Average distances were comparable to 3D distances in fixed cells and depletion of Scc2 or Rad61 had little impact. We then calculated the MSCD, which measures the change in distance for increasing steps in time (Δt) (Marshall et al., 1997; Miné-Hattab & Rothstein, 2012). Freely diffusing particles will display a linear increase of MSCD with increasing Δt, while tethered particles (such as two loci on the same chromosome) will reach a plateau that correlates with the radius of confinement (Qian et al., 1991). MSCD measurements revealed that the Scc2-depleted cells reached a higher plateau, suggesting they are less restricted in their movement than WT. This result indicates that loop extrusion restricts chromosome dynamics.

While imaging the fluorescently marked loci on chromosome IV, we noticed a subset of cells with more than 2 dots. The multiple dots were found in close proximity, limited to either centromere proximal or distal dots, and more common in the centromere proximal (GFP) dots. This suggests it is not due to a complete loss of sister cohesion. Live cell imaging revealed dynamic splitting of dots, where dots would sometimes separate for a few frames before merging again. Splitting events were more common after Scc2 depletion. This could be caused by a local loss of cohesion, presumably from the extended G2/M arrest (Vas et al., 2007). It could also be an effect of less constricted movement of chromosomes in the absence of loop extrusion. Each dot consists of two sister chromatids held together by cohesin and can likely be incorporated into loops as even 200 nm particles fused to DNA can be incorporated into loops by cohesin *in vitro* (Pradhan et al., 2022). To better understand the effect of loop extrusion on chromosome dynamics in yeast, it might be necessary to utilize super resolution microscopy, as the physical distances are at the limits of what can be observed by conventional light microscopy. Another source of error comes from the observation of cohesed sisters. Blocking replication by Cdc45 inactivation results in cells that go through mitosis without replication (Tercero et al., 2000). However, those unreplicated chromosomes are compacted in a cohesin-dependent manner, like wildtype G2/M cells (Schalbetter et al., 2017). This would increase the accuracy of the assay by measuring chromosome dynamics of individual chromosomes rather than sister chromatids.

5 Concluding remarks and future perspectives

The aim of this thesis has been to better understand spindle and chromosome dynamics during mitosis in budding yeast. To this end, I have investigated two modes of regulating the spindle: via MAPs as well as PTMs of the γ-TuSC anchor Spc110.

We have identified a novel role of Bik1 in chromosome congression and linked it to the kinesin Cin8, although the exact mechanism through which Bik1 mediates this remains elusive. Future studies will hopefully shed light on key aspects of Bik1 and other MAP's functions at the spindle:

Does Bik1 affect tension generation or biorientation of chromosomes? While we have not observed unattached kinetochores in Bik1-NES cells, we have not investigated if biorientation of sister chromatids is affected.

Is the kinetochore phenotype of Bik1-NES cells mediated via MT dynamics or kinetochore defects? It would seem likely that Bik1 regulates MT dynamics at the KT MTs, as both aMTs and KT MTs are affected when Bik1 is deleted or overexpressed (Berlin et al., 1990). However, FRAP analysis of spindle MTs have reported that loss of Bik1 does not affect the rate of Tub1 recovery, although 20% less is recovered than in WT cells (Wolyniak et al., 2006). Similarly, loss of Bim1 has little effect on Tub1 recovery rates, however, loss of both results in a two-fold decrease in recovery rate. These studies suggest that Bik1 and Bim1 are partially redundant for normal KT MT dynamics. With the potential role of Bik1 and Bim1 in Dam1 oligomerization (Dudziak et al., 2021) and the finding that Cin8 can bind directly to Dam1 *in vitro* (Suzuki et al., 2018), the distribution of other KT proteins, such as components of the Dam1 complex, should be addressed in cells lacking Bik1.

Budding yeast +TIPs Bik1, Bim1, Stu2, and Kar9 have been shown to interact, directly or indirectly (Stangier et al., 2018; Wolyniak et al., 2006), and localize to aMTs, SPBs, KTs, KT MTs and interpolar MTs. A future challenge is elucidating their roles at these different localizations. Careful characterization of Bik1 and Bim1 interactions have revealed that the CG domain of Bik1 interacts with a C-terminal motif in Bim1 (Stangier et al., 2018). This direct binding allows Bik1 to bind Kar9 indirectly. Conversely, the coiled-coil domain of Bik1 interacts with the C-terminal end of Stu2, allowing indirect interactions with Bim1. Do different pairwise interactions determine their localization? Are specific interactions mediated via PTMs? The usual suspects, Aurora B, Mps1 and Cdc14 are prime candidates as they all regulate spindles throughout mitosis.

We have also explored phosphorylations of old (from maternal origin) Spc110. Using RITE, we could separate old and new Spc110 and identify an uncharacterized phosphosite, S11, in old Spc110. Although hundreds of phosphorylation sites of SPB proteins have been identified, few have been functionally characterized. Like other phosphosites in the N-

terminal of Spc110, S11 appears to function in regulating spindle MTs. It remains unclear when S11 becomes phosphorylated and by what kinase, although a phosphoproteomic analysis suggests that Cdc5 is involved in dephosphorylation of this site (Zhou et al., 2021). Future work will hopefully further characterize the functions of SPB phosphorylation.

The study also establishes RITE as a technique to study post-translational modifications of pre-existing and newly synthesized subunits. This could prove a valuable tool to understand modifications that are involved in establishing SPB identity during spindle positioning. Previous studies have identified MEN components Dbf2/20 and Cdc15 as key regulators of spindle alignment by stabilizing Kar9 at the old SPB (Hotz et al., 2012). Additionally, Swe1 has been shown to phosphorylate Nud1 in G1 establish SPB identity (Lengefeld et al., 2017). Whether other modifications differ between old and new SPBs is not known and could be identified by our assay.

We have also established an assay to study chromosome dynamics in live yeast cells using microscopy. We used this to investigate the impact of loop extruding cohesin on intra-chromosomal distances in G2/M. We did not find that loop extrusion by cohesin had a major effect on chromosomal distances, however, it is possible that more subtle differences are masked by the fact that we observed sister chromatids that may be looped individually. By refining the assay, either through increased resolution (by for example super resolution microscopy), or by studying unreplicated chromosomes, it could be possible to observe loop extrusion in real-time, as has been done in mammalian cells (Gabriele et al., 2022; Mach et al., 2022).

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7 References

Agapite, J., Albou, L. P., Aleksander, S. A., Alexander, M., Anagnostopoulos, A. v., Antonazzo, G., Argasinska, J., Arnaboldi, V., Attrill, H., Becerra, A., Bello, S. M., Blake, J. A., Blodgett, O., Bradford, Y. M., Bult, C. J., Cain, S., Calvi, B. R., Carbon, S., Chan, J., … Zytkovicz, M. (2022). Harmonizing model organism data in the Alliance of Genome Resources. *Genetics*, *220*(4). https://doi.org/10.1093/genetics/iyac022

Aiken, J., Moore, J. K., & Bates, E. A. (2019). TUBA1A mutations identified in lissencephaly patients dominantly disrupt neuronal migration and impair dynein activity. *Human Molecular Genetics*, *28*(8). https://doi.org/10.1093/hmg/ddy416

Akhmanova, A., & Steinmetz, M. O. (2008). Tracking the ends: A dynamic protein network controls the fate of microtubule tips. In *Nature Reviews Molecular Cell Biology* (Vol. 9, Issue 4). https://doi.org/10.1038/nrm2369

Akiyoshi, B., Sarangapani, K. K., Powers, A. F., Nelson, C. R., Reichow, S. L., Arellano-Santoyo, H., Gonen, T., Ranish, J. A., Asbury, C. L., & Biggins, S. (2010). Tension directly stabilizes reconstituted kinetochore-microtubule attachments. *Nature*, *468*(7323). https://doi.org/10.1038/nature09594

Arumugam, P., Gruber, S., Tanaka, K., Haering, C. H., Mechtler, K., & Nasmyth, K. (2003). ATP Hydrolysis Is Required for Cohesin's Association with Chromosomes. *Current Biology*, *13*(22). https://doi.org/10.1016/j.cub.2003.10.036

Avena, J. S., Burns, S., Yu, Z., Ebmeier, C. C., Old, W. M., Jaspersen, S. L., & Winey, M. (2014). Licensing of Yeast Centrosome Duplication Requires Phosphoregulation of Sfi1. *PLoS Genetics*, *10*(10). https://doi.org/10.1371/journal.pgen.1004666

Avunie-Masala, R., Movshovich, N., Nissenkorn, Y., Gerson-Gurwitz, A., Fridman, V., Kõivomägi, M., Loog, M., Hoyt, M. A., Zaritsky, A., & Gheber, L. (2011). Phospho-regulation of kinesin-5 during anaphase spindle elongation. *Journal of Cell Science*, *124*(6). https://doi.org/10.1242/jcs.077396

Bardin, A. J., Visintin, R., & Amon, A. (2000). A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell*, *102*(1). https://doi.org/10.1016/S0092-8674(00)00007-6

Barford, D. (2011). Structural insights into anaphase-promoting complex function and mechanism. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 366, Issue 1584). https://doi.org/10.1098/rstb.2011.0069

Bausch, C., Noone, S., Henry, J. M., Gaudenz, K., Sanderson, B., Seidel, C., & Gerton, J. L. (2007). Transcription Alters Chromosomal Locations of Cohesin in Saccharomyces cerevisiae . *Molecular and Cellular Biology*, *27*(24). https://doi.org/10.1128/mcb.01007-07

Bean, J. M., Siggia, E. D., & Cross, F. R. (2006). Coherence and timing of cell cycle start examined at single-cell resolution. *Molecular Cell*, *21*(1). https://doi.org/10.1016/j.molcel.2005.10.035

Bembenek, J., Kang, J., Kurischko, C., Li, B., Raab, J. R., Belanger, K. D., Luca, F. C., & Yu, H. (2005). Crm1-mediated nuclear export of Cdc14 is required for the completion of cytokinesis in budding yeast. *Cell Cycle*, *4*(7). https://doi.org/10.4161/cc.4.7.1798

Ben-Shahar, T. R., Heeger, S., Lehane, C., East, P., Flynn, H., Skehel, M., & Uhlmann, F. (2008). Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. *Science*, *321*(5888). https://doi.org/10.1126/science.1157774

Benzi, G., Camasses, A., Atsunori, Y., Katou, Y., Shirahige, K., & Piatti, S. (2020). A common molecular mechanism underlies the role of Mps1 in chromosome biorientation and the spindle assembly checkpoint. *EMBO Reports*, *21*(6). https://doi.org/10.15252/embr.202050257

Bergen, L. G., & Borisy, G. G. (1980). Head-to-tail polymerization of microtubules in vitro: Electron microscope analysis of seeded assembly. *Journal of Cell Biology*, *84*(1). https://doi.org/10.1083/jcb.84.1.141

Bergman, Z. J., Wong, J., Drubin, D. G., & Barnes, G. (2019). Microtubule dynamics regulation reconstituted in budding yeast lysates. *Journal of Cell Science*, *132*(4). https://doi.org/10.1242/jcs.219386

Berlin, V., Styles, C. A., & Fink, G. R. (1990). BIK1, a protein required for microtubule function during mating and mitosis in Saccharomyces cerevisiae, colocalizes with tubulin. *Journal of Cell Biology*, *111*(6 I). https://doi.org/10.1083/jcb.111.6.2573

Bertazzi, D. T., Kurtulmus, B., & Pereira, G. (2011). The cortical protein Lte1 promotes mitotic exit by inhibiting the spindle position checkpoint kinase Kin4. *Journal of Cell Biology*, *193*(6). https://doi.org/10.1083/jcb.201101056

Biggins, S., & Murray, A. W. (2001). The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. *Genes and Development*, *15*(23). https://doi.org/10.1101/gad.934801

Biggins, S., Severin, F. F., Bhalla, N., Sassoon, I., Hyman, A. A., & Murray, A. W. (1999). The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes and Development*, *13*(5). https://doi.org/10.1101/gad.13.5.532

Bintu, B., Mateo, L. J., Su, J. H., Sinnott-Armstrong, N. A., Parker, M., Kinrot, S., Yamaya, K., Boettiger, A. N., & Zhuang, X. (2018). Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science*, *362*(6413). https://doi.org/10.1126/science.aau1783

Blake-Hodek, K. A., Cassimeris, L., & Huffaker, T. C. (2010). Regulation of microtubule dynamics by Bim1 and

Bik1, the budding yeast members of the EB1 and CLIP-170 families of plus-end tracking proteins. *Molecular Biology of the Cell*, *21*(12). https://doi.org/10.1091/mbc.E10-02-0083

Blat, Y., & Kleckner, N. (1999). Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell*, *98*(2). https://doi.org/10.1016/S0092-8674(00)81019-3

Bloom, K. S., & Carbon, J. (1982). Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. *Cell*, *29*(2). https://doi.org/10.1016/0092-8674(82)90147-7

Bock, L. J., Pagliuca, C., Kobayashi, N., Grove, R. A., Oku, Y., Shrestha, K., Alfieri, C., Golfieri, C., Oldani, A., Dal Maschio, M., Bermejo, R., Hazbun, T. R., Tanaka, T. U., & de Wulf, P. (2012). Cnn1 inhibits the interactions between the KMN complexes of the yeast kinetochore. *Nature Cell Biology*, *14*(6). https://doi.org/10.1038/ncb2495

Braun, M., Lansky, Z., Fink, G., Ruhnow, F., Diez, S., & Janson, M. E. (2011). Adaptive braking by Ase1 prevents overlapping microtubules from sliding completely apart. *Nature Cell Biology*, *13*(10). https://doi.org/10.1038/ncb2323

Broad, A. J., & DeLuca, J. G. (2020). The right place at the right time: Aurora B kinase localization to centromeres and kinetochores. In *Essays in Biochemistry* (Vol. 64, Issue 2). https://doi.org/10.1042/ebc20190081

Bullitt, E., Rout, M. P., Kilmartin, J. v., & Akey, C. W. (1997). The yeast spindle pole body is assembled around a central crystal of Spc42p. *Cell*, *89*(7). https://doi.org/10.1016/S0092-8674(00)80295-0

Busslinger, G. A., Stocsits, R. R., van der Lelij, P., Axelsson, E., Tedeschi, A., Galjart, N., & Peters, J. M. (2017). Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl. *Nature*, *544*(7651). https://doi.org/10.1038/nature22063

Buvelot, S., Tatsutani, S. Y., Vermaak, D., & Biggins, S. (2003). The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. *Journal of Cell Biology*, *160*(3). https://doi.org/10.1083/jcb.200209018

Carbon, J., & Clarke, L. (1984). Structural and functional analysis of a yeast centromere (CEN3). In *Journal of Cell Science: Vol. SUPPL. 1*. https://doi.org/10.1242/jcs.1984.supplement_1.4

Carlton, P. M., Boulanger, J., Kervrann, C., Sibarita, J. B., Salamero, J., Gordon-Messer, S., Bressan, D., Haber, J. E., Haase, S., Shao, L., Winoto, L., Matsuda, A., Kner, P., Uzawa, S., Gustafsson, M., Kam, Z., Agard, D. A., & Sedat, J. W. (2010). Fast live simultaneous multiwavelength four-dimensional optical microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(37). https://doi.org/10.1073/pnas.1004037107

Carmena, M., Wheelock, M., Funabiki, H., & Earnshaw, W. C. (2012). The chromosomal passenger complex (CPC): From easy rider to the godfather of mitosis. *Nature Reviews Molecular Cell Biology*, *13*(12). https://doi.org/10.1038/nrm3474

Carvalho, P., Gupta, M. L., Hoyt, M. A., & Pellman, D. (2004). Cell cycle control of kinesin-mediated transport of Bik1 (CLIP-170) regulates microtubule stability and dynein activation. *Developmental Cell*, *6*(6). https://doi.org/10.1016/j.devcel.2004.05.001

Castillo, A. R., Meehl, J. B., Morgan, G., Schutz-Geschwender, A., & Winey, M. (2002). The yeast protein kinase Mps1p is required for assembly of the integral spindle pole body component Spc42p. *Journal of Cell Biology*, *156*(3). https://doi.org/10.1083/jcb.200111025

Caydasi, A. K., & Pereira, G. (2009). Spindle Alignment Regulates the Dynamic Association of Checkpoint Proteins with Yeast Spindle Pole Bodies. *Developmental Cell*, *16*(1). https://doi.org/10.1016/j.devcel.2008.10.013

Cepeda-García, C., Delgehyr, N., Ortiz, M. A. J., ten Hoopen, R., Zhiteneva, A., & Segal, M. (2010). Actin-mediated delivery of astral microtubules instructs Kar9p asymmetric loading to the bud-ward spindle pole. *Molecular Biology of the Cell*, *21*(15). https://doi.org/10.1091/mbc.E10-03-0197

Chacón, J. M., Mukherjee, S., Schuster, B. M., Clarke, D. J., & Gardner, M. K. (2014). Pericentromere tension is self-regulated by spindle structure in metaphase. *Journal of Cell Biology*, *205*(3). https://doi.org/10.1083/jcb.201312024

Chan, K. L., Roig, M. B., Hu, B., Beckouët, F., Metson, J., & Nasmyth, K. (2012). Cohesin's DNA exit gate is distinct from its entrance gate and is regulated by acetylation. *Cell*, *150*(5). https://doi.org/10.1016/j.cell.2012.07.028

Cheeseman, I. M., Anderson, S., Jwa, M., Green, E. M., Kang, J. seog, Yates, J. R., Chan, C. S. M., Drubin, D. G., & Barnes, G. (2002). Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell*, *111*(2). https://doi.org/10.1016/S0092-8674(02)00973-X

Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M., & Desai, A. (2006). The Conserved KMN Network Constitutes the Core Microtubule-Binding Site of the Kinetochore. *Cell*, *127*(5). https://doi.org/10.1016/j.cell.2006.09.039

Cheeseman, I. M., Drubin, D. G., & Barnes, G. (2002). Simple centromere, complex kinetochore: Linking spindle microtubules and centromeric DNA in budding yeast. In *Journal of Cell Biology* (Vol. 157, Issue 2). https://doi.org/10.1083/jcb.200201052

Chen, X., Widmer, L. A., Stangier, M. M., Steinmetz, M. O., Stelling, J., & Barral, Y. (2019). Remote control of microtubule plus-end dynamics and function from the minus-end. *ELife*, *8*. https://doi.org/10.7554/eLife.48627

Cherry, J. M., Hong, E. L., Amundsen, C., Balakrishnan, R., Binkley, G., Chan, E. T., Christie, K. R., Costanzo, M. C., Dwight, S. S., Engel, S. R., Fisk, D. G., Hirschman, J. E., Hitz, B. C., Karra, K., Krieger, C. J., Miyasato, S. R., Nash, R. S., Park, J., Skrzypek, M. S., … Wong, E. D. (2012). Saccharomyces Genome Database: The genomics resource of budding yeast. *Nucleic Acids Research*, *40*(D1). https://doi.org/10.1093/nar/gkr1029

Choudhary, K., & Kupiec, M. (2022). The cohesin complex of yeasts: sister chromatid cohesion and beyond. *FEMS Microbiology Reviews*, fuac045. https://doi.org/10.1093/femsre/fuac045

Chrétien, D., & Wade, R. H. (1991). New data on the microtubule surface lattice. *Biology of the Cell*, *71*(1–2). https://doi.org/10.1016/0248-4900(91)90062-R

Ciosk, R., Shirayama, M., Shevchenko, A., Tanaka, T., Toth, A., Shevchenko, A., & Nasmyth, K. (2000). Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. *Molecular Cell*, *5*(2). https://doi.org/10.1016/S1097-2765(00)80420-7

Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M., & Nasmyth, K. (1998). An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell*, *93*(6). https://doi.org/10.1016/S0092-8674(00)81211-8

Clarke, L., & Carbon, J. (1980). Isolation of a yeast centromere and construction of functional small circular chromosomes. *Nature*, *287*(5782). https://doi.org/10.1038/287504a0

Cohen-Fix, O., Peters, J. M., Kirschner, M. W., & Koshland, D. (1996). Anaphase initiation in saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes and Development*, *10*(24). https://doi.org/10.1101/gad.10.24.3081

Collins, K. A., Castillo, A. R., Tatsutani, S. Y., & Biggins, S. (2005). De novo kinetochore assembly requires the centromeric histone H3 variant. *Molecular Biology of the Cell*, *16*(12). https://doi.org/10.1091/mbc.E05-08-0771

Coombes, C. E., Yamamoto, A., Kenzie, M. R., Odde, D. J., & Gardner, M. K. (2013). Evolving tip structures can explain age-dependent microtubule catastrophe. *Current Biology*, *23*(14). https://doi.org/10.1016/j.cub.2013.05.059

Costantino, L., Hsieh, T. H. S., Lamothe, R., Darzacq, X., & Koshland, D. (2020). Cohesin residency determines chromatin loop patterns. *ELife*, *9*. https://doi.org/10.7554/eLife.59889

Cote, R. H., & Borisy, G. G. (1981). Head-to-tail polymerization of microtubules in vitro. *Journal of Molecular Biology*, *150*(4), 577–599. https://doi.org/https://doi.org/10.1016/0022-2836(81)90382-X

Cross, R. A. (2004). The kinetic mechanism of kinesin. In *Trends in Biochemical Sciences* (Vol. 29, Issue 6). https://doi.org/10.1016/j.tibs.2004.04.010

Dallai, R., Lupetti, P., & Mencarelli, C. (2006). Unusual Axonemes of Hexapod Spermatozoa. In *International Review of Cytology* (Vol. 254). https://doi.org/10.1016/S0074-7696(06)54002-1

D'Ambrosio, C., Kelly, G., Shirahige, K., & Uhlmann, F. (2008). Condensin-Dependent rDNA Decatenation Introduces a Temporal Pattern to Chromosome Segregation. *Current Biology*, *18*(14). https://doi.org/10.1016/j.cub.2008.06.058

D'Amours, D., Stegmeier, F., & Amon, A. (2004). Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA. *Cell*, *117*(4). https://doi.org/10.1016/S0092-8674(04)00413-1

D'Aquino, K. E., Monje-Casas, F., Paulson, J., Reiser, V., Charles, G. M., Lai, L., Shokat, K. M., & Amon, A. (2005). The protein kinase Kin4 inhibits exit from mitosis in response to spindle position defects. *Molecular Cell*, *19*(2). https://doi.org/10.1016/j.molcel.2005.06.005

Dauban, L., Montagne, R., Thierry, A., Lazar-Stefanita, L., Bastié, N., Gadal, O., Cournac, A., Koszul, R., & Beckouët, F. (2020). Regulation of Cohesin-Mediated Chromosome Folding by Eco1 and Other Partners. *Molecular Cell*, *77*(6). https://doi.org/10.1016/j.molcel.2020.01.019

Demirel, P. B., Keyes, B. E., Chaterjee, M., Remington, C. E., & Burke, D. J. (2012). A redundant function for the N-terminal tail of Ndc80 in kinetochore-microtubule interaction in Saccharomyces cerevisiae. *Genetics*, *192*(2). https://doi.org/10.1534/genetics.112.143818

Dewar, H., Tanaka, K., Nasmyth, K., & Tanaka, T. U. (2004). Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. *Nature*, *428*(6978). https://doi.org/10.1038/nature02328

Dhatchinamoorthy, K., Shivaraju, M., Lange, J. J., Rubinstein, B., Unruh, J. R., Slaughter, B. D., & Gerton, J. L. (2017). Structural plasticity of the living kinetochore. *Journal of Cell Biology*, *216*(11). https://doi.org/10.1083/jcb.201703152

Dimitrov, A., Quesnoit, M., Moutel, S., Cantaloube, I., Poüs, C., & Perez, F. (2008). Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. *Science*, *322*(5906). https://doi.org/10.1126/science.1165401

Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J. S., & Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*, *485*(7398). https://doi.org/10.1038/nature11082

Donaldson, A. D., & Kilmartin, J. v. (1996). Spc42p: A phosphorylated component of the S. cerevisiae spindle pole body (SPB) with an essential function during SPB duplication. *Journal of Cell Biology*, *132*(5). https://doi.org/10.1083/jcb.132.5.887

Douzery, E. J. P., Snell, E. A., Bapteste, E., Delsuc, F., & Philippe, H. (2004). The timing of eukaryotic evolution: Does a relaxed molecular clock reconcile proteins and fossils? *Proceedings of the National Academy of Sciences of the United States of America*, *101*(43). https://doi.org/10.1073/pnas.0403984101

Dudziak, A., Engelhard, L., Bourque, C., Klink, B. U., Rombaut, P., Kornakov, N., Jänen, K., Herzog, F., Gatsogiannis, C., & Westermann, S. (2021). Phospho-regulated Bim1/EB1 interactions trigger Dam1c ring assembly at the budding yeast outer kinetochore. *The EMBO Journal*, *40*(18). https://doi.org/10.15252/embj.2021108004

Duellberg, C., Cade, N. I., Holmes, D., & Surrey, T. (2016). The size of the EB cap determines instantaneous microtubule stability. *ELife*, *5*(APRIL2016). https://doi.org/10.7554/eLife.13470

Edamatsu, M. (2014). Bidirectional motility of the fission yeast kinesin-5, Cut7. *Biochemical and Biophysical Research Communications*, *446*(1). https://doi.org/10.1016/j.bbrc.2014.02.106

Elserafy, M., Šarić, M., Neuner, A., Lin, T. C., Zhang, W., Seybold, C., Sivashanmugam, L., & Schiebel, E. (2014). Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Current Biology*, *24*(13). https://doi.org/10.1016/j.cub.2014.05.032

Eshel, D., Urrestarazu, L. A., Vissers, S., Jauniaux, J. C., van Vliet-Reedijk, J. C., Planta, R. J., & Gibbons, I. R. (1993). Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(23). https://doi.org/10.1073/pnas.90.23.11172

Falk, J. E., Chan, L. Y., & Amon, A. (2011). Lte1 promotes mitotic exit by controlling the localization of the spindle position checkpoint kinase Kin4. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(31). https://doi.org/10.1073/pnas.1107784108

Fernius, J., & Marston, A. L. (2009). Establishment of cohesion at the pericentromere by the Ctf19 kinetochore subcomplex and the replication fork-associated factor, Csm3. *PLoS Genetics*, *5*(9). https://doi.org/10.1371/journal.pgen.1000629

Finn, E. H., Pegoraro, G., Brandão, H. B., Valton, A. L., Oomen, M. E., Dekker, J., Mirny, L., & Misteli, T. (2019). Extensive Heterogeneity and Intrinsic Variation in Spatial Genome Organization. *Cell*, *176*(6). https://doi.org/10.1016/j.cell.2019.01.020

Fischböck-Halwachs, J., Singh, S., Potocnjak, M., Hagemann, G., Solis-Mezarino, V., Woike, S., Ghodgaonkar-Steger, M., Weissmann, F., Gallego, L. D., Rojas, J., Andreani, J., Köhler, A., & Herzog, F. (2019). The COMA complex interacts with Cse4 and positions Sli15/ipl1 at the budding yeast inner kinetochore. *ELife*, *8*. https://doi.org/10.7554/eLife.42879

Flory, M. R., Moser, M. J., Monnat, R. J., & Davis, T. N. (2000). Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(11). https://doi.org/10.1073/pnas.97.11.5919

Flyamer, I. M., Gassler, J., Imakaev, M., Brandão, H. B., Ulianov, S. v., Abdennur, N., Razin, S. v., Mirny, L. A., & Tachibana-Konwalski, K. (2017). Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. *Nature*, *544*(7648). https://doi.org/10.1038/nature21711

Fong, K. K., Zelter, A., Graczyk, B., Hoyt, J. M., Riffle, M., Johnson, R., MacCoss, M. J., & Davis, T. N. (2018). Novel phosphorylation states of the yeast spindle pole body. *Biology Open*, *7*(10). https://doi.org/10.1242/bio.033647

Franck, A. D., Powers, A. F., Gestaut, D. R., Gonen, T., Davis, T. N., & Asbury, C. L. (2007). Tension applied through the Dam1 complex promotes microtubule elongation providing a direct mechanism for length control in mitosis. *Nature Cell Biology*, *9*(7). https://doi.org/10.1038/ncb1609

Fridman, V., Gerson-Gurwitz, A., Shapira, O., Movshovich, N., Lakämper, S., Schmidt, C. F., & Gheber, L. (2013). Kinesin-5 Kip1 is a bi-directional motor that stabilizes microtubules and tracks their plus-ends in vivo. *Journal of Cell Science*, *126*(18). https://doi.org/10.1242/jcs.125153

Friedman, D. B., Kern, J. W., Huneycutt, B. J., Vinh, D. B. N., Crawford, D. K., Steiner, E., Scheiltz, D., Yates, J., Resing, K. A., Ahn, N. G., Winey, M., & Davis, T. N. (2001). Yeast Mps1p Phosphorylates the Spindle Pole Component Spc110p in the N-terminal Domain. *Journal of Biological Chemistry*, *276*(21). https://doi.org/10.1074/jbc.M010461200

Friedman, D. B., Sundberg, H. A., Huang, E. Y., & Davis, T. N. (1996). The 110-kD spindle pole body component of Saccharomyces cerevisiae is a phosphoprotein that is modified in a cell cycle-dependent manner. *Journal of Cell Biology*, *132*(5). https://doi.org/10.1083/jcb.132.5.903

Fudenberg, G., Abdennur, N., Imakaev, M., Goloborodko, A., & Mirny, L. A. (2017). Emerging Evidence of Chromosome Folding by Loop Extrusion. *Cold Spring Harbor Symposia on Quantitative Biology*, *82*. https://doi.org/10.1101/sqb.2017.82.034710

Gabriele, M., Brandão, H. B., Grosse-Holz, S., Jha, A., Dailey, G. M., Cattoglio, C., Hsieh, T.-H. S., Mirny, L., Zechner, C., & Hansen, A. S. (2022). Dynamics of CTCF- and cohesin-mediated chromatin looping revealed by live-cell imaging. *Science*, *376*(6592), 496–501. https://doi.org/10.1126/science.abn6583

Gandhi, R., Gillespie, P. J., & Hirano, T. (2006). Human Wapl Is a Cohesin-Binding Protein that Promotes Sister-Chromatid Resolution in Mitotic Prophase. *Current Biology*, *16*(24). https://doi.org/10.1016/j.cub.2006.10.061

Ganji, M., Shaltiel, I. A., Bisht, S., Kim, E., Kalichava, A., Haering, C. H., & Dekker, C. (2018). Real-time

imaging of DNA loop extrusion by condensin. *Science*, *360*(6384). https://doi.org/10.1126/science.aar7831

García-Rodríguez, L. J., Kasciukovic, T., Denninger, V., & Tanaka, T. U. (2019). Aurora B-INCENP Localization at Centromeres/Inner Kinetochores Is Required for Chromosome Bi-orientation in Budding Yeast. *Current Biology*, *29*(9). https://doi.org/10.1016/j.cub.2019.03.051

Gardner, M. K., Bouck, D. C., Paliulis, L. v., Meehl, J. B., O'Toole, E. T., Haase, J., Soubry, A., Joglekar, A. P., Winey, M., Salmon, E. D., Bloom, K., & Odde, D. J. (2008). Chromosome Congression by Kinesin-5 Motor-Mediated Disassembly of Longer Kinetochore Microtubules. *Cell*, *135*(5). https://doi.org/10.1016/j.cell.2008.09.046

Gardner, M. K., Haase, J., Mythreye, K., Molk, J. N., Anderson, M. B., Joglekar, A. P., O'Toole, E. T., Winey, M., Salmon, E. D., Odde, D. J., & Bloom, K. (2008). The microtubule-based motor Kar3 and plus end-binding protein Bim1 provide structural support for the anaphase spindle. *Journal of Cell Biology*, *180*(1). https://doi.org/10.1083/jcb.200710164

Gassaway, B. M., Paulo, J. A., & Gygi, S. P. (2021). Categorization of Phosphorylation Site Behavior during the Diauxic Shift in Saccharomyces cerevisiae. *Journal of Proteome Research*, *20*(5). https://doi.org/10.1021/acs.jproteome.0c00943

Gergely, Z. R., Crapo, A., Hough, L. E., Richard McIntosh, J., & Betterton, M. D. (2016). Kinesin-8 effects on mitotic microtubule dynamics contribute to spindle function in fission yeast. *Molecular Biology of the Cell*, *27*(22). https://doi.org/10.1091/mbc.E15-07-0505

Gerson-Gurwitz, A., Movshovich, N., Avunie, R., Fridman, V., Moyal, K., Katz, B., Hoyt, M. A., & Gheber, L. (2009). Mid-anaphase arrest in S. cerevisiae cells eliminated for the function of Cin8 and dynein. *Cellular and Molecular Life Sciences*, *66*(2). https://doi.org/10.1007/s00018-008-8479-2

Gerson-Gurwitz, A., Thiede, C., Movshovich, N., Fridman, V., Podolskaya, M., Danieli, T., Lakämper, S., Klopfenstein, D. R., Schmidt, C. F., & Gheber, L. (2011). Directionality of individual kinesin-5 Cin8 motors is modulated by loop 8, ionic strength and microtubule geometry. *EMBO Journal*, *30*(24). https://doi.org/10.1038/emboj.2011.403

Geymonat, M., Peng, Q., Guo, Z., Yu, Z., Unruh, J. R., Jaspersen, S. L., & Segal, M. (2020). Orderly assembly underpinning built-in asymmetry in the yeast centrosome duplication cycle requires cyclin-dependent kinase. *ELife*, *9*. https://doi.org/10.7554/ELIFE.59222

Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Véronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., André, B., Arkin, A. P., Astromoff, A., el Bakkoury, M., Bangham, R., Benito, R., Brachat, S., Campanaro, S., Curtiss, M., Davis, K., … Johnston, M. (2002). Functional profiling of the Saccharomyces cerevisiae genome. *Nature*, *418*(6896). https://doi.org/10.1038/nature00935

Gibcus, J. H., Samejima, K., Goloborodko, A., Samejima, I., Naumova, N., Nuebler, J., Kanemaki, M. T., Xie, L., Paulson, J. R., Earnshaw, W. C., Mirny, L. A., & Dekker, J. (2018). A pathway for mitotic chromosome formation. *Science*, *359*(6376). https://doi.org/10.1126/science.aao6135

Gillett, E. S., Espelin, C. W., & Sorger, P. K. (2004). Spindle checkpoint proteins and chromosome-microtubule attachment in budding yeast. *Journal of Cell Biology*, *164*(4). https://doi.org/10.1083/jcb.200308100

Glynn, E. F., Megee, P. C., Yu, H. G., Mistrot, C., Unal, E., Koshland, D. E., DeRisi, J. L., & Gerton, J. L. (2004). Genome-wide mapping of the cohesin complex in the yeast Saccharomyces cerevisiae. *PLoS Biology*, *2*(9). https://doi.org/10.1371/journal.pbio.0020259

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H., & Oliver, S. G. (1996). Life with 6000 genes. *Science (New York, N.Y.)*, *274*(5287), 546, 563–567. http://www.ncbi.nlm.nih.gov/pubmed/8849441

Goshima, G., & Yanagida, M. (2000). Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. *Cell*, *100*(6). https://doi.org/10.1016/S0092-8674(00)80699-6

Grishchuk, E. L., Molodtsov, M. I., Ataullakhanov, F. I., & McIntosh, J. R. (2005). Force production by disassembling microtubules. *Nature*, *438*(7066). https://doi.org/10.1038/nature04132

Gruneberg, U., Campbell, K., Simpson, C., Grindlay, J., & Schiebel, E. (2000). Nud1p links astral microtubule organization and the control of exit from mitosis. *EMBO Journal*, *19*(23). https://doi.org/10.1093/emboj/19.23.6475

Gu, B., Comerci, C. J., McCarthy, D. G., Saurabh, S., Moerner, W. E., & Wysocka, J. (2020). Opposing Effects of Cohesin and Transcription on CTCF Organization Revealed by Super-resolution Imaging. *Molecular Cell*, *80*(4). https://doi.org/10.1016/j.molcel.2020.10.001

Guacci, V., Hogan, E., & Koshland, D. (1994). Chromosome condesation and sister chromatid pairing in budding yeast. *Journal of Cell Biology*, *125*(3). https://doi.org/10.1083/jcb.125.3.517

Guacci, V., Hogan, E., & Koshland, D. (1997). Centromere position in budding yeast: Evidence for anaphase A. *Molecular Biology of the Cell*, *8*(6). https://doi.org/10.1091/mbc.8.6.957

Gudimchuk, N. B., & McIntosh, J. R. (2021). Regulation of microtubule dynamics, mechanics and function through the growing tip. In *Nature Reviews Molecular Cell Biology* (Vol. 22, Issue 12). https://doi.org/10.1038/s41580-021-00399-x

Gupta, K. K., Paulson, B. A., Folker, E. S., Charlebois, B., Hunt, A. J., & Goodson, H. v. (2009). Minimal plus-end tracking unit of the cytoplasmic linker protein

CLIP-170. *Journal of Biological Chemistry*, *284*(11). https://doi.org/10.1074/jbc.M807675200

Gupta, M. L., Carvalho, P., Roof, D. M., & Pellman, D. (2006). Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. *Nature Cell Biology*, *8*(9). https://doi.org/10.1038/ncb1457

Haarhuis, J. H. I., van der Weide, R. H., Blomen, V. A., Yáñez-Cuna, J. O., Amendola, M., van Ruiten, M. S., Krijger, P. H. L., Teunissen, H., Medema, R. H., van Steensel, B., Brummelkamp, T. R., de Wit, E., & Rowland, B. D. (2017). The Cohesin Release Factor WAPL Restricts Chromatin Loop Extension. *Cell*, *169*(4). https://doi.org/10.1016/j.cell.2017.04.013

Haering, C. H., Farcas, A. M., Arumugam, P., Metson, J., & Nasmyth, K. (2008). The cohesin ring concatenates sister DNA molecules. *Nature*, *454*(7202). https://doi.org/10.1038/nature07098

Hagan, I., & Yanagida, M. (1990). Novel potential mitotic motor protein encoded by the fission yeast cut7+ gene. *Nature*, *347*(6293). https://doi.org/10.1038/347563a0

Hauf, S., Waizenegger, I. C., & Peters, J. M. (2001). Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. *Science*, *293*(5533). https://doi.org/10.1126/science.1061376

Hayashi, I., & Ikura, M. (2003). Crystal structure of the amino-terminal microtubule-binding domain of end-binding protein 1 (EB1). *Journal of Biological Chemistry*, *278*(38). https://doi.org/10.1074/jbc.M305773200

Heck, M. M. S., Pereira, A., Pesavento, P., Yannoni, Y., Spradling, A. C., & Goldstein, L. S. B. (1993). The kinesin-like protein KLP61F is essential for mitosis in Drosophila. *Journal of Cell Biology*, *123*(3). https://doi.org/10.1083/jcb.123.3.665

Hepperla, A. J., Willey, P. T., Coombes, C. E., Schuster, B. M., Gerami-Nejad, M., McClellan, M., Mukherjee, S., Fox, J., Winey, M., Odde, D. J., O'Toole, E., & Gardner, M. K. (2014). Minus-end-directed kinesin-14 motors align antiparallel microtubules to control metaphase spindle length. *Developmental Cell*, *31*(1). https://doi.org/10.1016/j.devcel.2014.07.023

Herbert, S., Brion, A., Arbona, J., Lelek, M., Veillet, A., Lelandais, B., Parmar, J., Fernández, F. G., Almayrac, E., Khalil, Y., Birgy, E., Fabre, E., & Zimmer, C. (2017). Chromatin stiffening underlies enhanced locus mobility after DNA damage in budding yeast . *The EMBO Journal*, *36*(17). https://doi.org/10.15252/embj.201695842

Heun, P., Laroche, T., Shimada, K., Furrer, P., & Gasser, S. M. (2001). Chromosome dynamics in the yeast interphase nucleus. *Science, 294*(5549). https://doi.org/10.1126/science.1065366

Hibbel, A., Bogdanova, A., Mahamdeh, M., Jannasch, A., Storch, M., Schäffer, E., Liakopoulos, D., & Howard, J. (2015). Kinesin Kip2 enhances microtubule growth in vitro through length-dependent feedback on polymerization and catastrophe. *ELife*, *4*(NOVEMBER2015). https://doi.org/10.7554/eLife.10542

Higuchi, T., & Uhlmann, F. (2005). Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. *Nature*, *433*(7022). https://doi.org/10.1038/nature03240

Hildebrandt, E. R., & Hoyt, M. A. (2000). Mitotic motors in Saccharomyces cerevisiae. In *Biochimica et Biophysica Acta - Molecular Cell Research* (Vol. 1496, Issue 1). https://doi.org/10.1016/S0167-4889(00)00012-4

Hinshaw, S. M., & Harrison, S. C. (2019). The structure of the ctf19c/ccan from budding yeast. *ELife*, *8*. https://doi.org/10.7554/eLife.44239

Hinshaw, S. M., Makrantoni, V., Harrison, S. C., & Marston, A. L. (2017). The Kinetochore Receptor for the Cohesin Loading Complex. *Cell*, *171*(1). https://doi.org/10.1016/j.cell.2017.08.017

Hinshaw, S. M., Makrantoni, V., Kerr, A., Marston, A. L., & Harrison, S. C. (2015). Structural evidence for Scc4-dependent localization of cohesin loading. *ELife*, *4*(JUNE). https://doi.org/10.7554/eLife.06057

Hirano, T., & Mitchison, T. J. (1994). A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. *Cell*, *79*(3). https://doi.org/10.1016/0092-8674(94)90254-2

Höckner, S., Neumann-Arnold, L., & Seufert, W. (2016). Dual control by Cdk1 phosphorylation of the budding yeast APC/C ubiquitin ligase activator Cdh1. *Molecular Biology of the Cell*, *27*(14). https://doi.org/10.1091/mbc.E15-11-0787

Holinger, E. P., Old, W. M., Giddings, T. H., Wong, C., Yates, J. R., & Winey, M. (2009). Budding yeast centrosome duplication requires stabilization of Spc29 via Mps1-mediated phosphorylation. *Journal of Biological Chemistry*, *284*(19). https://doi.org/10.1074/jbc.M900088200

Hornung, P., Troc, P., Malvezzi, F., Maier, M., Demianova, Z., Zimniak, T., Litos, G., Lampert, F., Schleiffer, A., Brunner, M., Mechtler, K., Herzog, F., Marlovits, T. C., & Westermann, S. (2014). A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. *Journal of Cell Biology*, *206*(4). https://doi.org/10.1083/jcb.201403081

Hotz, M., & Barral, Y. (2014). The Mitotic Exit Network: New turns on old pathways. In *Trends in Cell Biology* (Vol. 24, Issue 3). https://doi.org/10.1016/j.tcb.2013.09.010

Hotz, M., Lengefeld, J., & Barral, Y. (2012). The MEN mediates the effects of the spindle assembly checkpoint on Kar9-dependent spindle pole body inheritance in budding yeast. *Cell Cycle (Georgetown, Tex.)*, *11*(16), 3109–3116. https://doi.org/10.4161/cc.21504

Howes, S. C., Geyer, E. A., LaFrance, B., Zhang, R., Kellogg, E. H., Westermann, S., Rice, L. M., & Nogales, E. (2018). Structural and functional differences between

porcine brain and budding yeast microtubules. *Cell Cycle*, *17*(3). https://doi.org/10.1080/15384101.2017.1415680

Hu, F., Wang, Y., Liu, D., Li, Y., Qin, J., & Elledge, S. J. (2001). Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell*, *107*(5). https://doi.org/10.1016/S0092-8674(01)00580-3

Huang, J., & Moazed, D. (2003). Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. *Genes and Development*, *17*(17). https://doi.org/10.1101/gad.1108403

Huh, W. K., Falvo, J. v., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S., & O'Shea, E. K. (2003). Global analysis of protein localization in budding yeast. *Nature*, *425*(6959). https://doi.org/10.1038/nature02026

Huisman, S. M., Bales, O. A. M., Bertrand, M., Smeets, M. F. M. A., Reed, S. I., & Segal, M. (2004). Differential contribution of Bud6p and Kar9p to microtubule capture and spindle orientation in S. cerevisiae. *Journal of Cell Biology*, *167*(2). https://doi.org/10.1083/jcb.200407167

Huisman, S. M., Smeets, M. F. M. A., & Segal, M. (2007). Phosphorylation of Spc110p by Cdc28p-Clb5p kinase contributes to correct spindle morphogenesis in S. cerevisiae. *Journal of Cell Science*, *120*(3). https://doi.org/10.1242/jcs.03342

Huyett, A., Kahana, J., Silver, P., Zeng, X., & Saunders, W. S. (1998). The Kar3p and Kip2p motors function antagonistically at the spindle poles to influence cytoplasmic microtubule numbers. *Journal of Cell Science*, *111*(3). https://doi.org/10.1242/jcs.111.3.295

Hyman, A. A., Salser, S., Drechsel, D. N., Unwin, N., & Mitchison, T. J. (1992). Role of GTP hydrolysis in microtubule dynamics: Information from a slowly hydrolyzable analogue, GMPCPP. *Molecular Biology of the Cell*, *3*(10). https://doi.org/10.1091/mbc.3.10.1155

Irniger, S., & Nasmyth, K. (1997). The anaphase-promoting complex is required in G1 arrested yeast cells to inhibit B-type cyclin accumulation and to prevent uncontrolled entry into S-phase. *Journal of Cell Science*, *110*(13). https://doi.org/10.1242/jcs.110.13.1523

Jakobs, S., Schauss, A. C., & Hell, S. W. (2003). Photoconversion of matrix targeted GFP enables analysis of continuity and intermixing of the mitochondrial lumen. *FEBS Letters*, *554*(1–2). https://doi.org/10.1016/S0014-5793(03)01170-0

Janke, C., Ortíz, J., Tanaka, T. U., Lechner, J., & Schiebel, E. (2002). Four new subunits of the Dam1-Duo1 complex reveal novel functions in sister kinetochore biorientation. *EMBO Journal*, *21*(1–2). https://doi.org/10.1093/emboj/21.1.181

Jaspersen, S. L., & Winey, M. (2004). The budding yeast spindle pole body: Structure, duplication, and function. In *Annual Review of Cell and Developmental Biology* (Vol. 20). https://doi.org/10.1146/annurev.cellbio.20.022003.114106

Játiva, S., Calabria, I., Moyano-Rodriguez, Y., Garcia, P., & Queralt, E. (2019). Cdc14 activation requires coordinated Cdk1-dependent phosphorylation of Net1 and PP2A–Cdc55 at anaphase onset. *Cellular and Molecular Life Sciences*, *76*(18). https://doi.org/10.1007/s00018-019-03086-5

Jenni, S., & Harrison, S. C. (2018). Structure of the DASH/Dam1 complex shows its role at the yeast kinetochore-microtubule interface. *Science*, *360*(6388). https://doi.org/10.1126/science.aar6436

Jeppsson, K., Carlborg, K. K., Nakato, R., Berta, D. G., Lilienthal, I., Kanno, T., Lindqvist, A., Brink, M. C., Dantuma, N. P., Katou, Y., Shirahige, K., & Sjögren, C. (2014). The Chromosomal Association of the Smc5/6 Complex Depends on Cohesion and Predicts the Level of Sister Chromatid Entanglement. *PLoS Genetics*, *10*(10). https://doi.org/10.1371/journal.pgen.1004680

Jeppsson, K., Sakata, T., Nakato, R., Milanova, S., Shirahige, K., & Björkegren, C. (2022). Cohesin-dependent chromosome loop extrusion is limited by transcription and stalled replication forks. *Science Advances*, *8*(23), eabn7063. https://doi.org/10.1126/sciadv.abn7063

Ji, Z., Gao, H., & Yu, H. (2015). Kinetochore attachment sensed by competitive Mps1 and microtubule binding to Ndc80C. *Science*, *348*(6240). https://doi.org/10.1126/science.aaa4029

Jin, Q. W., Fuchs, J., & Loidl, J. (2000). Centromere clustering is a major determinant of yeast interphase nuclear organization. *Journal of Cell Science*, *113*(11). https://doi.org/10.1242/jcs.113.11.1903

Joglekar, A. P., Bouck, D. C., Molk, J. N., Bloom, K. S., & Salmon, E. D. (2006). Molecular architecture of a kinetochore-microtubule attachment site. *Nature Cell Biology*, *8*(6). https://doi.org/10.1038/ncb1414

Jones, G. M., Stalker, J., Humphray, S., West, A., Cox, T., Rogers, J., Dunham, I., & Prelich, G. (2008). A systematic library for comprehensive overexpression screens in Saccharomyces cerevisiae. *Nature Methods*, *5*(3). https://doi.org/10.1038/nmeth.1181

Juanes, M. A., Twyman, H., Tunnacliffe, E., Guo, Z., ten Hoopen, R., & Segal, M. (2013). Spindle pole body history intrinsically links pole identity with asymmetric fate in budding yeast. *Current Biology*, *23*(14). https://doi.org/10.1016/j.cub.2013.05.057

Juang, Y. L., Huang, J., Peters, J. M., McLaughlin, M. E., Tai, C. Y., & Pellman, D. (1997). APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. *Science*, *275*(5304). https://doi.org/10.1126/science.275.5304.1311

Kachroo, A. H., Laurent, J. M., Yellman, C. M., Meyer, A. G., Wilke, C. O., & Marcotte, E. M. (2015). Systematic humanization of yeast genes reveals conserved

functions and genetic modularity. *Science*, *348*(6237). https://doi.org/10.1126/science.aaa0769

Kahana, J. A., Schnapp, B. J., & Silver, P. A. (1995). Kinetics of spindle pole body separation in budding yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(21). https://doi.org/10.1073/pnas.92.21.9707

Kapitein, L. C., Peterman, E. J. G., Kwok, B. H., Kim, J. H., Kapoor, T. M., & Schmidt, C. F. (2005). The bipolar mitotic kinesin Eg5 moves on both microtubules that it crosslinks. *Nature*, *435*(7038). https://doi.org/10.1038/nature03503

Keck, J. M., Jones, M. H., Wong, C. C. L., Binkley, J., Chen, D., Jaspersen, S. L., Holinger, E. P., Xu, T., Niepel, M., Rout, M. P., Vogel, J., Sidow, A., Yates, J. R., & Winey, M. (2011). A cell cycle phosphoproteome of the yeast centrosome. *Science*, *332*(6037). https://doi.org/10.1126/science.1205193

Khmelinskii, A., Keller, P. J., Bartosik, A., Meurer, M., Barry, J. D., Mardin, B. R., Kaufmann, A., Trautmann, S., Wachsmuth, M., Pereira, G., Huber, W., Schiebel, E., & Knop, M. (2012). Tandem fluorescent protein timers for in vivo analysis of protein dynamics. *Nature Biotechnology*, *30*(7). https://doi.org/10.1038/nbt.2281

Khmelinskii, A., Lawrence, C., Roostalu, J., & Schiebel, E. (2007). Cdc14-regulated midzone assembly controls anaphase B. *Journal of Cell Biology*, *177*(6). https://doi.org/10.1083/jcb.200702145

Khorasanizadeh, S. (2004). The Nucleosome: From Genomic Organization to Genomic Regulation. In *Cell* (Vol. 116, Issue 2). https://doi.org/10.1016/S0092-8674(04)00044-3

Kilmartin, J. v., & Adams, A. E. M. (1984). Structural rearrangements of tubulin and actin during the cell cycle of the yeast Saccharomyces. *Journal of Cell Biology*, *98*(3). https://doi.org/10.1083/jcb.98.3.922

Kim, J. O., Zelter, A., Umbreit, N. T., Bollozos, A., Riffle, M., Johnson, R., MacCoss, M. J., Asbury, C. L., & Davis, T. N. (2017). The Ndc80 complex bridges two dam1 complex rings. *ELife*, *6*. https://doi.org/10.7554/eLife.21069

Kim, T. H., Abdullaev, Z. K., Smith, A. D., Ching, K. A., Loukinov, D. I., Green, R. D. D., Zhang, M. Q., Lobanenkov, V. v., & Ren, B. (2007). Analysis of the Vertebrate Insulator Protein CTCF-Binding Sites in the Human Genome. *Cell*, *128*(6). https://doi.org/10.1016/j.cell.2006.12.048

Kim, Y., Shi, Z., Zhang, H., Finkelstein, I. J., & Yu, H. (2019). Human cohesin compacts DNA by loop extrusion. *Science*, *366*(6471). https://doi.org/10.1126/science.aaz4475

Kitamura, E., Tanaka, K., Kitamura, Y., & Tanaka, T. U. (2007). Kinetochore-microtubule interaction during S phase in Saccharomyces cerevisiae. *Genes and Development*, *21*(24). https://doi.org/10.1101/gad.449407

Knop, M. (1997). Spc98p and Spc97p of the yeast gamma -tubulin complex mediate binding to the spindle pole body via their interaction with Spc110p. *The EMBO Journal*, *16*(23). https://doi.org/10.1093/emboj/16.23.6985

Knop, M., & Schiebel, E. (1998). Receptors determine the cellular localization of a γ-tubulin complex and thereby the site of microtubule formation. *EMBO Journal*, *17*(14). https://doi.org/10.1093/emboj/17.14.3952

Kollman, J. M., Greenberg, C. H., Li, S., Moritz, M., Zelter, A., Fong, K. K., Fernandez, J. J., Sali, A., Kilmartin, J., Davis, T. N., & Agard, D. A. (2015). Ring closure activates yeast γTuRC for species-specific microtubule nucleation. *Nature Structural and Molecular Biology*, *22*(2). https://doi.org/10.1038/nsmb.2953

Kollman, J. M., Polka, J. K., Zelter, A., Davis, T. N., & Agard, D. A. (2010). Microtubule nucleating γ 3-TuSC assembles structures with 13-fold microtubule-like symmetry. *Nature*, *466*(7308). https://doi.org/10.1038/nature09207

Kollman, J. M., Zelter, A., Muller, E. G. D., Fox, B., Rice, L. M., Davis, T. N., & Agard, D. A. (2008). The structure of the γ-tubulin small complex: Implications of its architecture and flexibility for microtubule nucleation. *Molecular Biology of the Cell*, *19*(1). https://doi.org/10.1091/mbc.E07-09-0879

Kops, G. J. P. L., Saurin, A. T., & Meraldi, P. (2010). Finding the middle ground: How kinetochores power chromosome congression. In *Cellular and Molecular Life Sciences* (Vol. 67, Issue 13). https://doi.org/10.1007/s00018-010-0321-y

Korinek, W. S., Copeland, M. J., Chaudhuri, A., & Chant, J. (2000). Molecular linkage underlying microtubule orientation toward cortical sites in yeast. *Science*, *287*(5461). https://doi.org/10.1126/science.287.5461.2257

Kornakov, N., Möllers, B., & Westermann, S. (2020). The EB1-Kinesin-14 complex is required for efficient metaphase spindle assembly and kinetochore bi-orientation. *The Journal of Cell Biology*, *219*(12). https://doi.org/10.1083/jcb.202003072

Kotwaliwale, C. v., Frei, S. B., Stern, B. M., & Biggins, S. (2007). A Pathway Containing the Ipl1/Aurora Protein Kinase and the Spindle Midzone Protein Ase1 Regulates Yeast Spindle Assembly. *Developmental Cell*, *13*(3). https://doi.org/10.1016/j.devcel.2007.07.003

Kramer, E. R., Scheuringer, N., Podtelejnikov, A. v., Mann, M., & Peters, J. M. (2000). Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Molecular Biology of the Cell*, *11*(5). https://doi.org/10.1091/mbc.11.5.1555

Kruitwagen, T., Denoth-Lippuner, A., Wilkins, B. J., Neumann, H., & Barral, Y. (2015). Axial contraction and short-range compaction of chromatin synergistically promote mitotic chromosome condensation. *ELife*, *4*(NOVEMBER2015). https://doi.org/10.7554/eLife.10396

Kueng, S., Hegemann, B., Peters, B. H., Lipp, J. J., Schleiffer, A., Mechtler, K., & Peters, J. M. (2006). Wapl Controls the Dynamic Association of Cohesin with Chromatin. *Cell*, *127*(5). https://doi.org/10.1016/j.cell.2006.09.040

Lampert, F., Mieck, C., Alushin, G. M., Nogales, E., & Westermann, S. (2013). Molecular requirements for the formation of a kinetochore-microtubule interface by Dam1 and Ndc80 complexes. *Journal of Cell Biology*, *200*(1). https://doi.org/10.1083/jcb.201210091

Lanz, M. C., Yugandhar, K., Gupta, S., Sanford, E. J., Faça, V. M., Vega, S., Joiner, A. M. N., Fromme, J. C., Yu, H., & Smolka, M. B. (2021). In-depth and 3-dimensional exploration of the budding yeast phosphoproteome. *EMBO Reports*, *22*(2). https://doi.org/10.15252/embr.202051121

Lara-Gonzalez, P., Pines, J., & Desai, A. (2021). Spindle assembly checkpoint activation and silencing at kinetochores. In *Seminars in Cell and Developmental Biology* (Vol. 117). https://doi.org/10.1016/j.semcdb.2021.06.009

Lauzé, E., Stoelcker, B., Luca, F. C., Weiss, E., Schutz, A. R., & Winey, M. (1995). Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. *EMBO Journal*, *14*(8). https://doi.org/10.1002/j.1460-2075.1995.tb07154.x

Lazar-Stefanita, L., Scolari, V. F., Mercy, G., Muller, H., Guérin, T. M., Thierry, A., Mozziconacci, J., & Koszul, R. (2017). Cohesins and condensins orchestrate the 4D dynamics of yeast chromosomes during the cell cycle. *The EMBO Journal*, *36*(18). https://doi.org/10.15252/embj.201797342

le Guellec, R., Paris, J., Couturier, A., Roghi, C., & Philippe, M. (1991). Cloning by differential screening of a Xenopus cDNA that encodes a kinesin-related protein. *Molecular and Cellular Biology*, *11*(6). https://doi.org/10.1128/mcb.11.6.3395-3398.1991

Lechner, J., & Carbon, J. (1991). A 240 kd multisubunit protein complex, CBF3, is a major component of the budding yeast centromere. *Cell*, *64*(4). https://doi.org/10.1016/0092-8674(91)90501-O

Lee, L., Tirnauer, J. S., Li, J., Schuyler, S. C., Liu, J. Y., & Pellman, D. (2000). Positioning of the mitotic spindle by a cortical-microtubule capture mechanism. *Science (New York, N.Y.)*, *287*(5461), 2260–2262. http://www.ncbi.nlm.nih.gov/pubmed/10731147

Lee, W. L., Oberle, J. R., & Cooper, J. A. (2003). The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. *Journal of Cell Biology*, *160*(3). https://doi.org/10.1083/jcb.200209022

Lengefeld, J., Hotz, M., Rollins, M., Baetz, K., & Barral, Y. (2017). Budding yeast Wee1 distinguishes spindle pole bodies to guide their pattern of age-dependent segregation. *Nature Cell Biology*, *19*(8). https://doi.org/10.1038/ncb3576

Lengronne, A., Katou, Y., Mori, S., Yokobayashi, S., Kelly, G. P., Ito, T., Watanabe, Y., Shirahige, K., & Uhlmann, F. (2004). Cohesin relocation from sites of chromosomal loading to places of convergent transcription. *Nature*, *430*(6999). https://doi.org/10.1038/nature02742

Li, R., & Murray, A. W. (1991). Feedback control of mitosis in budding yeast. *Cell*, *66*(3). https://doi.org/10.1016/0092-8674(81)90015-5

Li, Y. Y., Yeh, E., Hays, T., & Bloom, K. (1993). Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(21). https://doi.org/10.1073/pnas.90.21.10096

Liakopoulos, D., Kusch, J., Grava, S., Vogel, J., & Barral, Y. (2003). Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. *Cell*, *112*(4), 561–574. http://www.ncbi.nlm.nih.gov/pubmed/12600318

Liang, F., Richmond, D., & Wang, Y. (2013). Coordination of Chromatid Separation and Spindle Elongation by Antagonistic Activities of Mitotic and S-Phase CDKs. *PLoS Genetics*, *9*(2). https://doi.org/10.1371/journal.pgen.1003319

Lieberman-Aiden, E., van Berkum, N. L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B. R., Sabo, P. J., Dorschner, M. O., Sandstrom, R., Bernstein, B., Bender, M. A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, L. A., Lander, E. S., & Dekker, J. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science*, *326*(5950). https://doi.org/10.1126/science.1181369

Lim, H. H., Goh, P. Y., & Surana, U. (1998). Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Clb2 during M phase in budding yeast. *Current Biology*, *8*(4). https://doi.org/10.1016/s0960-9822(98)70088-0

Lin, T. C., Neuner, A., Schlosser, Y., Scharf, A., Weber, L., & Schiebel, E. (2014). Cell-cycle dependent phosphorylation of yeast pericentrin regulates γ-TUSC-mediated microtubule nucleation. *ELife*, *2014*(3). https://doi.org/10.7554/eLife.02208

Lin, T. chen, Gombos, L., Neuner, A., Sebastian, D., Olsen, J. v., Hrle, A., Benda, C., & Schiebel, E. (2011). Phosphorylation of the yeast γ-tubulin tub4 regulates microtubule function. *PLoS ONE*, *6*(5). https://doi.org/10.1371/journal.pone.0019700

Liu, D., Vader, G., Vromans, M. J. M., Lampson, M. A., & Lens, S. M. A. (2009). Sensing chromosome bi-orientation by spatial separation of Aurora B kinase from kinetochore substrates. *Science*, *323*(5919). https://doi.org/10.1126/science.1167000

London, N., & Biggins, S. (2014). Mad1 kinetochore recruitment by Mps1-mediated phosphorylation of Bub1 signals the spindle checkpoint. *Genes and Development*, *28*(2). https://doi.org/10.1101/gad.233700.113

London, N., Ceto, S., Ranish, J. A., & Biggins, S. (2012). Phosphoregulation of Spc105 by Mps1 and PP1 regulates Bub1 localization to kinetochores. *Current*

Biology, *22*(10). https://doi.org/10.1016/j.cub.2012.03.052

Luppino, J. M., Park, D. S., Nguyen, S. C., Lan, Y., Xu, Z., Yunker, R., & Joyce, E. F. (2020). Cohesin promotes stochastic domain intermingling to ensure proper regulation of boundary-proximal genes. *Nature Genetics*, *52*(8). https://doi.org/10.1038/s41588-020-0647-9

Mach, P., Kos, P. I., Zhan, Y., Cramard, J., Gaudin, S., Tünnermann, J., Marchi, E., Eglinger, J., Zuin, J., Kryzhanovska, M., Smallwood, S., Gelman, L., Roth, G., Nora, E. P., Tiana, G., & Giorgetti, L. (2022). Live-cell imaging and physical modeling reveal control of chromosome folding dynamics by cohesin and CTCF. *BioRxiv*.

Maciejowski, J., Drechsler, H., Grundner-Culemann, K., Ballister, E. R., Rodriguez-Rodriguez, J. A., Rodriguez-Bravo, V., Jones, M. J. K., Foley, E., Lampson, M. A., Daub, H., McAinsh, A. D., & Jallepalli, P. v. (2017). Mps1 Regulates Kinetochore-Microtubule Attachment Stability via the Ska Complex to Ensure Error-Free Chromosome Segregation. *Developmental Cell*, *41*(2). https://doi.org/10.1016/j.devcel.2017.03.025

Maddox, P. S., Salmon, K. S., & Bloom, E. D. (2000). The polarity and dynamics of microtubule assembly in the budding yeast Saccharomyces cerevisae. *Nature Cell Biology*, *2*(1). https://doi.org/10.1038/71357

Maekawa, H., Priest, C., Lechner, J., Pereira, G., & Schiebel, E. (2007). The yeast centrosome translates the positional information of the anaphase spindle into a cell cycle signal. *Journal of Cell Biology*, *179*(3). https://doi.org/10.1083/jcb.200705197

Maekawa, H., & Schiebel, E. (2004). Cdk1-Clb4 controls the interaction of astral microtubule plus ends with subdomains of the daughter cell cortex. *Genes and Development*, *18*(14). https://doi.org/10.1101/gad.298704

Maekawa, H., Usui, T., Knop, M., & Schiebel, E. (2003). Yeast Cdk1 translocates to the plus end of cytoplasmic microtubules to regulate bud cortex interactions. *EMBO Journal*, *22*(3). https://doi.org/10.1093/emboj/cdg063

Malvezzi, F., Litos, G., Schleiffer, A., Heuck, A., Mechtler, K., Clausen, T., & Westermann, S. (2013). A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors. *EMBO Journal*, *32*(3). https://doi.org/10.1038/emboj.2012.356

Mann, B. J., & Wadsworth, P. (2019). Kinesin-5 Regulation and Function in Mitosis. In *Trends in Cell Biology* (Vol. 29, Issue 1). https://doi.org/10.1016/j.tcb.2018.08.004

Marco, E., Dorn, J. F., Hsu, P. H., Jaqaman, K., Sorger, P. K., & Danuser, G. (2013). S. cerevisiae chromosomes biorient via gradual resolution of syntely between S phase and anaphase. *Cell*, *154*(5). https://doi.org/10.1016/j.cell.2013.08.008

Markus, S. M., & Lee, W. L. (2011). Regulated offloading of cytoplasmic dynein from microtubule plus ends to the cortex. *Developmental Cell*, *20*(5). https://doi.org/10.1016/j.devcel.2011.04.011

Markus, S. M., Plevock, K. M., st. Germain, B. J., Punch, J. J., Meaden, C. W., & Lee, W. L. (2011). Quantitative analysis of Pac1/LIS1-mediated dynein targeting: Implications for regulation of dynein activity in budding yeast. *Cytoskeleton*, *68*(3). https://doi.org/10.1002/cm.20502

Marsden, M. P. F., & Laemmli, U. K. (1979). Metaphase chromosome structure: Evidence for a radial loop model. *Cell*, *17*(4). https://doi.org/10.1016/0092-8674(79)90325-8

Marshall, W. F., Straight, A., Marko, J. F., Swedlow, J., Dernburg, A., Belmont, A., Murray, A. W., Agard, D. A., & Sedat, J. W. (1997). Interphase chromosomes undergo constrained diffusional motion in living cells. *Current Biology*, *7*(12). https://doi.org/10.1016/S0960-9822(06)00412-X

Marsoner, T., Yedavalli, P., Masnovo, C., Fink, S., Schmitzer, K., & Campbell, C. S. (2022). Aurora B activity is promoted by cooperation between discrete localization sites in budding yeast. *Molecular Biology of the Cell*, *33*(9), ar85. https://doi.org/10.1091/mbc.E21-11-0590

Matellán, L., Manzano-López, J., & Monje-Casas, F. (2020). Polo-like kinase acts as a molecular timer that safeguards the asymmetric fate of spindle microtubule-organizing centers. *ELife*, *9*. https://doi.org/10.7554/eLife.61488

Maure, J. F., Kitamura, E., & Tanaka, T. U. (2007). Mps1 Kinase Promotes Sister-Kinetochore Bi-orientation by a Tension-Dependent Mechanism. *Current Biology*, *17*(24). https://doi.org/10.1016/j.cub.2007.11.032

Maurer, S. P., Fourniol, F. J., Bohner, G., Moores, C. A., & Surrey, T. (2012). EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell*, *149*(2). https://doi.org/10.1016/j.cell.2012.02.049

Menendez-Benito, V., van Deventer, S. J., Jimenez-Garcia, V., Roy-Luzarraga, M., van Leeuwen, F., & Neefjes, J. (2013). Spatiotemporal analysis of organelle and macromolecular complex inheritance. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(1), 175–180. https://doi.org/10.1073/pnas.1207424110

Meziane, M., Genthial, R., & Vogel, J. (2021). Kar9 symmetry breaking alone is insufficient to ensure spindle alignment. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-83136-w

Miller, M. P., Asbury, C. L., & Biggins, S. (2016). A TOG protein confers tension sensitivity to kinetochore-microtubule attachments. *Cell*, *165*(6). https://doi.org/10.1016/j.cell.2016.04.030

Miller, M. P., Evans, R. K., Zelter, A., Geyer, E. A., MacCoss, M. J., Rice, L. M., Davis, T. N., Asbury, C. L., & Biggins, S. (2019). Kinetochore-associated Stu2 promotes

chromosome biorientation in vivo. *PLoS Genetics*, *15*(10). https://doi.org/10.1371/journal.pgen.1008423

Miller, R. K., Cheng, S. C., & Rose, M. D. (2000). Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. *Molecular Biology of the Cell*, *11*(9), 2949–2959. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=14967&tool=pmcentrez&rendertype=abstract

Miller, R. K., Matheos, D., & Rose, M. D. (1999). The cortical localization of the microtubule orientation protein, Kar9p, is dependent upon actin and proteins required for polarization. *Journal of Cell Biology*, *144*(5). https://doi.org/10.1083/jcb.144.5.963

Miller, R. K., & Rose, M. D. (1998). Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. *Journal of Cell Biology*, *140*(2). https://doi.org/10.1083/jcb.140.2.377

Miné-Hattab, J., & Rothstein, R. (2012). Increased chromosome mobility facilitates homology search during recombination. *Nature Cell Biology*, *14*(5). https://doi.org/10.1038/ncb2472

Miranda, J. J. L., de Wulf, P., Sorger, P. K., & Harrison, S. C. (2005). The yeast DASH complex forms closed rings on microtubules. *Nature Structural and Molecular Biology*, *12*(2). https://doi.org/10.1038/nsmb896

Mitchison, T., & Kirschner, M. (1984a). Dynamic instability of microtubule growth. *Nature*, *312*(5991). https://doi.org/10.1038/312237a0

Mitchison, T., & Kirschner, M. (1984b). Microtubule assembly nucleated by isolated centrosomes. *Nature*, *312*(5991). https://doi.org/10.1038/312232a0

Moffat, J., & Andrews, B. (2004). Late-G1 cyclin-CDK activity is essential for control of cell morphogenesis in budding yeast. *Nature Cell Biology*, *6*(1). https://doi.org/10.1038/ncb1078

Molk, J. N., Schuyler, S. C., Liu, J. Y., Evans, J. G., Salmon, E. D., Pellman, D., & Bloom, K. (2004). The Differential Roles of Budding Yeast Tem1p, Cdc15p, and Bub2p Protein Dynamics in Mitotic Exit. *Molecular Biology of the Cell*, *15*(4). https://doi.org/10.1091/mbc.E03-09-0708

Molodtsov, M. I., Mieck, C., Dobbelaere, J., Dammermann, A., Westermann, S., & Vaziri, A. (2016). A Force-Induced Directional Switch of a Molecular Motor Enables Parallel Microtubule Bundle Formation. *Cell*, *167*(2). https://doi.org/10.1016/j.cell.2016.09.029

Moore, J. K., D'Silva, S., & Miller, R. K. (2006). The CLIP-170 homologue Bik1p promotes the phosphorylation and asymmetric localization of Kar9p. *Molecular Biology of the Cell*, *17*(1). https://doi.org/10.1091/mbc.E05-06-0565

Moore, J. K., & Miller, R. K. (2007). The cyclin-dependent kinase Cdc28p regulates multiple aspects of Kar9p function in yeast. *Molecular Biology of the Cell*, *18*(4). https://doi.org/10.1091/mbc.E06-04-0360

Moore, J. K., Stuchell-Brereton, M. D., & Cooper, J. A. (2009). Function of dynein in budding yeast: Mitotic spindle positioning in a polarized cell. In *Cell Motility and the Cytoskeleton* (Vol. 66, Issue 8). https://doi.org/10.1002/cm.20364

Morgan, D. O. (1997). Cyclin-dependent kinases: Engines, clocks, and microprocessors. In *Annual Review of Cell and Developmental Biology* (Vol. 13). https://doi.org/10.1146/annurev.cellbio.13.1.261

Muller, E. G. D., Snydsman, B. E., Novik, I., Hailey, D. W., Gestaut, D. R., Niemann, C. A., O'Toole, E. T., Giddings, T. H., Sundin, B. A., & Davis, T. N. (2005). The organization of the core proteins of the yeast spindle pole body. *Molecular Biology of the Cell*, *16*(7). https://doi.org/10.1091/mbc.E05-03-0214

Muñoz-Barrera, M., & Monje-Casas, F. (2017). Analysis of cell cycle progression in saccharomyces cerevisiae using the budding index and tubulin staining. In *Methods in Molecular Biology* (Vol. 1505). https://doi.org/10.1007/978-1-4939-6502-1_3

Murayama, Y., & Uhlmann, F. (2014). Biochemical reconstitution of topological DNA binding by the cohesin ring. *Nature*, *505*(7483). https://doi.org/10.1038/nature12867

Nannas, N. J., O'Toole, E. T., Winey, M., & Murray, A. W. (2014). Chromosomal attachments set length and microtubule number in the Saccharomyces cerevisiae mitotic spindle. *Molecular Biology of the Cell*, *25*(25). https://doi.org/10.1091/mbc.E14-01-0016

Nasmyth, K. (1996). At the heart of the budding yeast cell cycle. In *Trends in Genetics* (Vol. 12, Issue 10). https://doi.org/10.1016/0168-9525(96)10041-X

Nasmyth, K. (2001). Disseminating the genome: Joining, resolving, and separating sister chromatids during mitosis and meiosis. In *Annual Review of Genetics* (Vol. 35). https://doi.org/10.1146/annurev.genet.35.102401.091334

Nasmyth, K., & Haering, C. H. (2009). Cohesin: Its roles and mechanisms. In *Annual Review of Genetics* (Vol. 43). https://doi.org/10.1146/annurev-genet-102108-134233

Naumova, N., Imakaev, M., Fudenberg, G., Zhan, Y., Lajoie, B. R., Mirny, L. A., & Dekker, J. (2013). Organization of the mitotic chromosome. *Science*, *342*(6161). https://doi.org/10.1126/science.1236083

Nazarova, E., O'Toole, E., Kaitna, S., Francois, P., Winey, M., & Vogel, J. (2013). Distinct roles for antiparallel microtubule pairing and overlap during early spindle assembly. *Molecular Biology of the Cell*, *24*(20). https://doi.org/10.1091/mbc.E13-05-0232

Neurohr, G., Naegeli, A., Titos, I., Theler, D., Greber, B., Díez, J., Gabaldón, T., Mendoza, M., & Barral, Y. (2011). A midzone-based ruler adjusts chromosome compaction to anaphase spindle length. *Science*, *332*(6028). https://doi.org/10.1126/science.1201578

Ng, C. T., Deng, L., Chen, C., Lim, H. H., Shi, J., Surana, U., & Gan, L. (2019). Electron cryotomography analysis of Dam1C/DASH at the kinetochore-spindle interface in situ. *Journal of Cell Biology*, *218*(2). https://doi.org/10.1083/jcb.201809088

Ng, R., & Carbon, J. (1987). Mutational and in vitro protein-binding studies on centromere DNA from Saccharomyces cerevisiae. *Molecular and Cellular Biology*, *7*(12). https://doi.org/10.1128/mcb.7.12.4522-4534.1987

Nsamba, E. T., Bera, A., Costanzo, M., Boone, C., & Gupta, M. L. (2021). Tubulin isotypes optimize distinct spindle positioning mechanisms during yeast mitosis. *Journal of Cell Biology*, *220*(12). https://doi.org/10.1083/jcb.202010155

Nsamba, E. T., & Gupta Jr, M. L. (2022). Tubulin isotypes – functional insights from model organisms. *Journal of Cell Science*, *135*(9), jcs259539. https://doi.org/10.1242/jcs.259539

Olins, A. L., & Olins, D. E. (1974). Spheroid chromatin units (v bodies). *Science*, *183*(4122). https://doi.org/10.1126/science.183.4122.330

Ono, T., Losada, A., Hirano, M., Myers, M. P., Neuwald, A. F., & Hirano, T. (2003). Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. *Cell*, *115*(1). https://doi.org/10.1016/S0092-8674(03)00724-4

O'Toole, E. T., Winey, M., & McIntosh, J. R. (1999). High-voltage electron tomography of spindle pole bodies and early mitotic spindles in the yeast Saccharomyces cerevisiae. *Molecular Biology of the Cell*, *10*(6). https://doi.org/10.1091/mbc.10.6.2017

Ou, H. D., Phan, S., Deerinck, T. J., Thor, A., Ellisman, M. H., & O'Shea, C. C. (2017). ChromEMT: Visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science*, *357*(6349). https://doi.org/10.1126/science.aag0025

Paldi, F., Alver, B., Robertson, D., Schalbetter, S. A., Kerr, A., Kelly, D. A., Baxter, J., Neale, M. J., & Marston, A. L. (2020). Convergent genes shape budding yeast pericentromeres. *Nature*, *582*(7810). https://doi.org/10.1038/s41586-020-2244-6

Pandey, H., Popov, M., Goldstein-levitin, A., & Gheber, L. (2021). Mechanisms by which kinesin-5 motors perform their multiple intracellular functions. In *International Journal of Molecular Sciences* (Vol. 22, Issue 12). https://doi.org/10.3390/ijms22126420

Parelho, V., Hadjur, S., Spivakov, M., Leleu, M., Sauer, S., Gregson, H. C., Jarmuz, A., Canzonetta, C., Webster, Z., Nesterova, T., Cobb, B. S., Yokomori, K., Dillon, N., Aragon, L., Fisher, A. G., & Merkenschlager, M. (2008). Cohesins Functionally Associate with CTCF on Mammalian Chromosome Arms. *Cell*, *132*(3). https://doi.org/10.1016/j.cell.2008.01.011

Pearson, C. G., Maddox, P. S., Zarzar, T. R., Salmon, E. D., & Bloom, K. (2003). Yeast kinetochores do not stabilize Stu2p-dependent spindle microtubule dynamics. *Molecular Biology of the Cell*, *14*(10). https://doi.org/10.1091/mbc.E03-03-0180

Pearsona, C. G., Maddoxa, P. S., Salmona, E. D., & Blooma, K. (2001). Budding yeast chromosome structure and dynamics during mitosis. *Journal of Cell Biology*, *152*(6). https://doi.org/10.1083/jcb.152.6.1255

Pellman, D., Bagget, M., Tu, H., & Fink, G. R. (1995). Two microtubule-associated proteins required for anaphase spindle movement in Saccharomyces cerevisiae. *Journal of Cell Biology*, *130*(6). https://doi.org/10.1083/jcb.130.6.1373

Pereira, G., Höfken, T., Grindlay, J., Manson, C., & Schiebel, E. (2000). The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Molecular Cell*, *6*(1). https://doi.org/10.1016/S1097-2765(05)00017-1

Pereira, G., Knop, M., & Schiebel, E. (1998). Spc98p directs the yeast γ-tubulin complex into the nucleus and is subject to cell cycle-dependent phosphorylation on the nuclear side of the spindle pole body. *Molecular Biology of the Cell*, *9*(4). https://doi.org/10.1091/mbc.9.4.775

Pereira, G., & Schiebel, E. (2003). Separase Regulates INCENP-Aurora B Anaphase Spindle Function Through Cdc14. *Science*, *302*(5653). https://doi.org/10.1126/science.1091936

Pereira, G., & Schiebel, E. (2005). Kin4 kinase delays mitotic exit in response to spindle alignment defects. *Molecular Cell*, *19*(2). https://doi.org/10.1016/j.molcel.2005.05.030

Pereira, G., Tanaka, T. U., Nasmyth, K., & Schiebel, E. (2001). Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex. *The EMBO Journal*, *20*(22), 6359–6370. https://doi.org/10.1093/emboj/20.22.6359

Peterson, J. B., & Ris, H. (1976). Electron microscopic study of the spindle and chromosome movement in the yeast Saccharomyces cerevisiae. *Journal of Cell Science*, *22*(2).

Pierre, P., Scheel, J., Rickard, J. E., & Kreis, T. E. (1992). CLIP-170 links endocytic vesicles to microtubules. *Cell*, *70*(6). https://doi.org/10.1016/0092-8674(92)90240-D

Pinsky, B. A., Kung, C., Shokat, K. M., & Biggins, S. (2006). The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. *Nature Cell Biology*, *8*(1). https://doi.org/10.1038/ncb1341

Pinsky, B. A., Nelson, C. R., & Biggins, S. (2009). Protein Phosphatase 1 Regulates Exit from the Spindle Checkpoint in Budding Yeast. *Current Biology*, *19*(14). https://doi.org/10.1016/j.cub.2009.06.043

Pradhan, B., Barth, R., Kim, E., Davidson, I. F., Bauer, B., van Laar, T., Yang, W., Ryu, J.-K., van der Torre, J., Peters, J.-M., & Dekker, C. (2022). SMC complexes can traverse physical roadblocks bigger than their ring size. *Cell Reports*, *41*(3), 111491.

https://doi.org/https://doi.org/10.1016/j.celrep.2022.111491

Primorac, I., Weir, J. R., Chiroli, E., Gross, F., Hoffmann, I., van Gerwen, S., Ciliberto, A., & Musacchio, A. (2013). Bub3 reads phosphorylated MELT repeats to promote spindle assembly checkpoint signaling. *ELife*, *2013*(2). https://doi.org/10.7554/eLife.01030

Qian, H., Sheetz, M. P., & Elson, E. L. (1991). Single particle tracking. Analysis of diffusion and flow in two-dimensional systems. *Biophysical Journal*, *60*(4). https://doi.org/10.1016/S0006-3495(91)82125-7

Queralt, E., Lehane, C., Novak, B., & Uhlmann, F. (2006). Downregulation of PP2ACdc55 Phosphatase by Separase Initiates Mitotic Exit in Budding Yeast. *Cell*, *125*(4). https://doi.org/10.1016/j.cell.2006.03.038

Queralt, E., & Uhlmann, F. (2008). Separase cooperates with Zds1 and Zds2 to activate Cdc14 phosphatase in early anaphase. *Journal of Cell Biology*, *182*(5). https://doi.org/10.1083/jcb.200801054

Rao, S. S. P., Huang, S. C., Glenn St Hilaire, B., Engreitz, J. M., Perez, E. M., Kieffer-Kwon, K. R., Sanborn, A. L., Johnstone, S. E., Bascom, G. D., Bochkov, I. D., Huang, X., Shamim, M. S., Shin, J., Turner, D., Ye, Z., Omer, A. D., Robinson, J. T., Schlick, T., Bernstein, B. E., … Aiden, E. L. (2017). Cohesin Loss Eliminates All Loop Domains. *Cell*, *171*(2). https://doi.org/10.1016/j.cell.2017.09.026

Rao, S. S. P., Huntley, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., Robinson, J. T., Sanborn, A. L., Machol, I., Omer, A. D., Lander, E. S., & Aiden, E. L. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell*, *159*(7). https://doi.org/10.1016/j.cell.2014.11.021

Ricci, M. A., Manzo, C., García-Parajo, M. F., Lakadamyali, M., & Cosma, M. P. (2015). Chromatin fibers are formed by heterogeneous groups of nucleosomes in vivo. *Cell*, *160*(6). https://doi.org/10.1016/j.cell.2015.01.054

Roberts, A. J., Goodman, B. S., & Reck-Peterson, S. L. (2014). Reconstitution of dynein transport to the microtubule plus end by kinesin. *ELife*, *3*. https://doi.org/10.7554/elife.02641

Roccuzzo, M., Visintin, C., Tili, F., & Visintin, R. (2015). FEAR-mediated activation of Cdc14 is the limiting step for spindle elongation and anaphase progression. *Nature Cell Biology*, *17*(3). https://doi.org/10.1038/ncb3105

Rock, J. M., & Amon, A. (2011). Cdc15 integrates Tem1 GTPase-mediated spatial signals with Polo kinase-mediated temporal cues to activate mitotic exit. *Genes and Development*, *25*(18). https://doi.org/10.1101/gad.17257711

Rock, J. M., Lim, D., Stach, L., Ogrodowicz, R. W., Keck, J. M., Jones, M. H., Wong, C. C. L., Yates, J. R., Winey, M., Smerdon, S. J., Yaffe, M. B., & Amon, A. (2013). Activation of the yeast Hippo pathway by phosphorylation-dependent assembly of signaling complexes. *Science*, *340*(6134). https://doi.org/10.1126/science.1235822

Roostalu, J., Hentrich, C., Bieling, P., Telley, I. A., Schiebel, E., & Surrey, T. (2011). Directional switching of the kinesin Cin8 through motor coupling. *Science*, *332*(6025). https://doi.org/10.1126/science.1199945

Roostalu, J., Thomas, C., Cade, N. I., Kunzelmann, S., Taylor, I. A., & Surrey, T. (2020). The speed of GTP hydrolysis determines GTP cap size and controls microtubule stability. *ELife*, *9*. https://doi.org/10.7554/eLife.51992

Rosenberg, J. S., Cross, F. R., & Funabiki, H. (2011). KNL1/Spc105 recruits PP1 to silence the spindle assembly checkpoint. *Current Biology*, *21*(11). https://doi.org/10.1016/j.cub.2011.04.011

Rowland, B. D., Roig, M. B., Nishino, T., Kurze, A., Uluocak, P., Mishra, A., Beckouët, F., Underwood, P., Metson, J., Imre, R., Mechtler, K., Katis, V. L., & Nasmyth, K. (2009). Building Sister Chromatid Cohesion: Smc3 Acetylation Counteracts an Antiestablishment Activity. *Molecular Cell*, *33*(6). https://doi.org/10.1016/j.molcel.2009.02.028

Roy, B., Han, S. J. Y., Fontan, A. N., Jema, S., & Joglekar, A. P. (2022). Aurora B phosphorylates Bub1 to promote spindle assembly checkpoint signaling. *Current Biology*, *32*(1). https://doi.org/10.1016/j.cub.2021.10.049

Roy, B., Verma, V., Sim, J., Fontan, A., & Joglekar, A. P. (2019). Delineating the contribution of Spc105-bound PP1 to spindle checkpoint silencing and kinetochore microtubule attachment regulation. *Journal of Cell Biology*, *218*(12). https://doi.org/10.1083/JCB.201810172

Rudner, A. D., & Murray, A. W. (2000). Phosphorylation by Cdc28 activates the Cdc20-dependent activity of the anaphase-promoting complex. *Journal of Cell Biology*, *149*(7). https://doi.org/10.1083/jcb.149.7.1377

Samejima, K., Samejima, I., Vagnarelli, P., Ogawa, H., Vargiu, G., Kelly, D. A., Alves, F. de L., Kerr, A., Green, L. C., Hudson, D. F., Ohta, S., Cooke, C. A., Farr, C. J., Rappsilber, J., & Earnshaw, W. C. (2012). Mitotic chromosomes are compacted laterally by KIF4 and condensin and axially by topoisomerase IIα. *Journal of Cell Biology*, *199*(5). https://doi.org/10.1083/jcb.201202155

Santaguida, S., Tighe, A., D'Alise, A. M., Taylor, S. S., & Musacchio, A. (2010). Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *Journal of Cell Biology*, *190*(1). https://doi.org/10.1083/jcb.201001036

Sarangapani, K. K., Koch, L. B., Nelson, C. R., Asbury, C. L., & Biggins, S. (2021). Kinetochore-bound Mps1 regulates kinetochore–microtubule attachments via Ndc80 phosphorylation. *Journal of Cell Biology*, *220*(12). https://doi.org/10.1083/jcb.202106130

Saunders, W. S., & Hoyt, M. A. (1992). Kinesin-related proteins required for structural integrity of the

mitotic spindle. *Cell*, *70*(3). https://doi.org/10.1016/0092-8674(92)90169-D

Saunders, W. S., Koshland, D., Eshel, D., Gibbons, I. R., & Hoyt, M. A. (1995). Saccharomyces cerevisiae kinesin- and dynein-related proteins required for anaphase chromosome segregation. *Journal of Cell Biology*, *128*(4). https://doi.org/10.1083/jcb.128.4.617

Saurin, A. T., van der Waal, M. S., Medema, R. H., Lens, S. M. A., & Kops, G. J. P. L. (2011). Aurora B potentiates Mps1 activation to ensure rapid checkpoint establishment at the onset of mitosis. *Nature Communications*, *2*(1). https://doi.org/10.1038/ncomms1319

Schalbetter, S. A., Goloborodko, A., Fudenberg, G., Belton, J. M., Miles, C., Yu, M., Dekker, J., Mirny, L., & Baxter, J. (2017). SMC complexes differentially compact mitotic chromosomes according to genomic context. *Nature Cell Biology*, *19*(9). https://doi.org/10.1038/ncb3594

Schatz, P. J., Solomon, F., & Botstein, D. (1986). Genetically essential and nonessential alpha-tubulin genes specify functionally interchangeable proteins. *Molecular and Cellular Biology*, *6*(11). https://doi.org/10.1128/mcb.6.11.3722-3733.1986

Schleiffer, A., Maier, M., Litos, G., Lampert, F., Hornung, P., Mechtler, K., & Westermann, S. (2012). CENP-T proteins are conserved centromere receptors of the Ndc80 complex. *Nature Cell Biology*, *14*(6). https://doi.org/10.1038/ncb2493

Schutz, A. R., & Winey, M. (1998). New alleles of the yeast MPS1 gene reveal multiple requirements in spindle pole body duplication. *Molecular Biology of the Cell*, *9*(4). https://doi.org/10.1091/mbc.9.4.759

Schuyler, S. C., Liu, J. Y., & Pellman, D. (2003). The molecular function of Ase1p: Evidence for a MAP-dependent midzone-specific spindle matrix. *Journal of Cell Biology*, *160*(4). https://doi.org/10.1083/jcb.200210021

Schwab, M., Neutzner, M., Möcker, D., & Seufert, W. (2001). Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC. *EMBO Journal*, *20*(18). https://doi.org/10.1093/emboj/20.18.5165

Schwarzer, W., Abdennur, N., Goloborodko, A., Pekowska, A., Fudenberg, G., Loe-Mie, Y., Fonseca, N. A., Huber, W., Haering, C. H., Mirny, L., & Spitz, F. (2017). Two independent modes of chromatin organization revealed by cohesin removal. *Nature*, *551*(7678). https://doi.org/10.1038/nature24281

Segal, M., Bloom, K., & Reed, S. I. (2000). Bud6 directs sequential microtubule interactions with the bud tip and bud neck during spindle morphogenesis in Saccharomyces cerevisiae. *Molecular Biology of the Cell*, *11*(11). https://doi.org/10.1091/mbc.11.11.3689

Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A., & Cavalli, G. (2012). Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell*, *148*(3). https://doi.org/10.1016/j.cell.2012.01.010

Shapira, O., & Gheber, L. (2016). Motile properties of the bi-directional kinesin-5 Cin8 are affected by phosphorylation in its motor domain. *Scientific Reports*, *6*. https://doi.org/10.1038/srep25597

Shapira, O., Goldstein, A., Al-Bassam, J., & Gheber, L. (2017). A potential physiological role for bi-directional motility and motor clustering of mitotic kinesin-5 Cin8 in yeast mitosis. *Journal of Cell Science*, *130*(4). https://doi.org/10.1242/jcs.195040

Shaw, S. L., Yeh, E., Maddox, P., Salmon, E. D., & Bloom, K. (1997). Astral microtubule dynamics in yeast: A microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. *Journal of Cell Biology*, *139*(4). https://doi.org/10.1083/jcb.139.4.985

Sherwin, D., Huetteman, A., & Wang, Y. (2022). Yeast Kinesin-5 Motor Protein CIN8 Promotes Accurate Chromosome Segregation. *Cells*, *11*(14). https://doi.org/10.3390/cells11142144

Shou, W., Azzam, R., Chen, S. L., Huddleton, M. J., Baskerville, C., Charbonneau, H., Annan, R. S., Carr, S. A., & Deshaies, R. J. (2002). Cdc5 influences phosphorylation of Net1 and disassembly of the RENT complex. *BMC Molecular Biology*, *3*. https://doi.org/10.1186/1471-2199-3-3

Shou, W., Seol, J. H., Shevchenko, A., Baskerville, C., Moazed, D., Susan Chen, Z. W., Jang, J., Shevchenko, A., Charbonneau, H., & Deshaies, R. J. (1999). Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell*, *97*(2). https://doi.org/10.1016/S0092-8674(00)80733-3

Shteinberg, M., Protopopov, Y., Listovsky, T., Brandeis, M., & Hershko, A. (1999). Phosphorylation of the cyclosome is required for its stimulation by Fizzy/cdc20. *Biochemical and Biophysical Research Communications*, *260*(1). https://doi.org/10.1006/bbrc.1999.0884

Shyu, Y. J., & Hu, C. D. (2008). Fluorescence complementation: an emerging tool for biological research. In *Trends in Biotechnology* (Vol. 26, Issue 11). https://doi.org/10.1016/j.tibtech.2008.07.006

Slangy, A., Lane, H. A., d'Hérin, P., Harper, M., Kress, M., & Niggt, E. A. (1995). Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell*, *83*(7). https://doi.org/10.1016/0092-8674(95)90142-6

Srinivasan, M., Scheinost, J. C., Petela, N. J., Gligoris, T. G., Wissler, M., Ogushi, S., Collier, J. E., Voulgaris, M., Kurze, A., Chan, K. L., Hu, B., Costanzo, V., & Nasmyth, K. A. (2018). The Cohesin Ring Uses Its Hinge to Organize DNA Using Non-topological as well as Topological Mechanisms. *Cell*, *173*(6). https://doi.org/10.1016/j.cell.2018.04.015

Stangier, M. M., Kumar, A., Chen, X., Farcas, A. M., Barral, Y., & Steinmetz, M. O. (2018). Structure-Function Relationship of the Bik1-Bim1 Complex. *Structure*, *26*(4). https://doi.org/10.1016/j.str.2018.03.003

Stegmeier, F., & Amon, A. (2004). Closing mitosis: The functions of the Cdc14 phosphatase and its regulation. In *Annual Review of Genetics* (Vol. 38). https://doi.org/10.1146/annurev.genet.38.072902.093051

Steinkraus, K. A., Kaeberlein, M., & Kennedy, B. K. (2008). Replicative aging in yeast: The means to the end. In *Annual Review of Cell and Developmental Biology* (Vol. 24). https://doi.org/10.1146/annurev.cellbio.23.090506.123509

Stephens, A. D., Haase, J., Vicci, L., Taylor, R. M., & Bloom, K. (2011). Cohesin, condensin, and the intramolecular centromere loop together generate the mitotic chromatin spring. *Journal of Cell Biology*, *193*(7). https://doi.org/10.1083/jcb.201103138

Stirling, D. A., & Stark, M. J. R. (1996). The phosphorylation state of the 110 kDa component of the yeast spindle pole body shows cell cycle dependent regulation. *Biochemical and Biophysical Research Communications*, *222*(2). https://doi.org/10.1006/bbrc.1996.0728

Stoepel, J., Ottey, M. A., Kurischko, C., Hieter, P., & Luca, F. C. (2005). The mitotic exit network Mob1p-Dbf2p kinase complex localizes to the nucleus and regulates passenger protein localization. *Molecular Biology of the Cell*, *16*(12). https://doi.org/10.1091/mbc.E05-04-0337

Storchová, Z., Becker, J. S., Talarek, N., Kögelsberger, S., & Pellman, D. (2011). Bub1, Sgo1, and Mps1 mediate a distinct pathway for chromosome biorientation in budding yeast. *Molecular Biology of the Cell*, *22*(9). https://doi.org/10.1091/mbc.E10-08-0673

Straight, A. F., Belmont, A. S., Robinett, C. C., & Murray, A. W. (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Current Biology*, *6*(12). https://doi.org/10.1016/S0960-9822(02)70783-5

Straight, A. F., Marshall, W. F., Sedat, J. W., & Murray, A. W. (1997). Mitosis in living budding yeast: Anaphase a but no metaphase plate. *Science*, *277*(5325). https://doi.org/10.1126/science.277.5325.574

Straight, A. F., Sedat, J. W., & Murray, A. W. (1998). Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. *Journal of Cell Biology*, *143*(3). https://doi.org/10.1083/jcb.143.3.687

Straight, A. F., Shou, W., Dowd, G. J., Turck, C. W., Deshaies, R. J., Johnson, A. D., & Moazed, D. (1999). Net1, a Sir2-associated nucleolar protein required for rDNA silencing and nucleolar integrity. *Cell*, *97*(2). https://doi.org/10.1016/S0092-8674(00)80734-5

Strick, T. R., Kawaguchi, T., & Hirano, T. (2004). Real-time detection of single-molecule DNA compaction by condensin I. *Current Biology*, *14*(10). https://doi.org/10.1016/j.cub.2004.04.038

Sullivan, M., Higuchi, T., Katis, V. L., & Uhlmann, F. (2004). Cdc14 phosphatase induces rDNA condensation and resolves cohesin-independent cohesion during budding yeast anaphase. *Cell*, *117*(4). https://doi.org/10.1016/S0092-8674(04)00415-5

Sundberg, H. A., & Davis, T. N. (1997). A mutational analysis identifies three functional regions of the spindle pole component Spc110p in Saccharomyces cerevisiae. *Molecular Biology of the Cell*, *8*(12). https://doi.org/10.1091/mbc.8.12.2575

Suzuki, A., Badger, B. L., Haase, J., Ohashi, T., Erickson, H. P., Salmon, E. D., & Bloom, K. (2016). How the kinetochore couples microtubule force and centromere stretch to move chromosomes. *Nature Cell Biology*, *18*(4). https://doi.org/10.1038/ncb3323

Suzuki, A., Gupta, A., Long, S. K., Evans, R., Badger, B. L., Salmon, E. D., Biggins, S., & Bloom, K. (2018). A Kinesin-5, Cin8, Recruits Protein Phosphatase 1 to Kinetochores and Regulates Chromosome Segregation. *Current Biology*, *28*(17). https://doi.org/10.1016/j.cub.2018.08.038

Szabo, Q., Donjon, A., Jerković, I., Papadopoulos, G. L., Cheutin, T., Bonev, B., Nora, E. P., Bruneau, B. G., Bantignies, F., & Cavalli, G. (2020). Regulation of single-cell genome organization into TADs and chromatin nanodomains. *Nature Genetics*, *52*(11). https://doi.org/10.1038/s41588-020-00716-8

Talbert, P. B., & Henikoff, S. (2020). What makes a centromere? *Experimental Cell Research*, *389*(2). https://doi.org/10.1016/j.yexcr.2020.111895

Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E. K., Prescott, A. R., Antony, C., & Tanaka, T. U. (2005). Molecular mechanisms of kinetochore capture by spindle microtubules. *Nature*, *434*(7036). https://doi.org/10.1038/nature03483

Tanaka, T., Cosma, M. P., Wirth, K., & Nasmyth, K. (1999). Identification of cohesin association sites at centromeres and along chromosome arms. *Cell*, *98*(6). https://doi.org/10.1016/S0092-8674(00)81518-4

Tanaka, T., Fuchs, J., Loidl, J., & Nasmyth, K. (2000). Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. *Nature Cell Biology*, *2*(8). https://doi.org/10.1038/35019529

Tanaka, T. U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M. J. R., & Nasmyth, K. (2002). Evidence that the Ipl1-Sli15 (Aurora Kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell*, *108*(3). https://doi.org/10.1016/S0092-8674(02)00633-5

ten Hoopen, R., Cepeda-García, C., Fernández-Arruti, R., Juanes, M. A., Delgehyr, N., & Segal, M. (2012). Mechanism for astral microtubule capture by cortical Bud6p priming spindle Polarity in S. cerevisiae. *Current Biology*, *22*(12). https://doi.org/10.1016/j.cub.2012.04.059

Terakawa, T., Bisht, S., Eeftens, J. M., Dekker, C., Haering, C. H., & Greene, E. C. (2017). The condensin complex is a mechanochemical motor that translocates along

DNA. *Science*, *358*(6363). https://doi.org/10.1126/science.aan6516

Tercero, J. A., Labib, K., & Diffley, J. F. X. (2000). DNA synthesis at individual replication forks requires the essential initiation factor Cdc45p. *EMBO Journal*, *19*(9). https://doi.org/10.1093/emboj/19.9.2082

Thiede, C., Fridman, V., Gerson-Gurwitz, A., Gheber, L., & Schmidt, C. F. (2012). Regulation of bi-directional movement of single kinesin-5 Cin8 molecules. *BioArchitecture*, *2*(2). https://doi.org/10.4161/bioa.20395

Thomas, E. C., Ismael, A., & Moore, J. K. (2020). Ase1 domains dynamically slow anaphase spindle elongation and recruit Bim1 to the midzone. *Molecular Biology of the Cell*, *31*(24). https://doi.org/10.1091/mbc.E20-07-0493-T

Tien, J. F., Umbreit, N. T., Gestaut, D. R., Franck, A. D., Cooper, J., Wordeman, L., Gonen, T., Asbury, C. L., & Davis, T. N. (2010). Cooperation of the Dam1 and Ndc80 kinetochore complexes enhances microtubule coupling and is regulated by aurora B. *Journal of Cell Biology*, *189*(4). https://doi.org/10.1083/jcb.200910142

Tomishige, M., Stuurman, N., & Vale, R. D. (2006). Single-molecule observations of neck linker conformational changes in the kinesin motor protein. *Nature Structural and Molecular Biology*, *13*(10). https://doi.org/10.1038/nsmb1151

Tóth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., & Nasmyth, K. (1999). Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes and Development*, *13*(3). https://doi.org/10.1101/gad.13.3.320

Tovey, C. A., & Conduit, P. T. (2018). Microtubule nucleation by γ-tubulin complexes and beyond. In *Essays in Biochemistry* (Vol. 62, Issue 6). https://doi.org/10.1042/EBC20180028

Trelles-Sticken, E., Loidl, J., & Scherthan, H. (1999). Bouquet formation in budding yeast: Initiation of recombination is not required for meiotic telomere clustering. *Journal of Cell Science*, *112*(5). https://doi.org/10.1242/jcs.112.5.651

Tropini, C., Roth, E. A., Zanic, M., Gardner, M. K., & Howard, J. (2012). Islands containing slowly hydrolyzable GTP analogs promote microtubule rescues. *PLoS ONE*, *7*(1). https://doi.org/10.1371/journal.pone.0030103

Trueheart, J., Boeke, J. D., & Fink, G. R. (1987). Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. *Molecular and Cellular Biology*, *7*(7). https://doi.org/10.1128/mcb.7.7.2316-2328.1987

Tytell, J. D., & Sorger, P. K. (2006). Analysis of kinesin motor function at budding yeast kinetochores. *Journal of Cell Biology*, *172*(6). https://doi.org/10.1083/jcb.200509101

Uhlmann, F., Lottspelch, F., & Nasmyth, K. (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature*, *400*(6739). https://doi.org/10.1038/21831

Uhlmann, F., & Nasmyth, K. (1998). Cohesion between sister chromatids must be established during DNA replication. *Current Biology*, *8*(20). https://doi.org/10.1016/s0960-9822(98)70463-4

Uhlmann, F., Wernic, D., Poupart, M. A., Koonin, E. v., & Nasmyth, K. (2000). Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell*, *103*(3). https://doi.org/10.1016/S0092-8674(00)00130-6

Ünal, E., Heidinger-Pauli, J. M., Kim, W., Guacci, V., Onn, I., Gygi, S. P., & Koshland, D. E. (2008). A molecular determinant for the establishment of sister chromatid cohesion. *Science*, *321*(5888). https://doi.org/10.1126/science.1157880

van der Vaart, B., Fischböck, J., Mieck, C., Pichler, P., Mechtler, K., Medema, R. H., & Westermann, S. (2017). TORC1 signaling exerts spatial control over microtubule dynamics by promoting nuclear export of Stu2. *Journal of Cell Biology*, *216*(11). https://doi.org/10.1083/jcb.201606080

Vas, A. C. J., Andrews, C. A., Matesky, K. K., & Clarke, D. J. (2007). In vivo analysis of chromosome condensation in Saccharomyces cerevisiae. *Molecular Biology of the Cell*, *18*(2). https://doi.org/10.1091/mbc.E06-05-0454

Verzijlbergen, K. F., Menendez-Benito, V., van Welsem, T., van Deventer, S. J., Lindstrom, D. L., Ovaa, H., Neefjes, J., Gottschling, D. E., & van Leeuwen, F. (2010). Recombination-induced tag exchange to track old and new proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(1), 64–68. https://doi.org/10.1073/pnas.0911164107

Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M., & Amon, A. (1998). The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Molecular Cell*, *2*(6). https://doi.org/10.1016/S1097-2765(00)80286-5

Visintin, R., Hwang, E. S., & Amon, A. (1999). Cfi 1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. *Nature*, *398*(6730). https://doi.org/10.1038/19775

Visintin, R., Stegmeier, F., & Amon, A. (2003). The Role of the Polo Kinase Cdc5 in Controlling Cdc14 Localization. *Molecular Biology of the Cell*, *14*(11). https://doi.org/10.1091/mbc.E03-02-0095

Vizeacoumar, F. J., van Dyk, N., Vizeacoumar, F. S., Cheung, V., Li, J., Sydorskyy, Y., Case, N., Li, Z., Datti, A., Nislow, C., Raught, B., Zhang, Z., Frey, B., Bloom, K., Boone, C., & Andrews, B. J. (2010). Integrating high-throughput genetic interaction mapping and high-content screening to explore yeast spindle morphogenesis. *Journal of Cell Biology*, *188*(1). https://doi.org/10.1083/jcb.200909013

Vogel, J., Drapkin, B., Oomen, J., Beach, D., Bloom, K., & Snyder, M. (2001). Phosphorylation of γ-Tubulin Regulates Microtubule Organization in Budding Yeast. *Developmental Cell*, *1*(5). https://doi.org/10.1016/S1534-5807(01)00073-9

Waizenegger, I. C., Hauf, S., Meinke, A., & Peters, J. M. (2000). Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell*, *103*(3). https://doi.org/10.1016/S0092-8674(00)00132-X

Walker, R. A., Inoue, S., & Salmon, E. D. (1989). Asymmetric behavior of severed microtubule ends after ultraviolet-microbeam irradiation of individual microtubules in vitro. *Journal of Cell Biology*, *108*(3). https://doi.org/10.1083/jcb.108.3.931

Walker, R. A., O'Brien, E. T., Pryer, N. K., Soboeiro, M. F., Voter, W. A., Erickson, H. P., & Salmon, E. D. (1988). Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *The Journal of Cell Biology*, *107*(4). https://doi.org/10.1083/jcb.107.4.1437

Wang, E., Ballister, E. R., & Lampson, M. A. (2011). Aurora B dynamics at centromeres create a diffusion-based phosphorylation gradient. *Journal of Cell Biology*, *194*(4). https://doi.org/10.1083/jcb.201103044

Wargacki, M. M., Tay, J. C., Muller, E. G., Asbury, C. L., & Davis, T. N. (2010). Kip3, the yeast kinesin-8, is required for clustering of kinetochores at metaphase. *Cell Cycle*, *9*(13). https://doi.org/10.4161/cc.9.13.12076

Weiner, B. M., & Kleckner, N. (1994). Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. *Cell*, *77*(7). https://doi.org/10.1016/0092-8674(94)90438-3

Weiss, E., & Winey, M. (1996). The Saccharomyces cerevisiae spindle pole body duplication gene MPS1 is part of a mitotic checkpoint. *Journal of Cell Biology*, *132*(1–2). https://doi.org/10.1083/jcb.132.1.111

Weitzer, S., Lehane, C., & Uhlmann, F. (2003). A Model for ATP Hydrolysis-Dependent Binding of Cohesin to DNA. *Current Biology*, *13*(22). https://doi.org/10.1016/j.cub.2003.10.030

Welburn, J. P. I., Vleugel, M., Liu, D., Yates, J. R., Lampson, M. A., Fukagawa, T., & Cheeseman, I. M. (2010). Aurora B Phosphorylates Spatially Distinct Targets to Differentially Regulate the Kinetochore-Microtubule Interface. *Molecular Cell*, *38*(3). https://doi.org/10.1016/j.molcel.2010.02.034

Wendt, K. S., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., Yahata, K., Imamoto, F., Aburatani, H., Nakao, M., Imamoto, N., Maeshima, K., Shirahige, K., & Peters, J. M. (2008). Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature*, *451*(7180). https://doi.org/10.1038/nature06634

Werner-Washburne, M., Braun, E. L., Crawford, M. E., & Peck, V. M. (1996). Stationary phase in Saccharomyces cerevisiae. In *Molecular Microbiology* (Vol. 19, Issue 6). https://doi.org/10.1111/j.1365-2958.1996.tb02461.x

Westermann, S., Cheeseman, I. M., Anderson, S., Yates, J. R., Drubin, D. G., & Barnes, G. (2003). Architecture of the budding yeast kinetochore reveals a conserved molecular core. *Journal of Cell Biology*, *163*(2). https://doi.org/10.1083/jcb.200305100

Westermann, S., Drubin, D. G., & Barnes, G. (2007). Structures and functions of yeast kinetochore complexes. In *Annual Review of Biochemistry* (Vol. 76). https://doi.org/10.1146/annurev.biochem.76.052705.160607

Wickstead, B., & Gull, K. (2007). Dyneins across eukaryotes: A comparative genomic analysis. *Traffic*, *8*(12). https://doi.org/10.1111/j.1600-0854.2007.00646.x

Wiese, C., & Zheng, Y. (2000). A new function for the γ-tubulin ring complex as a microtubule minus-end cap. *Nature Cell Biology*, *2*(6). https://doi.org/10.1038/35014051

Winey, M., Goetsch, L., Baum, P., & Byers, B. (1991). MPS1 and MPS2: Novel yeast genes defining distinct steps of spindle pole body duplication. *Journal of Cell Biology*, *114*(4). https://doi.org/10.1083/jcb.114.4.745

Winey, M., Mamay, C. L., O'Toole, E. T., Mastronarde, D. N., Giddings, T. H., McDonald, K. L., & McIntosh, J. R. (1995). Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. *Journal of Cell Biology*, *129*(6). https://doi.org/10.1083/jcb.129.6.1601

Winey, M., & O'Toole, E. (2014). Centriole structure. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 369, Issue 1650). https://doi.org/10.1098/rstb.2013.0457

Winey, M., & O'Toole, E. T. (2001). The spindle cycle in budding yeast. In *Nature Cell Biology* (Vol. 3, Issue 1). https://doi.org/10.1038/35050663

Wolyniak, M. J., Blake-Hodek, K., Kosco, K., Hwang, E., You, L., & Huffaker, T. C. (2006). The regulation of microtubule dynamics in Saccharomyces cerevisiae by three interacting plus-end tracking proteins. *Molecular Biology of the Cell*, *17*(6). https://doi.org/10.1091/mbc.E05-09-0892

Woodbury, E. L., & Morgan, D. O. (2007). Cdk and APC activities limit the spindle-stabilizing function of Fin1 to anaphase. *Nature Cell Biology*, *9*(1). https://doi.org/10.1038/ncb1523

Woodcock, C. L., & Horowitz, R. A. (1995). Chromatin organization re-viewed. In *Trends in Cell Biology* (Vol. 5, Issue 7). https://doi.org/10.1016/S0962-8924(00)89038-8

Wutz, G., Várnai, C., Nagasaka, K., Cisneros, D. A., Stocsits, R. R., Tang, W., Schoenfelder, S., Jessberger, G., Muhar, M., Hossain, M. J., Walther, N., Koch, B., Kueblbeck, M., Ellenberg, J., Zuber, J., Fraser, P., &

Peters, J. (2017). Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. *The EMBO Journal*, *36*(24). https://doi.org/10.15252/embj.201798004

Xiangwei, H., Saurabh, A., & Sorger, P. K. (2000). Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. *Cell*, *101*(7). https://doi.org/10.1016/s0092-8674(00)80888-0

Xiao, H., Wang, F., Wisniewski, J., Shaytan, A. K., Ghirlando, R., Fitzgerald, P. C., Huang, Y., Wei, D., Li, S., Landsman, D., Panchenko, A. R., & Wu, C. (2017). Molecular basis of CENP-C association with the CENP-A nucleosome at yeast centromeres. *Genes and Development*, *31*(19). https://doi.org/10.1101/gad.304782.117

Yeh, E., Haase, J., Paliulis, L. v., Joglekar, A., Bond, L., Bouck, D., Salmon, E. D., & Bloom, K. S. (2008). Pericentric Chromatin Is Organized into an Intramolecular Loop in Mitosis. *Current Biology*, *18*(2). https://doi.org/10.1016/j.cub.2007.12.019

Yeh, E., Yang, C., Chin, E., Maddox, P., Salmon, E. D., Lew, D. J., & Bloom, K. (2000). Dynamic positioning of mitotic spindles in yeast: Role of microtubule motors and cortical determinants. *Molecular Biology of the Cell*, *11*(11). https://doi.org/10.1091/mbc.11.11.3949

Yin, H., Pruyne, D., Huffaker, T. C., & Bretscher, A. (2000). Myosin V orientates the mitotic spindle in yeast. *Nature*, *406*(6799). https://doi.org/10.1038/35023024

Yoshida, S., Asakawa, K., & Toh-e, A. (2002). Mitotic Exit Network controls the localization of Cdc14 to the Spindle Pole Body in Saccharomyces cerevisiae. *Current Biology*, *12*(11). https://doi.org/10.1016/S0960-9822(02)00870-9

Yukawa, M., Yamada, Y., Yamauchi, T., & Toda, T. (2018). Two spatially distinct kinesin-14 proteins, Pkl1 and Klp2, generate collaborative inward forces against kinesin-5 Cut7 in S. pombe. *Journal of Cell Science*, *131*(1). https://doi.org/10.1242/jcs.210740

Zachariae, W., Schwab, M., Nasmyth, K., & Seufert, W. (1998). Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. *Science*, *282*(5394). https://doi.org/10.1126/science.282.5394.1721

Zhou, X., Li, W., Liu, Y., & Amon, A. (2021). Cross-compartment signal propagation in the mitotic exit network. *ELife*, *10*. https://doi.org/10.7554/eLife.63645

Zimniak, T., Stengl, K., Mechtler, K., & Westermann, S. (2009). Phosphoregulation of the budding yeast EB1 homologue Bim1p by Aurora/Ipl1p. *Journal of Cell Biology*, *186*(3). https://doi.org/10.1083/jcb.200901036