Dissertation zur Erlangung des Doktorgrades
der Fakultät für Chemie und Pharmazie
der Ludwig-Maximilians-Universität München

Role of spindle forces in centromeric cohesin deprotection and chromosome segregation at meiosis II

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2023

Erklärung

Diese Dissertation wurde im Sinne von § 7 der Promotionsordnung vom 28. November 2011 von Herrn Prof. Dr. Franz-Ulrich Hartl betreut.

Eidesstattliche Versicherung

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

München, 19. 01. 2023

Oleksii Lyzak

Dissertation eingereicht am: 19. 01. 2023

1. Gutachter: Prof. Dr. Franz-Ulrich Hartl

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Mündliche Prüfung am: 27. 03. 2023

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Summary

Chromosome segregation during meiosis relies on cohesin protein complexes, whose sequential loss allows homologous chromosomes to be separated at meiosis I, while sister chromatids disjoin only at meiosis II. Cohesin is removed by the separase protease, which is activated at each anaphase by a ubiquitin-ligase known as the anaphase-promoting complex (APC/C). The selective removal of cohesin from chromosome arms but not centromeres at anaphase I depends on the protection of centromeric cohesin by the shugoshin-PP2A phosphatase complex. The role of shugoshin-PP2A in protection is evolutionarily conserved, while the mechanism of deprotection required for cohesin removal at meiosis II has remained unclear. There are several models developed for different model systems. In mammalian oocytes, bipolar spindle forces are believed to deprotect centromeric cohesin by physically creating a tension separating shugoshin-PP2A from cohesin. On the other hand, the protector was shown to be destroyed in an APC/C-dependent manner in yeast. In this work, we tested the role of spindle forces in cohesin deprotection using yeast as a model system. Firstly, we developed a new system to manipulate tension specifically at meiosis II. We demonstrated that activation of the APC/C in the absence of tension results in efficient cleavage of centromeric cohesin. On the other hand, bipolar spindle forces promote chromatid separation by facilitating the decatenation of centromeric sister DNAs. Finally, we discovered a new mechanism for how spindle forces control APC/C activity in addition to the canonical spindle assembly checkpoint.

Here, we show that spindle forces are not required for the deprotection of cohesin when APC/C is activated in the absence of the spindle tension at meiosis II. We also demonstrate that both, protection of Rec8 and inhibition of separase, are required for the stability of centromeric cohesin at metaphase II. In addition, we show that spindle forces are nevertheless important for chromosome segregation by helping topoisomerase II to resolve the catenation of centromeric DNA.

1. Introduction

1.1. Overview of the mitotic meiotic cell division programs

Mitosis is a type of a cell division in which a cell divides to produce two genetically identical daughter cells. The mitotic cell cycle starts with G1-phase, during which the cell grows, and is followed by S-phase, in which DNA replication occurs, converting each chromosome into two identical sister chromatids linked through a protein complex, called cohesin (Uhlmann and Nasmyth, 1998). As the cell enters metaphase, it starts to form a mitotic spindle. The spindle is a bipolar arrangement of microtubules organized by centrioles, which attaches to chromosomes via kinetochores, a protein complex that links centromeric DNA to spindle microtubules to transmit forces generated by the spindle (Biggins, 2013). The sister kinetochores of each chromatid pair should attach to microtubules emanating from opposite poles to ensure that each daughter cell receives one copy of each chromosome. Since sister chromatids are physically bound together by cohesin. Therefore, the correct attachment of sister chromatids to opposite poles of the spindle creates a tension, which stabilizes microtubule-kinetochores attachments and allows the cell to proceed to anaphase (Nicklas, 1997). During anaphase, cohesion between sister chromatids is destroyed; this allows the mitotic spindle to segregate sister chromatids towards opposite poles of the dividing cell. The spindle disassembles at the end of mitosis, and the cell exits mitosis and restarts a new cell cycle with entry into the G1-phase (Sullivan and Morgan, 2007).

Meiosis is characterized by a single round of DNA replication followed by two rounds of chromosome segregation. It uses the same general principles and machinery as mitosis. However, there are many modifications specific to the meiotic program to support proper chromosome segregation through two consecutive divisions (Marston and Amon, 2004). Firstly, during meiosis, the first prophase is prolonged and includes the process of meiotic recombination. Recombination provides the exchange of DNA segments between homologous chromosomes and thereby physically links them to ensure proper segregation of homologs (Lee and Orr-Weaver, 2001). Secondly, the first meiotic division is special as homologous chromosomes are bi-oriented and segregated, contrary to the mitotic and the second meiotic division (Petronczki et al., 2003). The bi-orientation of homologous chromosomes, i.e. mono-orientation of sister

chromatids, in meiosis I is provided by monopolar attachment, in which two sister kinetochores face the same spindle pole. In yeast, monopolar attachment is conferred by the monopolin complex (Petronczki *et al.*, 2003; Toth et al., 2000). Thirdly, cohesin is lost in two steps. The physical linkage of chromosomes via the cohesin complex is vital for proper chromosome segregation (Petronczki *et al.*, 2003). During mitosis in yeast, all cohesin is cleaved at anaphase. If the same would happen during meiosis at the onset of anaphase I, there would be no cohesin left to support meiosis II. Indeed, not all cohesin is cleaved at meiosis I. At anaphase I, cohesin along chromosomal arms is cleaved, allowing the segregation of homologous chromosomes. However, the centromeric fraction is protected from cleavage. At anaphase II, centromeric cohesin is cleaved, enabling sister chromatid segregation (Petronczki *et al.*, 2003). Finally, there must be mechanisms enabling a second division to occur after the first one, without exit from division or re-replication of DNA (Marston and Amon, 2004).

From a biochemical point of view, progression through the cell cycle is controlled by oscillations in the activities of two enzymatic complexes: cell cycle kinases (Cdks) and the anaphase-promoting complex (APC/C), which is a ubiquitin ligase (Nasmyth, 1996; Nigg, 2001; Peters, 2006). Cdks phosphorylate other key regulators of cell division progression, thereby promoting processes such as DNA replication, spindle assembly, chromosome condensation, and segregation. Cdk requires a regulatory subunit – a cyclin – for its activity (Murray, 2004). The cell cycle in the budding yeast *Saccharomyces cerevisiae* is controlled by a single Cdk1 (Cdc28) in combination with several cyclins (Kuntzel et al., 1996). DNA replication is governed by Cdk1 bound to Clb5 and Clb6, while metaphase is induced by Cdk1 complexes with M-phase cyclins: Clb1, Clb2, Clb3, and Clb4. The latter is required for spindle formation and the alignment of chromosomes on the spindle (Bloom and Cross, 2007). High Cdk1 activity is required for the S and M phases, while low Cdk1 activity is necessary for maintaining a prolonged G1- phase and for exit from mitosis (Wolf et al., 2007). Low Cdk1 activity is achieved through cyclin degradation, which is triggered by the APC/C, while the levels of the Cdk1 protein remain constant (Irniger et al., 1995; King et al., 1995).

APC/C ubiquitinates its substrates, thereby targeting them for degradation by the 26S proteasome (Zachariae et al., 1996). APC/C activity is mediated by three co-activators: Cdc20, Cdh1, and the meiosis-specific Ama1. The most prominent

activity is shown by APC/C-Cdc20 as it is responsible for triggering anaphase. This activity consists of two parts: degradation of cyclins, hence, lowering Cdk activity and induction of cohesin cleavage, required for chromosome segregation. Other APC/C activators are required for exit from mitotic (Cdh1) and meiotic (Ama1) divisions as well as maintaining a G1 phase (Oelschlaegel et al., 2005; Okaz et al., 2012; Yeong et al., 2000).

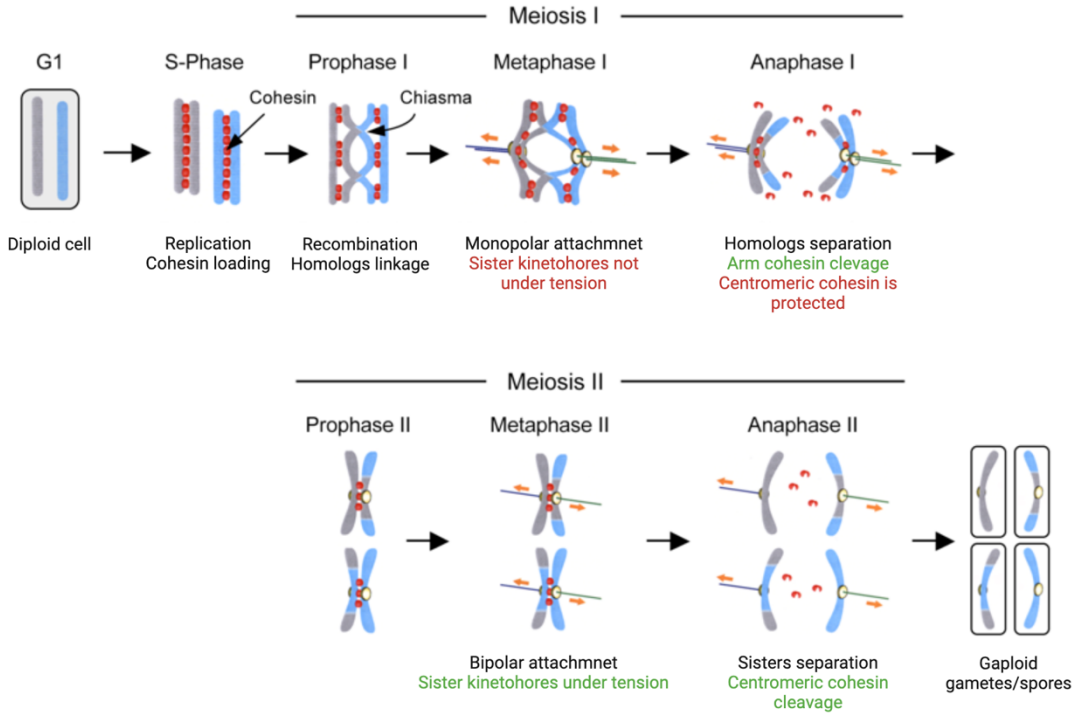

Figure 1. Overview of meiosis, adapted from (Okaz *et al.*, 2012).
Meiosis starts similarly to mitosis with the replication of DNA, followed by cohesin loading. At prophase I recombination takes place. During recombination, homologue chromosomes exchange DNA fragments and make the physical link required for correct segregation at anaphase I. At metaphase I sister kinetochores are mono-oriented and homologs are segregated at anaphase I after arm cohesin cleavage. At metaphase II sister kinetochores are bi-oriented and remaining centromeric cohesin ensures correct segregation of sister chromatids at anaphase II onset after its cleavage. As result four haploid cells are formed from one parental diploid cell. Further, these cells undergo differentiation into gametes (like in higher eukaryotes) or spores (like budding yeast).

1.2. Structure and function of cohesin proteins

Structural maintenance of chromosomes (SMC) is essential for proper chromosome segregation. There are two main SMC complexes: condensin and cohesin. Cohesin is the complex that holds sister chromatids together and is therefore the main regulator of their segregation (Nasmyth and Haering, 2005).

The cohesin complex consists of four core subunits. In budding yeast, mitotic cohesin is represented by the proteins Smc1, Smc3, Scc1, and Scc3, whereas

during meiosis, the Scc1 component of the cohesin complex is replaced with Rec8 (Nasmyth and Haering, 2009; Onn et al., 2008). Smc1 and Smc3 form long anti-parallel coiled-coil structures that are able to connect to each other with ends opposite to their ATPase heads. Scc1 and Rec8 are members of the kleisin family of proteins and they bridge the ATPase head domains of Smc1 and Smc3 subunits to form a ring that entraps chromatids (Nasmyth and Haering, 2009; Schleiffer et al., 2003). The fourth subunit of the core cohesin complex, Scc3, is associated with kleisin (Gruber et al., 2003).

Cohesin is loaded on chromatin by the Scc2–Scc4 protein complex in *S. cerevisiae* (Ciosk et al., 2000). It has been demonstrated that the Scc2–Scc4 complex physically interacts with the cohesin, therefore it is suggested that the Scc2–Scc4 complex is able to open the cohesin ring to load it onto the chromosomes (Arumugam et al., 2003; Toth et al., 1999).

Once chromosomes are properly attached and oriented on the spindle, tension, generated by spindle forces pulling against cohesins holding chromatids together, activates APC/C-Cdc20. Active APC/C induces rapid degradation of Pds1 (securin), which activates separase (Zachariae and Nasmyth, 1999). Activate separase cleaves the kleisin subunit of the cohesin complex, which leads to the dissociation of cohesin from the chromosomes and the separation of sister chromatids or homologues chromosomes at meiosis I.

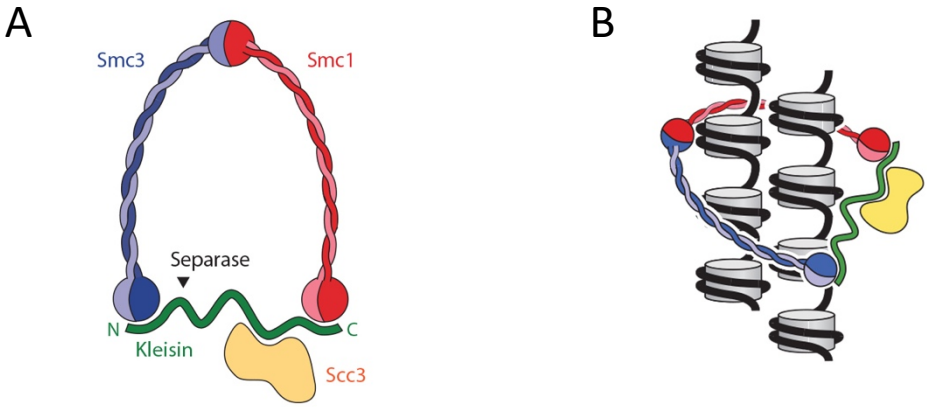

Figure 2. Structure of cohesin complex.
(A) Structure of cohesin complex, kleisin subunit is presented by Scc1 and Rec8 during mitosis and meiosis, respectively. **(B)** The ring model of cohesin function. Cohesin ring entraps chromatids until anaphase onset. At anaphase, the kleisin subunit is cleaved, releasing chromatids. Adapted from (Nasmyth and Haering, 2009).

1.3. Role of DNA catenation and chromatin remodeling in chromosomes segregation

DNA catenation is a natural by-product of DNA replication (Postow et al., 2001). Therefore, to correctly segregate chromosomes at anaphase, this catenation has to be resolved in a timely manner (Holm et al., 1989). Topoisomerase II (Topo II) is an enzyme required for DNA decatenation (Baldi et al., 1980) and is highly conserved among various groups of eukaryotes (Strumberg et al., 1999).

Topo II can both catenate and decatenate DNA, hence, based only on Topo II activity, there would always be some catenation left (Hardy et al., 2004; Rybenkov et al., 1997). Therefore, full decatenation requires not only Topo II activity, but additional external cues. The interplay between SMC complexes and Topo II at chromosome arms helps to resolve catenation before anaphase: loop extrusion by condensin may generate positive supercoiling which then is recognized by Topo II (Baxter et al., 2011). At the same time, catenation at centromeres is present at metaphase and is maintained by cohesin (Farcas et al., 2011), therefore, can be resolved only after its removal (Wang et al., 2010a). In this scenario, tension forces may provide directionality for Topo II activity (Baxter *et al.*, 2011; Farcas *et al.*, 2011) as Topo II is biased to resolve catenation when it is bound to extremely bent DNA (Dong and Berger, 2007; Vologodskii et al., 2001). It was noted that centromeric DNA is present as so-called ultrafine anaphase bridges (UFBs) due to catenation at the onset of anaphase without nucleosomes (Baumann et al., 2007). Topo II was shown to be recruited to these UBFs and this interaction was promoted by nucleosome eviction (Sperling et al., 2011). There are a few factors working at centromeric and pericentromeric DNA supporting Topo II activity to resolve catenation with nucleosome-less DNA at the centromeric region. BLM and PICH helicases are required to resolve UFBs by restriction of histone incorporation into DNA, which allows UFBs to stretch over long distance as well as provide sufficient substrate for Topo II (Rouzeau et al., 2012).

1.4. Structure and function of kinetochores

Segregation of chromosomes depends on their connection to microtubules. Kinetochores are the protein complexes responsible for these connections. The kinetochore is assembled at a specific part of the chromosome, called the

centromere (Westermann et al., 2007). In budding yeast, each kinetochore binds to a single microtubule (Peterson and Ris, 1976; Winey et al., 1995).

The yeast centromere is specified by DNA sequence and organized as a single unique nucleosome containing a specialized variant of histone H3 called CENP-A or Cse4 in yeast (Clarke, 1998; Cole et al., 2011; Furuyama and Biggins, 2007; Lefrancois et al., 2009). A group of proteins, which form the inner part of the kinetochore, is called the constitutive centromere-associated network (CCAN). Proteins in this group are able to recognize both centromeric DNA and the Cse4 nucleosome and function as a platform for assembling the outer kinetochore (Santaguida and Musacchio, 2009). The essential components of CCAN are the yeast-specific Cbf3 complex which binds directly to centromeric DNA (Jehn et al., 1991; Lechner and Carbon, 1991) and Mif2, Ame1 and Okp1 (the two last proteins are part of COMA subcomplex), which link Cse4 with the Mtw1 complex (Dimitrova et al., 2016; Westermann et al., 2003).

The outer kinetochore is presented by the KMN network and the Dam1 complex. KMN network consists of 3 subcomponents: the Mtw1 complex (MIND or Mis12 complex), the Spc105 complex (or Knl1 complex), and the Ndc80 complex (Biggins, 2013). The Mtw1 complex does not exhibit microtubule-binding activity on its own but links Spc105 and Ndc80 complexes with the inner part of the kinetochore through Mif2 and the COMA complex (Biggins, 2013; Cheeseman et al., 2006; De Wulf et al., 2003; Hornung et al., 2011). Spc105, the main component of the Spc105 complex, is a scaffold for spindle assembly checkpoint (SAC) proteins in budding yeast (Aravamudhan et al., 2015; Kiyomitsu et al., 2007; Liu et al., 2010). The Ndc80 complex provides the major microtubule-binding activity within KMN (Cheeseman *et al.*, 2006; Ciferri et al., 2008; DeLuca et al., 2006; Hornung *et al.*, 2011). The interaction between Ndc80 and microtubules is largely electrostatic (Ciferri *et al.*, 2008). Dam1 is the outermost kinetochore complex in yeast; it has 10 structural components; 16 Dam1 complexes can assemble into a ring around microtubules, at least *in vitro* (De Wulf *et al.*, 2003; Lampert et al., 2013; Miranda et al., 2005).

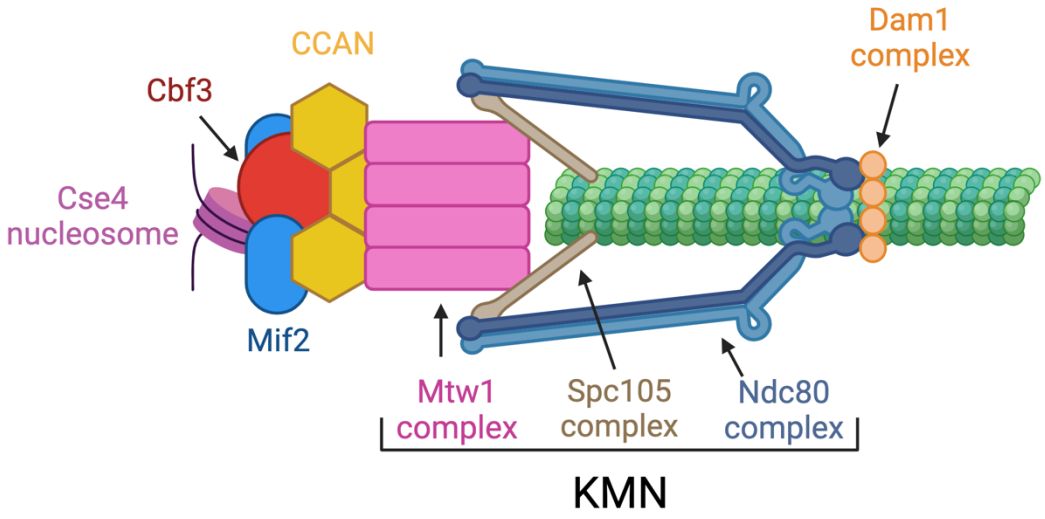

Figure 3. A structure of yeast kinetochore.
The kinetochore complex provides a connection between the centromere and spindle microtubule. It is organized hierarchically from several subcomplexes, where more inner subcomplex has multiple binding sites for proteins from more outer one. Adapted from (Biggins, 2013).

1.5. Mono-orientation of sister kinetochores

To achieve bi-orientation of homologous chromosomes at meiosis I, kinetochores of sister chromatids must be mono-oriented to the same pole of the spindle. This is achieved through the monopolin complex. The core of monopolin consists of two proteins: Csm1 and Lrs4 (Rabitsch et al., 2003). They form a V-shaped structure in which Csm1 dimers are bridged by two Lrs4 molecules. Each Csm1 dimer binds to the Dsn1 protein (part of the Mtw1 complex at kinetochore), thereby fusing sister kinetochores (Corbett et al., 2010).

There are two additional components in the monopolin complex – Mam1 and Hrr25 (Petronczki et al., 2006; Rabitsch *et al.*, 2003; Toth *et al.*, 2000). Mam1 is meiosis-specific and present at kinetochores only at meiosis I (Toth *et al.*, 2000). It flexibly links Hrr25 to Csm1, which allows the former to access its potential substrates within the kinetochore as well as the monopolin complex itself. For example, Dsn1 can be phosphorylated by Hrr25 *in vitro* (Corbett and Harrison, 2012; Ye et al., 2016). Additionally, Hrr25 kinase activity may help in stabilizing the monopolin complex (Petronczki *et al.*, 2006).

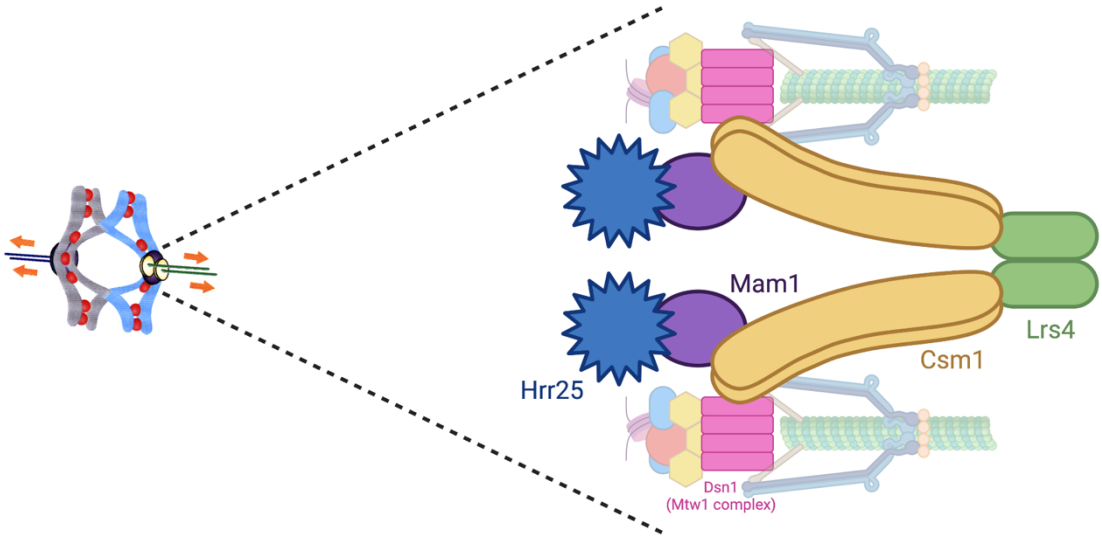

Figure 4 A structure of monopolin complex.
Lrs4 and Csm1 are core monopolin complex proteins. Csm1 dimers bind to Dsn1 protein in kinetochore and are bridges by Lrs4 dimer. Mam1 and Hrr25 are additional components and have regulatory functions. Adapted from (Corbett and Harrison, 2012).

1.6. Chromosomal passenger complex

The chromosomal passenger complex (CPC) is composed of the Ipl1 protein kinase (Aurora B), Sli15 (INCENP), Bir1 (Survivin), and Nbl1 (Borealin). This complex is associated with the inner kinetochore until anaphase (Carmena et al., 2012; Widlund et al., 2006) and its main function is the correction of wrong attachments between chromosomes and spindle microtubules. CPC is targeted to the inner kinetochore via interactions with the Cbf3 complex through Bir1 and the COMA complex via Sli15 (Knockleby and Vogel, 2009). It was also shown that CPC requires the phosphorylation of two histone tails for proper localization: histone H3 Thr3 by Haspin kinase (recently it was shown to be relevant to yeasts) (Dai and Higgins, 2005; Edgerton et al., 2016) and histone H2A Thr120 by the kinetochore-associated Bub1 kinase (Kawashima et al., 2007; Yamagishi et al., 2010).

The model of CPC function in attachment correction is based on the spatial separation of Aurora kinase from its substrates – proteins of the KMN network (Tanaka et al., 2002). Incorrect attachments to kinetochores keep the KMN network in Aurora's vicinity to be phosphorylated. This phosphorylation strongly decreases kinetochores' microtubule-binding activity (Cheeseman *et al.*, 2006; Ciferri *et al.*, 2008; Liu et al., 2009; Welburn et al., 2010). When a correct

attachment is established and kinetochores are pulled apart, the resulting tension moves the KMN network away from Aurora, whereas phosphorylation is removed by the PP1 phosphatase. In addition, interactions between the Dam1 complex and the Ndc80 complex are also negatively regulated by Aurora B-dependent phosphorylation (Tien et al., 2010).

1.7. Spindle assembly checkpoint

The Spindle assembly checkpoint (SAC) monitors the correctness of chromosome-microtubule attachment by their ability to generate tension due to spindle forces trying to pull chromosomes apart and cohesin holding them together. Only when all sister chromatids (or homologous chromosomes at meiosis I) are attached to microtubules emanating from opposite spindle poles, anaphase is triggered and cohesin is cleaved. As a result, each half of a pair would be equally divided among daughter cells. In a nutshell, unattached kinetochores catalyze the formation of an inhibitor, called the mitotic checkpoint complex (MCC), which inhibits the APC/C, hence, anaphase, through the SAC regulatory cascade (Hardwick et al., 2000; Sudakin et al., 2001). Even a single unattached kinetochore can delay cell cycle progression. Experiments in which unattached kinetochores were micromanipulated with a needle or destroyed with a laser demonstrated their ability to produce the inhibiting signal that delays anaphase (Li and Nicklas, 1995; Rieder et al., 1995).

In *S. cerevisiae*, two groups of proteins were discovered in screens looking for genes involved in the response to spindle damage – Mitotic Arrest Deficient – Mad1, Mad2, Mad3 and Budding Uninhibited by Benzimidazole – Bub1, Bub3 (Hoyt et al., 1991; Li and Murray, 1991). Later, other SAC components, like the Mps1 kinase, were identified (Weiss and Winey, 1996).

The most upstream regulator of the SAC is the Mps1 kinase (Abrieu et al., 2001; Liu and Winey, 2012; London et al., 2012; Shepperd et al., 2012). Mps1 is recruited to the Ndc80 complex in the outer kinetochore, while its main target is Spc105 (Hiruma et al., 2015; Ji et al., 2015; Kemmler et al., 2009). Its recruitment to kinetochores may be the result of the absence of microtubules at their binding sites (Hiruma *et al.*, 2015; Ji *et al.*, 2015) and is promoted by CDK1 and the Aurora B kinases (Hayward et al., 2019a; Santaguida et al., 2010; Saurin et al., 2011). When Mps1 is located at an unattached kinetochore, it is able to phosphorylate

MELT (Met-Glu-Leu-Thr) motifs in Spc105 (Aravamudhan *et al.*, 2015; London *et al.*, 2012). This phosphorylation is recognized by the Bub3-Bub1 protein complex, which binds to it (London *et al.*, 2012; Overlack et al., 2015; Shepperd *et al.*, 2012; Vleugel et al., 2013). Additionally, Mps1 phosphorylates Bub1, which helps it to bind the Mad1-Mad2 complex (Chen et al., 1999; Jia et al., 2016; London and Biggins, 2014). The Bub3-Bub1 complex, located on MELT repeats, functions as a scaffold for binding the executive components of the SAC – Mad3(BubR1), Cdc20, and Mad1-Mad2 complex to catalyze the formation of the MCC complex (Di Fiore et al., 2015; Faesen et al., 2017; Ji et al., 2017; Jia *et al.*, 2016). It consists of the Mad2, Mad3, Bub3, and Cdc20 (Sudakin *et al.*, 2001; Visintin et al., 1997). The Mad3-Bub3 part of the MCC probably is not regulated and persists throughout cell division, while the Mad2-Cdc20 part is formed by SAC activity (Chen, 2002; Hardwick *et al.*, 2000; Musacchio and Salmon, 2007). The Mad1-Mad2 complex at the kinetochore has just a catalytical function for free Mad2 protein incorporation into the MCC and not becoming part of the MCC itself (De Antoni et al., 2005). The formation of MCC is explained through the 'Mad2 template model'. Mad2 has two conformations - when unbound, it has an "open" conformation (O-Mad2) but when it binds to Mad1 or Cdc20, it changes to a "closed" conformation (C-Mad2), with Mad1/Cdc20 now trapped within it (De Antoni *et al.*, 2005; Luo et al., 2004; Luo et al., 2002; Sironi et al., 2002). The Mad1-Mad2 complex then works as a "template" for the conversion of other molecules of Mad2 from the open to the closed state capturing Cdc20 (De Antoni *et al.*, 2005). The MCC may inhibit APC/C in several ways – first of all, MCC acts as an inhibitor of APC/C. Second, MCC can also affect the conformation of the APC/C to disrupt its interaction with an E2 enzyme, UBE2C/UBCH10, which prevents APC/C's function as a ubiquitin-ligase. As a result, APC/C is not able to initiate cyclin B and securin degradation, thereby halting progression through cell division at metaphase (Alfieri et al., 2016; Yamaguchi et al., 2016).

In addition to Mps1, other kinases are also involved in SAC. It was suggested that the CPC might create unattached kinetochores, which are subsequently recognized by the SAC pathway (Pinsky et al., 2006). In addition, it enhances SAC signaling by promoting Mps1 recruitment (Hayward *et al.*, 2019a; Santaguida et al., 2011; Saurin *et al.*, 2011). It was shown that Aurora is required not only as a part of the error-correction machinery but also has a synergistic effect with Mps1 on SAC (Santaguida *et al.*, 2011).

Mps1 localization and activation are also controlled by Cdk (Hayward *et al.*, 2019a; Morin et al., 2012). This allows licensing the SAC at specific times within cell division when it is required (during metaphase), but also prevents SAC activation at other stages of cell division, for example, SAC is not re-activated during anaphase (Kops, 2014). In addition, the outer kinetochore is assembled only in a high Cdk activity state and is disassembled when the cyclin B level drops (Gascoigne and Cheeseman, 2013). Cdk was also shown to phosphorylate Bub1, thereby promoting Bub1-Mad1 interaction (Ji *et al.*, 2017; Zhang et al., 2017).

There is evidence that the formation of the MCC is not the only way to inhibit APC/C and delay anaphase onset in the presence of incorrect attachments. Firstly, some observations suggest Mad3 (BubR1) can bind Cdc20 and inhibit APC/C in the absence of Mad2, at least *in vitro*, as it has another Cdc20 binding site (Davenport et al., 2006; Tang et al., 2001). Secondly, Bub1 and Bub3 were shown to be able to delay anaphase independently of Mad-proteins suggesting that the SAC represents a combination of several mechanisms (Kim et al., 2017; Proudfoot et al., 2019).

When the proper attachment of microtubules to kinetochores is established, SAC is rapidly inactivated. Under tension, kinetochores stretch and this disrupts Mps1's ability to phosphorylate its substrates (Aravamudhan *et al.*, 2015). In addition, a microtubule can compete with Mps1 for localization (Hiruma *et al.*, 2015). Mps1 removal allows the phosphatases PP1 and PP2A-B56 (PP2A-Rts1 in *S. cerevisiae*) to counteract phosphorylation. There are different pools of PP2A-Rts1 at kinetochores and PP2A-Rts1 recruited by kinetochore-bound BubR1 (Espert et al., 2014; Hertz et al., 2016; Wang et al., 2016a; Wang et al., 2016b) can dephosphorylate the Mad1 docking motif in Bub1 and Mps1 activation loop (Espert *et al.*, 2014; Hayward et al., 2019c; Maciejowski et al., 2017; Qian et al., 2017). In addition, PP2A counteracts Aurora kinase activity by dephosphorylation RVSF motifs, which is required for PP1 localization, after which PP1 is recruited to Spc105 and counteracts Mps1 activity (London *et al.*, 2012; Moura et al., 2017; Smith et al., 2019).

Other phosphatases may affect SAC indirectly. PP2A-B55 (PP2A-Cdc55 in *S. cerevisiae*) has been identified as one of the phosphatases opposing Cdk, specifically in the case of the phosphorylation of Mps1. Thus, it terminates the SAC-permissive time window. When Cdk activity drops at anaphase, Mps1

cannot be recruited to the kinetochore anymore, so the SAC cannot be reactivated (Hayward *et al.*, 2019a). The Cdc14 phosphatase is also implicated in the inactivation of the checkpoint as it dephosphorylates Sli15, part of CPC, which promotes Sli15/Aurora re-localization to the spindle (Mirchenko and Uhlmann, 2010).

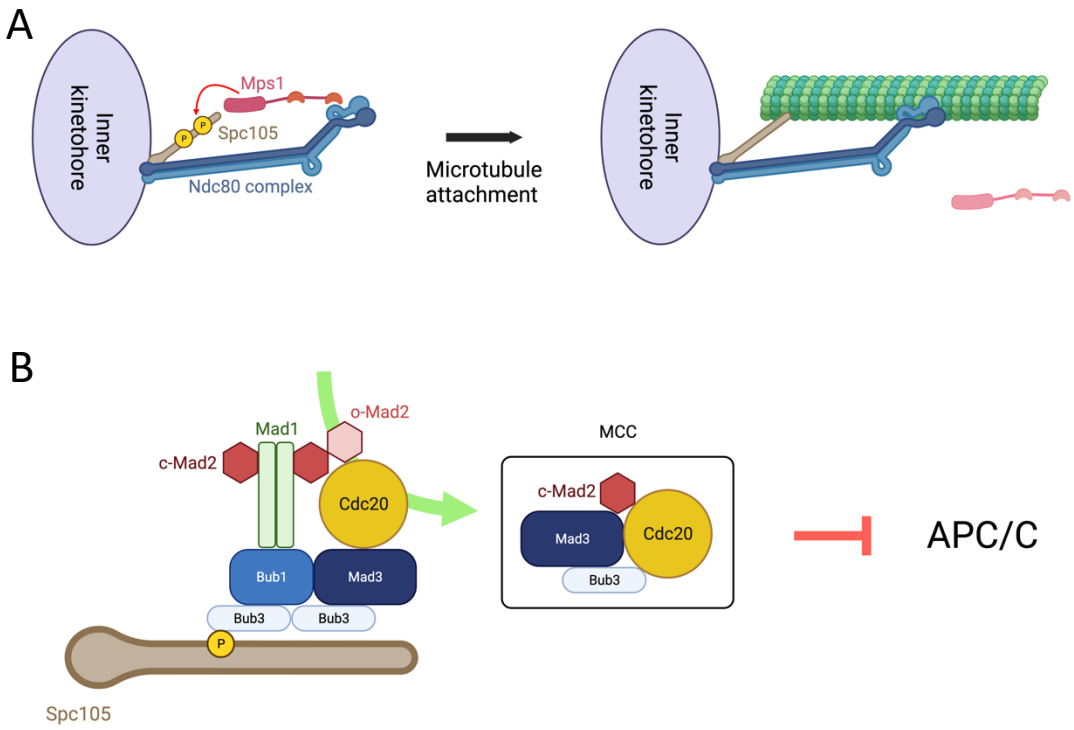

Figure 5. Spindle assembly checkpoint.
(A) At an unattached kinetochore Mps1 is localizing at the Ndc80 complex and is able to phosphorylate Spc105. When proper attachment between a microtubule and a kinetochore is established Mps1 is not localized at the kinetochore anymore and Spc105 is dephosphorylated switching SAC off. **(B)** Phosphorylation of Spc105 functions as a docking platform to assemble the catalytic part of SAC for MCC generation, MCC is a direct inhibitor of APC/C, adapted from (Musacchio, 2015).

1.8. Protection of centromeric cohesin

During meiosis, sister chromatids are held together by cohesin complexes containing the meiosis-specific Rec8 subunit. In meiosis I, homologous chromosomes are linked by chiasmata and sister kinetochores attach to microtubules emanating from the same spindle pole. Separase cleaves the Rec8 on chromosome arms. However, Rec8 at centromeres is protected from cleavage. The protein Mei-S332 was identified as a crucial component for protecting centromeric cohesin until meiosis II in *Drosophila melanogaster* (Goldstein, 1980;

Kerrebrock et al., 1995). Later, various studies identified homologs of Mei-S332 in budding yeast, fission yeast, and vertebrate. This protein is called Sgo1 in budding yeast; cells depleted of Sgo1 cannot retain pericentromeric Rec8 during meiosis I, resulting in random chromosome segregation at meiosis II (Katis et al., 2004; Kitajima et al., 2004; Marston et al., 2004; McGuinness et al., 2005; Salic et al., 2004). Later it was found that a) phosphatase PP2A is a binding partner of Sgo1 in yeasts during meiosis (Kitajima et al., 2006; Riedel et al., 2006) and b) Rec8 phosphorylation is required for cleavage by separase (Ishiguro et al., 2010; Katis et al., 2010; Rogers et al., 2002; Rumpf et al., 2010). Thus, the protection of centromeric cohesin relies on PP2A counteracting phosphorylation of Rec8, which makes cohesin cleavable (Kawashima et al., 2010; Kitajima *et al.*, 2006; Riedel *et al.*, 2006; Tang et al., 2006). There are two kinases, which promote cohesin cleavage in meiosis I – Hrr25 and Cdc7-Dbf4 (Ishiguro *et al.*, 2010; Katis *et al.*, 2010). Inactivation of both of them blocks cohesin cleavage, similar to a non-phosphorylatable Rec8 mutant. Additionally, the combination of kinases inactivation with Sgo1 depletion does not restore Rec8 cleavage. Thus Sgo1 counteracts the activity of these kinases (Katis *et al.*, 2010). Another support for this model is the fact that mitotic Scc1 cannot support protection if expressed instead of Rec8 in meiosis. Indeed, when Rec8 is replaced by Scc1 in meiosis (which does not require phosphorylation prior to cleavage), the cells lose two-step cleavage of cohesin and destroy all cohesin at anaphase I (Katis *et al.*, 2004; Katis *et al.*, 2010; Toth *et al.*, 2000).

Localization of Sgo1, hence PP2A, depends on H2A histone phosphorylation made by Bub1 kinase (Kawashima *et al.*, 2010) while during mitosis, Sgo1 is localized at centromeres by the Mps1 kinase in addition to Bub1 (Storchova et al., 2011). It was shown in budding yeast, that Bub1 is required for protection only during meiosis I. However, in meiosis II, Bub1 seems to be dispensable, while Mps1 is required for Sgo1 and PP2A localization. This may be important for the deprotection of centromeric cohesin at meiosis II as Mps1 degradation depends on APC/C-Cdc20 in yeast (Arguello-Miranda et al., 2017; Palframan et al., 2006)

1.9. Deprotection of centromeric cohesin at meiosis II

Even though the mechanism of protection is well understood, it is still unclear how protection stops at meiosis II, i.e., how centromeric cohesin becomes deprotected (Arguello-Miranda *et al.*, 2017; Chambon et al., 2013; Jonak et al.,

2017; Wassmann, 2013). There are several models proposed to explain the deprotection of centromeric cohesin specifically in meiosis II.

The first model is called deprotection-by-tension or deprotection-by-spatial separation (further referred to as "the tension model") and it proposes that Sgo1 and PP2A are physically separated from centromeric cohesin by spindle forces when sister chromatids are bioriented at meiosis II. This was suggested for both male and female meiosis II (Gomez et al., 2007; Lee et al., 2008). Meanwhile, at meiosis I this separation cannot happen because of sister kinetochores' mono-orientation. This model is very attractive due to several reasons. In mitotic cells, Sgo1 is removed from kinetochores in a tension-dependent manner as chromosomes bi-orient on the spindle at metaphase. (Eshleman and Morgan, 2014; Nerusheva et al., 2014). Additionally, Mps1 is known to be responsive to tension, as it happens in SAC (Aravamudhan *et al.*, 2015; Maure et al., 2007). The spatial separation of Sgo1-PP2A and Rec8 at metaphase II has also been observed on chromosome spreads from yeast (Arguello-Miranda *et al.*, 2017; Katis *et al.*, 2004). However, this model has never been experimentally tested in meiosis in any model organism. In addition, it also suggests that centromeric cohesin is deprotected already in metaphase II, which might negatively affect oocytes, which have a prolonged metaphase II-arrest stage.

Another model, based on studies in oocytes, proposed that the conserved histone chaperone SET/TAF-1β inhibits PP2A at centromeres at meiosis II (Chambon *et al.*, 2013). However, the mechanism of how SET affects PP2A's activity and how it is limited to meiosis II is unclear. Furthermore, the SET orthologues of budding yeast are not required for meiotic chromosome segregation (Jonak *et al.*, 2017).

The last model is based on observations in budding yeast – the deprotection-by-APC/C model – suggests that protection is removed by APC/C-Cdc20, which mediates the degradation of Sgo1 and Mps1, thus making centromeric cohesin vulnerable to phosphorylation (further referred to as "the APC model") (Arguello-Miranda *et al.*, 2017; Jonak *et al.*, 2017). This model explains the coordination of deprotection of centromeric cohesin with separase activation and also proposes that cohesin is protected until entry into anaphase II. While this model cannot specifically explain why deprotection is limited to meiosis II, it seems to be beneficial for oocytes, as it suggests that during prolonged metaphase II cohesin stays protected.

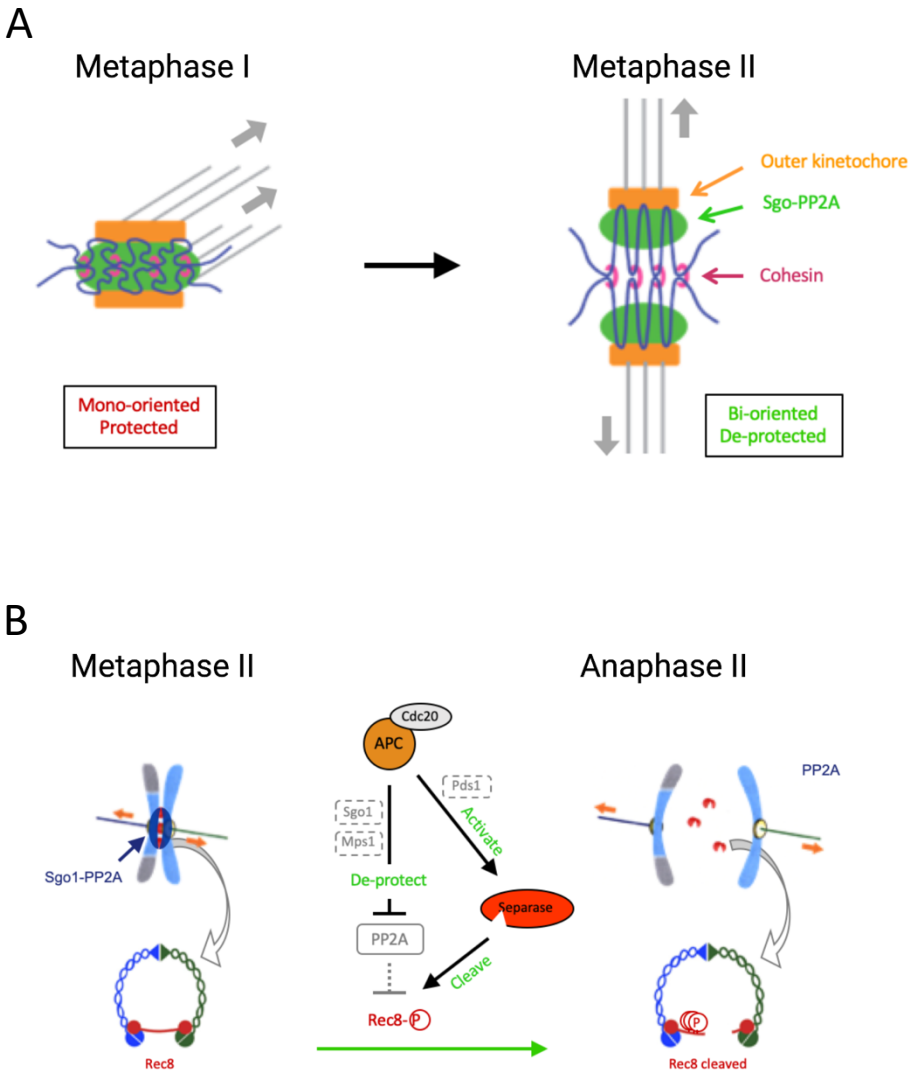

Figure 6. Models of cohesin deprotection.
(A) The tension model (Lee *et al.*, 2008) At metaphase I centromeric cohesin is protected because the Sgo-PP2A complex (protector) is colocalizing with cohesin. At metaphase II spindle pulls the protector away from cohesin, making it vulnerable to cleavage. **(B)** The APC model (Arguello-Miranda *et al.*, 2017). Cleavage of centromeric cohesin at anaphase II requires active separase and deprotection. Both these requirements are fulfilled with APC/C-Cdc20 activity. APC/C-Cdc20 induces degradation of separase inhibitor Pds1 as well as components of protector – Sgo1 and Mps1 at anaphase II onset when APC/C becomes active. Thus, it leads to rapid cohesin removal at anaphase II, but not earlier.

Here, we investigate the role of tension created by spindle forces in centromeric cohesin deprotection and chromosome segregation at meiosis II using yeast as a model system.

2. Results

Protection of centromeric cohesin is essential to ensure correct chromosome segregation at meiosis II. Previously, we demonstrated the role of APC/C-Cdc20 in the deprotection of centromeric cohesin using yeast *S. cerevisiae* (Arguello-Miranda *et al.*, 2017). However, how deprotection is timely restricted only to anaphase II is still unclear. A hypothesis, proposed for oocytes, suggests protection to be removed by the tension created by the division spindle (the tension model), however, this model was never tested. Yeast separates Sgo1 signals from cohesin during meiosis. Therefore, tension may play a role directly in deprotection in yeast, and *S. cerevisiae* can be used as a model organism to test the tension model. In this work, we tested in yeast, if tension, generated by the spindle, is required not only as a read-out for the SAC but also specifically for cohesion deprotection.

2.1. Development of the assay to manipulate tension at meiosis II

2.1.1. *spo12Δ* leads to spindle morphology defect at meiosis II

One of the possible approaches to studying the role of spindle forces in the deprotection of centromeric cohesin is to create a situation where no tension is applied to kinetochores and test if centromeric cohesion is cleaved in these conditions. The golden standard to abolish spindle tension is to use spindle poisons, like nocodazole. However, during yeast meiosis, functional doses of nocodazole are too high and would cause side effects. Thus, in this work, we used a genetic approach by creating defect spindles due to blocked SPB reduplication at entry into metaphase II. In *S. cerevisiae*, a spindle during meiosis can be formed only when SPBs duplicate (entry into meiosis I) or reduplicate (entry into meiosis II). If reduplication is blocked, it can be expected that a cell would go through first division unaffected. However, when it enters meiosis II, it would not be able to form a proper spindle because of this block of SPB reduplication. Furthermore, it is also expected not to be able to reform the spindle as well as SPB are far apart after anaphase I (**Figure 7A**).

We used the deletion of the *SPO12* gene to block SPB reduplication (Buonomo et al., 2003). To monitor spindle formation, we performed a live-cell imaging experiment in which we tagged one of the SPB proteins, Spc42 with GFP and α-tubulin, the main component of microtubules, with RFP in both wild-type and

spo12Δ strains to be able to track the behavior of SPBs and spindle, respectively (**Figure 7B**). SPB duplication occurs during S-phase. However, separate SPBs become visible only upon entry into metaphase I (Jaspersen and Winey, 2004). In wild-type, cells enter metaphase I with a short single bipolar spindle, then, when they proceed through anaphase I, it elongates and, upon entry into MII, disassembles. When cells enter MII, SPBs reduplicate and each pair of daughter SPBs forms a short bipolar metaphase II spindle, which elongates through anaphase II and disassembles when division ends.

spo12Δ cells enter metaphase I similarly to wild-type with a single short bipolar spindle. However, after spindle elongation indicating anaphase I, there is no subsequent SPB reduplication and cells keep only two parental SPBs. Post anaphase I, ~45% of cells presented spindles as short fragments connected to only one SPB (further referred to as half-spindles). The remaining ~55% of cells were able to eventually reconnect these half-spindles into one spindle structure. However, this spindle demonstrated a much weaker midzone compared to the spindles in wild-type cells. Because there is no distinction between anaphase I and metaphase II in *spo12Δ* when only SPBs and spindle are monitored in live-cell imaging, we compared the total time spent by wild-type or *spo12Δ* cells from entry into metaphase I, when the metaphase I spindle forms, to the end of a division when disassembly of spindle/s occurs. For the wild-type strain, it takes 102 ± 11 min while *spo12Δ* spends 179 ± 36 min from entry into metaphase I until exit from anaphase II. This data demonstrates that *spo12Δ* cells are delayed in progression through meiosis compared to wild-type.

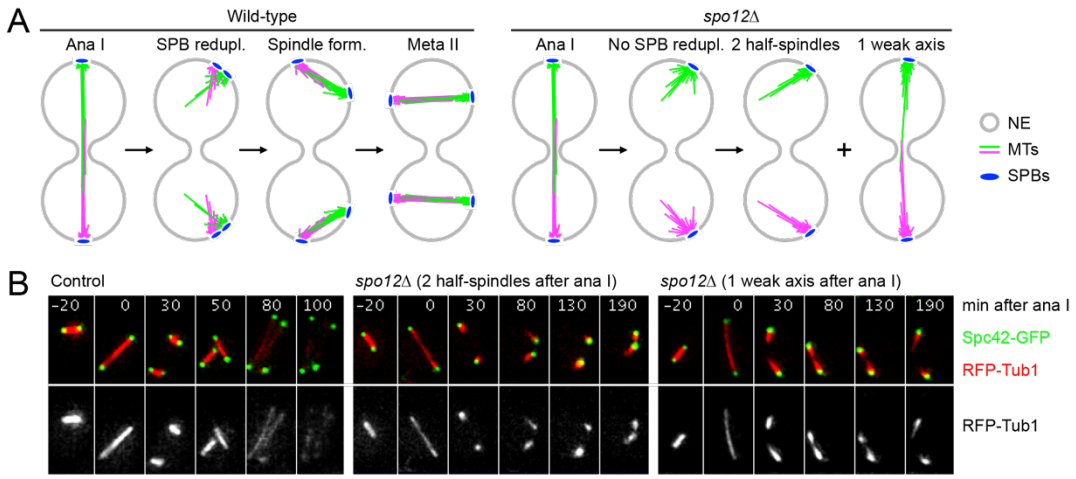

Figure 7. *spo12Δ* leads to spindle morphology defect at meiosis II.
(A) Cartoon of microtubule structures at meiosis II in the wild-type and a mutant (*spo12Δ*) defective in SPB reduplication. NE, nuclear envelope; MTs, microtubules. **(B)** wild-type (control, Z33310) and *spo12Δ* (Z33309) strains expressing Spc42-GFP and Tub1-RFP were filmed every 10 min for 12 hours.

2.1.2. *spo12Δ* leads to increased SAC activity at metaphase II

Spindles, not being able to create tension at kinetochores, should lead to SAC activation and consequent delay in progression through meiosis II. To investigate if the delay observed in the *spo12Δ* strain is SAC-dependent, we performed live-cell imaging with one of the SAC proteins, Mad2, fused with GFP. In the absence of tension, when the SAC is active, Mad2 forms clusters, and can be observed as bright foci at kinetochores (Musacchio, 2015). In yeast, kinetochores are located at the SPB, so we used Cnm67, a SPB component, fused with RFP to visualize foci localization. In the wild-type, Mad2-GFP foci appear at the SPBs for a short time prior to each metaphase, persisting for 19 ± 6 min at meiosis I and for 13 ± 5 min at meiosis II (**Figure 8**). In *spo12Δ* cells, Mad2-GFP foci persistence at meiosis I takes the same time as in wild-type (19 ± 6 min). This supports the expectation, that meiosis I is not affected by *spo12Δ*. However, at meiosis II, the time of Mad2 loci persistence is extended to 88 ± 30 min. This demonstrates that, indeed, SAC activity is prolonged in *spo12Δ* cells. In addition to time extension, the Mad2 foci signal in *spo12Δ* is also much brighter compared to the wild-type, which suggests stronger SAC activation. Therefore, we show that spindles in *spo12Δ* are not functional at meiosis II. Furthermore, there were no cells in *spo12Δ* which

demonstrated wild-type timing of SAC activity in meiosis II and this indicates that both spindle types (half spindles and 1 axis spindle with a weak midzone) in *spo12Δ* are not functional spindle structures.

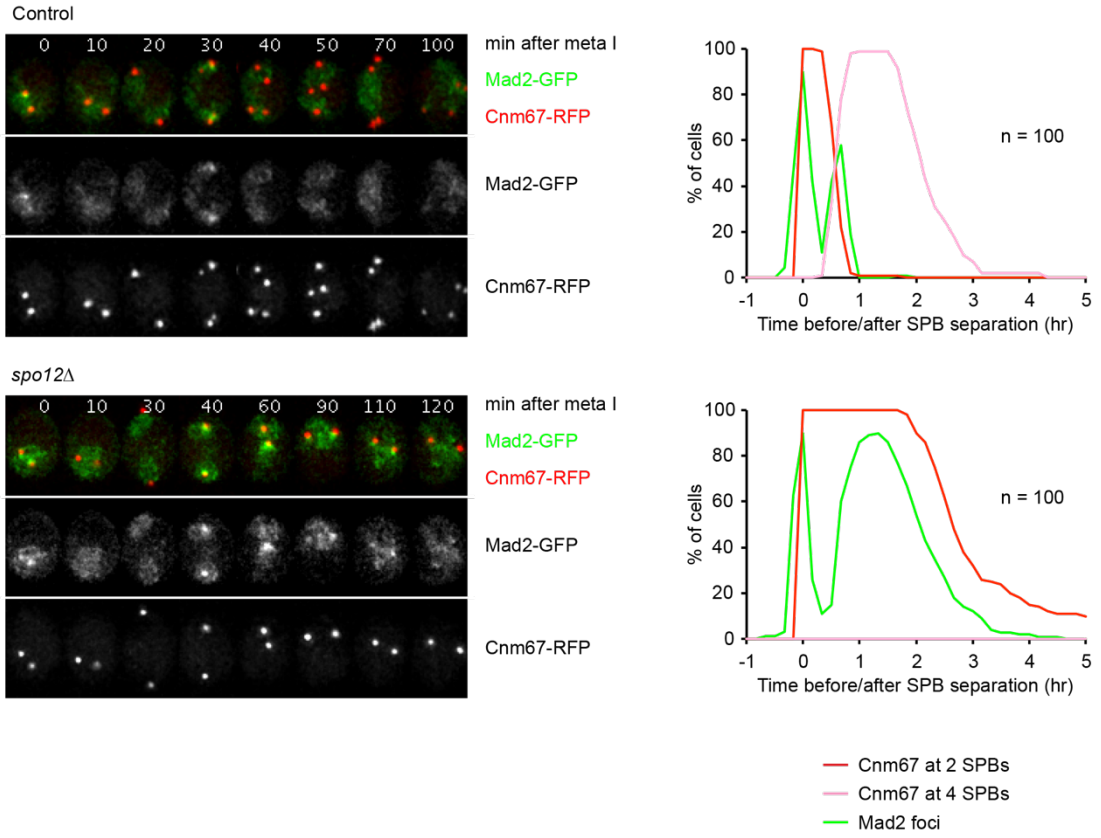

Figure 8. *spo12Δ* leads to increased SAC activity at metaphase II.
Wild-type (control, Z33261) and *spo12Δ* (Z33262) strains expressing Mad2-GFP and Cnm67-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the left and the quantification of Mad2 foci persistence on the right.

2.1.3 *spo12Δ* delays centromeric cohesin cleavage in meiosis II

To distinguish meiosis I and meiosis II in *spo12Δ* cells, we performed live-cell imaging with the cohesin subunit Rec8 tagged with GFP and α-tubulin-RFP. Cells enter metaphase I with a so-called full Rec8 signal when cohesin decorates the entire chromatin (chromosome arms and centromeres). When cells proceed through anaphase I, arm cohesin is cleaved by separase, therefore, the disappearance of a major fraction of the cohesin signal indicates anaphase I. Both, wild-type and *spo12Δ* cells enter meiosis I with a full Rec8 signal (**Figure 9**). When a cell proceeds to anaphase, most Rec8 signal disappears, leaving a small fraction of centromeric cohesion which accumulates as a dot. When a wild-type cell goes

through metaphase II this dot signal is localized in the middle of meiosis II spindles due to spindle tension and persists for 47 ± 10 min. In *spo12Δ*, centromeric cohesin cleavage is delayed and persists for 138 ± 54 min. In addition, the Rec8 signal is localized at the spindle's poles (~60 %) or diffusely along the spindle axis, contrary to wild-type, due to the absence of the tension. Similar to our previous observation, spindle disassembles in *spo12Δare* delayed as well: 178 ± 78 min after anaphase compared to 98 ± 19 min in the wild-type. Thus, this result supports the idea of APC/C activation, responsible for cohesin cleavage, is delayed at meiosis II, because of prolonged activity of the SAC.

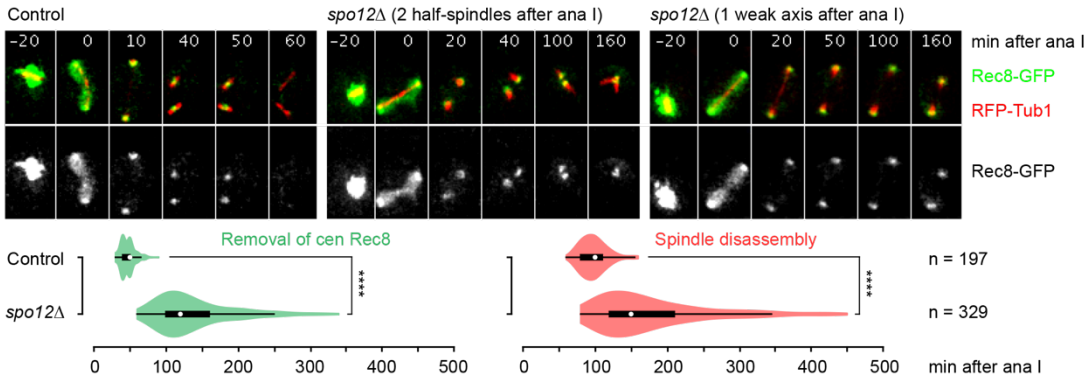

Figure 9. *spo12Δ* delays centromeric cohesin cleavage in meiosis II.
Wild-type (control, Z32945) and *spo12Δ* (Z32944) strains expressing Rec8-GFP and Tub1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the top and the quantification of Rec8 and spindle persistence on the bottom.

2.1.4. *spo12Δ* leads to delay in APC/C dependent protein degradation at anaphase II

Next, we wanted to confirm our conclusion that APC/C is inhibited on the protein level as well, by monitoring the degradation of proteins, known to be specific substrates of APC/C. We used a prophase (Ndt80) arrest-release synchronization system to have better timing resolution of the degradation of specific proteins. Cells were depleted of the Ndt80 transcriptional factor and this leads to an arrest in prophase. In addition, cells have the *NDT80* gene under the control of the estradiol-inducible promoter. When estradiol is added to the media, *NDT80* would be expressed and, as a result, would activate the expression of genes required for progression through meiosis (cyclins, *MAM1*, *CDC5*, etc.). This causes cells to progress through meiosis synchronously. Both control and *spo12Δ* cells degrade meiosis I specific APC/C targets, such as Spo13, and arm

cohesin with similar timing when most cells exit meiosis I (**Figure 10**). This again demonstrates that meiosis I is not affected by deleting *SPO12*. However, at meiosis II, substrates of both APC/C-Cdc20 (Clb1, Clb3, Sgo1) and APC/C-Ama1 (Cdc5) are degraded much later in *spo12Δ* cells. This demonstrates that in *spo12Δ* the SAC inhibits proteolysis mediated by APC/C-Cdc20 as well as APC/C-Ama1 (which depends on APC/C-Cdc20 (Oelschlaegel *et al.*, 2005)). Taking together, live-cell imaging and protein extract data show that deletion of *SPO12* leads to unfunctional spindles and tension defect, and subsequential SAC-induced delay of all APC/C-dependent events at meiosis II.

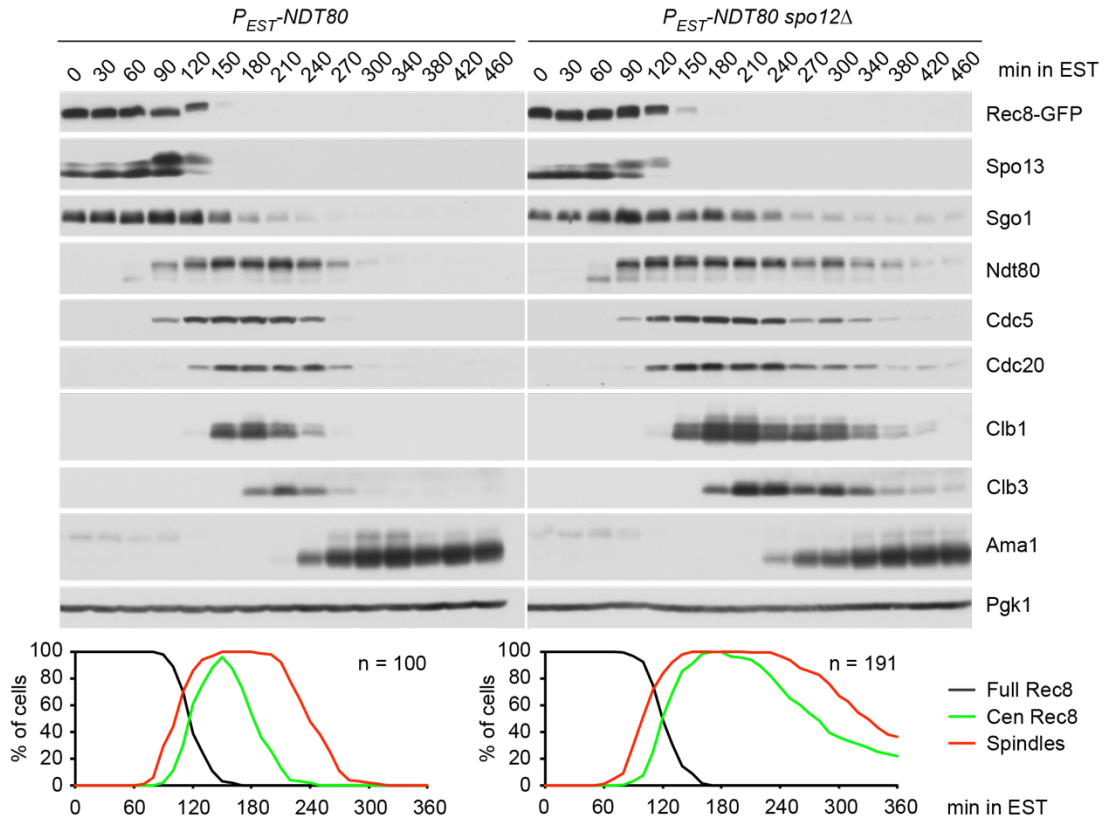

Figure 10. *spo12Δ* leads to delay in all APC/C dependent protein degradation at anaphase II. Immunoblot detection of proteins in P_{EST}-*NDT80* (Z33877) and P_{EST}-*NDT80 spo12Δ* (Z33878) strains. Cells were arrested in prophase I and released from arrest at t = 7 h by addition of 10 μM of estradiol (EST) and then samples were taken every 30 min.

2.1.5. Cohesin and protection colocalize in *spo12Δ*

The key assumption of the tension model is that the relative localization of cohesin and its protector is essential for protection. To assess the localization of protection proteins in relation to centromeric cohesin, we used live-cell imaging of one of the kinetochore proteins – Mtw1 tagged with RFP together with Rec8 or Rts1 subunit of phosphatase PP2A, tagged with GFP. As was previously mentioned, yeast kinetochores cluster at SPBs. Centromeric cohesion is localized in between kinetochore clusters in wild-type cells (**Figure 11A**, left), while PP2A subunit Rts1 is colocalizing with them (**Figure 11B**, left). This is in line with the tension model, which suggests that PP2A is separated from cohesion by spindle tension. On the other hand, there is no separation between cohesin and phosphatase in *spo12Δ* – both cohesin (**Figure 11A**, right) and Rts1 (**Figure 11B**, right) respectively, colocalize with kinetochore clusters.

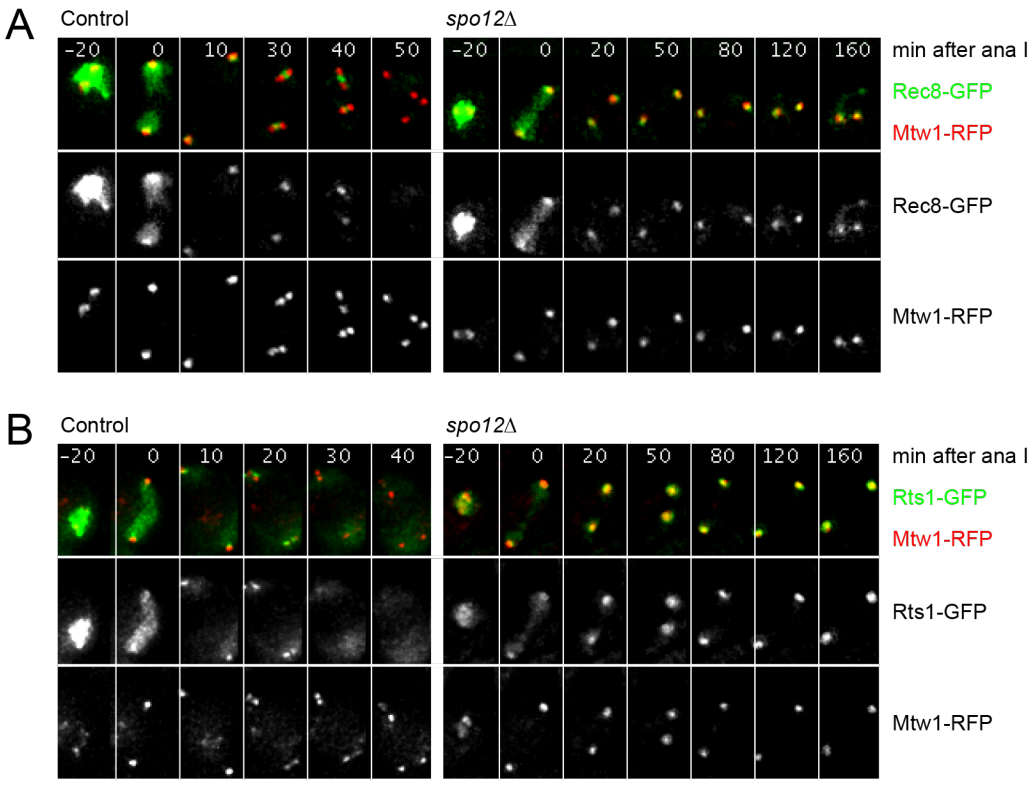

Figure 11. In *spo12Δ* cohesin and protection colocalize.
(A) Wild-type (control, Z33305) and *spo12Δ* (Z33506) strains expressing Rec8-GFP and Mtw1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain. **(B)** Wild-type (control, Z15736) and *spo12Δ* (Z34178) strains expressing Rts1-GFP and Mtw1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain.

2.1.6. The *MAM1* deletion rescues spindle defect in the *spo12Δ* mutant

The actual functions of *SPO12* are not well studied yet, therefore, the observed delay in centromeric cohesin cleavage in *spo12Δ* cells could be a result not only of SAC activity caused by spindle defects but also by other unaccounted effects. For instance, Spo12 is a component of the FEAR network regulating the activity of Cdc14 phosphatase, responsible for exit from mitosis. Hence, deleting *SPO12* may lead to a prolonged M-phase due to the inability of cells to exit from a division. If the observed delay in cohesin cleavage and spindle disassembly is a result only of spindle defect, restoring the tension in cells with *SPO12* deletion should fully rescue this phenotype. We hypothesized that the *spo12Δ* phenotype is a result of SPBs separating too far at meiosis I. Thus, if SPBs would be kept closer together during meiosis I, it may help to restore a functional spindle between them. To achieve this, we used the deletion of *MAM1*. This mutation leads to chromosome biorientation in meiosis I, which blocks nuclear division at meiosis I, but does not affect the general progression of meiosis. Therefore, cells combining *spo12Δ* deletion with *mam1Δ* may rebuild a functional spindle at meiosis II (**Figure 12**).

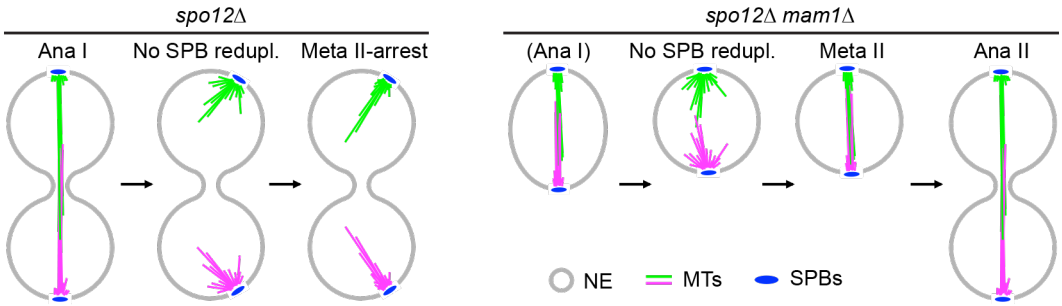

Figure 12. Cartoon showing how the *MAM1* deletion restores the formation of a functional spindle at meiosis II in *spo12Δ* cells. NE, nuclear envelope; MTs, microtubules.

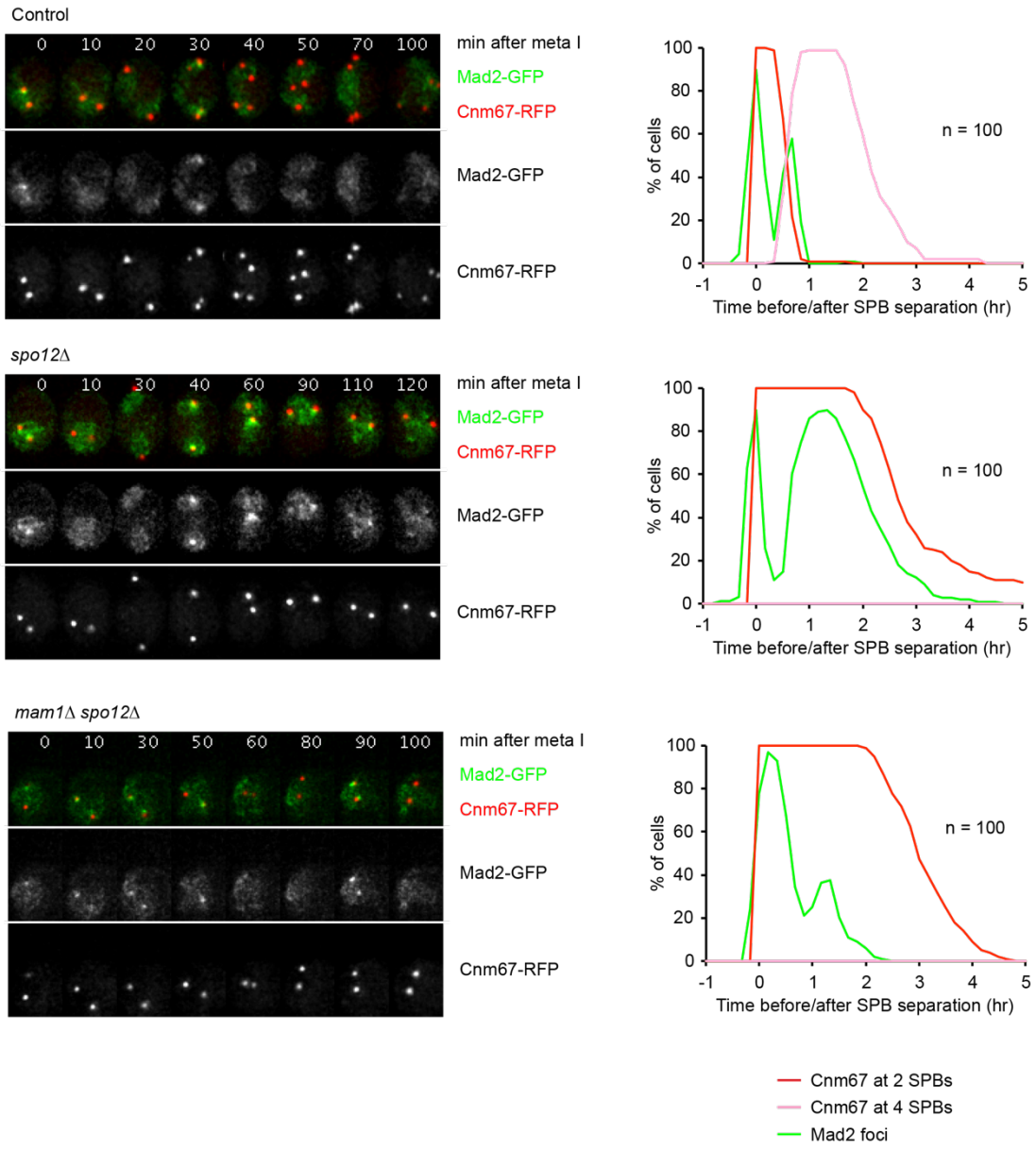

Figure 13. *spo12Δ mam1Δ* has reduced SAC activity at meiosis II.
Wild-type (control, Z33261), *spo12Δ* (Z33262), and *spo12Δ mam1Δ* (Z33263) strains expressing Mad2-GFP and Cnm67-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the left and the quantification of Mad2-foci persistence on the right.

2.1.7. *spo12Δ mam1Δ* has reduced SAC activity at meiosis II

At first, we compared SAC activity in *spo12Δ* and *spo12Δ mam1Δ* strains. If spindle integrity is restored by the *MAM1* deletion, SAC activity should be reduced. To evaluate this, we used the Mad2-GFP assay again (**Figure 13**). At meiosis II, SAC activity in *spo12Δ mam1Δ* cells lasts for 16±7 min, which is significantly reduced compared to the *spo12Δ* single mutant and is similar to wild-type SAC duration (13 ± 5 min), while both strains keep only two SPBs. This suggests that, indeed, in *spo12Δ mam1Δ* double mutant spindle can create enough tension to satisfy the SAC even though reduplication of SPBs is blocked.

2.1.8. *mam1Δ* restores timing of cohesin cleavage and spindle disassembly in *spo12Δ cells*

Similar to *spo12Δ*, *spo12Δ mam1Δ* double mutant has the reduplication defect and keeps only two parental SPBs through both meiotic divisions. However, due to biorientation at meiosis I caused by *mam1Δ*, the double mutant keeps a short bipolar spindle until entry into anaphase II (**Figure 14**), even when arm cohesin is cleaved at anaphase I. When *spo12Δ mam1Δ* cells enter the second division, they form a normal-looking one-axis spindle in contrast to a spindle with a weak midzone in *spo12Δ*. Additionally, no cells with half-spindles were observed. Centromeric Rec8 signal also accumulates as a dot in the middle of the spindle similar to wild-type, which is an indication that the spindle can create the tension. In addition, Rec8 is removed as in wild-type after 56 ± 12 min, which is almost twice as fast as in *spo12Δ*, because the SAC is satisfied. Disassembly of the spindle is also advanced compared to *spo12Δ* cells (99 ± 18 min after anaphase I).

Thus, we concluded that the observed *spo12Δ* phenotype is a result of only tension defect. In addition, we also by manipulating *SPO12* and *MAM1*, we developed a new instrument to study allowing manipulating SAC specifically at meiosis II.

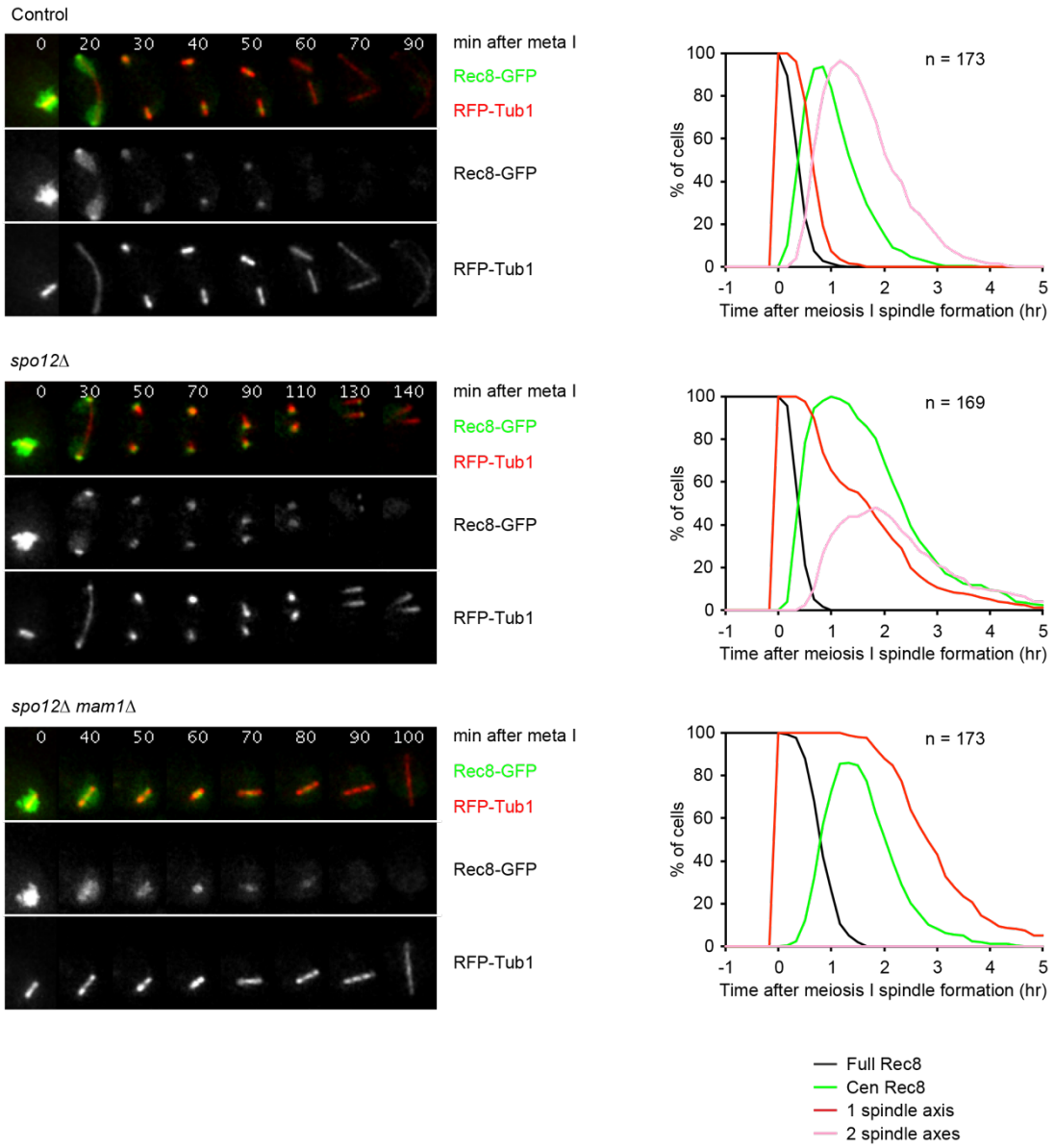

Figure 14. *mam1Δ* restores timing of cohesin cleavage and spindle disassembly in *spo12Δ*. Wild-type (control, Z32945), *spo12Δ* (Z32944), and *spo12Δ mam1Δ* (Z35329) strains expressing Rec8-GFP and Tub1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the left and the quantification of Rec8 and spindle persistence on the right.

2.2. Activation of the APC/C at meiosis II in *spo12Δ* cells

In the previous part of this work, we developed an instrument to manipulate the spindle tension specifically at meiosis II. Here, we use this instrument to test the role of tension specifically in the deprotection of centromeric cohesin. In *spo12Δ*, cohesin removal is delayed as well as the degradation of other APC/C substrates due to SAC activity. SAC inactivation in *spo12Δ* would lead to APC/C activation in cells without spindle tension. This would reveal whether APC/C can induce cohesin removal alone or requires tension for deprotection. If tension is required for deprotection, as the tension model suggests, disabling the SAC would lead to the advance of degradation of APC/C substrates compared to the *spo12Δ* single mutant, while centromeric cohesin removal would remain delayed. If tension is not required for deprotection specifically downstream of APC/C, both APC/C substrate degradation and cohesin removal would be advanced. To achieve APC/C activation in cells with spindle defects, we disabled the SAC by deleting its component Mad2.

2.2.1. In *spo12Δ mad2Δ* chromosomes fail to biorient similarly to *spo12Δ*

Firstly, we wanted to confirm that *spo12Δ mad2Δ* cells fail to biorient sister kinetochores similarly to *spo12Δ* single mutant. To do so, we quantified splitting sister centromeres of one chromosome (**Figure 15**). When a chromosome is properly attached to a spindle, the spindle can separate centromeric regions of sister chromatids despite the presence of cohesin. To analyze this, we used live-cell imaging of cells containing an array of *tet* operators integrated at the centromere of one copy of chromosome V together with *tet* repressor fused with RFP and Rec8-GFP and compared the splitting of centromeres at metaphase II in the presence of centromeric Rec8. During meiosis I, only one dot is observed in all strains as sister kinetochores are mono-oriented, thus, not under tension. At metaphase II, there is a splitting of a dot into two signals in almost all wild-type cells (92%). By contrast, in *spo12Δ* cells centromeric dot splits much less frequently in the presence of Rec8 - only up to 23% even though metaphase II in these cells is prolonged. Interestingly, in *spo12Δ mad2Δ* this rate is even less – less than 1%.

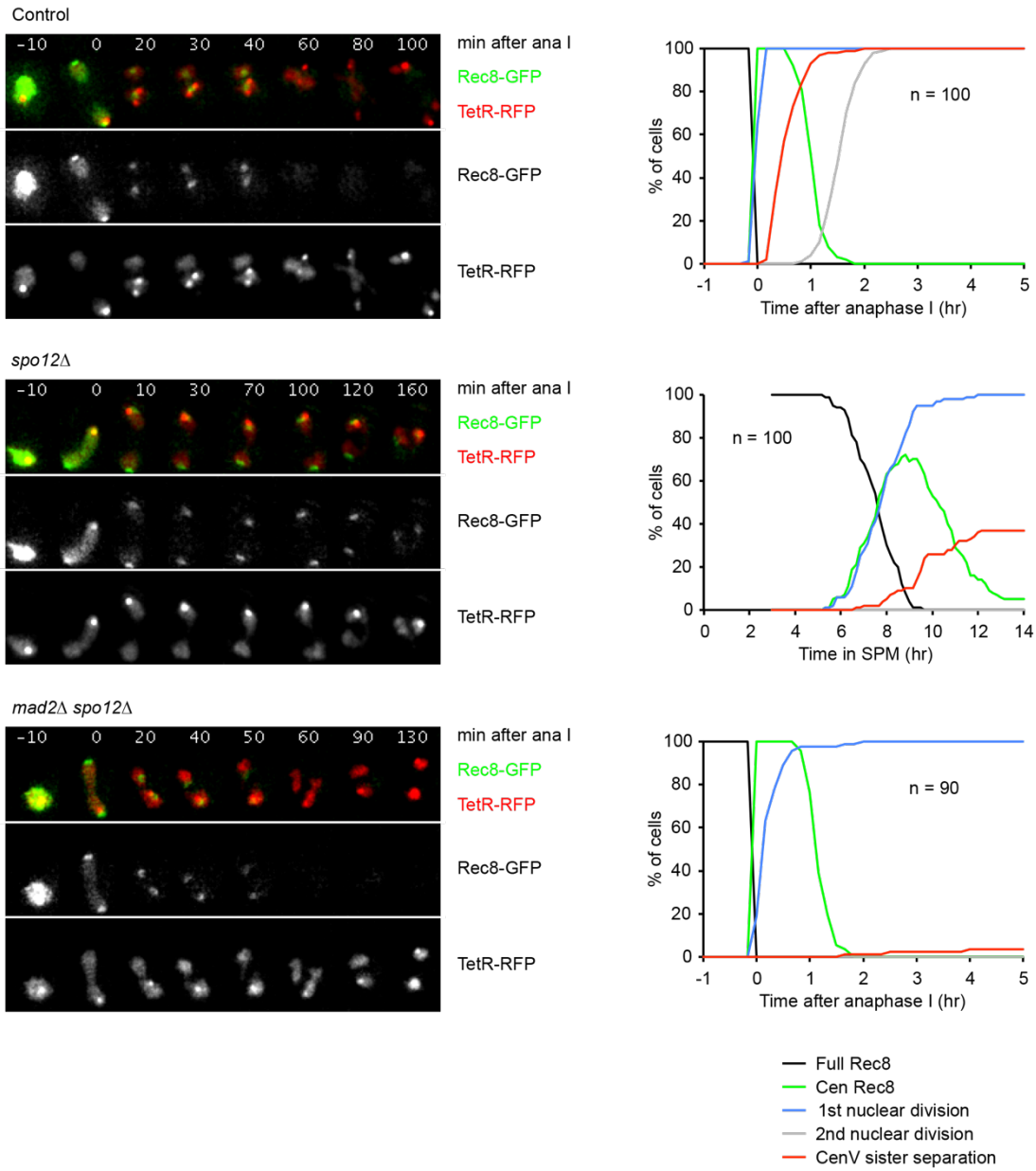

Figure 15. In *spo12Δ mad2Δ* chromosomes fail to biorient similarly to *spo12Δ*.
Wild-type (control, Z33612), *spo12Δ* (Z33613), and *spo12Δ mad2Δ* (Z33828) strains expressing Rec8-GFP and TetR-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the left and the quantification of Rec8 persistence and centromeric dots separation on the right.

2.2.2. *mad2Δ* rescues delay of cohesin cleavage and spindle disassembly in *spo12Δ*

Knowing that in *spo12Δ mad2Δ* cells are not able to biorient sister kinetochores similarly to *spo12Δ* we could compare cohesin removal and spindle disassembly in *spo12Δ* and *spo12Δ, mad2Δ*. This would reveal the answer to our main question – can cohesin be cleaved in the absence of tension when APC/C, and thus

separase, is active. Even though the double mutant shows the same defective spindle morphology as *spo12Δ*, both centromeric cohesin cleavage and spindle disassembly occurs faster compared to *spo12Δ*: centromeric Rec8 is removed at 59 ± 17 min which is ~ 80 min earlier than in *spo12Δ* cells, and spindle disassembly happens 106 ± 34 min after anaphase I which is ~ 75 min earlier comparing to *spo12Δ* (**Figure 16**). In addition, these timings are similar to corresponding timing in wild-type cells, therefore can conclude that tension is not required for the deprotection of centromeric cohesin. Rather than this, the role of tension is only to satisfy the SAC and thereby activate the APC/C.

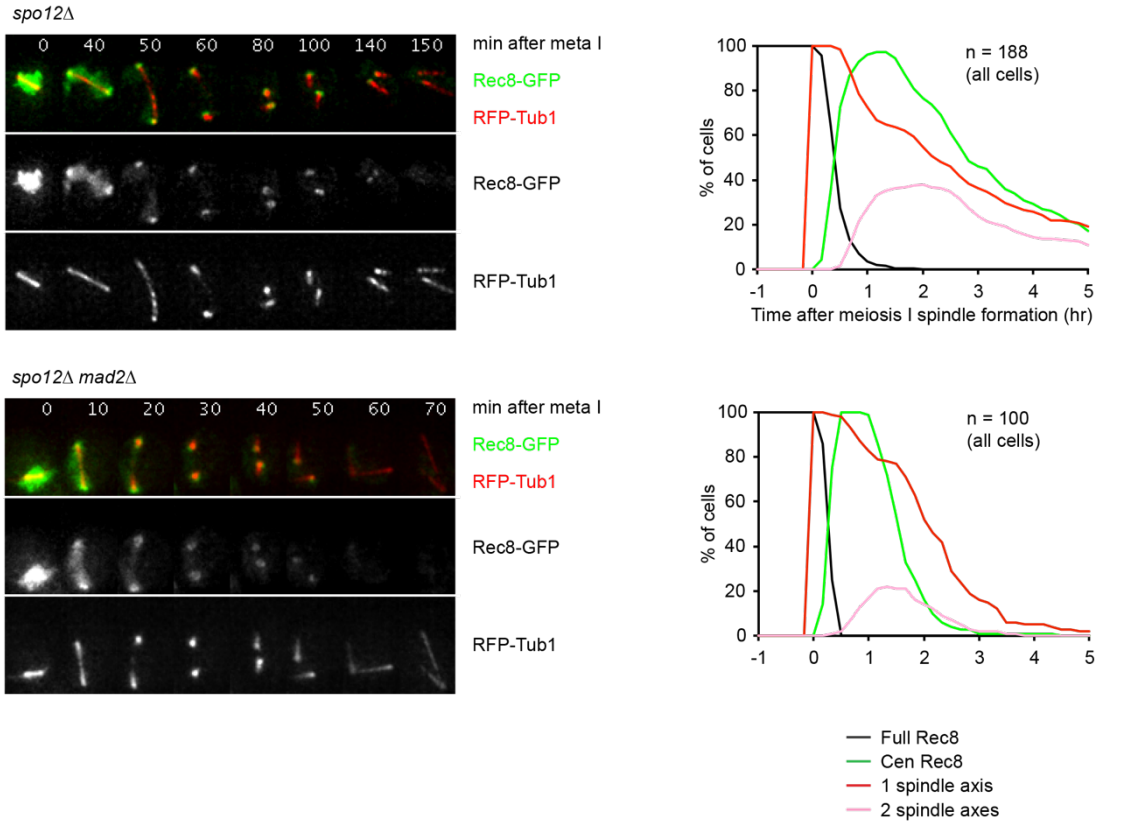

Figure 16. *mad2Δ* rescues delay of cohesin cleavage and spindle disassembly in *spo12Δ*. *spo12Δ* (Z32944) and *spo12Δ mad2Δ* (Z30454) strains expressing Rec8-GFP and Tub1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the left and the quantification of Rec8 and spindle persistence on the right.

2.2.3. In *spo12Δ mad2Δ* cohesin cleavage is not affected by protector co-localization

Interestingly, while in *spo12Δ mad2Δ* cells Rec8 removal is occurring with the same timing as in wild-type strain, the Rts1 subunit of PP2A is still colocalizing

with Rec8 similarly to *spo12Δ* single mutant (**Figure 17A** and **B**). This observation also contradicts the tension model, as this model suggests that PP2A colocalizing with cohesion is the key factor for cohesin protection from cleavage. On the other hand, this can also mean that when APC/C becomes active at anaphase onset, colocalization of cohesion with PP2A does not matter or there are multiple pools of PP2A at a kinetochore with different functions, therefore observed Rts1 may not be involved in protection.

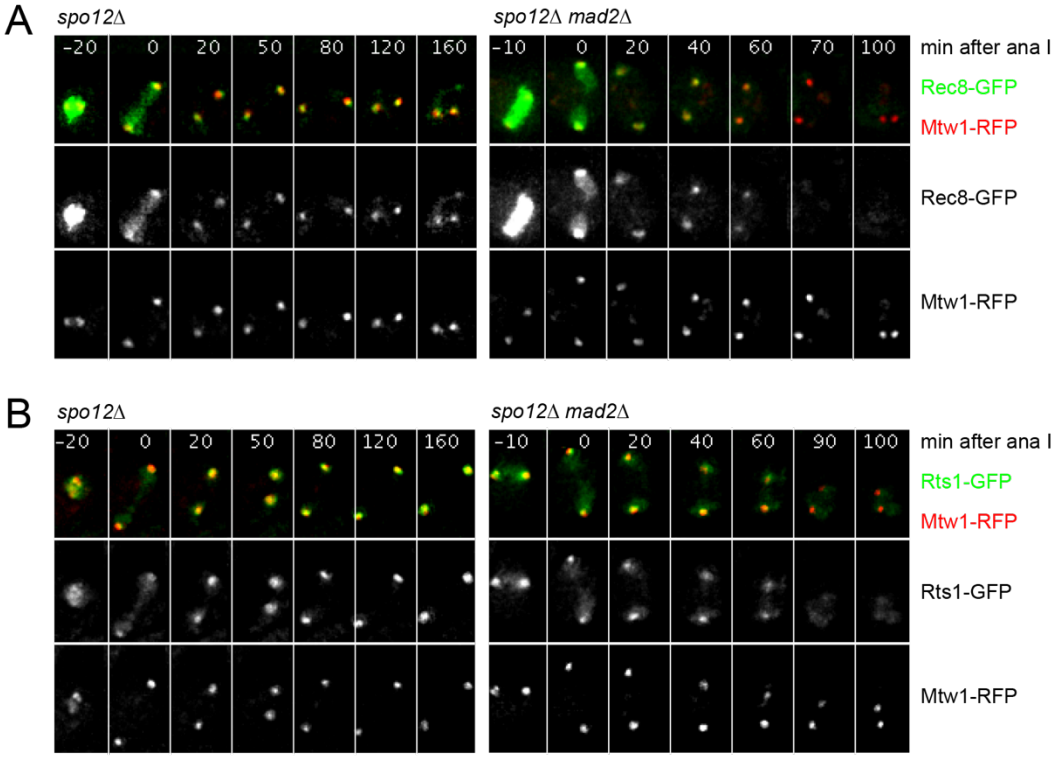

Figure 17. In *spo12Δ mad2Δ* cohesin cleavage is not affected by protector co-localization.
(A) *spo12Δ* (Z33506) and *spo12Δ mad2Δ* (Z33507) strains expressing Rec8-GFP and Mtw1-RFP were filmed every 10 min for 12 hours. **(B)** *spo12Δ* (Z34178) and *spo12Δ mad2Δ* (Z34176) strains expressing Rts1-GFP and Mtw1-RFP were filmed every 10 min for 12 hours.

2.2.4. Sister centromeres are connected after cohesin cleavage in the absence of tension

Centromeric dots are not splitting in *spo12Δ mad2Δ* strain as well as in *spo12Δ* in the presence of cohesin as there is no tension to pull them apart. However, in *spo12Δ mad2Δ* they virtually do not split even when cohesion is removed (**Figure 15**). We assumed this is a result of the fact that without spindle forces sister centromeres would hardly separate within the small volume of nuclei. To investigate this further, we perform an immunofluorescence analysis of chromatin spreads. By using this approach, we could evaluate if centromeres are not separated just due to limited space. Our result supports live-cell imaging data that in the presence of cohesin, centromeric dots do not split (**Figure 18A**). On the other hand, after cohesin removal, dots split with a higher rate on chromatin spreads compared to live-cell imaging, albeit inefficiently – in 51% of cells. In addition to separation being inefficient, dots also never separate far from each other. In contrast, GFP dots marking the arm region of chromosome V, 394 kb away from the centromere, split four times farther on average in the same conditions (**Figure 18B**). This suggests that even after cohesin cleavage, in *spo12Δ mad2Δ* cells centromeres stay loosely connected. We hypothesized that in the *spo12Δ mad2Δ* strain, this connection is a result of the inability of topoisomerase II to effectively resolve DNA catenation (a consequence of DNA replication). Catenanes at chromosome arms are resolved mostly before entry into the M phase, thus when arm cohesin is removed at anaphase I arms become fully separated and dots can freely split. In contrast, centromeres' catenation is resolved only after centromeric cohesin cleavage and spindle forces aid this process by providing direction to the reaction catalyzed by topoisomerase II. Thus, without the tension, this process would be inefficient, leading to chromosomes staying linked (Charbin et al., 2014; Farcas *et al.*, 2011; Wang et al., 2010b).

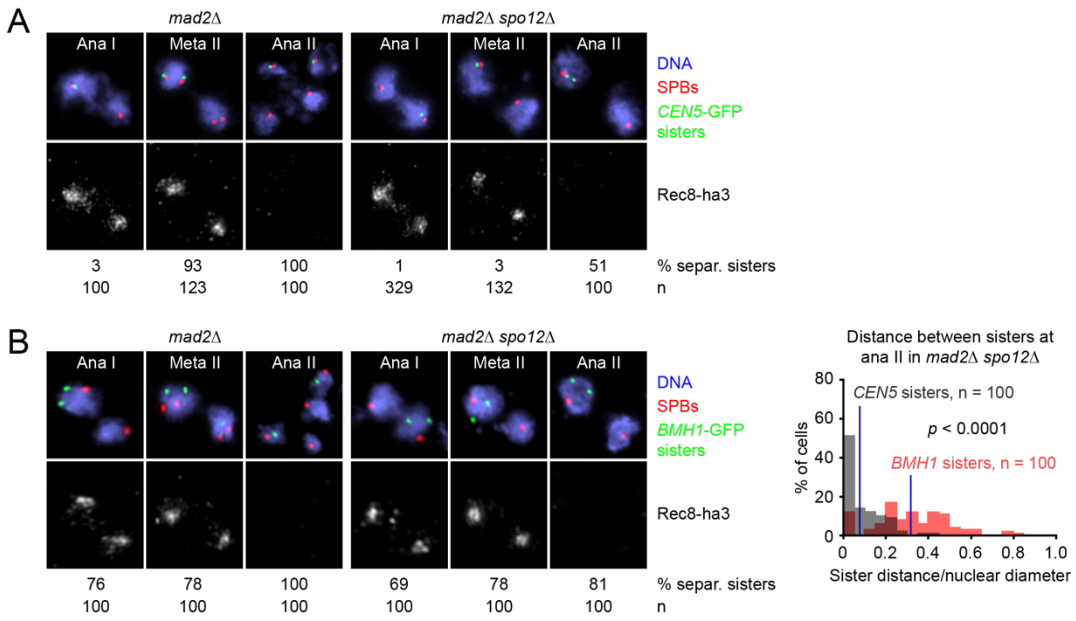

Figure 18. Sister centromeres are connected after cohesin cleavage in the absence of tension. Representative pictures of the spread nuclei, stained with anti-Ha, anti-GFP, and anti-Tub4 (SPB) antibodies. **(A)** *mad2Δ* (Z34890) and *spo12Δ mad2Δ* (Z34891) with *CEN5* dots. **(B)** *mad2*Δ (Z37473) *spo12*Δ *mad2*Δ (37472) with *BMH1* dots.

2.2.5. in *spo12Δ mad2Δ* APC/C activity is regulated by tension even without MCC formation

Deletion of *SPO12* leads to a significant delay in cohesin removal during meiosis II, which is rescued by *MAD2* deletion to wild-type timing. These data show that spindle tension is not required for deprotection. However, when we compared *spo12Δ mad2Δ* to *mad2Δ* single mutant, turned out that cohesin cleavage in *mad2Δ* is faster by ~20-30 min (**Figure 19**).

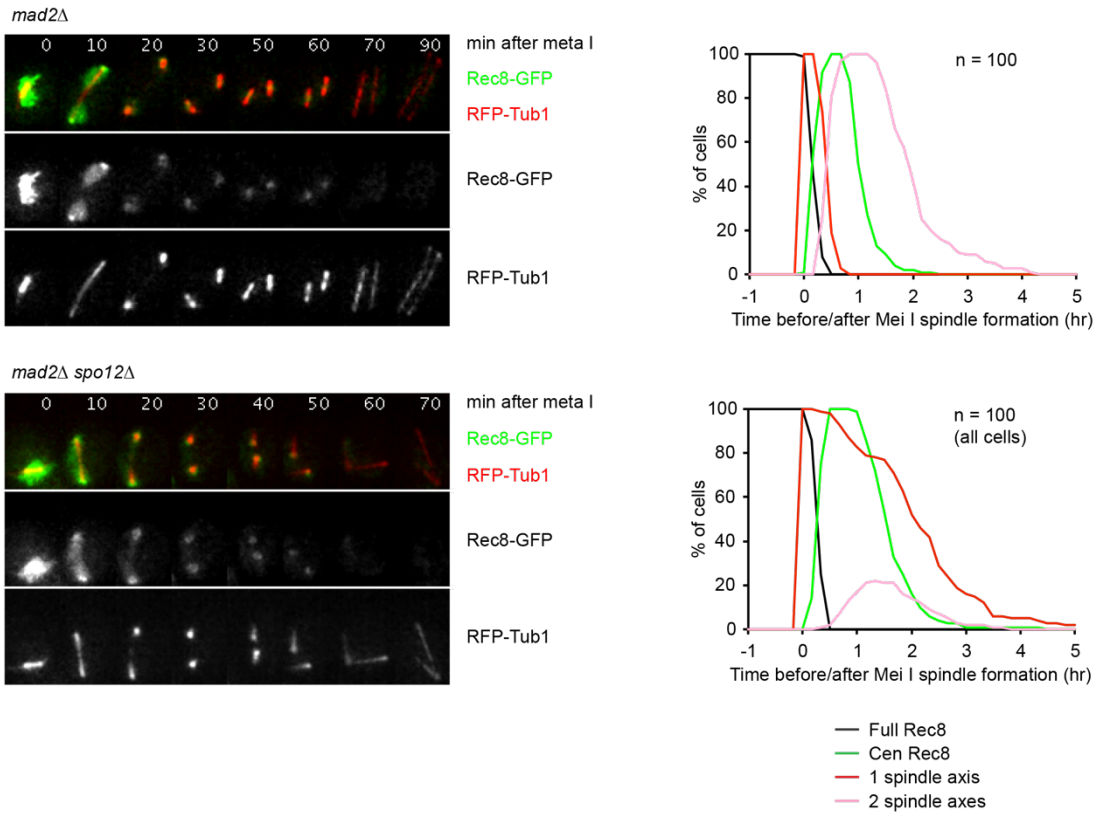

Figure 19. Removal of cohesin is delayed in *spo12Δ mad2Δ* compared to *mad2Δ*.
mad2Δ (Z30453) and *spo12Δ mad2Δ* (Z30454) strains expressing Rec8-GFP and Tub1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the left and the quantification of Rec8 and spindle persistence on the right.

In addition, we observed a similar effect on protein level in extracts from Ndt80 synchronized cultures – APC/C dependent proteolysis at meiosis II in *spo12Δ mad2Δ* strain happens 20-30 min later compared to *mad2Δ* (**Figure 20**). This observation does not contradict our main conclusion that tension is not required for deprotection. Instead, it may suggest that there is a mechanism to translate the presence of tension into APC/C activation in addition to the SAC - in *spo12Δ mad2Δ* strain, all APC/C-dependent events were delayed compared to *mad2Δ* single mutant.

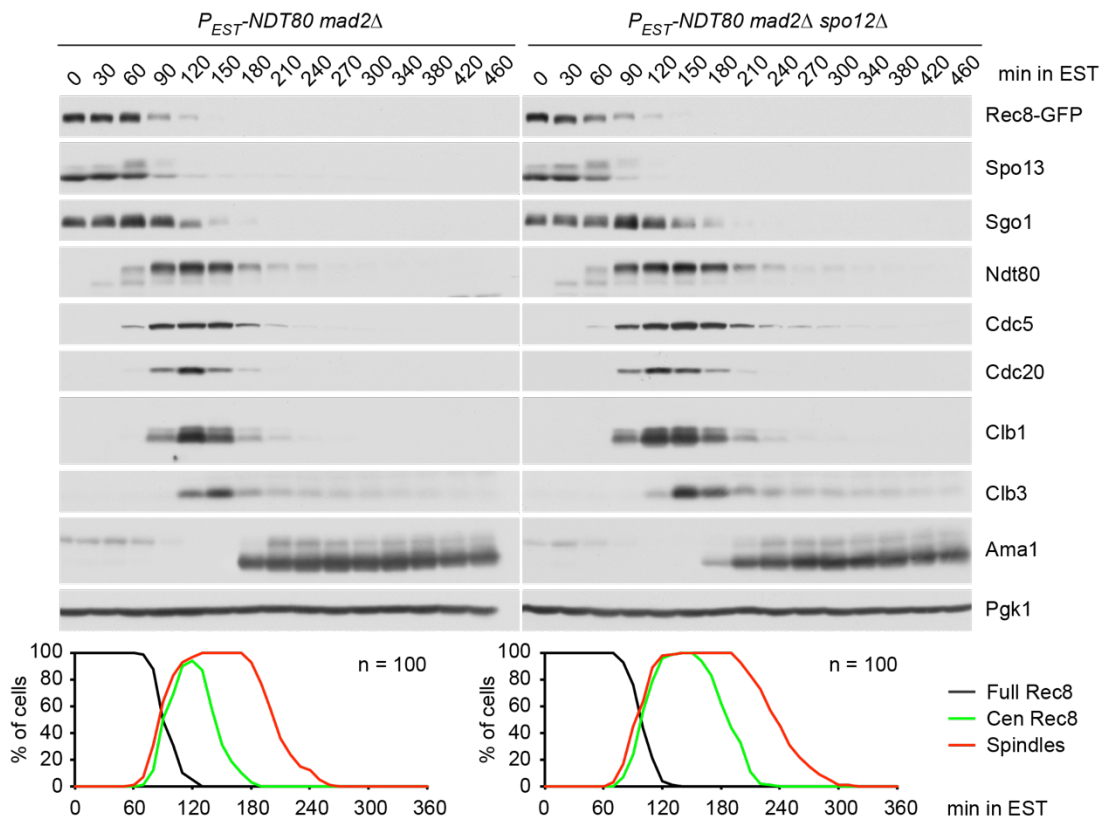

Figure 20. Degradation of APC/C substrates is delayed in *spo12Δ mad2Δ* compared to *mad2Δ*. Immunoblot detection of proteins in P_{EST}-*NDT80 mad2Δ* (Z34026) and P_{EST}-*NDT80 mad2Δ spo12Δ* (Z34025) were arrested in prophase I and released from arrest at t = 7 h by addition of 10 μM of estradiol (EST) and then samples were taken every 30 min.

Similar to *spo12Δ* single mutant, if this phenotype is the only result of spindle defect, deletion of *MAM1* would rescue it. *spo12Δ mad2Δ mam1Δ* triple mutant demonstrates similar timing of centromeric cohesin removal, as well as spindle disassembly, to *mad2Δ* single mutant. This confirms our hypothesis that the observed delay is caused by tension defect alone and in this way is similar to the SAC but does not depend on MCC (**Figure 21**).

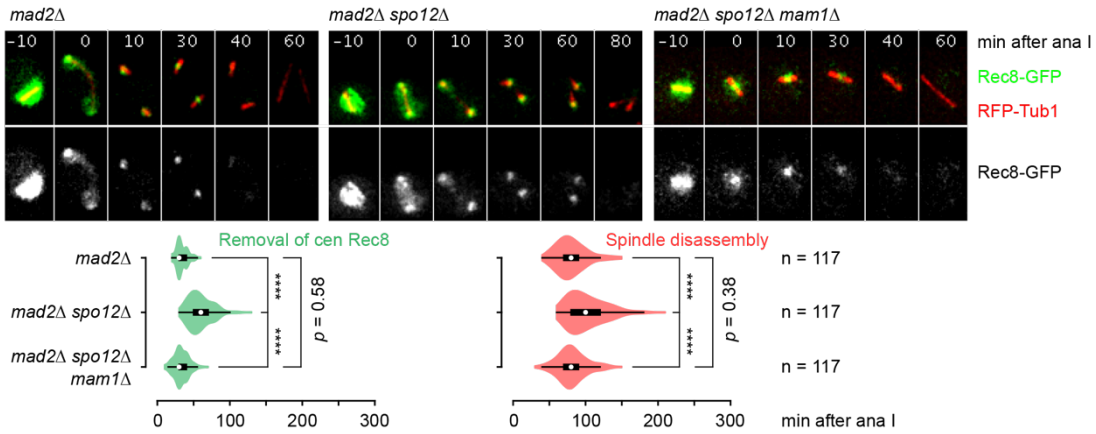

Figure 21. *spo12Δ* delays cohesin cleavage and spindle disassembly in a tension-dependent manner in cells without SAC.
mad2Δ (Z30453), *spo12Δ mad2Δ* (Z30454) and *spo12Δ mad2Δ mam1Δ* (Z30618) strains expressing Rec8-GFP and Tub1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the top and the quantification of Rec8 and spindle persistence on the bottom.

There is evidence that the conventional SAC can be active even in absence of Mad2 (Tang *et al.*, 2001). To test if our delay is still related to SAC or at least to MCC formation, we deleted all three effector components of SAC – *MAD2, MAD3,* and *MAD1* (**Figure 22**). But, even in this strain, deletion of *spo12Δ* would lead to delay in cohesin cleavage and spindle disassembly, rescued with *mam1Δ*. This proved that the observed phenomenon is not a result of residual SAC activity but rather a new entity: yeast can induce a SAC-like delay in response to spindle damage without MCC. Similar observations were made for other model organisms. Furthermore, it is hypothesized that the tension might directly regulate Cdc20 binding to the APC/C core through Cdc20 phosphorylation (Kim *et al.*, 2017).

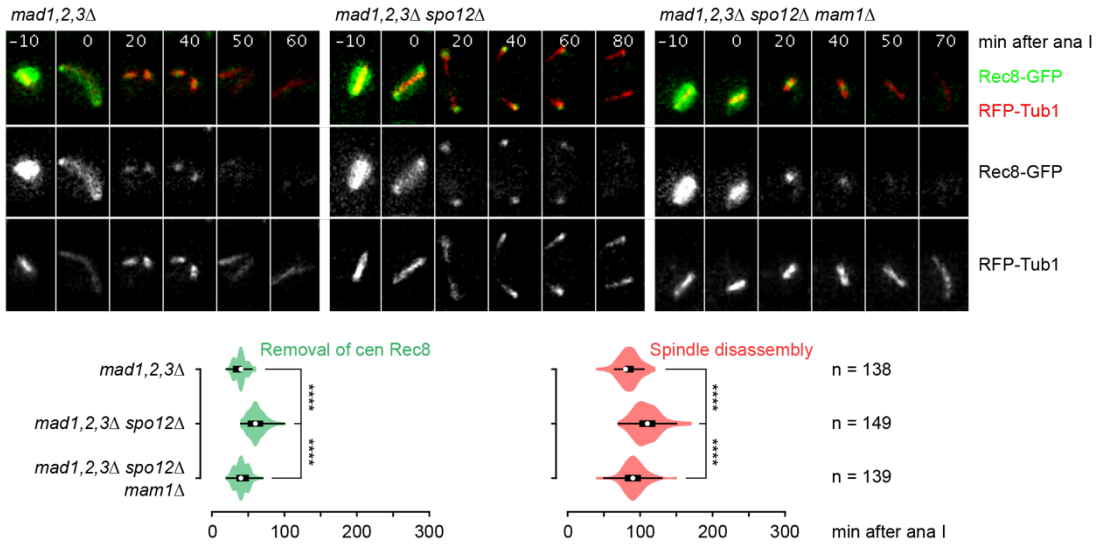

Figure 22. *spo12Δ* cells have delayed centromeric cohesion cleavage at meiosis II in absence of MCC.
mad1Δ mad2Δ mad3Δ (Z35132), *mad1Δ mad2Δ mad3Δ spo12Δ* (Z35133), and *mad1Δ mad2Δ mad3Δ spo12Δ mam1Δ* (Z35134) strains expressing Rec8-GFP and Tub1-RFP were filmed every 10 min for 12 hours. Representative time-lapse images of each strain are presented on the top and the quantification of Rec8 and spindle persistence on the bottom.

2.3. Protection of centromeric cohesin at metaphase II

In the previous part, we showed that tension created by spindle forces is required for deprotection only to activate APC/C-Cdc20 via SAC silencing, while does not have a specific role in deprotection itself. However, the tension model suggests another hypothesis: at metaphase II, while APC/C is still not active, cohesion should be already deprotected by tension.

To assess protection status specifically at metaphase II we used the metaphase II arrest system (Jonak, 2020; Mengoli et al., 2021). Cells stay arrested in metaphase II due to APC/C-cdc20-3 inactivity at an increased temperature (Shirayama et al., 1998). Similar to oocytes, these cells stay in prolonged metaphase II with bioriented chromosomes. To achieve APC/C-independent activation of separase, we used an auxin-inducible degradation system. This system relays on two components - plant F-box protein (in this work osTir1) and the AID domain: when *osTIR1* is expressed, any protein tagged with the AID domain would be quickly destroyed in a proteasome-dependent way (Morawska and Ulrich, 2013). Thus, using Pds1-AID* would allow to uncouple separase activation from APC/C activity. Active separase can cleave only deprotected cohesin, therefore we can assess if centromeric cohesin is protected on chromosomes under tension. If cohesin under tension is deprotected as the tension model suggests, it would be quickly destroyed and disappear from cells. To evaluate the cleavage of cohesin we used chromosome spreads.

Cells enter into metaphase II with centromeric cohesin located in between SPB, similar to live cell imaging and high levels of Pds1-AID* (**Figures 23A** and **B**). Most control cells, containing only Pds1-AID* keep centromeric cohesin for at least 2 h (apparent half-life t½ = 240 min, **Figure 23C**). Cells with Pds1-AID* expressing OsTir1 quickly lose Pds1. However, this does not lead to major cohesin loss (t½ = 143 min) and suggests that cohesin is protected when kinetochores are bioriented, even though not perfectly.

To deprotect cohesin we used inducible degradation of Sgo1-AID* (**Figures 24A** and **B**). Cells with Sgo1-AID* were retaining most of the cohesin even when osTir1 was expressed. However, apparent half-life t½ was reduced from 258 min to 161 min when depletion of Sgo1-AID* is induced, which suggested that during metaphase II separase is not completely inhibited, even when levels of Pds1 are high.

Only when both degradation of Sgo1-AID* and degradation of Pds1-AID* are combined, cells quickly lose cohesin (initial t½ = 24 min, **Figures 24C** and **D**). Furthermore, these cells were able to perform a second nuclear division.

This indicates that cleavage of centromeric cohesin in presence of tension requires not only active separase but also removal of components of protection machinery. Thus, this result contradicts the tension model and supports our conclusion, that tension is responsible only for APC/C-Cdc20 activation, while both deprotection and cleavage of cohesin depend only on APC/C-Cdc20 activity. In addition, our data suggest, that to ensure faithful chromosome segregation at meiosis II both: active protection and separase are required until APC/C would be activated.

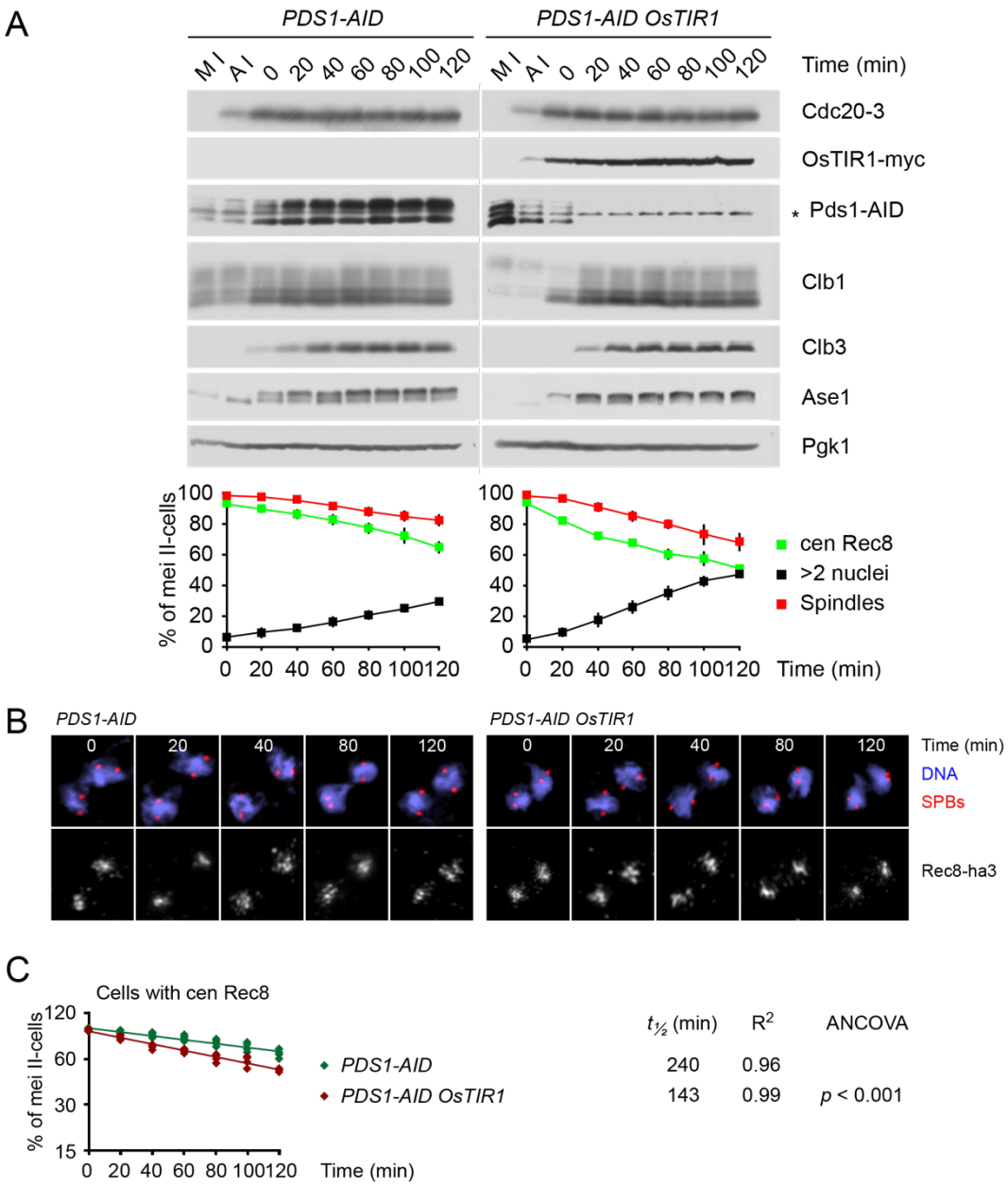

Figure 23. Centromeric cohesin is protected at metaphase II.
*cdc20ts-mAR ama1Δ PDS1-AID** (Z36435) and *cdc20ts-mAR ama1Δ PDS1-AID* CUP1p-osTIR1* (Z35713) strains were arrested at metaphase II and treated with IAA at t = 0 to deplete Pds1-AID in cells expressing OsTir1. **(A)** Top, protein blots. Bottom, percentages of meiosis II cells (four SPBs) with spindles, nuclear division (> 2 nuclei), and centromeric Rec8 (from chromatin spreads). **(B)** Chromatin spreads from meiosis II (four SPBs) stained for DNA, γ-tubulin/SPBs, and Rec8-ha3. **(C)** Semi-log plot of the percentages of meiosis II cells with centromeric Rec8. Half-lives were calculated from exponential regression of the mean values. Slopes were compared with ANCOVA.

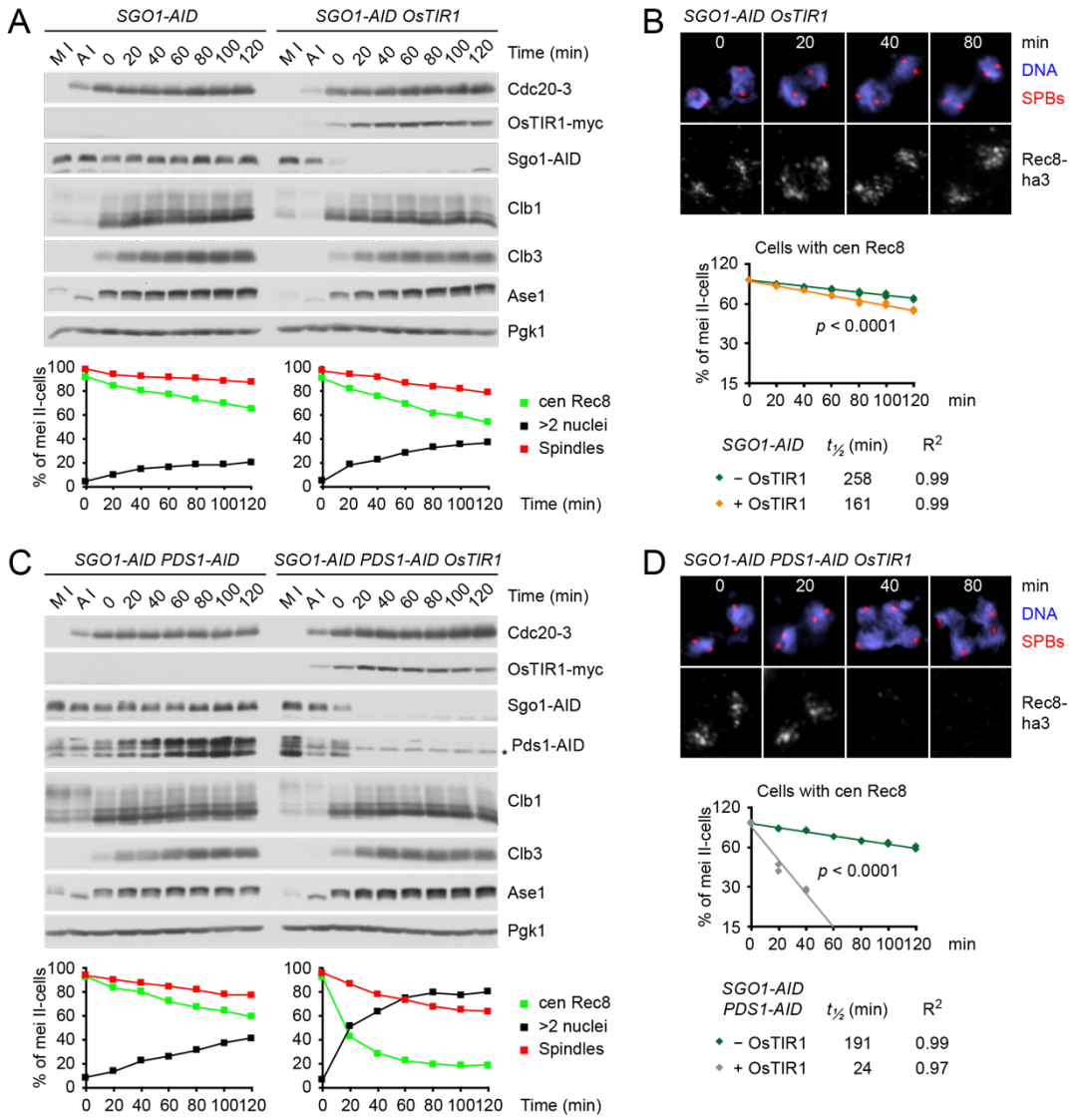

Figure 24. Protection of centromeric cohesin at metaphase II depends on Sgo1.
A, B. *cdc20ts-mAR ama1Δ SGO1-AID** (Z37076) and *cdc20ts-mAR ama1Δ SGO1-AID* CUP1p-osTIR1* (Z37077) strains were arrested at metaphase II and treated with IAA at t = 0 to deplete Sgo1-AID* in cells expressing OsTir1. **(A)** Top, protein blots. Bottom, percentages of meiosis II cells (four SPBs) with spindles, nuclear division (> 2 nuclei), and centromeric Rec8 (from chromatin spreads). **(B)** Top, chromatin spreads from meiosis II (four SPBs) stained for DNA, γ-tubulin/SPBs, and Rec8-ha3. Bottom, semi-log plot of the percentages of meiosis II cells with centromeric Rec8. Half-lives were calculated from exponential regression of the mean values. Slopes were compared with ANCOVA.
C, D. *cdc20ts-mAR ama1Δ SGO1-AID* PDS1-AID** (Z37078) and *cdc20ts-mAR ama1Δ SGO1-AID* PDS1-AID* CUP1p-osTIR1* (Z37079) strains were arrested at metaphase II as above and treated with IAA at t = 0 to deplete Sgo1-AID* and Pds1-AID* in cells expressing OsTir1. **(C)** Protein blots and quantification of spindles, nuclear division, and centromeric Rec8 in meiosis II cells as in **(A)**. **(D)** Chromatin spreads and half-lives of centromeric Rec8 as in **(B)**.

3. Discussion

3.1. Protection of centromeric cohesin at meiosis II

Every cell division relays on a tension force created between centromeric cohesion and the spindle, as it is the only way for a cell to verify proper chromosome attachment to the spindle. If a kinetochore is not properly attached to the spindle, the absence of tension would activate the SAC, which delays anaphase onset by inhibiting APC/C-Cdc20. Only when tension is applied to all kinetochores, the SAC is silenced, APC/C-Cdc20 becomes active and the cell proceeds to anaphase. This induces the degradation of securin, an inhibitor of separase, which leads to its activation. Active separase cleaves cohesin, allowing the spindle to distribute chromosomes equally into daughter cells. Thus, the tension is responsible for cohesin cleavage as an activator of the APC/C.

Faithful chromosome segregation during meiosis II relays on centromeric cohesin being protected from cleavage by separase at anaphase I. The general mechanism of this protection during meiosis I is conserved among various eukaryotes and depends on the complex of Sgo-proteins with phosphatase PP2A. On the other hand, there is no unified explanation for the deprotection of cohesin during meiosis II, required to make cohesin cleavable at anaphase II.

There are a few hypotheses proposed for different model systems. Previous work in our lab demonstrated the importance of APC/C-Cdc20 for the inactivation of Sgo1-PP2A and interlinks the deprotection with separase activation in *S. cerevisiae.* Another model, proposed for mammalian oocytes and spermatocytes, designates spindle forces as a key component of the deprotection mechanism as they may physically separate Sgo1-PP2A and cohesin by pulling them apart during meiosis II (Gomez *et al.*, 2007; Lee *et al.*, 2008). This model explains how deprotection is restricted specifically to meiosis II. Additionally, this tension model might be relevant to yeast to explain the separation of Sgo1-PP2A from cohesin observed at metaphase II by live-cell imaging and immunofluorescence analysis. On the other hand, the yeast APC/C model might be attractive for oocytes as well as they have prolonged arrest at metaphase II and would benefit from increased cohesin stability provided by protection. Sister centromere cohesion was shown to be very sensitive to decreased cohesin levels in aged mouse oocytes (Chiang et al., 2010; Lister et al., 2010) so keeping protection

during metaphase II may be an additional mechanism against low levels of separase activity and ensuring cohesin stability.
It is important to note that neither one of these hypotheses contradicts the other. Furthermore, the tension-based model derived from oocyte studies may overcome the limitations of the APC/C-based model by explaining deprotection specificity to metaphase II. Therefore, in this work, we tested a key prediction of the deprotection-by-tension model using the yeast system, namely whether the tension has a function in the deprotection of centromeric cohesin downstream of the APC/C in addition to SAC silencing and APC/C activation.

3.2. Role of tension in deprotection of centromeric cohesin in yeast

The tension model predicts that cohesin cannot be cleaved without tension even when APC/C is active, as, in the absence of tension, Sgo1-PP2A would be in close proximity to centromeric cohesin and would keep it protected. To prevent the biorientation of sister kinetochores in meiosis II and eliminate spindle forces, we deleted *SPO12*. This mutation causes blockage of SPBs reduplication in meiosis II and, as a result, defects in spindle functionality restricted specifically to meiosis II. These defects lead to SAC activation in response to the absence of tension at kinetochores and, consequently, to inhibition of APC/C-Cdc20 and a delay of anaphase II onset. Removal of cohesin depends on APC/C mediated activation of separase, thus, when the SAC is active, this removal is not possible. We induced APC/C activity by inactivation of the SAC by removing its essential components. As a result, in cells combining the absence of tension (spo12*Δ*) and active APC/C (deletion of *MAD* genes) cohesin was cleaved at a rate similar to that in wild-type cells. This suggests that tension is not essential for the deprotection of centromeric cohesin and proves that the tension model is not applicable to yeast. In addition, we observed that in the *spo12Δ mad2Δ* -mutant, cohesin is cleaved while it is not separated from the PP2A signal on live-cell imaging. This implies that spatial separation of cohesin and PP2A phosphatase is not required for deprotection if APC/C is active. We hypothesize that there may be different pools of the Sgo1-PP2A complex with different functions and the visualized signal of PP2A is not necessarily representing the protector (Indjeian et al., 2005; Peplowska et al., 2014).
Another prediction, that can be made according to the tension model, is that at metaphase II, cohesin is already deprotected. At this stage, biorientation has been

already established and sister kinetochores are under tension, thus PP2A-Sgo1 is expected to be separated from centromeric cohesin. Thus, the presence of centromeric cohesin relies only on the inhibition of separase by Pds1. If separase would be activated in this condition, it should lead to cohesin cleavage similarly as it occurs naturally. To evaluate this idea we used yeast cells with inactive APC/C-Cdc20 precisely in meiosis II (Mengoli *et al.*, 2021). These cells do not progress into anaphase II due to the inactivation of Cdc20, thus keeping a prolonged metaphase II arrest with bioriented chromosomes, similar to the oocyte arrest. To activate separase in the absence of active APC/C-Cdc20, we used ectopic degradation of Pds1. However, even with active separase, cells were not able to significantly remove cohesin, which suggests that even under tension centromeric cohesin is still protected. Only when separase was activated coupled with inactivation of protection components by degradation of Sgo1 (or inhibiting of Mps1 kinase), cohesin was cleaved in a fast and efficient manner. This result is the evidence of active protection during metaphase II. The presence of protection may be especially beneficial during metaphase II as it was previously shown that securin levels are lower in meiosis II compared to meiosis I (Jonak *et al.*, 2017). Therefore, separase is probably inhibited less efficiently in addition to some molecules of separase being active as a consequence of the law of mass action. Oocytes stay for a long time in the metaphase II-arrest with chromosomes already bioriented, which would mean they are deprotected according to the tension model, thus relying only on separase inhibition. In oocytes, securin levels are also lower during the metaphase II arrest compared to metaphase I (Marangos and Carroll, 2008; Nabti et al., 2008) similar to yeast. Thus, to keep centromeric cohesin intact, it might rely on protection as well. In support of this hypothesis, both Sgol2 and Mps1 are present not only at anaphase I but during the metaphase II arrest (El Yakoubi et al., 2017; Lee *et al.*, 2008).

Additionally, the role of tension in the deprotection of centromeric cohesin in mouse oocytes was assessed using an approach based on similar assumptions (Mengoli *et al.*, 2021). Combining tension defect, created by spindle depolymerization drugs or inducing monopolar spindle, together with APC/C activation by inhibiting SAC with reversine leads to cohesin removal in oocytes, similar to yeasts. This suggests that spindle tension is not required for deprotection in oocytes similar to yeasts. Surprisingly, the separation of sister chromatids was less efficient in the absence of tension even when centromeric

cohesin had already been removed. This suggested that tension has an additional function regarding chromosome segregation, other than just SAC inactivation as discussed below.

Furthermore, the tension model does not limit deprotection to metaphase II, but rather to the state of biorientation. Thus, whenever sister kinetochores would be bioriented, cohesin located at corresponding centromeres would become deprotected irrespective of the stage of cell division. This suggests, that deprotection of centromeric cohesin can occur, for example, even during SAC at entry into metaphase II. At least some of the chromosomes would already be properly connected and under tension, thus, they would be also deprotected. Any fluctuation of separase inhibition would lead to loss of centromeric cohesin there, and, as centromeric cohesin cannot be restored at centromeres if it has been lost, this would lead to premature disjoint of sister chromatids and the inability of proper segregation (Katis *et al.*, 2010).

3.3. Deprotection of centromeric cohesin at meiosis II

Protection of centromeric cohesin in yeast relies on Sgo1 and Mps1, thus deprotection is based on APC/C-dependent degradation of both Sgo1 and Mps1. The inability to degrade both of these proteins delays the cleavage of centromeric cohesin beyond spindle disassembly, leading to the development of diploid dyads (Arguello-Miranda *et al.*, 2017; Jonak *et al.*, 2017). In contrast, protection at anaphase I depends on Spo13, which is degraded during anaphase I and is not present in metaphase II (Arguello-Miranda *et al.*, 2017; Katis *et al.*, 2004; Lee et al., 2004).

This indicates that both Spo13 and Mps1 may be parts of the switch mechanism which makes deprotection possible only at anaphase II, but not earlier.

In mammalian oocytes, on the other hand, deprotection does not depend on the removal of Sgo2 and Mps1, as they stay on chromatin even after sister chromatids are separated (Gryaznova et al., 2021). This observation suggests that instead of being removed, Sgo2 and Mps1 are rather inactivated in some other way. The possible additional player here is Cdk1-cyclin B, as its activity is regulated by APC-mediated degradation of cyclin B. Cdk1 activity is required for Mps1 activity and localization(Hayward et al., 2019b; Morin *et al.*, 2012). It is also indicated that it may be important for Sgo2 ability to localize PP2A to cohesin similarly to Sgo1 during mitosis (Liu et al., 2013a; Liu et al., 2013b). Thus, the

inactivation of Cdk1 by APC/C-dependent degradation of cyclins may lead to the deprotection of centromeric cohesin by inactivating or releasing Sgol1-PP2A.

3.4. Regulation of the SAC at meiosis

We expected *mad2Δ* and *spo12Δ mad2Δ* strains to have similar timing of centromeric cohesin removal because both strains have the SAC compromised, therefore, the kinetics of cohesin removal are expected to be similar. However, in the *spo12Δ mad2Δ* strain, centromeric cohesin removal was delayed by 20 min, compared to *mad2Δ* single mutant. Furthermore, restoring tension back in *spo12Δ mad2Δ* removes this delay. This result may suggest that cohesin removal is requiring tension, as in cells without tension (*spo12Δ mad2Δ*) it is slower than in cells with (*mad2Δ*). However, we showed that in *spo12Δ mad2Δ* cells, all APC/C dependent events are delayed, not only cohesin removal. This means that cells lacking conventional SAC are still able to respond to tension defects with a SAC-like mechanism, by delaying APC/C activation. Recently, data from worms and yeasts proposed that Bub1 and Bub3 have separate functions in SAC-APC/C activity control in addition to their role in MCC formation (Kim *et al.*, 2017). Removing them did not lead to faster anaphase onset, as it happens in the case of Mad-proteins. Bub1-Bub3 are responsible for the recruitment of Cdc20 at the kinetochore, which can lead to two different results depending on tension status. In absence of tension, Cdc20 is directed to form MCC while when tension is present, Cdc20 would be dephosphorylated by the phosphatase PP1 present at kinetochores. Dephosphorylated Cdc20 is more likely to interact with APC/C, thereby promoting APC/C activity (Labit et al., 2012). As a result, in *spo12Δ mad2Δ cells*, APC/C-Cdc20 is less active and needs more time for activation, as Cdc20 is phosphorylated (Bancroft et al., 2020) and cannot be effectively dephosphorylated. Thus, we discovered another proof that SAC is more complicated than it was viewed before and consists of two branches: conventional SAC which is responsible for the formation of the MCC and the "new" mechanism, which regulates APC/C-Cdc20 activity through phosphorylation of Cdc20. Therefore, when SAC is active it produces an inhibitor MCC and keeps APC/C-Cdc20 less active, while when it is inactivated – it does not only stop inhibitor production but also promotes fast activation of APC/C by directing Cdc20 for dephosphorylation (Jia *et al.*, 2016). Interestingly, this

"new" SAC seems to take place only at meiosis II, but not at meiosis I (Mengoli *et al.*, 2021). It can be speculated that this difference depends on the different behavior of sister kinetochores during first and second meiotic divisions. At meiosis II they are bioriented, similarly to mitosis, and in both cases there is this dependence of APC/C activity on tension. On the other hand, at meiosis I sister kinetochores are monooriented, which might render this mechanism not functional.

3.5. The role of tension in chromatid segregation at meiosis II

As was previously mentioned our results demonstrate that the sole role of tension in the deprotection of centromeric cohesin is to activate APC/C-Cdc20 through silencing SAC and regulation of Cdc20 phosphorylation. However, cohesin cleavage is only one part of chromosome segregation. While it is not required for deprotection, we noticed that tension has an additional function in supporting the segregation of sister chromatids at anaphase II. We observed that in the absence of tension, chromatids disjoin less efficiently even when cohesin is fully removed. This suggests that even after cohesin cleavage, they remain loosely connected. It can be explained if taken into account that after replication sister chromatids are connected not only by cohesin but by DNA catenation as well. DNA catenation is resolved by topoisomerase II. There is evidence that catenation can provide some level of cohesion between sister chromatids when topoisomerase II is inhibited (Toyoda and Yanagida, 2006). Different parts of chromosomes are decatenating at different times: on chromosome arms, catenation is resolved when cells enter M phase, decatenation at centromeres can occur only after cohesin cleavage (Farcas *et al.*, 2011; Wang *et al.*, 2010b). Only under tension created by the spindle, the catenation of sisters can be effectively resolved, as the reaction catalyzed by topoisomerase II has no direction *per se* and can lead to both decatenation as well as catenation (Nitiss, 2009). In the absence of tension, a reaction catalyzed by topoisomerase II is not specifically directed towards decatenation, which reduces its efficiency. Additionally, tension may facilitate the binding of topoisomerase II to DNA by bending DNA strands, which would not happen in absence of bipolar spindle forces (Dong and Berger, 2007; Vologodskii *et al.*, 2001). As result, sister chromatids remain linked by DNA catenation even when cohesin is removed.

4. Materials and Methods

4.1. Yeast strains

Diploid *Saccharomyces cerevisiae* strains of the fast-sporulating SK1 genetic background (*MATa/MATα ho::LYS2 lys2 ade2Δ::hisG trp1::hisG leu2::hisG his3Δ::hisG ura3*) (Kane and Roth, 1974) were used to perform all experiments. These strains were obtained via mating of corresponding haploids. All mutations in experimental strains are homozygous unless stated otherwise. Strains genotypes are listed in Table 1. The next alleles have been described previously: *SPC42-eGFP*, *CNM67-tdTomato*, *CEN5-tetO224*, P_{URA3}*-tetR-tdTomato*, P_{GAL1}*-NDT80* (Matos et al., 2008), *ndt80Δ* (Okaz *et al.*, 2012), *REC8-mNeonGreen* (Arguello-Miranda *et al.*, 2017), *RTS1-eGFP, MTW1-mCherry* (Katis *et al.*, 2010), *spo12Δ*, *mam1Δ* (Buonomo *et al.*, 2003), *mad2Δ* (Chen *et al.*, 1999), *MAD2-mNeonGreen* (Rojas, 2019), *REC8-ha3*, P_{URA3}*-tetR-eGFP*, *BMH1-tetO224* (Oelschlaegel *et al.*, 2005), *mad1Δ* (Hardwick and Murray, 1995), *mad3Δ (Hardwick et al., 2000)* P_{GPD1}*-GAL4-ER* (Benjamin et al., 2003), P_{HIS3}*-mCherry-TUB1* (Khmelinskii et al., 2007), P_{CUP1}*-cdc20-3*, P_{HSL1}*-CDC20* (Jonak, 2020), P_{DMC1}*-cAMA1* (Arguello-Miranda *et al.*, 2017), *ama1Δ* (Oelschlaegel *et al.*, 2005)

To visualize specific proteins, they were tagged with RFP: mCherry or tdTomato (Shaner et al., 2004) or GFP: eGFP (Yang et al., 1996) or mNeonGreen (Shaner et al., 2013).

4.1.1. Strains constructions

SK1 strain containing P_{CUP1}*-OsTIR1-myc3* at *ura3* locus was used as parental to introduce *OsTIR1* into strains used in metaphase II arrest experiment (a gift from Neil Hunter; (Tang et al., 2015)).

For auxin-inducible degradation, *PDS1* and *SGO1* were C-terminally tagged with the fragment of *IAA17* (71–114 aa) – AID* (Morawska and Ulrich, 2013) together with a 30 amino acid linker.

Table 1. *Saccharomyces cerevisiae* SK1 strains used in this study

Figure	Strain[1]	Genotype[2]
7	Z33310	*SPC42/SPC42-eGFP::HIS3MX6 ura3::P_{HIS3}-mCherry-TUB1::URA3*
7	Z33309	*SPC42/SPC42-eGFP::HIS3MX6 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4*
8, 13	Z33261	*MAD2-mNeonGreen::KlTRP1 CNM67-tdTomato::NatMX4*
8, 13	Z33262	*MAD2-mNeonGreen::KlTRP1 CNM67-tdTomato::NatMX4 spo12Δ::NatMX4*
9, 14	Z32945	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3*
9, 14, 16	Z32944	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4*
10	Z33877	*ndt80Δ::NatMX4 leu2::P_{GAL1}-NDT80::LEU2 his3::P_{GPD1}-GAL4484-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3*
10	Z33878	*ndt80Δ::NatMX4 leu2::P_{GAL1}-NDT80::LEU2 his3::P_{GPD1}-GAL4484-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4*
11	Z33505	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4*
11	Z15736	*RTS1-eGFP::KanMX4 MTW1-mCherry::HphMX4*
11, 17	Z33506	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4 spo12Δ::NatMX4*
11, 17	Z34178	*RTS1-eGFP::KanMX4 MTW1-mCherry::HphMX4 spo12Δ::NatMX4*
13	Z33263	*MAD2-mNeonGreen::KlTRP1 CNM67-tdTomato::NatMX4 spo12Δ::NatMX4 mam1Δ::HIS3*
14	Z35329	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4 mam1Δ::HIS3*

15	Z33612	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO224::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2*
15	Z33613	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO224::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 spo12Δ::NatMX4*
15	Z33828	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 CEN5/CEN5::tetO224::HIS3 leu2/leu2::P_{URA3}-tetR-tdTomato::LEU2 spo12Δ::NatMX4 mad2Δ::KlURA3*
16, 19, 21	Z30454	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 spo12Δ::NatMX4 mad2Δ::KlURA3*
17	Z33507	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 MTW1-mCherry::HphMX4 spo12Δ::NatMX4 mad2Δ::KlURA3*
17	Z34176	*RTS1-eGFP::KanMX4 MTW1-mCherry::HphMX4 spo12Δ::NatMX4 mad2Δ::KlURA3*
18	Z34890	*REC8-ha3::URA3 CEN5/CEN5::tetO224::HIS3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KlURA3*
18	Z34891	*REC8-ha3::URA3 CEN5/CEN5::tetO224::HIS3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KlURA3 spo12Δ::NatMX4*
18	Z37473	*REC8-ha3::URA3 BMH1/BMH1:: tetO224::URA3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KlURA3*
18	Z37472	*REC8-ha3::URA3 BMH1/BMH1:: tetO224::URA3 leu2/leu2::P_{URA3}-tetR-eGFP::LEU2 mad2Δ::KlURA3 spo12Δ::NatMX4*
19, 21	Z30453	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KlURA3*
20	Z34026	*ndt80Δ::NatMX4 leu2::P_{GAL1}-NDT80::LEU2 his3::P_{GPD1}-GAL4484-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KlURA3*

20	Z34025	*ndt80Δ::NatMX4 leu2::P_{GAL1}-NDT80::LEU2 his3::P_{GPD1}-GAL4484-ER::HIS3 rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KlURA3 spo12Δ::NatMX4*
21	Z30618	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad2Δ::KlURA3 spo12Δ::NatMX4 mam1Δ::HIS3*
22	Z35132	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad1Δ::KanMX4 mad2Δ::KlURA3 mad3Δ::KlTRP1*
22	Z35133	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad1Δ::KanMX4 mad2Δ::KlURA3 mad3Δ::KlTRP1 spo12Δ::NatMX4*
22	Z35134	*rec8Δ::KanMX4::REC8-mNeonGreen::LEU2 ura3::P_{HIS3}-mCherry-TUB1::URA3 mad1Δ::KanMX4 mad2Δ::KlURA3 mad3Δ::KlTRP1 spo12Δ::NatMX4 mam1Δ::HIS3*
23	Z36435	*cdc20::P_{HSL}1-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4*
23	Z35713	*cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1Δ::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 PDS1-AID*::KanMX4 ura3::P_{CUP1}- OsTIR-myc3::URA3*
24	Z37076	*cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1D::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4*
24	Z37077	*cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1D::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4 ura3::P_{CUP1}- OsTIR-myc3::URA3*

24	Z37078	*cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1D::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4 PDS1- AID*::KanMX4*
24	Z37079	*cdc20::P_{HSL1}-CDC20::HphMX4 trp1::P_{CUP1}-cdc20-3::TRP1 ama1D::NatMX4 leu2::P_{DMC1}-cAMA1::LEU2 REC8-ha3::URA3 SGO1-AID*::KanMX4 PDS1- AID*::KanMX4 ura3::P_{CUP1}-OsTIR-myc3::URA3*

4.2. Time course experiment of synchronous meiosis

Entry into meiosis was induced at 30 °C as described before (Oelschlaegel *et al.*, 2005). Corresponding haploids were mated on YPD media and then streaked on YPG plates to pick healthy diploids for ~40 h. Then, single colonies were moved on fresh YPD plates to grow as 2×2cm patches for 24-28 h. Later, these patches were plated as even monolayers on full or half- YPD plates until they grew into the lawn (23-24 h). Cells were inoculated into 250 ml of YEPA in 2.8L flasks if protein samples were taken, or into 30 ml of YEPA in 500mL flasks, if only live-cell imaging was performed, to OD_{600} ~0.3 and incubated in an orbital shaker at 200 rpm for 12 h. After this, when cells reached OD600 ~1.6-1.8 and were arrested transiently in G1, cells were washed with one volume of SPM at 3600 rpm 3min to remove residues of YEPA and reinoculated in 100ml/10 ml of fresh SPM, resulting in final OD_{600} ~3.5. Samples for trichloroacetic acid (TCA) protein extracts, immunofluorescence, live-cell imaging, and cellular DNA content were taken at the indicated time points. All Od measurements were made on Ultrospec 2100pro UV/Visible Spectrophotometer (Biochrom) after cells were briefly sonicated.

Strains for the Ndt80 arrest-release experiment were induced to enter meiosis similarly to progressive cells. To release from Ndt80 arrest cells were treated with 5 μM estradiol at 7 h (at time point 0). Protein samples were taken every 30 min for 5 h and then every 40 min. A 5 mM stock solution of ß-estradiol (Sigma) was made in DMSO and stored at -20 °C until use.

Strains for the metaphase II arrest experiment were induced to enter meiosis at 25°C. To release from metaphase I arrest cells were treated with 10 μM $CuSO_4$ at 8h and 50 min later, cultures were shifted to 37°C to arrest cells in metaphase II

by inactivation of Cdc20-3. 70 min after release (at time point 0) cells were treated with 2mM indole-3-acetic acid (to induce auxin-inducible degradation) dissolved in DMSO.

4.3. Live-cell imaging

4.3.1. Experimental setup

Imaging of living cells undergoing meiosis was performed essentially as described (Okaz *et al.*, 2012). Cultures were induced to enter meiosis as described above. 250 µl of meiotic cell cultures were applied to 8-well Ibidi chamber slides (Ibidi, Ibitreat 80826) coated with Concanavalin A (Sigma C5275, 0.5 mg/ml in PBS) to give a density of 20-30 cells per field of view. Imaging was performed on a DeltaVision Ultra system composed of an Olympus IX71 microscope with autofocus, solid-state illumination, UplanSApo 100×/1.4 oil objective, DeltaVision filters, CoolSnap HQ2 camera, and an environmental chamber set to 30 °C.). For each frame stack of 8 1-µm intervals was taken in green and red/orange channels every 10 minutes with an intensity of 5-10% for green and 10-32% for red with an exposure time of 0.05-0.4 s. Additionally, a single image in white light was taken as a reference. After the experiment, acquired data were deconvolved and projected into a single 2-D image via SoftWoRx 5.0.

4.3.2. Data analysis

Images were analyzed and processed with Fiji software (https://fiji.sc/). For quantifications, cells in 6-12 fields (depending on cell density) of view were individually followed through meiosis. Percentages of meiotic events were then calculated for each time point using Microsoft Excel. The resulting graphs show percentages of cells passing specific meiotic events over time. In addition, sometimes these percentages were normalized to a specific event like entry into metaphase I or anaphase I as t=0. Representative cells were picked out from the original file and processed in Fiji using a stack combiner, time stamper, and montage tools.

4.4. Indirect immunofluorescence microscopy of chromosome spreads

Chromosome spreading was performed essentially as described (Loidl et al., 1998). 1ml of cell culture was collected and resuspended in 200µl of

spheroplasting solution (SPM+1.2M sorbitol) and incubated for 15 min at 30°C with 10 mM DTT. Then, cells were spheroplasted with zymolyase 100T (Seikagaku, 50 μg/ml) until 80-90% of cells burst when 2%SDS was added to the cell suspension. The process of spheroplasting was stopped with the addition of 1ml cold stop solution (0.1 M MES, 1mM EDTA, 0.5 mM $MgCl_2$, pH 6.4) and spheroplasts were centrifuged for 1 min, 3000 rpm, and gently resuspended in 100 μl of Stop solution. Then, on glass slides were added in succession: 20 μl of spheroplast suspension, 40 μl of fixative solution (4% paraformaldehyde, 3.4% sucrose), 80 μl of Lipsol (1%), and 80 μl of fixative solution. After each step of addition, samples were mixed briefly. Then spheroplasts were gently spread over the slide with a glass rod. Slides were dried at room temperature overnight and later kept at -20°C. For analysis, slides were washed in PBS buffer (50 mM potassium phosphate buffer pH 7.4, 0.15 M NaCl) for 15 min and then 3 times for 5 min. After, they were blocked in PBS containing 1% BSA for 1h. 100 μl of primary antibody dilutions were added on slides and incubated overnight at 4°C in a humid chamber. Then slides were washed 3 times in PBS buffer for 5 min, reblocked in PBS+1%BSA for 30 min, and incubated with 100 μl of secondary antibodies dilution for 2h. After this slides were washed 2 times in PBS, then mounted with 30 μl pd-DAPI (10% PBS, 1mg/ml phenylenediamine, 0.05 μg/ml DAPI, 90% glycerol, pH 8.0) and analyzed under a microscope.

The following primary antibodies were used: rat monoclonal antibodies to Ha (Roche 3F10, 1:40), rabbit antibodies against GFP ((Okaz *et al.*, 2012), 1:200), and mouse monoclonal antibodies against Tub4 ((Okaz *et al.*, 2012), 1:100). Secondary antibodies from goat anti-rat conjugated with CY3, goat anti-mouse CY5 (Abcam, 1:300 and 1:200 respectively), and goat anti-rabbit conjugated with Alexa488 (Invitrogen, 1:300) were used for detection. Cells were observed on an Axioskop 2 epifluorescence microscope with a 100x plan-apochromat 1.40 NA oil immersion objective lens (Carl Zeiss). Pictures were taken with a SPOT RT210 CCD camera (Diagnostic Instruments, Sterling Heights, MI) controlled by IPLab 3.5 software (Scanalytics) or with a Retiga Exi controlled by QCapture 2.9.12 software (QImaging) and processed with Adobe Photoshop. For quantifications, at least 100 spreads per sample were counted.

4.5. Analysis of Proteins

4.5.1. Preparation of Protein Extracts

To analyze protein levels, extracts prepared by trichloroacetic acid (TCA) precipitation were separated in SDS polyacrylamide gels followed by immunoblot detection of proteins. For each sample, cells from 8-10 ml of meiotic culture (OD_{600} ~3.5) were collected by centrifugation at 4000rpm for 2 min, washed in 1 ml 10% (TCA), and frozen in liquid nitrogen. Later, samples were thawed on ice and resuspended in 200 µl 10%TCA and an equal volume of zirconium beads (diameter 0.5mm) (Roth, 11079105z) was added to the samples. Cells were homogenized by shaking at 30 Hertz for 6 min at 4 C with a mixer mill (MM400 Retsch). Then 800ul of TCA was added and samples were moved to new tubes and centrifugated at 3000rpm for 10 min. the resulting pellet was resuspended in reducing sample (Laemmli) buffer (62.5 mMTris-HCl pH 6.8, 10% Glycerol, 2% SDS, 0.01% bromophenol blue, 30 mM β-mercaptoethanol) and then neutralized with 1 M Tris base solution. Samples were boiled at 95 ◦C for 10 min, then centrifuged at 14000 rpm for 10 min. Protein concentration in the supernatant was determined with a colorimetric Bio-Rad Protein Assay. Absorbance was measured at 595 nm with an Ultrospec 3100pro UV/Visible Spectrophotometer (Amersham Biocsience). Then samples were loaded on polyacrylamide SDS (SDS-PAGE) gels or were frozen in liquid nitrogen and stored at -80C.

4.5.2. SDS-PAGE and Western Blotting

Samples of 60-120µg of protein were separated in 8% (or 10% to detect Spo13) polyacrylamide SDS gels as described (Matos *et al.*, 2008). Gels were run at 35-45V for 12-16h. Semi-dry blotting (0.45 mA/cm2 for 1 h) was used to transfer proteins to a PVDF membrane (Immobilon P, Millipore). After transfer membranes were blocked in phosphate-buffer saline (PBS) with 0.1% Tween 20(PBS-T) and 4% non-fat milk powder (PBS-T/milk) for 1h and then were incubated with primary antibodies. After incubation membranes were washed 3 times in PBS-T/milk for 6-10 min each time and then incubated with secondary antibodies conjugated to horseradish peroxidase (1:5000) in PBS-T/milk. After, membranes were washed in PBS-T 3 times for 6-10 min each time and then incubated for 30 sec with ECL (ECL detection system, GE Healthcare) for 15-30

sec. Membranes were exposed to X-rayfilm and developed using an Optimax 2010 machine (Protec). The following primary antibodies were used:

- mouse antibodies were used to detect Pgk1 (Invitrogen, 1:40000)
- Rabbit antibodies were used to detect Ase1 (a gift from David Pellman, (Juang et al., 1997), 1:1000), Cdc5 ((Matos *et al.*, 2008), 1:5000), Cdc20 ((Camasses et al., 2003), 1:2000), Clb3 ((Schwickart et al., 2004), 1:3000), Ndt80 (a gift from Kirsten Benjamin, (Benjamin *et al.*, 2003), 1:5000), Sgo1 (a gift from Adam Rudner, (Lianga et al., 2013), 1:1,000), Spo13 ((Matos *et al.*, 2008), 1:5,000) and Rec8 ((Matos *et al.*, 2008), 1:10,000)
- goat antibodies were used to detect Clb1 Santa Cruz, sc-7647; 1:300) and Cdc14 (1:1000, sc-12045 Santa Cruz)

The primary antibodies were diluted in PBS-T/milk with 0.01% sodium azide and stored at -20 C.

List of abbreviations

AID – auxin-inducible degron
APC/C – anaphase-promoting complex/cyclosome
BSA – bovine serum albumin
CCAN – constitutive centromere associated network
COMA – Ctf19/Okp1/Mcm21/Ame1 complex
CPC – chromosomal passenger complex
DAPI - 4′,6-diamidino-2-phenylindole
DMSO – dimethyl sulfoxide
EDTA – Ethylenediaminetetraacetic acid
FEAR – Cdc fourteen early anaphase release
GFP – green fluorescent protein
IAA – indole-3-acetic acid
MCC – mitotic checkpoint complex
OD – optical density
PAGE - Polyacrylamide gel electrophoresis
PBS – phosphate buffered saline
RFP – red fluorescent protein
SAC – spindle assemble checkpoint
SDS – sodium dodecylsulfate
SMC – structural maintenance of chromosomes
SPB – spindle pole body
SPM – sporulation medium
TCA – trichloroacetic acid
UFB – ultrafine anaphase bridges
YEPA– yeast extract/peptone/potassium acetate medium
YPD – yeast extract/peptone/glucose medium
YPG – yeast extract/peptone/glycerol medium

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6. Contributions

Dr. Valentina Mengoli contributed to experiments presented in figures 10, 15, 16, 19, and 20.

Dr. Katarzyna Jonak contributed to experiments presented in figures 10, 20, 23, and 24.

The rest of the work presented in this thesis is my own.

Figures 3-5 were created with BioRender.com

7. Acknowledgment

Above all, I would like to thank my supervisor Dr. Wolfgang Zachariae for giving me a chance to join his lab, for scientific training and advice in writing this thesis.
I would like to thank Dr. Valentina Mengoli and Dr. Katarzyna Jonak, without whom this project would not be possible.
I would like to thank all other members of Zachariae's lab for their help, support, and fruitful discussions.
I would like to thank my thesis advisory committee, Dr. Christian Biertümpfel and Prof. Dr. Marc Bramkamp for their scientific expertise and support. As well, I thank Prof. Dr. Franz-Ulrich Hartl, Prof. Dr. Julian Stingele, Prof. Dr. Christof Osman, Prof. Dr. Johannes Stigler, Prof. Dr. Lucas Jae, and Prof. Dr. Klaus Förstemann for reviewing this thesis.
I am grateful to International Max Planck Research School for Molecular and Cellular Life Sciences for giving me an opportunity to do my PhD as a part of this graduate program.
I would like to thank my family and friends for their unlimited support, understanding, and care.