Chromosome Segregation in Budding Yeast: Sister Chromatid Cohesion and Related Mechanisms

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ABSTRACT Studies on budding yeast have exposed the highly conserved mechanisms by which duplicated chromosomes are evenly distributed to daughter cells at the metaphase–anaphase transition. The establishment of proteinaceous bridges between sister chromatids, a function provided by a ring-shaped complex known as cohesin, is central to accurate segregation. It is the destruction of this cohesin that triggers the segregation of chromosomes following their proper attachment to microtubules. Since it is irreversible, this process must be tightly controlled and driven to completion. Furthermore, during meiosis, modifications must be put in place to allow the segregation of maternal and paternal chromosomes in the first division for gamete formation. Here, I review the pioneering work from budding yeast that has led to a molecular understanding of the establishment and destruction of cohesion.

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DURING cell division, chromosomes must be replicated exactly and accurately distributed into daughter cells. The regulated sequence of events that leads to cell division is known as the cell cycle. In S phase of the cell cycle, DNA synthesis and the establishment of sister chromatid cohesion generates two identical sister chromatids that are held tightly together by a conserved protein complex, known as cohesin. In mitosis, the cohesin linkages provide resistance and generate tension to facilitate the attachment of sister chromatids to microtubules emanating from opposite poles. Once all the chromosomes have properly attached to microtubules, an enzyme known as separase becomes active and cleaves cohesin, thereby triggering the separation of sister chromatids to opposite poles (Figure 1). This process is modified during meiosis, which produces haploid gametes from a diploid progenitor cell. During meiosis, two rounds of chromosome segregation follow a single S phase. In meiosis I, the maternal and paternal chromosomes, called homologs, are segregated, whereas sister chromatids are segregated during meiosis II, which resembles mitosis (Figure 2). To achieve this, an additional layer of regulation must be introduced. While conserved in eukaryotes, what we know about the molecular biology of chromosome segregation is derived largely from work on the budding yeast *Saccharomyces cerevisiae*. Here I review the discoveries in budding yeast that led to an understanding of the molecular biology of chromosome segregation together with the exquisite controls that ensure its accuracy and the modifications that take place to generate gametes.

Building Mitotic Chromosomes

Structure and function of the cohesin complex

Discovery of cohesion: Pioneering studies in budding yeast were instrumental in the discovery of the chromosome segregation machinery that is conserved in all eukaryotes. Early on, it was recognized that the two sister chromatids must be held tightly together at metaphase to resist spindle forces, thereby allowing their attachment to microtubules from opposite poles. However, the nature of this cohesion was not known and two general models were put forward. One model postulated that the intertwining of sister DNA molecules, perhaps due to the persistence of catenations after DNA replication, might provide cohesion (Murray and Szostak 1985). Another, not mutually exclusive, model proposed the existence of proteins that generate molecular bridges between sister chromatids. However, testing these models relied on the establishment of an assay for sister chromatid cohesion. This was initially achieved by the development of a fluorescence *in situ* hybridization assay

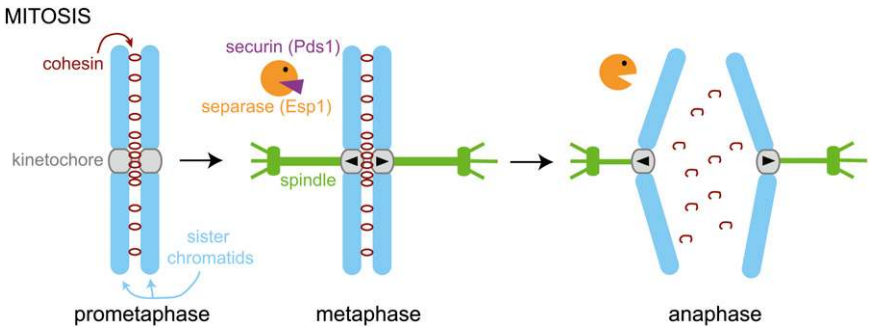

Figure 1 Chromosome segregation during mitosis. Schematic diagram showing the key features of chromosome segregation during budding yeast mitosis.

(FISH) in yeast (Koshland and Hartwell 1987). Using this assay, it was shown that minichromosomes are cohesed at metaphase even though they lack catenations, dispelling the idea that DNA catenation was sufficient to provide the "glue" (Koshland and Hartwell 1987; Guacci *et al.* 1994). This prompted the search for proteins that might mediate cohesion. A key technical development was the ingenious development of a method to label a single chromosome by integration of tandem repeats of bacterial *lacO*, to which ectopically produced LacI–GFP binds (Straight *et al.* 1996). A similar system was developed using *tetO* and TetR–GFP (Michaelis *et al.* 1997). The availability of these methods to label single chromosomes enabled the first cohesion proteins to be identified (Guacci *et al.* 1997; Michaelis *et al.* 1997). These elegant studies isolated mutants incapable of maintaining sister chromatid cohesion when arrested in mitosis. Subsequent studies revealed that sister chromatid cohesion genes fall into functional classes (Table 1). One class of genes encodes the proteins that make up the structural component of cohesion, called cohesin. Others are accessory, loading, or establishment factors. Remarkable progress has been made in understanding how these many gene products interact to generate sister chromatid cohesion.

The cohesin ring: The core structural component of cohesin forms a ring, composed of two structural maintenance of chromosome (SMC) proteins, Smc1 and Smc3, and a "kleisin" (from the Greek for closure) subunit, Scc1/Mcd1 (Guacci *et al.* 1997; Michaelis *et al.* 1997; Losada *et al.* 1998) (Figure 3). A meiosis-specific kleisin, Rec8, replaces Scc1 in meiotic cells and plays several roles important for the segregation of homologous chromosomes (see below). SMC proteins are conserved from prokaryotes to eukaryotes and are composed of globular N and C termini, joined by a large coiled-coil domain that is separated by a central "hinge" domain (Nasmyth and Haering 2005). Like bacterial SMC proteins, insect cell-produced yeast Smc1 and Smc3 fold back on themselves at the hinge region to form antiparallel intramolecular coiled coils (Melby *et al.* 1998; Haering *et al.* 2002). This arrangement juxtaposes the Walker A-containing N terminus and Walker B-containing C terminus of a single SMC protein to generate an ATP nucleotide binding domain (NBD) of the ABC family (Hopfner *et al.* 2000; Lowe *et al.* 2001). The N terminus of each SMC protein also contains a signature motif that is required for the activity of ABC family ATPases. Smc1 NBD crystallized as a dimer with ATP sandwiched between the Walker A motif of one monomer and the signature motif on the other. In reality, Smc1 and Smc3 heterodimerize at their hinge domains to create a V-shaped structure (Anderson *et al.* 2002; Haering *et al.* 2002). Therefore, the most likely arrangement is that two molecules of ATP are sandwiched between the Smc1 and Smc3 NBDs. Consistently, fluorescence resonance energy transfer (FRET) experiments indicated that Smc1 and Smc3 NBD domains are in close proximity (Mc Intyre *et al.* 2007).

The kleisin subunit, Scc1, forms a bridge between the NBDs of the Smc1–Smc3 heterodimer, making contacts with Smc3 at its N terminus and Smc1 at its C terminus (Haering *et al.* 2002). A crystal structure revealed that the Scc1 C terminus forms a winged helix domain that contacts the Smc1 NBD and mutations in this interface demonstrated that this interaction is essential (Haering *et al.* 2004). Interestingly, prior binding of the Scc1 C terminus to the Smc1 NBD is required for the Scc1 N terminus to bind the Smc3 NBD (Arumugam *et al.* 2003; Haering *et al.* 2004). This may ensure that a single molecule of Scc1 binds to the Smc1–Smc3 heterodimer. Although ATP binding to Smc1's NBD is required for binding to the Scc1 C terminus, the interaction of Scc1's N terminus with Smc3 does not require ATP (Arumugam *et al.* 2003; Gruber *et al.* 2003). An explanation for this observation is offered by the arrangement of a bacterial Smc–kleisin complex. While the C terminus of a bacterial kleisin contacts the ATPase head of one Smc protein, its N terminus associates with the coil-coiled domain of the other Smc subunit (Bürmann *et al.* 2013). It seems likely that Scc1 adopts a similar asymmetric arrangement in eukaryotic cohesin; however, confirmation will await structural analysis of the Smc3–Scc1 interaction.

The Scc3 subunit binds to the central domain of Scc1 and completes cohesin (Haering *et al.* 2002). Scc3 is essential for the establishment, though not the maintenance, of cohesion (Toth *et al.* 1999; Kulemzina *et al.* 2012). Similarly, Pds5 protein is also associated with cohesin and important for cohesion establishment (Hartman *et al.* 2000; Panizza *et al.* 2000; Kulemzina *et al.* 2012). Live cell imaging measurements of fluorescently tagged proteins suggest that Pds5, Smc3, and Scc3 exist on chromosomes in a 1:1:1 ratio (Chan

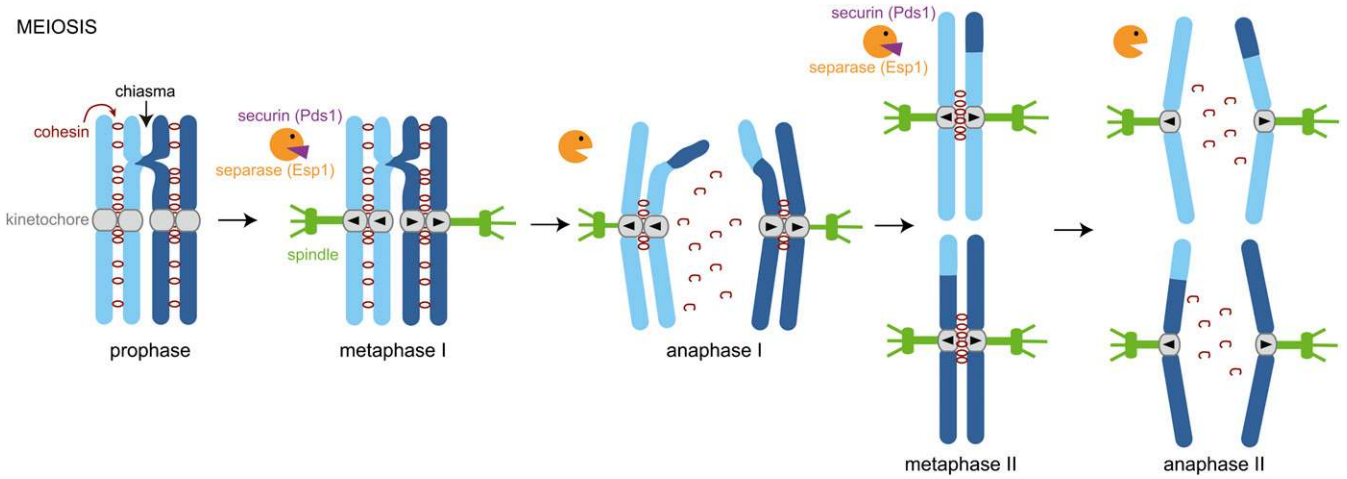

Figure 2 Chromosome segregation during meiosis. Schematic diagram showing the key features of chromosome segregation during budding yeast meiosis.

et al. 2012). However, the structures of Pds5 and Scc3 and their molecular function in cohesion establishment are not yet known.

How does cohesin hold chromosomes together?: The realization that the Smc1, Smc3, and Scc1 cohesin subunits form a ring-like structure *in vitro* led to the "embrace" model for cohesion (Haering *et al.* 2002). This model proposes that cohesion is the result of topologically embracing the two sister DNA molecules within a cohesin ring and that opening of the ring, due to cleavage of its Scc1 subunit by separase, liberates the sister chromatids, thereby destroying cohesion. Although this model has an attractive simplicity, others have argued that the ring structure of cohesin may not be the relevant cohesive form on chromatin and alternative models have been suggested (Haering *et al.* 2002; Milutinovich and Koshland 2003; Huang *et al.* 2005). Rather than interacting topologically with the DNA, these models suggest that cohesin binds to the DNA of one sister chromatid and then oligomerizes with one or more cohesin molecules bound to the other sister chromatid. Variations of these models include the "snap" model and "bracelet" models which postulate that cohesin oligomerization occurs through the coiled coil or hinge domain, respectively (Milutinovich and Koshland 2003; Huang *et al.* 2005).

Support for the idea that chromosome-bound cohesin is a ring came with the finding that cohesin subunits remain associated with each other, but not with the chromosomes, after cleavage within the coiled-coil domain of Smc3 or at the separase recognition sites in Scc1 (Gruber *et al.* 2003). Evidence that cohesion interacts topologically with the DNA came from experiments showing that cohesin is released from purified and cohesed circular minichromosomes after cleavage of either the DNA or cohesin (Ivanov and Nasmyth 2005, 2007). A more rigorous demonstration that cohesin interacts topologically with DNA came from experiments where all three interfaces in the cohesin ring were covalently sealed either by use of fusion proteins or the introduction of side chains that allowed specific chemical cross-linking of cohesin subunits (Haering *et al.* 2008). After protein denaturation, this chemically closed cohesin ring maintained its association with 2.3-kb or 26-kb circular minichromosomes, but not with a 42-kb linear minichromosome (Haering *et al.* 2008; Farcas *et al.* 2011). This provides further support for the topological embrace model and is consistent with the idea that sliding of cohesin along chromatin fibers is normally prevented by the presence of chromatin-bound proteins.

The fact that 26-kb circular and 42-kb linear minichromosomes, which, unlike 2.3-kb minichromosomes, are catenated *in vivo*, allowed Nasmyth and colleagues to finally test the contribution of DNA catenations to cohesion. Importantly, they found that the persistence of catenanes after S phase is dependent on cohesin (Farcas *et al.* 2011). Therefore, cohesin holds sister chromosomes together by preventing the resolution of catenanes, as well as through a direct topological embrace. Nevertheless, cohesin is sufficient to hold sister chromatids together in the absence of catenations, whereas the reverse is not true (Farcas *et al.* 2011). This argues that the direct topological embrace of sister chromatids by cohesin is its critical physical property.

Loading cohesin onto chromosomes

To provide cohesion, cohesin must first be loaded onto chromosomes before S phase. Loading of cohesin onto chromosomes requires a separate "loader" complex composed of Scc2 and Scc4 proteins (Ciosk *et al.* 2000). A DNA replication-coupled process converts loaded cohesin into functional cohesion (Uhlmann and Nasmyth 1998) after which Scc2/Scc4 are no longer required (Ciosk *et al.* 2000). Recently it has become apparent that cohesin loading occurs at preferred chromosomal sites that are recognized by Scc2/Scc4. Analysis of mutants in the Smc subunits of cohesin that disrupt ATP binding or hydrolysis have provided insight into the cohesin loading reaction.

Table 1 Genes involved in generating cohesion

Function	Gene	Features
Core cohesin subunit	Smc1	Coiled-coil ATPase
	Smc3	Coiled-coil ATPase
	Scc1/Mcd1/Rad21	Kleisin subunit, cleaved by separase
	Rec8	Meiosis-specific kleisin, replaces Scc1
Cohesin associated	Scc3	Cohesion establishment
	Pds5	Cohesion establishment
	Wpl1	Destabilizes cohesin's association with chromosomes
Cohesin loading	Scc2	Required for cohesin's association with chromosomes
	Scc4	Required for cohesin's association with chromosomes
Cohesion establishment	Eco1	Acetyl transferase, acetylates Smc3

The pattern of cohesin localization on chromosomes: Genome-wide studies have examined the localization of cohesion and its loader. Cohesin is present along chromosomes; however, it is not uniformly associated with all regions of the genome. Cohesin-associated regions (CARs) typically extend for 1–4 kb, are spaced at 2- to 35-kb intervals, and tend to correlate with intergenic regions between convergent genes (Blat and Kleckner 1999; Hartman *et al.* 2000; Laloraya *et al.* 2000; Glynn *et al.* 2004; Lengronne *et al.* 2004). However, the most notable feature of cohesin binding to chromosomes is its enrichment in a large (20–50 kb) region surrounding the small (~125 bp) centromere (Blat and Kleckner 1999; Megee *et al.* 1999; Tanaka *et al.* 1999; Glynn *et al.* 2004; Weber *et al.* 2004; Kiburz *et al.* 2005). This region of cohesin enrichment surrounding the centromere defines the budding yeast pericentromere, which differs from that in other eukaryotes in that it lacks heterochromatin. The enrichment of cohesin within the pericentromere is functionally important, as its absence leads to increased chromosome loss (Tanaka *et al.* 2000; Eckert *et al.* 2007; Fernius and Marston 2009; Ng *et al.* 2009). One clear role of pericentromeric cohesion is to facilitate the proper biorientation of sister chromatids on the metaphase spindle, perhaps by generating the appropriate geometry for this interaction (Ng *et al.* 2009). Additionally, pericentromeric cohesion is critical for accurate segregation during meiosis (see below).

Genome-wide mapping of Scc2 and Scc4 association reported a pattern that was distinct from that of cohesin (Lengronne *et al.* 2004). These sites of Scc2/Scc4 association likely represent cohesin-loading sites as it is here that cohesin is first detected upon cell cycle entry (Lengronne *et al.* 2004). Subsequently, cohesin translocates away from its loading sites to generate the pattern observed at metaphase (Lengronne *et al.* 2004). Transcription has been suggested to contribute to cohesin translocation and the chromosomal locations of cohesin are indeed altered by transcription, though it is unclear if this occurs as a result of cohesin sliding along the chromatin fiber or some other method of translocation (Lengronne *et al.* 2004; Bausch *et al.* 2007; Ocampo-Hafalla *et al.* 2007).

The chromosomal features that are recognized by Scc2/Scc4 and therefore define the sites of cohesin loading are not well understood. However, the best-studied site for cohesin loading is at the centromere. Initially, centromere sequences were found to promote cohesin recruitment to minichromosomes (Megee and Koshland 1999; Megee *et al.* 1999). Moreover, relocation of a centromere to a chromosomal arm site set up a domain of enriched cohesin surrounding the ectopic centromere, while eliminating cohesin enrichment at the endogenous pericentromere (Tanaka *et al.* 1999; Weber *et al.* 2004). Consistent with the idea that the centromere is a cohesin-loading site, it shows robust association of Scc2/Scc4 (Lengronne *et al.* 2004; Kogut *et al.* 2009; Hu *et al.* 2011). However, the extent of the Scc2/Scc4-associated domain in centromeric regions has been debated. Although one report suggested a similar profile of Scc2 and cohesin throughout the pericentromere (Kogut *et al.* 2009), others have found that Scc2/Scc4 is localized predominantly within the core (~125 bp) centromere, in a much narrower domain than cohesin (Lengronne *et al.* 2004; Hu *et al.* 2011). While the former report implies that cohesin loading occurs throughout the pericentromere, the latter suggests that cohesin loaded at the core centromere translocates into the pericentromere. In live cells, GFP-tagged cohesin forms a pericentromeric barrel between clustered sister kinetochores (Yeh *et al.* 2008). In contrast, Scc2–GFP forms foci that colocalize with kinetochores, consistent with distinct localizations of cohesin and its loader (Hu *et al.* 2011). Furthermore, cohesin appears at the core centromere earlier in the cell cycle than at the pericentromere (Fernius *et al.* 2013). The available evidence for cohesin enrichment at the pericentromere is therefore most consistent with the loading and translocation model, though mechanistic details are still lacking.

The factors that attract Scc2/Scc4 to specific sites on chromosomes are poorly defined. Regions of high transcriptional activity by PolI (tRNA genes), PolII, and PolIII (rDNA) are correlated with Scc2/Scc4 localization and specific induction of gene expression leads to Scc2/Scc4 recruitment at that site (D'Ambrosio *et al.* 2008b). However, the low level of Scc2/Scc4 at these sites has hampered analysis and the factors involved in its recruitment are not known. Recently, however, factors required for Scc2/Scc4 association with centromeres have been identified. The centromere directs the assembly of the kinetochore, a large multisubunit complex that mediates the binding of chromosomes to

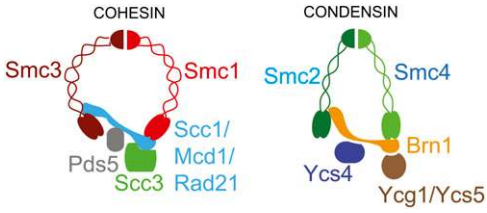

Figure 3 Cohesin and condensin structure. Models for the relative arrangements of subunits of cohesin and condensin are shown. Both complexes form a tripartite ring made up of two SMC proteins and a kleisin subunit, which is thought to make asymmetric contacts with the SMC proteins. Both complexes have additional subunits associated with the kleisin subunit, though their exact arrangement is not well defined.

microtubules (Westermann and Schleiffer 2013). Mutants lacking components of the Ctf19 kinetochore subcomplex (many of which are nonessential) fail to enrich cohesin within the pericentromere, though chromosome arm sites are not affected (Eckert *et al.* 2007; Fernius and Marston 2009; Ng *et al.* 2009; Fernius *et al.* 2013). Consistently, Ctf19 complex mutants show cohesion defects that are specific to centromere-proximal sites (Fernius and Marston 2009; Ng *et al.* 2009). Scc2/Scc4 fails to associate with the centromere in Ctf19 complex mutants (Ng *et al.* 2009; Fernius *et al.* 2013). However, artificially tethering Scc4 to the centromere rescues cohesin recruitment and the cohesion defects of Ctf19 complex mutants (Fernius *et al.* 2013). This demonstrates that the Ctf19 complex directs cohesin enrichment within the pericentromere by attracting Scc2/Scc4 to centromeres. Recently, the Ctf19 complex was shown to enable the association of the Dbf4-dependent kinase (DDK) with kinetochores from telophase to G1 phase (Natsume *et al.* 2013). Furthermore, kinetochore-associated DDK is required to attract Scc2/Scc4 to centromeres in late G1 to ensure proper cohesin enrichment in the pericentromere (Natsume *et al.* 2013). Interestingly, kinetochore-associated DDK is also independently important to promote the early replication of centromeres (Natsume *et al.* 2013). Centromeric DDK therefore couples early replication of the pericentromere with enhanced cohesin loading. However, it remains unclear how DDK attracts Scc2/Scc4 and cohesin loading to centromeres or whether DDK recruitment is the only critical role of the Ctf19 complex in cohesion establishment.

The cohesin loading reaction: How cohesin comes to topologically embrace DNA is still very mysterious but some critical steps in the loading reaction are beginning to emerge (Figure 4). The first step in cohesin loading is preassembly of the loading complex composed of cohesin and Scc2/Scc4. Although it was initially assumed that the Scc2/Scc4 loader complex was prebound to DNA, recently the association of Scc2 with centromeres, at least, was shown to require cohesin itself (Fernius *et al.* 2013). The complete tripartite cohesin ring, with ATP bound to both Smc heads (engaged state) together with Scc3, is required for cohesin loading (Arumugam *et al.* 2006; Hu *et al.* 2011). Cohesin ring assembly allows its interaction with Scc2/Scc4, which in turn enables the entire cohesin–Scc2/Scc4 complex to associate with centromeres and probably other loading sites too (Fernius *et al.* 2013). This explains why cohesin is not associated with chromosomes in early G1 or anaphase cells. Although Smc1, Smc3, and Scc2/Scc4 are all present in early G1 cells, Scc1 is produced only in late G1 and is cleaved in anaphase (Guacci *et al.* 1997; Michaelis *et al.* 1997; Ciosk *et al.* 2000; Uhlmann *et al.* 2000; Kogut *et al.* 2009). Therefore, Scc1 production upon cell cycle entry is the trigger for cohesin loading. The second step in cohesin loading is the transition from the state where the cohesin–Scc2/Scc4 complex has docked at its loading site to one where cohesin is encircling DNA and can translocate along it. This transition is blocked by mutations in the Smc1 and Smc3 ATPase heads that block ATP hydrolysis, demonstrating that ATP hydrolysis is important for this step (Hu *et al.* 2011). The notion that cohesin topologically embraces chromosomes predicts that the ring must be opened for its loading onto DNA. ATP hydrolysis is stimulated by Scc1 binding and this could facilitate cohesin ring opening (Arumugam *et al.* 2003). Interestingly, evidence suggests that the interfaces between the Smc1 and Smc3 NBD heads and Scc1 need not be opened for cohesin's association with DNA (Gruber *et al.* 2006). Instead, the most likely scenario is that cohesin opens at the interface between the hinge domains of Smc1 and Smc3 to allow DNA entry (Gruber *et al.* 2006). Given the separation of the site of ATP hydrolysis with the hinge domain by the long Smc coiled coils, this poses a conundrum: How could ATP hydrolysis influence opening of the hinge? To accommodate this idea, it has been suggested that the coiled-coil domains could fold back on themselves to bring the hinge in proximity of the site of ATP hydrolysis (Gruber *et al.* 2006; Hu *et al.* 2011). Intriguingly, insertional mutations in the loop structure of Smc1, situated close to the NBDs, do not affect cohesin's overall association with chromosomes but prevent cohesin enrichment in the pericentromere and CARs, in common with insertional hinge mutants (Milutinovich *et al.* 2007). This implicates the loop region in cohesin loading. Another possibility is that the NBD-proximal motifs in Smc1 are important for the interaction with Scc2/Scc4, which in turn enable ATP hydrolysis to drive a conformational change that leads to opening of the hinge (Figure 4). The hinge likely plays additional roles in cohesion establishment after loading since the crystal structure of the hinge revealed a positively charged channel in which neutralizing mutations caused loss of cohesion, though they did not affect either dimerization or cohesin loading (Kurze *et al.* 2011). Finally, fluorescence recovery after photobleaching (FRAP) experiments have shown that Scc2 turns over extremely rapidly at the centromere (Hu *et al.* 2011) and Scc2/Scc4 does not stably copurify with kinetochores (Fernius *et al.* 2013), suggesting that Scc2/Scc4 dissociates from the cohesin complex after the loading reaction is complete.

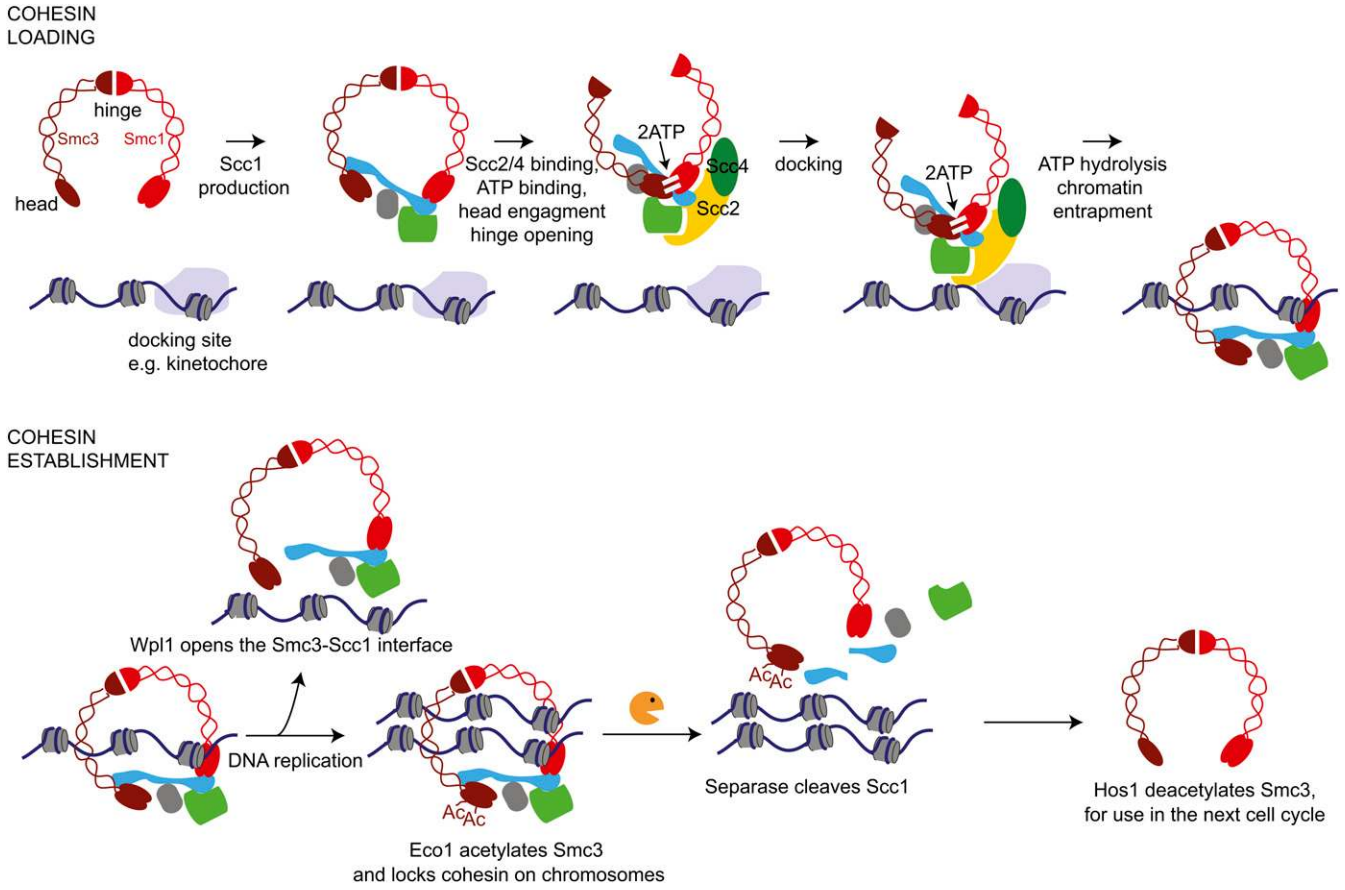

Figure 4 Cohesin cycle. Current model for cohesin loading and establishment. Cohesin loading involves opening of the Smc1 and Smc3 hinge and requires the Scc2/Scc4 protein and ATP binding to the SMC heads. ATP hydrolysis ensues and chromosome entrapment occurs. Cohesin is unstable on chromosomes due to the activity of Wpl1/Rad61, which opens the Smc3–Scc1 interface. Note that the contact point of Scc2/Scc4 on cohesin shown here is speculative. Eco1-dependent acetylation of Smc3 makes cohesin refractory to Wpl1, effectively locking the rings shut. Cohesin is destroyed during anaphase by cleavage of Scc1 by separase. Hos1 deacetylase recycles Smc1–Smc3 for use in the next cell cycle by removing the acetyl mark on Smc3.

Replication–coupled establishment of cohesion

Eco1-directed cohesion establishment: The Eco1 acetyltransferase (also called Ctf7) is not required for cohesin's association with chromosomes, but is needed during DNA replication for cohesion generation (Skibbens *et al.* 1999; Toth *et al.* 1999; Ivanov *et al.* 2002). A clue as to Eco1 function came from the observation that the lethality caused by deletion of the fission yeast *Schizosaccharomyces pombe* ortholog of *ECO1*, called *eso1*, is suppressed by loss of Pds5 function (which is nonessential in *S. pombe*) (Tanaka *et al.* 2001). This suggested a negative effect of Pds5 on cohesion establishment that is counteracted by Eco1. This idea was upheld in budding yeast with the identification of missense mutations in *SMC3*, *PDS5*, and *SCC3* as well as null alleles of *WPL1/RAD61*, all of which can suppress *eco1* loss-of-function mutations (Ben-Shahar *et al.* 2008; Unal *et al.* 2008; Zhang *et al.* 2008; Sutani *et al.* 2009; Rowland *et al.* 2009). The critical substrate of Eco1 is Smc3, which is acetylated on two residues K112 and K113 in its NBD (Ben-Shahar *et al.* 2008; Unal *et al.* 2008; Zhang *et al.* 2008; Rowland *et al.* 2009). Mutations in Smc3 K113 as well as nearby residues were among those found to suppress the loss of Eco1 function. This indicated that Smc3 is the only critical substrate of Eco1. The other three proteins in which mutations were found to suppress loss of Eco1 function, Pds5, Scc3, and Wpl1, form a complex that is loosely associated with cohesin. Based on the knowledge that Wpl1 is the ortholog of human WAPL1, which was known to promote cohesin's dissociation from chromosomes during prophase (Gandhi *et al.* 2006; Kueng *et al.* 2006), it was proposed that suppressor mutations in Pds5, Scc3, and Wpl1 abolish an "antiestablishment" activity of these proteins (Rowland *et al.* 2009). This model proposed that Pds5 and Scc3, while essential for cohesion establishment, would also have an additional cohesin-destabilizing effect by allowing access of Wpl1 to cohesin. Conversely, the Smc3 suppressor mutations are thought to make cohesin resistant to this destabilizing activity. This leads to the idea that acetylation of Smc3 by Eco1 counteracts the destabilizing activity of Wpl1 on cohesin, ensuring its maintenance on chromosomes (Figure 4).

Although the discovery of Smc3 as the key Eco1 substrate was a crucial step in understanding its molecular function,

how this contributes to cohesion establishment in S phase was still unexplained. Importantly, it was questionable whether Wpl1 really possessed a cohesin-destabilizing activity in budding yeast. Although loss of WAPL function in mammals leads to an increase in chromosomal cohesin, budding yeast lacking *WPL1* have impaired cohesion and cohesin levels are reduced on chromosomes (Warren *et al.* 2004; Rowland *et al.* 2009; Sutani *et al.* 2009). This contradiction was addressed by measuring the dynamicity of cohesin either using FRAP or its ability to be "anchored" outside the nucleus (Chan *et al.* 2012; Lopez-Serra *et al.* 2013). These studies showed that Wpl1 indeed promotes turnover of cohesin on chromosomes and that this is counteracted by Eco1-dependent acetylation of Smc3. The reduced levels of cohesin on chromosomes of *wpl1Δ* cells seem to be a result of generally decreased cellular levels, though the underlying cause remains unknown (Chan *et al.* 2012). Mutations in Scc3 and Pds5 that suppress loss of Eco1 function also reduce cohesin turnover on chromosomes (Chan *et al.* 2012). Some, but not all, of these mutations affect Wpl1 recruitment to cohesin (Sutani *et al.* 2009; Chan *et al.* 2012). Interestingly, measurements using GFP-tagged proteins suggested that although one molecule of each of Scc3 and Pds5 are associated with the cohesin ring, Wpl1 is substochiometric and highly dynamic (Chan *et al.* 2012). This suggests a catalytic mechanism of cohesin dissociation by Wpl1. Remarkably, fusing Scc1's N terminus to Smc3 suppresses lethality due to loss of Eco1 function and causes cohesin to be stable on chromosomes. This indicates that Wpl1 exerts its function by disrupting the interface between Scc1 and Smc3. Notably, this cohesin "exit gate" is distinct from the "entry gate," the hinge domain, involved in cohesin loading (Gruber *et al.* 2006; Chan *et al.* 2012). Acetylation of Smc3 by Eco1 locks the exit gate, thereby making cohesin refractory to the effects of Wpl1.

A key outstanding question was whether Wpl1 acts specifically to prevent the initial chromosome entrapment by cohesin (antiestablishment model) or whether it can also dismantle already established cohesin ("antimaintenance" model). Eco1 is not required after S phase for cohesion establishment (Skibbens *et al.* 1999; Toth *et al.* 1999) and is thought to travel with the replication fork, being recruited by PCNA (Lengronne *et al.* 2006; Moldovan *et al.* 2006). Moreover, Smc3 acetylation requires prior loading onto chromosomes and appears in S phase (Ben-Shahar *et al.* 2008; Unal *et al.* 2008; Zhang *et al.* 2008; Rowland *et al.* 2009; Sutani *et al.* 2009). Therefore, ordinarily, Eco1-dependent "locking" of cohesin at the exit gate is coupled to DNA replication. Although experiments in human cells had suggested that Eco1-dependent cohesin acetylation counteracts a replication fork-slowing activity of Wpl1 (Terret *et al.* 2009), there is no evidence for this in budding yeast (Lopez-Serra *et al.* 2013). Indeed, Wpl1 can cause dissociation of nonacetylated cohesin outside S phase, in G2, consistent with an antimaintenance activity of Wpl1 (Chan *et al.* 2012; Lopez-Serra *et al.* 2013). These experiments argue in favor of a model whereby competition between Scc2/Scc4-dependent cohesin loading and Wpl1-dependent cohesin dissociation creates a state of high cohesin dynamicity on chromosomes. Eco1-dependent cohesin acetylation at the replication fork locks cohesin around sister chromatids, rendering it refractory to Wpl1 activity and thereby stably bound to chromosomes.

What is the function of the dynamic nonacetylated pool of cohesin, since it is not participating in cohesion? Analysis of a *wpl1Δ* mutant, in which nonacetylated cohesin loses its dynamicity, revealed that chromosomes were more highly condensed. This suggests that cohesin turnover may be important to modulate the state of chromosome compaction. In contrast, Wpl1-dependent cohesin dynamicity does not contribute in a major way to cohesin's roles in transcription or meiosis (Lopez-Serra *et al.* 2013).

During anaphase, cohesin is deacetylated by the Hos1 deacetylase (Beckouët *et al.* 2010; Borges *et al.* 2010; Xiong *et al.* 2010). Cohesin release from chromosomes, as a result of its cleavage in anaphase, is essential for cohesin deacetylation, though Hos1 is present earlier (Beckouët *et al.* 2010; Borges *et al.* 2010). How chromosome-bound acetylated cohesin is "shielded" from Hos1 activity is unknown but Scc3 and Pds5 are likely to be involved in this. Cohesin deacetylation allows Smc1–Smc3 complexes to be recycled for cohesion establishment in the next S phase. Cohesin that is not deacetylated in anaphase, or a mutant version that mimics this state, loads onto chromosomes in the next cell cycle, but fails to establish cohesion. This indicates that cohesin must be acetylated *de novo* during S phase to lock rings shut.

Other factors involved in cohesion establishment: Eco1, by counteracting Wpl1, ensures the stability of cohesin on chromosomes, an activity that is likely to be important for the longevity of cohesion. However, in the absence of Eco1–Wpl1, budding yeast are viable and establish cohesion, albeit less robustly than that of wild-type cells (Ben-Shahar *et al.* 2008; Unal *et al.* 2008; Zhang *et al.* 2008; Rowland *et al.* 2009; Sutani *et al.* 2009). Furthermore, nonacetylatable fission yeast Smc3 (called Psm3) does not cause lethality, even in the presence of Wpl1 (Feytout *et al.* 2011). This reveals that cohesion exists without Eco1–Wpl1. If the fundamental process by which cohesion is established is independent of Eco1–Wpl1, how does this work? A simple explanation is that after loading, cohesin rings encircle a chromatin thread and that the DNA replication machinery passes through this ring to synthesize a sister chromatin thread, which is automatically contained within the ring. An alternative model is that factors associated with the replication machinery facilitate cohesin ring opening upon fork passage, and its reclosure in the wake of the polymerase. Several other factors are known to contribute to cohesion establishment, including Ctf18, Csm3, Tof1, Mrc1, Ctf4, and Chl1 but their roles are unknown (Hanna *et al.* 2001; Mayer *et al.* 2001; Mayer *et al.* 2004; Petronczki *et al.* 2004;

Skibbens 2004; Warren *et al.* 2004; Xu *et al.* 2007; Fernius and Marston 2009). Loss of each of these factors reduces cohesin acetylation to variable extents, but it is not known whether they are directly involved in facilitating Eco1 function or whether they participate in an unrelated establishment process that is a prerequisite for cohesin acetylation (Beckouët *et al.* 2010; Borges *et al.* 2013). Ctf18, at least may contribute to cohesin acetylation directly, since it is thought to help load PCNA, thereby providing a platform for Eco1 at the replication fork (Bermudez *et al.* 2003; Bylund and Burgers 2005; Lengronne *et al.* 2006). Other factors also function in replication-associated processes. Csm3, Tof1, and Mrc1 form a complex that travels with the replication fork and elicits stalling when barriers are encountered (Katou *et al.* 2003; Calzada *et al.* 2005; Tourriere *et al.* 2005; Bando *et al.* 2009). Ctf4 is an integral part of the replisome and helps to couple polymerase α/primase to the Mcm replicative helicase (Gambus *et al.* 2006, 2009). Chl1 is a likely DNA helicase whose molecular function is unknown (Gerring *et al.* 1990). Uncovering the molecular function of these factors in cohesion establishment will be an important priority for the future.

Recently, SUMOylation of several cohesin subunits has been found to occur in the window between cohesin loading and chromosome entrapment (Almedawar *et al.* 2012). SUMO, like ubiquitin, is a small protein modifier that is attached to lysines of target proteins, which can result in a range of effects including changes in localization, stability, or function (see Cubeñas-Potts and Matunis 2013 for review). A role for SUMOylation in the entrapment process was suggested by the observation that Scc1 fused to a SUMO-deconjugating enzyme reduced cohesin SUMOylation, which led to cohesion defects and lethality (Almedawar *et al.* 2012). SUMOylation is independent of acetylation and does not appear to counteract Wpl1, suggesting that it may function in an Eco1-independent pathway of cohesion establishment (Almedawar *et al.* 2012).

DNA damage-induced cohesion

Although cohesion establishment is ordinarily restricted to S phase, it can occur later in the cell cycle under conditions of DNA damage. Yeast cells that fail to establish cohesion in S phase are unable to repair damage caused by γ-irradiation (Sjogren and Nasmyth 2001). However, additional cohesin is also recruited post S phase to an ∼100-kb domain surrounding the damage site in a manner dependent on Scc2/Scc4 and the DNA damage checkpoint, and this is essential for repair (Strom *et al.* 2004; Unal *et al.* 2004). DNA damage not only triggers cohesin loading at the break site, but also, remarkably, genome-wide cohesion establishment in G2/M through Eco1 (Strom *et al.* 2007; Unal *et al.* 2007). However, the existence of separation-of-function mutations in *ECO1* that abolish damage-induced cohesion, but not S phase-induced cohesion suggested that Eco1 might work through different mechanisms in these two situations (Unal *et al.* 2007; Zhang *et al.* 2008). Indeed, rather than acetylate Smc3 as in S phase-generated cohesion, Scc1 is the likely target of Eco1 in G2/M in response to DNA damage (Heidinger-Pauli *et al.* 2008, 2009). The acetylation of Scc1 is thought to occur in response to its phosphorylation of the checkpoint kinase, Chk1 and like acetylation of Smc3, counteract Wpl1 activity (Heidinger-Pauli *et al.* 2008, 2009). Normally, Eco1 is limiting after S phase because Eco1 overexpression can cause cohesion establishment in G2/M phase (Unal *et al.* 2007). Stepwise phosphorylation of Eco1 by cyclin-dependent kinase (CDK), Dbf4-dependent Cdc7 kinase (DDK), and the GSK3 kinase, Mck1, triggers Eco1 ubiquitination by the SCF (Skp1, Cullin, F box) E3 ligase after S phase, leading to its degradation (Lyons and Morgan 2011; Lyons *et al.* 2013). A failure to degrade Eco1 increases cohesion at metaphase (Lyons and Morgan 2011). In the case of DNA damage, Eco1 degradation is prevented because Dbf4–Cdc7 is inhibited, probably as a result of its phosphorylation by the Rad53 checkpoint kinase (Lopez-Mosqueda *et al.* 2010; Zegerman and Diffley 2010).

Scc1 is also sumoylated in response to DNA damage by the SUMO E3 ligases Siz1, Siz2, and Nse2/Mms21 (a component of the cohesin-related Smc5–Smc6 complex) (McAleenan *et al.* 2012). The requirement for Scc1 SUMOylation in damage-induced cohesion seems to be at the establishment step, similar to its role in S phase cohesion (Almedawar *et al.* 2012; McAleenan *et al.* 2012).

Interestingly, a recent report implied that the cohesion that is induced genome-wide in response to DNA damage may have a different function to that that is built around the break site. The translesion synthesis polymerase, Polη (*RAD30* in budding yeast) is required for genome-wide, but not local, damage-induced cohesion, perhaps by facilitating Scc1 acetylation (Enervald *et al.* 2013). It was observed that genome-wide cohesion generation in G2/M appears to be important not for repair, but for segregation, leading to the proposal that it may be needed to reinforce S phase cohesion (Enervald *et al.* 2013). Consistent with this idea, cohesin, which established cohesion in S phase, has been reported to be removed genome-wide upon DNA damage in G2/M (McAleenan *et al.* 2013). This removal of cohesin seems to be required for the efficient repair of DNA lesions by allowing access of repair proteins. Surprisingly, damage-dependent removal of cohesin in G2/M was reported to depend on cleavage of its Scc1 subunit by separase (McAleenan *et al.* 2013). This is unexpected because global separase activation must be prevented until all chromosomes have achieved biorientation otherwise mis-segregation will occur, resulting in aneuploidy. The prediction is that separase activity or cohesin cleavage must be locally regulated to spare some cohesion from destruction. Future work will be required to fully illuminate the mechanisms underlying the role of cohesion in DNA-damage repair.

Other structural components of chromosomes

Following the duplication of chromosomes and the establishment of linkages between them, sister chromatids must

be prepared for their segregation during mitosis. Chromosomes are organized into rigid structures by condensation and DNA molecules are resolved from each other, while the linkages between sister chromatids are maintained. The key players in this process are the cohesin-related condensin complex and topoisomerase II (Earnshaw *et al.* 1985; Gasser *et al.* 1986; Hirano and Mitchison 1994; Strunnikov *et al.* 1995). Although dramatic structural changes in chromosome organization cannot be observed directly in the small yeast nucleus, both condensin and topoisomerase II are essential for chromosome segregation, suggesting they perform similar functions in yeast too (Dinardo *et al.* 1984; Holm *et al.* 1985; Strunnikov *et al.* 1995; Bhalla *et al.* 2002).

The condensin complex: The condensin complex is related to cohesin but it is much less well understood (Thadani *et al.* 2012). Defective condensin leads to reduced chromosome compaction and a failure of chromosomes to segregate during anaphase (Freeman *et al.* 2000; Bhalla *et al.* 2002; Lavoie *et al.* 2004). However, the molecular function of condensin in chromosome segregation remains unclear.

In mammals, two distinct condensin complexes (condensin I and condensin II) exist. Condensin II mediates the premitosis condensation of chromosomes, whereas condensin I assembles resolved metaphase chromosomes after nuclear envelope breakdown at the start of mitosis (Hirano 2005). Budding yeast possesses only condensin I, composed of two Smc subunits (Smc2 and Smc4), a kleisin (Brn1), and two HEAT (huntingtin–elongation factor 3–protein phosphatase 2A–TOR1) repeat-containing subunits (Ycs4 and Ycg1/Ycs5) (Figure 3) (reviewed in Hirano 2012).

Defective condensin causes severe chromosome segregation defects characterized by chromosome bridges during anaphase (Saka *et al.* 1994; Strunnikov *et al.* 1995; Bhat *et al.* 1996; Hudson *et al.* 2003; Ono *et al.* 2004; Oliveira *et al.* 2005; Gerlich *et al.* 2006). A likely activity of condensin that allows it to perform its function is the bringing together of distant DNA sequences of the same molecule. Thus, unlike cohesin, which forms intersister linkages, condensin is thought to build intrasister linkages and stabilize chromatin loops (Cuylen *et al.* 2011; Cuylen and Haering 2011; Thadani *et al.* 2012). Condensin forms a ring-like structure, similar to cohesin, although the hinge dimerization interface may adopt a distinct geometry (Anderson *et al.* 2002) (Figure 3). Furthermore, like cohesin, condensin can topologically embrace a minichromosome (Cuylen *et al.* 2011). Condensin can also bind DNA and introduce positive supercoils (Kimura *et al.* 1999; St-Pierre *et al.* 2009) This has led to the proposal that condensin may act enzymatically, rather than structurally, and drive chromosome compaction through the introduction of positive supercoils (Baxter and Aragón 2012). This idea has allowed an extension of the loop stabilization model that can explain why condensin specifically links regions of the same DNA molecule (Baxter and Aragón 2012).

Condensin is highly enriched at centromeres, pericentromeres, telomeres, and the rDNA (D'Ambrosio *et al.* 2008b). Condensin has also been reported to associate with genes transcribed by RNA PolI such as tRNAs and 5S rDNA, and the association with tRNAs was found to be partially dependent on the Scc2/Scc4 cohesin loader complex (D'Ambrosio *et al.* 2008b). However, overall chromosomal condensin levels are not grossly affected by Scc2 inactivation (Ciosk *et al.* 2000), suggesting that the relationship between Scc2/Scc4 and condensin may not be direct. The recruitment of condensin to the rDNA depends on the replication fork block protein, Fob1, as well as the monopolin proteins, Lrs4 and Csm1, which have roles also at the kinetochore during meiosis (see below) (Johzuka and Horiuchi 2009). Indeed, the fission yeast monopolin proteins, Mde4 and Pcs1, are important for condensin association with centromeric regions (Tada *et al.* 2011); however, this is not the case in budding yeast (Brito *et al.* 2010), and the factors responsible to centromeric/pericentromeric condensin association in budding yeast remain unknown. Notably, while condensin is associated with the rDNA throughout the cell cycle, it begins to colocalize with kinetochores from around the time of S phase but is absent at anaphase onset (Bachellier-Bassi *et al.* 2008).

Topoisomerase II: Topoisomerase II catalyzes the ATP-dependent transport of one DNA double helix through another to relieve both negative and positive supercoils (Wang 2002). In mammalian cells, topoisomerase II is required for the individualization of chromosomes prior to mitosis (Giménez-Abián *et al.* 2000) and evidence that budding yeast Top2 helps condense chromosomes was obtained using a *lacO*–LacI–GFP reporter system (Vas *et al.* 2007). The activity of Top2 is required prior to mitosis to remove catenates generated as a result of two converging replication forks colliding (Holm *et al.* 1985). A failure to remove these catenates is manifest during anaphase where chromosome bridges are observed (Holm *et al.* 1985). Interestingly, proper cohesion at centromeres depends on SUMOylation and Top2 appears to be an important target (Bachant *et al.* 2002). This implies that modulating chromosome topology through Top2 is also important for proper cohesion.

Establishment of Biorientation

Having prepared a pair of duplicated chromosomes, the next step in segregation is their attachment to the mitotic spindle. In budding yeast, each kinetochore has a binding site for a single microtubule (Winey *et al.* 1995). This means that the erroneous situation where a kinetochore attaches to microtubules from the same pole (merotelic attachment) is impossible in budding yeast. However, it is still possible that sister kinetochores attach to microtubules from the same pole (syntelic attachment) and this must be avoided. The equal segregation of sister chromatids to daughter cells will occur only when sister kinetochores are attached to microtubules from opposite poles (amphitelic attachment or biorientation) (Figure 5).

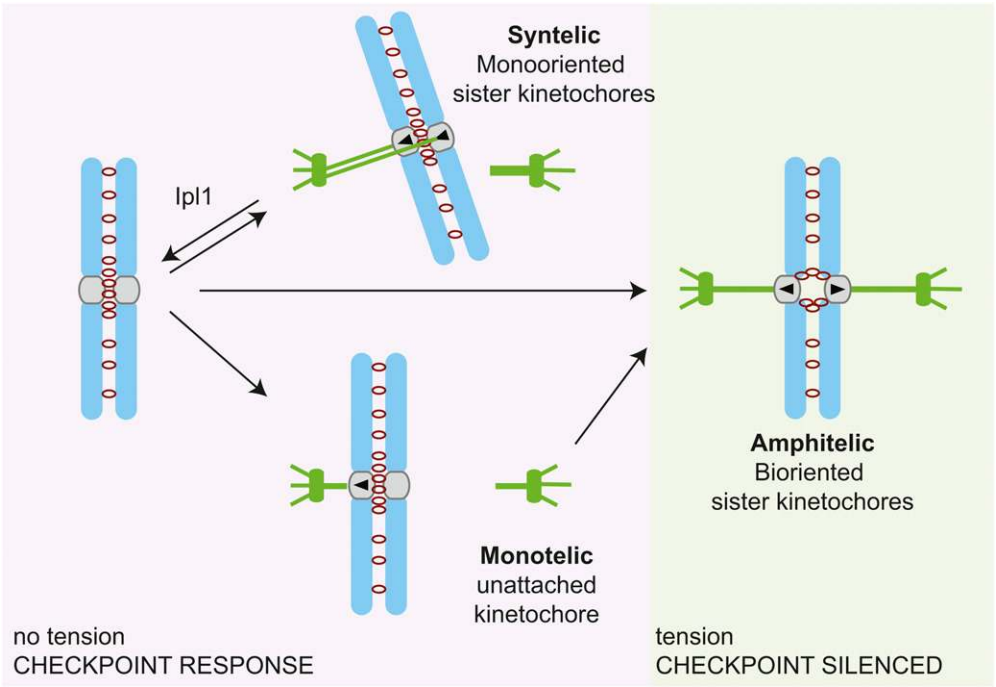

Figure 5 Establishment of biorientation. The possible modes of kinetochore attachment en route to biorientation in mitosis are shown schematically. For details see text.

The steps leading to kinetochore capture by microtubules have been reviewed recently and will be summarized only briefly here (Tanaka 2010). In budding yeast, kinetochores remain bound to microtubules throughout the cell cycle, detaching transiently only for a short window as DNA is replicated and kinetochores reassemble (Winey and O'Toole 2001; Kitamura *et al.* 2007). The majority of initial kinetochore–microtubule interactions are syntelic, and Ipl1 is required early in mitosis to sever these connections and provide an opportunity for amphitelic attachment to be established (He *et al.* 2000; Biggins and Murray 2001; Tanaka *et al.* 2002). Unattached kinetochores are captured as follows (Tanaka 2010). First, kinetochores attach to the lateral side of microtubules, either directly or via a microtubule nucleated from the kinetochore, a process that involves the XMAP215/ch-TOG protein, Stu2 (Kitamura *et al.* 2010; Gandhi *et al.* 2011). Second, the captured kinetochore is transported along the microtubule toward the spindle pole body (SPB) by the kinesin-14 protein, Kar3 (Tanaka *et al.* 2005, 2007). The lateral attachment of the kinetochore is then converted into an end-on attachment to a SPB-derived microtubule, which requires a conserved loop on the Ndc80 kinetochore protein (Hsu and Toda 2011; Maure *et al.* 2011; Zhang *et al.* 2012). Upon end-on attachment, loading of the Dam1 complex that can couple the kinetochore to a shrinking microtubule occurs (Westermann *et al.* 2006; Tanaka *et al.* 2007). Third, capture of the sister kinetochore occurs. The mechanisms that ensure that sister kinetochores are captured by microtubules from opposite poles to achieve biorientation are summarized below.

Role of kinetochore geometry and centromere structure

Do sister kinetochores adopt a particular geometry that helps facilitate their attachment to opposite poles? There is no doubt that the pericentromeric chromatin surrounding the kinetochore has specialized properties that could facilitate biorientation through establishment of a preferred geometry for capture by microtubules. The first indication of a specialized pericentromeric structure was the observation using GFP-labeled centromeres, that microtubule tension at metaphase was sufficient to pull sister centromeres apart prior to separase activity and cohesin cleavage (Goshima and Yanagida 2000; He *et al.* 2000; Tanaka *et al.* 2000). The domain of separation extends for ~10 kb on either side of the centromere and sister chromosomal arm sequences remain associated at metaphase. This observation has posed the question: What happens to cohesin during the tension-dependent separation of the pericentromere? One possibility is that cohesin is removed in this region. Indeed, chromatin immunoprecipitation (ChIP) experiments have suggested that pericentromeric cohesin levels are reduced when sister centromeres are under tension compared to those not under tension (Eckert *et al.* 2007; Ocampo-Hafalla *et al.* 2007). However, a pericentromeric barrel of cohesin is clearly visible by microscopy at metaphase, indicating that a substantial amount of cohesin remains associated with the pericentromere (Yeh *et al.* 2008; Hu *et al.* 2011). This poses a paradox: in this situation, where sister centromeres separate over distances of 2–4 μm, it seems impossible that they are trapped within the same cohesin ring. One proposal that could reconcile these observations is that pericentromeric cohesin forms intramolecular, rather than intermolecular, linkages. This would enable the pericentromere to adopt a cruciform structure with sister kinetochores protruding in opposite directions (Yeh *et al.* 2008). Cohesin and condensin together with pericentromeric chromatin constitute a "mitotic chromosome

spring" that balances spindle forces and allows the generation of tension (Stephens *et al.* 2011, 2013). The spring-like properties of the pericentromere are thought to be key to the detection of tension upon biorientation. A further attraction of the cruciform model is that it could be envisaged to facilitate a "back to back" geometry of sister kinetochores, thereby enabling their efficient capture from microtubules from opposite poles.

But is kinetochore geometry actually important for biorientation? The observation that sister kinetochores are inherently biased to biorient on the mitotic spindle argues for this possibility (Indjeian and Murray 2007). Defective kinetochore geometry could also explain why cells lacking cohesin enrichment within the pericentromere are slow to achieve biorientation and rely on the error correction machinery (Ng *et al.* 2009). However, it is equally possible that enriched pericentromere cohesin promotes biorientation by strengthening intersister cohesion to facilitate the generation of tension.

Although it is likely that kinetochore geometry facilitates biorientation, it is clear that it cannot be the only important factor and tension-sensing-based mechanisms exist. Indeed, tension is sufficient to allow biorientation in the absence of a back-to-back sister kinetochore configuration because dicentric chromosomes with physically separated kinetochores achieve biorientation (Dewar *et al.* 2004). Furthermore, reconstituted kinetochore–microtubule attachments persist longer under force, indicating that tension directly mechanically stabilizes them (Akiyoshi *et al.* 2010). In practice, sister kinetochore geometry is likely to increase the probability that initial attachments are made to opposite poles.

Error correction

While tension stabilizes kinetochore–microtubule attachments, conversely, a lack of tension leads to the destabilization of kinetochore–microtubule attachments, providing a further opportunity for the correct attachments to be made. Central to this "error correction" process is the Aurora B kinase (Biggins *et al.* 1999; Tanaka *et al.* 2002). Aurora B is the kinase constituent of the chromosomal passenger complex (CPC) that also contains INCENP (Sli15), Survivin (Bir1), and Borealin (Nbl1) (reviewed in Carmena *et al.* 2012). Ipl1 phosphorylates a number of substrates in the outer kinetochore that are thought to prevent interactions with microtubules (Cheeseman *et al.* 2002; Akiyoshi *et al.* 2009a; Demirel *et al.* 2012). Since Ipl1 is associated with the inner kinetochore, it has been proposed that tension physically separates Ipl1 from its substrates, thereby allowing their dephosphorylation and silencing of the error correction machinery (Tanaka *et al.* 2002; Keating *et al.* 2009). Although not directly tested in budding yeast, support for this model has been obtained from work in mammalian cells (Liu *et al.* 2009; Welburn *et al.* 2010). In contradiction of this model, Campbell and Desai (2013) recently described a truncated form of budding yeast Sli15, which does not accumulate at centromeres but rather associates with microtubules and chromatin, yet is proficient for tension-sensing and chromosome biorientation. Presumably, though not properly regulated, sufficient CPC accumulates at centromeres to disrupt incorrect attachments. This demonstrates that tight regulation of CPC localization at centromeres may not be essential under normal circumstances (Campbell and Desai 2013)

The Shugoshin (Sgo1) protein, which is associated with the budding yeast pericentromere (in the same region as the enriched cohesin) (Kiburz *et al.* 2005), also contributes to biorientation (Indjeian *et al.* 2005; Indjeian and Murray 2007). Sgo1 is recruited to the pericentromere through phosphorylation of H2A by Bub1 kinase (Kawashima *et al.* 2010). This explains a requirement for the Bub1 kinase domain and residue S121 on H2A as well as several residues on H3 in biorientation (Fernius and Hardwick 2007; Kawashima *et al.* 2010). In fission yeast the Shugoshin paralog, Sgo2, similarly promotes biorientation during mitosis, where its role appears to be recruitment of Aurora B (called Ark1 in *S. pombe*) to centromeric regions (Kawashima *et al.* 2007; Vanoosthuyse *et al.* 2007). Although CPC subunits colocalize with kinetochores in *sgo1Δ* cells in budding yeast (Kiburz *et al.* 2008; Storchová *et al.* 2011), it seems likely that Sgo1 affects biorientation through Ipl1 too. Indeed, the ability of truncated Sli15, which clusters on microtubules, to rescue the biorientation defects of *sgo1Δ* cells, is consistent with Sgo1 promoting biorientation through the CPC (Campbell and Desai 2013). One possible scenario is that there are multiple ways by which Ipl1 can be recruited to centromeres and that Sgo1 only promotes Ipl1 association under certain conditions, for example in response to a lack of tension. Indeed, Ipl1 is essential, presumably due to a need to sever the attachment of kinetochores to SPBs (Tanaka *et al.* 2002), whereas Sgo1 is not. Consistently, Bir1 CPC subunit is also recruited to the kinetochore through a direct interaction with the kinetochore protein, Ndc10 (Yoon and Carbon 1999; Cho and Harrison 2012).

The Mps1 kinase is also essential for biorientation and triggers checkpoint arrest both in response to unattached kinetochores and a lack of tension (Maure *et al.* 2007; Liu and Winey 2012). This can be explained, at least in part, by a requirement for Mps1 for Bub1 association with the kinetochore, allowing, in turn, canonical checkpoint activation and presumably Sgo1 and Ipl1 recruitment to centromeres (Fernius and Hardwick 2007; Storchová *et al.* 2011; London *et al.* 2012). However, Mps1 is likely to play additional, possibly more direct, functions in biorientation and, consistently, Mps1 substrates in the outer kinetochore have been identified (Shimogawa *et al.* 2006; Kemmler *et al.* 2009).

The destabilization of kinetochore–microtubule attachments that are not under tension not only provides an opportunity for reorienting these attachments but also serves to arrest the cell cycle until all errors are corrected. Neither Sgo1 nor Ipl1 are required to arrest the cell cycle in the presence of unattached kinetochores in budding yeast, though both proteins are required for a cell cycle delay in

response to a lack of tension (achieved experimentally by preventing replication or cohesion establishment) (Biggins and Murray 2001; Indjeian *et al.* 2005). This led to the proposal that Sgo1 and Ipl1 indirectly elicit a checkpoint response in response to tension defects by generating unattached kinetochores (Pinsky *et al.* 2006). Ipl1 may additionally potentiate the checkpoint signal in response to unattached kinetochores. Indeed, mutation of sites in Mad3 that are phosphorylated by Ipl1 *in vitro* abrogates the checkpoint response to lack of tension, but not to unattached kinetochores (King *et al.* 2007a).

Destruction of Sister Chromatid Cohesion and Anaphase Onset

The cohesion built in S phase holds sister chromatids together until their separation at anaphase onset. Crucially, cohesion provides the resistance to spindle microtubule forces, enabling sister chromatids to attach to opposite poles at metaphase. It is essential that cohesion is not destroyed until all chromosomes are properly attached to microtubules. A surveillance mechanism, known as the spindle checkpoint, senses improperly attached chromosomes and prevents separase activation to ensure that it is the case. Once biorientation is achieved, the spindle checkpoint is satisfied, separase is activated, and anaphase proceeds.

Cleavage of cohesin by separase

Cohesin destruction requires the activity of an E3 ubiquitin ligase known as the anaphase promoting complex, or cyclosome (APC/C) (Irniger *et al.* 1995). The APC/C is a huge molecular machine that attaches ubiquitin, a small protein modifier, to lysine residues of target proteins (see Peters 2006 for review). Polyubiquitinated substrates are recognized by the 26S proteasome, which mediates their destruction. The critical substrate of the APC/C at anaphase onset is not cohesin, but an anaphase inhibitor, known as securin or Pds1 (Cohen-Fix *et al.* 1996; Funabiki *et al.* 1996; Ciosk *et al.* 1998). The APC/C also targets mitotic cyclin for degradation at the metaphase–anaphase transition but this is not required for chromosome separation in budding yeast (Surana *et al.* 1993). Securin binds to and inhibits separase (Esp1), a cysteine protease that is required for sister chromatid separation (Ciosk *et al.* 1998). It is cleavage of the Scc1 subunit of cohesin by separase that is the trigger for sister chromatid separation. Mutation of the separase recognition sites in Scc1 prevents sister chromatid segregation, though securin is still destroyed (Uhlmann *et al.* 1999). Moreover, artificial production of the tobacco etch virus (TEV) protease in cells where the only copy of Scc1 has TEV-cleavage sites triggers chromatid separation (Uhlmann *et al.* 2000). Therefore, Scc1 cleavage is both necessary and sufficient for chromosome segregation. Given that cleavage of Scc1 will result in opening of the ring, it is easy to envisage why this causes the release of cohesin from chromosomes (Gruber *et al.* 2003).

The spindle checkpoint

Inhibition of APC–Cdc20: Broadly speaking, there are two elements to the surveillance mechanisms that ensure an accurate anaphase. First, cell cycle progression must be halted until all the proper attachments have been generated. This is the role of a checkpoint, known as the "spindle assembly checkpoint." Second, erroneous attachments, that is, where both sister kinetochores have attached to microtubules from the same pole (syntelic attachment), must be prevented or corrected. Syntelic attachments fail to generate tension and are destabilized by the error-correction machinery, providing a further opportunity for sister kinetochores to attach to microtubules from opposite poles (ampitelic attachment or biorientation).

The spindle checkpoint targets the APC to prevent anaphase onset in the presence of unattached kinetochores by stabilizing securin, thereby maintaining separase inhibition (reviewed in Lara-Gonzalez *et al.* 2012) (Figure 6). For the APC to be active, it must associate with a so-called "coactivator" that is thought to present specific substrates to the APC for ubiquitylation. In vegetatively growing budding yeast, there are two possible coactivators, Cdc20 and Cdh1. APC–Cdc20 is responsible for triggering securin degradation and, consequently, Cdc20 is essential for anaphase onset. Cdc20 is also the crucial target of the spindle checkpoint (Hwang *et al.* 1998). Cdh1 is not required for chromosome segregation, but is activated later in the cell cycle where it promotes mitotic exit by targeting cyclins for degradation (Visintin *et al.* 1997). Degron motifs known as D (destruction) boxes and KEN boxes on substrates are bound by recognition sites for these motifs on Cdc20 or Cdh1 (Peters 2006).

Genetic screens in budding yeast identified the components of the spindle checkpoint that are conserved throughout eukaryotes. The isolation of mutants that failed to arrest when microtubules were disrupted by drugs led to the identification of the "budding uninhibited by benzimidazole" (*BUB*) and "mitotic arrest deficient" (*MAD*) genes (Hoyt *et al.* 1991; Li and Murray 1991). Together with the Mps1 kinase (Winey *et al.* 1995; Weiss and Winey 1996) Mad1, Mad2, Mad3 (BubR1), Bub1, and Bub3 form the core spindle checkpoint components that inhibit the APC–Cdc20 in response to the presence of unattached kinetochores. The spindle checkpoint may also detect kinetochores that are attached to microtubules, but which lack intersister tension, though this is controversial. Nevertheless, it is clear that these components work together to generate a signal at the kinetochore that culminates in the inhibition of the APC; however, the mechanism is not completely understood. Briefly, and taking into account a great deal of work in other organisms (see Musacchio and Salmon 2007; Lara-Gonzalez *et al.* 2012 for more detailed reviews), the following general principles of spindle checkpoint function have emerged. The Aurora B and Mps1 kinases are the most upstream kinetochore components. In many organisms, including fission

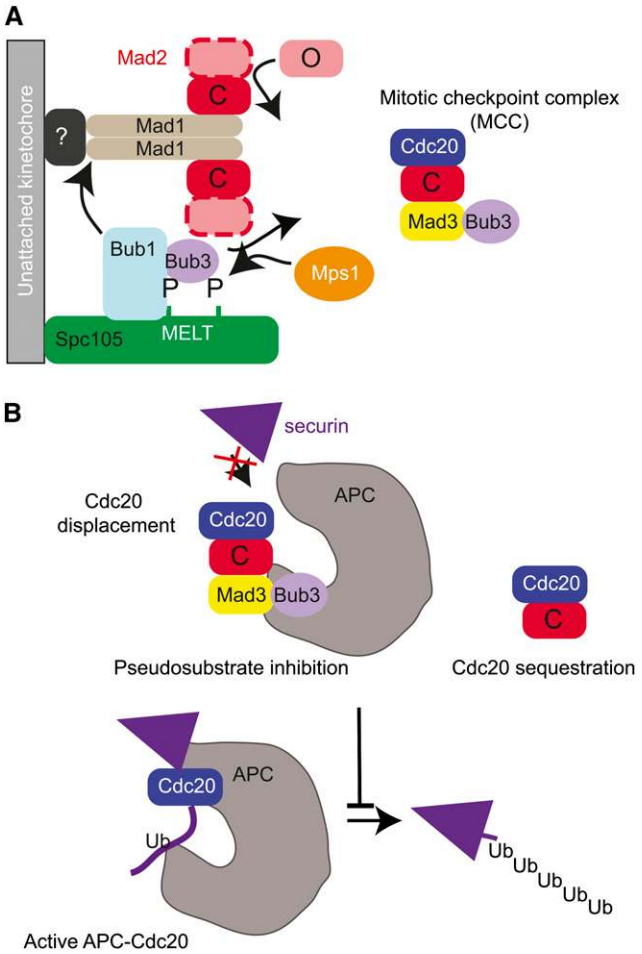

Figure 6 The spindle checkpoint. (A) Generation of the mitotic checkpoint complex (MCC) by an unattached kinetochore. (B) Modes of APC–Cdc20 inhibition by the spindle checkpoint. Pseudosubstrate inhibition, Cdc20 displacement, and Cdc20 sequestration are all thought to contribute to APC–Cdc20 inhibition.

yeast, Aurora B kinase enables Mps1 kinase association with kinetochores, which in turn enables recruitment of other checkpoint components (summarized in Heinrich *et al.* 2012). However, in budding yeast, Aurora B (Ipl1) and Mps1 appear to be recruited to kinetochores independently (Maure *et al.* 2007). Mps1 phosphorylates the kinetochore protein Spc105/KNL1/Blinkin on conserved MELT motifs to enable recruitment of a complex of Bub1 and Bub3 (London *et al.* 2012; Shepperd *et al.* 2012; Yamagishi *et al.* 2012). Bub1 is also a conserved kinase, though its kinase activity is not essential for checkpoint function and is rather required for biorientation through recruitment of Sgo1 (Warren *et al.* 2002; Peters 2006; Fernius and Hardwick 2007). The Bub1–Bub3 complex is present at kinetochores from S phase until metaphase of mitosis (Kerscher *et al.* 2003; Gillett *et al.* 2004). In contrast, Mad1 and Mad2 are visualized on kinetochores only under conditions where they are not expected to be attached to microtubules, whereas Mad3 has not been detected at kinetochores (Gillett *et al.* 2004). Importantly, Bub1 and Bub3 are required for the kinetochore association of Mad1 and Mad2 upon checkpoint activation (Gillett *et al.* 2004). How Bub1–Bub3 influences Mad1–Mad2 remains a mystery, although a Mad1–Bub1–Bub3 complex has been observed upon checkpoint activation and this appears to be functionally important in triggering cell cycle arrest (Brady and Hardwick 2000). Ipl1 is also required for Mad2 association with kinetochores during checkpoint activation (Gillett *et al.* 2004), perhaps due to a requirement for Ipl1 in generating unattached kinetochores to which Mad1–Mad2 can be recruited (Pinsky *et al.* 2006). Though the kinetochore receptor for Mad1 is not yet known, kinetochore-bound Mad1 plays a key role in generating the "wait anaphase" signal at kinetochores through recruitment of Mad2 from the soluble pool (Chen *et al.* 1998, 1999). These studies led to the Mad2 "template" model, which provides an explanation for the role of the Mad1–Mad2 interaction in generating the "mitotic checkpoint complex" (MCC) (De Antoni *et al.* 2005), a potent APC inhibitor, composed of Mad2, Mad3, Bub3, and Cdc20 (Hardwick *et al.* 2000; Brady and Hardwick 2000) (Figure 6A). Binding to Mad1 converts Mad2 from an "open" (O-Mad2) to a "closed" (C-Mad2) conformation. C-Mad2 that is already bound to Mad1 dimerizes with soluble O-Mad2, generating further C-Mad2. Since Cdc20 binds C-Mad2 in a similar way to Mad1, the Mad1 template catalyses Mad2 binding to Cdc20. Mad3 binds to the same interface of C-Mad2 as O-Mad2 bound to Mad1 (Chao *et al.* 2012; Mariani *et al.* 2012) and Mad3 binds to the same surface of Bub3 as Bub1 (Larsen *et al.* 2007). Exactly how these interactions lead to Mad3 and Bub3 incorporation into the MCC is not well understood.

What is the role of the MCC in APC inhibition? This question has been difficult to answer, not least because the exact identity of the downstream effector that inhibits the APC is uncertain. The picture that has emerged is that Bub3–Mad3 and Mad2 synergistically inhibit the APC, though the relative contribution of Mad3 and Mad2 is controversial (Fang *et al.* 1998; Tang *et al.* 2001; Fang 2002; Davenport *et al.* 2006; Burton and Solomon 2007; Kulukian *et al.* 2009; Foster and Morgan 2012; Lara-Gonzalez *et al.* 2012; Lau and Murray 2012). One potential mode of APC inhibition by the MCC, for which evidence is accumulating, is the "pseudosubstrate model." The possibility that Mad3 could act as a pseudosubstrate, blocking access of APC–Cdc20 to securin and cyclin was suggested following the realization that Mad3 has two KEN boxes that are important for APC inhibition (Burton and Solomon 2007; King *et al.* 2007b; Sczaniecka *et al.* 2008; Malureanu *et al.* 2009). Indeed, in the MCC crystal structure, a Mad3 KEN box is optimally positioned by Mad2 to obscure the recognition sites for the KEN box degron on Cdc20, providing support for the pseudosubstrate model (Chao *et al.* 2012). Additional mechanisms of APC inhibition are also likely. For instance, in a model where the MCC crystal structure was mapped onto an existing EM density map of the APC–MCC complex, Cdc20 was displaced away from contacts on the APC required to constitute its D box receptor (Chao *et al.* 2012).

Furthermore, in budding yeast, Mad2 and Cdc20 form a separate complex independent of MCC, suggesting that Cdc20 sequestration by Mad2 may also play a role in APC inhibition (Poddar *et al.* 2005). This idea is supported by the finding that Mad2 prevents Cdc20 binding to the APC in an *in vitro* assay using purified budding yeast components (Foster and Morgan 2012). In addition, tethering Mad2 to Cdc20 is sufficient to inhibit the APC in budding yeast, though the basis of the inhibition is not known (Lau and Murray 2012). Human Mad2 also interacts with Cdc20 through the same site it normally uses to bind the APC (Izawa and Pines 2012). Therefore, there is also substantial evidence that in the absence of BubR1/Mad3, the Mad2–Cdc20 complex fails to bind to, and activate, the APC. In summary, pseudosubstrate inhibition by Mad3 and disruption of key interfaces between Cdc20 and the APC by Mad2 are both likely to contribute to the inhibition of the APC by the spindle checkpoint (Figure 6B). Whether APC inhibition occurs solely in the context of the MCC, or if indeed different subcomplexes of checkpoint proteins elicit inhibition through different mechanisms, is a question that should be addressable using recently developed *in vitro* APC assays and structural analysis.

Checkpoint silencing: Once all sister kinetochores have bioriented, the inhibitory signals that prevent cohesin cleavage must be silenced to allow anaphase to proceed. Of paramount importance is the silencing of the spindle checkpoint to allow APC activation. Broadly, there are two types of reversals that must occur. First, the phosphorylations that are put in place by the checkpoint and error correction machinery must be removed. The protein phosphatase 1 (PP1) plays a central role in checkpoint silencing (Akiyoshi *et al.* 2009b; Pinsky *et al.* 2009). One role of PP1 is to reverse the Mps1-dependent phosphorylation of Spc105 to release Bub1 from the kinetochore (London *et al.* 2012), but there are likely to be many more important substrates and possibly other phosphatases that will be important too. Second, the MCC must be disassembled and recent data suggest that Cdc20 autoubiquitination in the context of the MCC plays a role in this process, allowing for rapid activation of the APC once the checkpoint is satisfied (Mansfeld *et al.* 2011; Chao *et al.* 2012; Foster and Morgan 2012; Uzunova *et al.* 2012). The Mnd2/Apc15 subunit of the APC is important for Cdc20 autoubiquitination, though not for securin or cyclin B ubiquitination (Mansfeld *et al.* 2011; Foster and Morgan 2012; Uzunova *et al.* 2012). Interestingly, free Cdc20 can also be ubiquitinated in an Mnd2/Apc15-independent manner (Foster and Morgan 2012; Uzunova *et al.* 2012). This leads to a model whereby ubiquitylation of free Cdc20 restricts its cellular levels, enabling a checkpoint response to be mounted, whereas ubiquitylation of Cdc20 in the context of the MCC serves to disable the checkpoint response (Musacchio and Ciliberto 2012). An implication of this model is that for a sustained checkpoint response, MCC must be constantly produced to counterbalance the effect of Cdc20 autoubiquitination. Interestingly, the most upstream checkpoint component, Mps1, is ubiquitinated by the APC–Cdc20 at anaphase onset, leading to its degradation (Palframan *et al.* 2006). This helps explain why no more MCC is produced once the checkpoint is satisfied, allowing rapid APC activation and anaphase onset once the last appropriate kinetochore–microtubule interaction is made (Musacchio and Ciliberto 2012).

It is also essential that the sudden loss of tension between sister kinetochores at anaphase does not reengage the error correction machinery or activate the spindle checkpoint. The Cdc14 phosphatase, which becomes active during anaphase (see below), dephosphorylates the CPC component Sli15 (INCENP), causing its dissociation from centromeres and relocalization at the spindle midzone, and this is important to prevent reengagement of the spindle checkpoint after anaphase onset (Mirchenko and Uhlmann 2010).

Other factors regulating anaphase onset

In addition to being targeted by the spindle checkpoint, in budding yeast, securin/Pds1 is also targeted by the DNA damage response machinery to prevent anaphase onset (Wang *et al.* 2001). The DNA damage checkpoint kinase, Chk1, phosphorylates Pds1, making it resistant to APC–Cdc20-dependent destruction (Sanchez *et al.* 1999; Wang *et al.* 2001). Therefore the DNA damage response and the spindle checkpoint both prevent anaphase onset by preventing Pds1 degradation, though the mechanism by which this is achieved is distinct.

Despite the convergence of regulatory networks on Pds1, cells lacking *PDS1* are viable and initiate anaphase onset with normal timing (Alexandru *et al.* 2001). At first impression, this is surprising, since separase would be expected to be hyperactive in *pds1Δ* cells, resulting in precocious loss of cohesion. However, this paradox can be explained because securin also plays a positive role in separase activation. Indeed, mice lacking securin are viable but only when separase activity is not also compromised (Wirth *et al.* 2006). In budding yeast, securin not only inhibits separase but also promotes its accumulation within the nucleus, and facilitates its rapid activation upon securin destruction (Agarwal and Cohen-Fix 2002; Hornig *et al.* 2002). Securin can therefore be thought of as an inhibitory chaperone for separase.

What controls the timing of anaphase onset in cells lacking securin? Since cohesin cleavage is sufficient to trigger chromosome segregation, anaphase onset could be additionally regulated by securin-independent separase inhibition or by making cohesin more resistant to cleavage. In the absence of securin, a particular form of the protein phosphatase 2A, containing its Cdc55 regulatory subunit, becomes essential (Tang and Wang 2006; Chiroli *et al.* 2007; Clift *et al.* 2009). Conditional mutants lacking both Cdc55 and securin/Pds1 cleave cohesin prematurely, implicating Cdc55 as a regulator of cohesin cleavage (Clift *et al.* 2009). Timely cohesin cleavage depends on phosphorylation of its Scc1 subunit within its separase-dependent cleavage sites by Polo kinase (Cdc5), so that regulation of cohesin

phosphorylation is one additional way to regulate anaphase onset (Alexandru *et al.* 2001). Recently, an elegant method to spatially measure separase activation at the single cell level has been developed. Morgan and colleagues engineered a fragment of the Scc1 cohesin subunit containing a separase cleavage site between LacI and GFP tethered to lacOs at a specific location on the chromosome (Yaakov *et al.* 2012). Cleavage of this "separase biosensor" leads to release of GFP from the tether and loss of specific fluorescence at this site. Additionally tethering Cdc55 to the biosensor delayed its cleavage in a similar way to blocking the Polo-dependent phosphorylation sites. This suggests that PP2A–Cdc55 prevents cohesin cleavage by counteracting the Polo-dependent phosphorylation of Scc1 (Yaakov *et al.* 2012). In addition to this regulation at the level of cohesin it is possible that Cdc55, or indeed other factors, prevents anaphase onset by regulating separase. In mammals, separase is additionally inhibited by the phosphorylation-dependent binding of Cdk1 (Stemmann *et al.* 2001; Gorr *et al.* 2006), though this is not known to occur in budding yeast, where downregulation of CDK activity is not required for anaphase onset (Surana *et al.* 1993). The separase biosensor will be invaluable in uncovering further mechanisms regulating cohesin cleavage.

Anaphase Progression

Once cohesin destruction is initiated, anaphase ensues and chromosomes begin to move apart. For successful cell division, chromosomes must be completely partitioned before cytokinesis occurs. Once initiated, several mechanisms govern safe passage through anaphase with the result that sister chromatids are fully partitioned to opposite poles of the cell in preparation for exit from mitosis.

Separase initiates mitotic exit

Cell cycle transitions are governed by fluctuations in CDK activity (reviewed in Morgan 2007). In budding yeast, there is a single CDK, Cdc28, which in turn associates with G1 cyclins (Cln1, Cln2, and Cln3) and S phase (Clb5 and Clb6) and M phase (Clb1, Clb2, Clb3, and Clb4) B type cyclins. Following cell cycle entry, S phase Clb–CDKs promote DNA replication and subsequently M phase Clb–CDKs trigger entry into mitosis. At the end of mitosis, Clb–CDKs are inactivated, to restore the G1 state. In budding yeast, Clb–CDK inactivation takes place in two waves. Concomitant with securin degradation at the metaphase–anaphase transition, the APC–Cdc20 targets a pool of Clbs for destruction; however, this is not essential for anaphase onset and Clb–CDK activity persists until the end of anaphase (Surana *et al.* 1993). The essential Cdc14 phosphatase triggers Clb–CDKs inactivation at the end of mitosis, both by triggering cyclin degradation and allowing the accumulation of the Clb–CDK inhibitor, Sic1 (Jaspersen *et al.* 1998; Visintin *et al.* 1998; Zachariae *et al.* 1998). First, Cdc14 dephosphorylates the APC/C activator, Cdh1, which in turn enables the degradation of Clb cyclins. Second, Cdc14 increases the stability and levels of Sic1 by dephosphorylating Sic1 and the transcription factor, Swi5. Cdc14 is controlled by its localization. For most of the cell cycle, Cdc14 is sequestered in the nucleolus through binding to its inhibitor, Cfi1/Net1 (Shou *et al.* 1999; Visintin *et al.* 1999). During anaphase, Cdc14 is released from the nucleolus into the nucleus and cytoplasm where it can dephosphorylate its substrates and trigger exit from mitosis. Two regulatory networks control Cdc14 localization and activity (reviewed in Weiss 2012). At the end of mitosis, a Ras-like GTPase cascade known as the mitotic exit network (MEN) becomes active and triggers the full activation of Cdc14 and exit from mitosis (reviewed in Stegmeier and Amon 2004). Migration of the SPB into the bud plays a critical role in MEN activation, enabling spindle orientation to be monitored to ensure equal nuclear division. The MEN is essential and in its absence, cells arrest with high CDK activity and fail to break down their spindles (reviewed in Caydasi and Pereira 2012). In early anaphase, Cdc14 is under control of the nonessential Cdc14 early anaphase release (FEAR) network. Cdc14 released via the FEAR network cannot by itself trigger CDK inactivation and exit from mitosis, but rather governs safe passage through anaphase.

The FEAR network is composed of a group of proteins that includes separase (Esp1), the separase-associated protein, Slk19, Polo kinase (Cdc5), Spo12, the nucleolar replication fork block protein, Fob1, the protein phosphatase 2A bound to its Cdc55 regulatory subunit, the PP2A regulators Zds1 and Zds2, and Clb–CDKs (reviewed in Rock and Amon 2009). Although it remains unclear precisely how these proteins work together to regulate Cdc14 in early anaphase, the overall picture that has emerged is as follows (Figure 7). Ultimately, Cdc14 activation occurs because phosphorylation destabilizes the Cdc14–Cfi1/Net1 interaction, leading to Cdc14 release (Azzam *et al.* 2004). Clb1–CDK, Clb2–CDK, Clb5–CDK, and Polo kinase (Cdc5) all contribute to destabilization of the Cdc14–Cfi1/Net1 interaction (Azzam *et al.* 2004; Manzoni *et al.* 2010). PP2A–Cdc55 prevents Cfi1/Net1 phosphorylation until anaphase, maintaining its tight association with Cdc14 for the rest of the cell cycle (Queralt *et al.* 2006). Fob1 also contributes to stabilization of the Cdc14–Cfi1/Net1 interaction (Stegmeier *et al.* 2004). Activation of separase at anaphase onset, together with Slk19, downregulates PP2A–Cdc55 through its Zds1 and Zds2 inhibitors (Queralt *et al.* 2006; Queralt and Uhlmann 2008; Rossio and Yoshida 2011; Calabria *et al.* 2012). This allows Clb1–CDK and Clb2–CDK to phosphorylate Cfi1–Net1, disrupting its interaction with Cdc14 (Queralt *et al.* 2006; Queralt and Uhlmann 2008). Interestingly, the protease activity of separase is not required for its function in the FEAR network (Sullivan and Uhlmann 2003). Spo12 is also phosphorylated by CDKs during anaphase and this is thought to somehow impair the ability of Fob1 to stabilize the Cdc14–Cfi1/Net1 interaction (Tomson *et al.* 2009). While the FEAR network initiates Cdc14 release, sustained

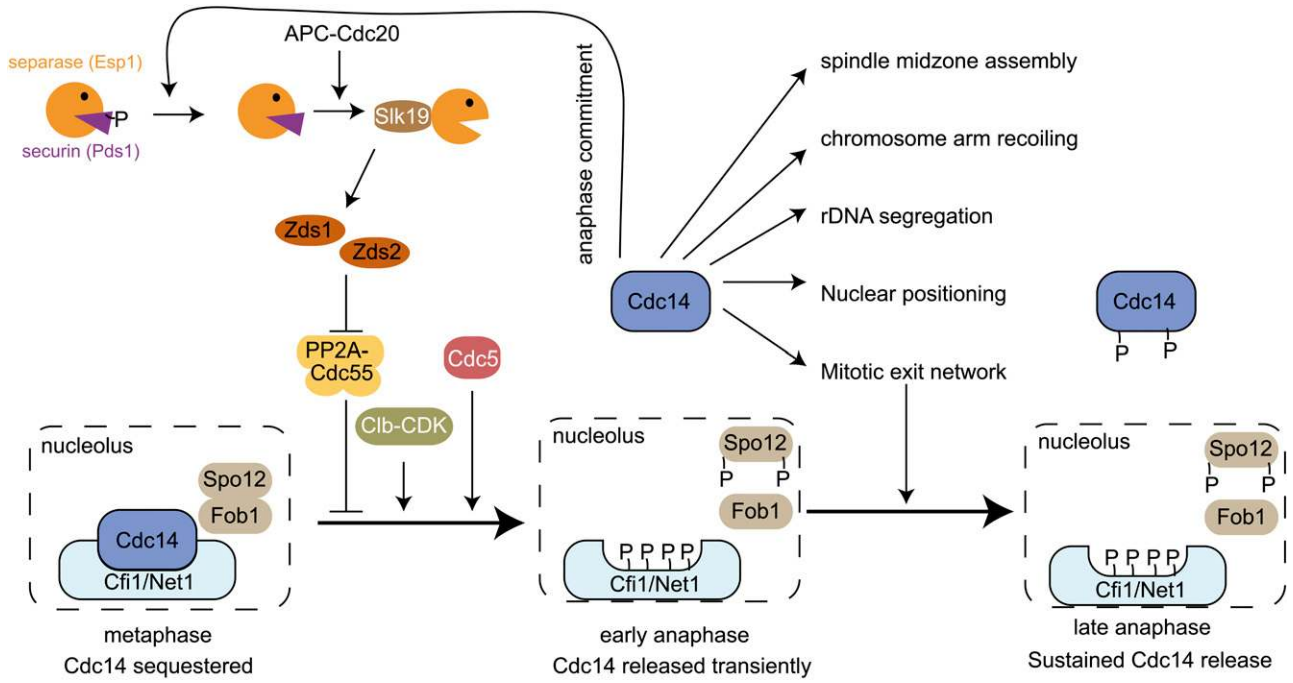

Figure 7 FEAR network and anaphase. Regulation and role of the FEAR network in completing chromosome segregation during early anaphase. For details see text.

release and export into the cytoplasm requires the activity of the MEN (Mohl *et al.* 2009).

Separase activation, therefore, not only triggers chromosome separation but also initiates mitotic exit through its role in the FEAR network. This provides a mechanism by which the onset of chromosome segregation is coordinated with mitotic exit. Importantly, assaults that result in securin stabilization, such as DNA damage or improperly attached kinetochores, will prevent both chromosome segregation and mitotic exit through separase inhibition.

Although Cdc14 released via the FEAR network is insufficient for mitotic exit, it plays numerous roles in ensuring safe passage through anaphase. These include: sharpening the anaphase switch, inactivation of mitotic surveillance mechanisms, segregation of the rDNA, nuclear positioning, spindle stabilization, spindle midzone assembly, and MEN activation. Consistently, Cdc14 interacts with numerous potential substrates in mitosis, although the functional importance of the majority of these has not been investigated (Bloom *et al.* 2011). These substrates must be dephosphorylated with carefully regulated timing to ensure ordered progression through anaphase. This order is due to the fact that substrates are predisposed to be dephosphorylated at a specific threshold of kinase/phosphatase ratio as this decreases during anaphase (Bouchoux and Uhlmann 2011). Perhaps this phenomenon could explain why Cdc14 is required for spindle stability in anaphase but, paradoxically, triggers spindle disassembly moments later during exit from mitosis.

Committing to anaphase: Once the decision to destroy cohesin has been made, the linkages between chromosomes are destroyed rapidly. Cdc14 facilitates rapid cohesin loss by removing stabilizing CDK-dependent phosphorylations on securin, accelerating its proteolysis (Holt *et al.* 2008).

Nuclear position: Due to the asymmetric nature of budding yeast cell division, correct nuclear position is particularly important for accurate segregation. One set of chromosomes must be partitioned into the bud, while the other set remains in the mother cell. Cdc14 released through the FEAR network affects nuclear position in anaphase by modulating the forces that cytoplasmic microtubules exert on the cell cortex. In the absence of FEAR-dependent Cdc14 activity, the entire nucleus migrates aberrantly into the bud, suggesting that forces at the mother cell cortex are weaker (Ross and Cohen-Fix 2004). How Cdc14 alters cortical spindle forces is, however, not known.

Completion of chromosome segregation: Chromosome segregation must also be driven to completion upon anaphase onset. Not all regions of the genome segregate simultaneously. Centromeres are the first to segregate, followed by chromosome arms, telomeres, and finally the rDNA during midanaphase (D'Amours *et al.* 2004; Sullivan *et al.* 2004; Renshaw *et al.* 2010). Cdc14 is required for efficient segregation of telomeres and is essential for removal of cohesin-independent linkages to allow segregation of the rDNA (D'Amours *et al.* 2004; Sullivan *et al.* 2004). Cdc14 shuts down transcription in the rDNA to allow condensin binding to the rDNA, which in turn enables compaction of the rDNA and its efficient segregation (D'Amours *et al.* 2004; Sullivan *et al.* 2004; Machín *et al.* 2006; Tomson

et al. 2006; Clemente-Blanco *et al.* 2009). The critical role of condensin in the rDNA may be to facilitate decatenation by topoisomerase II (D'Ambrosio *et al.* 2008a). In support of this, condensin, as well as spindle forces were shown to drive an increase in positive supercoiling that occurs as chromosomes segregate, and this was proposed to promote decatenation by topoisomerase II (Baxter *et al.* 2011). The supercoiling activity of condensin in anaphase is promoted by Polo kinase (Cdc5), which directly phosphorylates multiple condensin subunits (St-Pierre *et al.* 2009). Condensin also localizes to chromosome arms during anaphase and enables recoiling of stretched chromosomes, which promotes the removal of residual cohesin (Renshaw *et al.* 2010). Using a system to artifically open and re-close condensin rings, accurate segregation in anaphase was recently shown to require intact condensin rings. (Cuylen *et al.* 2013). The critical function of condensin in chromosome arm segregation in anaphase, therefore, may be mediated through the topological entrapment of chromosomes.

Maintaining spindle integrity: During anaphase, several proteins relocate from kinetochores to the spindle midzone or are newly recruited to the spindle and/or focused to the midzone to promote spindle stability and elongation. Cdc14 activation is key to this process as it dephosphorylates several substrates to enable their association with the spindle (Pereira and Schiebel 2003; Higuchi and Uhlmann 2005; Khmelinskii *et al.* 2007). The CPC components are so-called because of their conserved relocalization from kinetochores to the spindle midzone during anaphase (Carmena *et al.* 2012). Cdc14 dephosphorylates the CPC component Sli15 to enable this transition (Pereira and Schiebel 2003). Another CPC component, Ipl1, is also subject to Cdc14-dependent removal of CDK-directed phosphorylation, which enables its association with the microtubule plus end-tracking protein, Bim1 (yeast EB1 protein), leading to Ipl1 concentration at the spindle midzone (Nakajima *et al.* 2011; Zimniak *et al.* 2012). SUMOylation of the kinetochore component, Mcm21, is also important for CPC relocalization to the midzone (Vizeacoumar *et al.* 2010). The CPC additionally undergoes self-regulation in anaphase by Ipl1-dependent phosphorylation of Sli15, which directs it away from regions of microtubule dynamicity (Nakajima *et al.* 2011).

CPCs are important for midzone assembly, spindle stability, elongation, and disassembly. Although the mechanism by which they achieve these functions is unclear, the CPC is important for recruitment of many downstream effectors. Interestingly, distinct CPC subcomplexes exist that appear to carry out specific functions. Among the proteins recruited to the midzone in anaphase by CPC components are the kinetochore protein Ndc10 (Cbf2), which binds to Bir1 (Bouck and Bloom 2005; Widlund *et al.* 2006; Thomas and Kaplan 2007; Rozelle *et al.* 2011). Ndc10, Bir1, and Sli15 form an alternative CPC that lacks Ipl1 and regulates spindle elongation (Rozelle *et al.* 2011). Ndc10 must be SUMOylated for proper spindle stability, suggesting that SUMOylation might be generally important for spindle midzone assembly (Montpetit *et al.* 2006). A complex of Slk19–Esp1 additionally relocates to the spindle midzone in a manner dependent on Sli15, and this is also required for spindle stability (Khmelinskii *et al.* 2007).

Another key protein at the midzone is the microtubule-bundling protein, Ase1. Ase1 is focused at the midzone by Cdc14-dependent dephosphorylation where it recruits downstream components to enable midzone assembly, including the kinesin-5 protein, Cin8 (Khmelinskii *et al.* 2007, 2009). Cdc14 further dephosphorylates Fin1, a regulatory subunit of the protein phosphatase 1 (PP1) to trigger its association with spindle poles and microtubules and which is also important for spindle stability (Woodbury and Morgan 2007). Overall, Cdc14 ensures the integrity of the mitotic spindle in anaphase through dephosphorylation of multiple substrates.

Recently, CPC at the spindle midzone has been reported to be important in ensuring that chromosomes are clear of the division plane prior to cytokinesis as part of the "NoCut" pathway (Norden *et al.* 2006; Mendoza *et al.* 2009). However, several situations where chromosomes fail to clear the division plane do not lead to a delay in cytokinesis. Inactivation of topoisomerase II prior to anaphase prevents chromosome segregation, but not cytokinesis, resulting in the "cut" phenotype (abscission of the nucleus by the cleavage plane). (Baxter and Diffley 2008). A failure to resolve rDNA loci after Cdc14 inactivation results in the presence of a single unsegregated chromosome in the division plane, yet only a short delay in cytokinesis, leading to severing of the chromosome (Quevedo *et al.* 2012). Similarly, though artificial cleavage of condensin prior to anaphase prevents chromosome arm segregation, cytokinesis occurs with normal timing, generating DNA breaks that persist in the next cell cycle (Cuylen *et al.* 2013).

Chromosome Segregation During Meiosis

Meiosis is a specialized cell division, which produces gametes with half the ploidy of the progenitor cell. Two gametes fuse to restore normal ploidy in the offspring. Diploid budding yeast undergo meiosis to produce four haploid spores. To achieve the halving of ploidy, DNA replication is followed by two consecutive rounds of chromosome segregation. In meiosis I, the maternal and paternal chromosomes, or homologs, are segregated, whereas during meiosis II, which resembles mitosis, the sister chromatids are separated. This pattern of segregation requires several remarkable modifications to the segregation machinery. First, homologous chromosomes must be linked to ensure their accurate segregation in meiosis I. In budding yeast, meiotic recombination generates chiasmata that are important in holding homologs together. Second, sister chromatids must segregate to the same pole, rather than opposite poles during meiosis I. A protein complex known as monopolin ensures that sister kinetochores are monooriented during meiosis I. Third, sister chromatids must retain

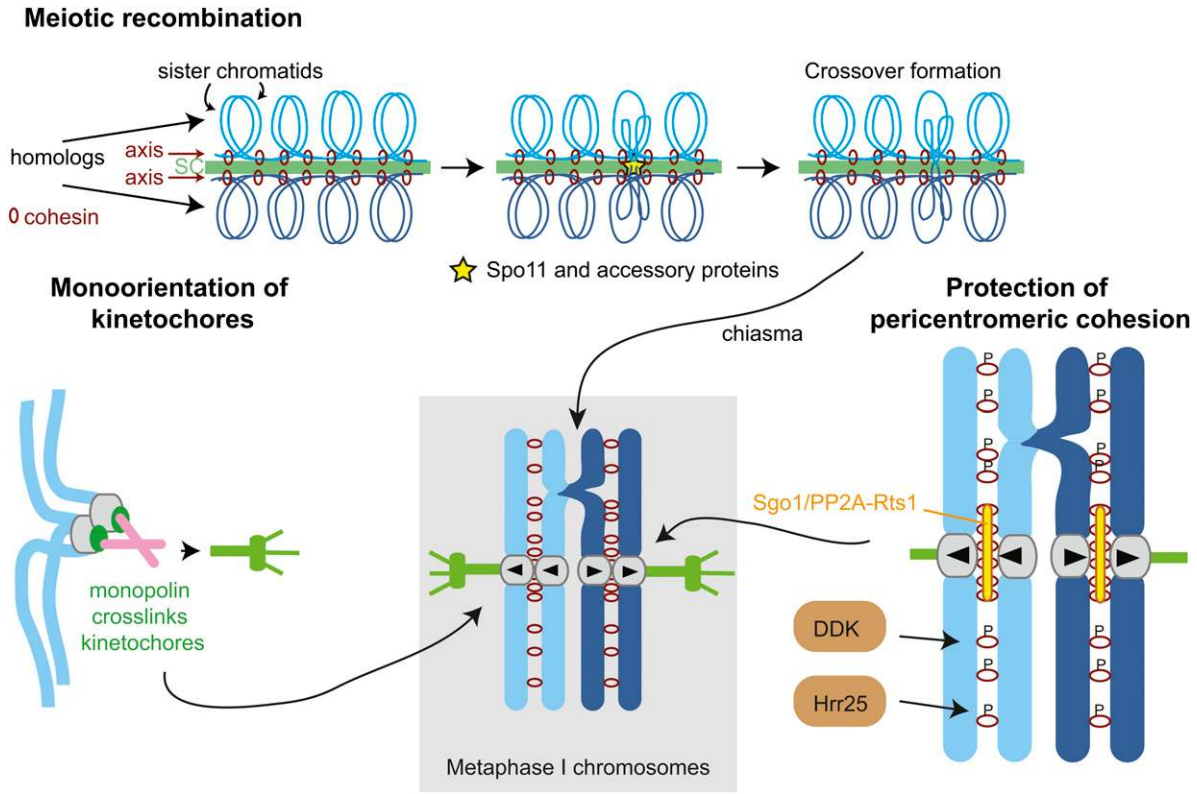

Figure 8 Modifications to chromosomes for segregating homologs during meiosis I. Meiotic recombination, monoorientation of kinetochores, and protection of pericentromeric cohesion specialize meiosis I chromosomes to enable homolog segregation.

cohesive linkages between them until their segregation to opposite poles during meiosis II. Therefore, although cohesion on chromosome arms is lost during meiosis I, cohesion around the centromere is protected until meiosis II. Finally, cell cycle controls must be modified so that meiosis I is followed not by DNA replication, but by another chromosome segregation phase, meiosis II. In recent years, our knowledge of the molecular basis for these changes has increased dramatically and much of what we have learned has been from work using budding yeast. These modifications are summarized briefly below (Figure 8). For more detailed discussion see Marston and Amon (2004) and Brar and Amon (2008).

Establishment of links between homologs during meiosis I

In budding yeast, the establishment of linkages between homologs to mediate their accurate segregation is dependent on meiotic recombination. Meiotic recombination produces a reciprocal exchange between the homologs, called crossovers (COs). COs in their mature state are known as chiasmata and hold homologs together owing to sister chromatid cohesion.

Homologous chromosome pairing: As a prerequisite to productive recombination, homologous chromosomes must find each other through a process known as pairing. In early meiotic prophase, centromeres detach from the spindle pole body and telomeres become tethered to the nuclear membrane. SUN proteins in the nuclear membrane link telomeres in the nucleus to motors in the cytoplasm (Hiraoka and Dernburg 2009). Chromosomes undergo rapid telomere-led prophase movements and this facilitates chromosome pairing (Conrad *et al.* 2008; Koszul *et al.* 2008; Lee *et al.* 2012). In budding yeast, pairing is initiated by nonhomologous coupling of the centromeres (Tsubouchi and Roeder 2005). This process requires the Zip1 protein, a major component of the synaptonemal complex (SC), a proteinaceous structure that forms a scaffold between the homologs (Tsubouchi and Roeder 2005). It is thought that nonhomologous pairing initiates a homology search, culminating in stabilization of homologous chromosome pairs. Consistently, synapsis is initiated at centromeres (Tsubouchi *et al.* 2008). The transition to homologous pairing requires the initiation of recombination, that is the introduction of double strand breaks (DSBs) by the Spo11 nuclease (Tsubouchi and Roeder 2005). The early telomere-led movements culminate in the clustering of telomeres at a common site, known as the bouquet stage (Scherthan 2001). Although the precise function of the bouquet is debated, it is thought to somehow optimize homolog interactions during meiotic recombination, perhaps by clustering centromeres to enable synapsis initiation (Subramanian and Hochwagen 2011).

Meiotic recombination: Meiotic recombination begins with the deliberate introduction of DSBs thoughout the genome

by the Spo11 endonuclease (Keeney *et al.* 1997). Meiotic chromosomes are organized in loops that radiate from a proteinaceous axis (Zickler and Kleckner 1999). The DSBs are made in the loops that become tethered to the axis by Spo11 accessory proteins (Blat *et al.* 2002; Panizza *et al.* 2011). DSBs are distributed nonrandomly throughout the genome (Gerton *et al.* 2000; Blitzblau *et al.* 2007; Buhler *et al.* 2007; Pan *et al.* 2011). Regions of the genome that are highly proficient for recombination are known as "hotspots," whereas recombination-poor zones, such as centromere and telomere-proximal regions, are known as "coldspots." The local structure of chromatin influences its susceptibility to Spo11-mediated breakage (Brachet *et al.* 2012). For example, histone H4 lysine 3 trimethylation has been identified as a predisposing mark for breakage (Borde *et al.* 2009).

Following DSB formation, 5′ ends are resected through the activity of the Exo1 exonuclease and bifunctional exo/endonuclease Mre11, leaving single strand 3′ ends that invade template DNA to form a so-called D-loop (Mimitou and Symington 2008; Zhu *et al.* 2008; Nicolette *et al.* 2010; Garcia *et al.* 2011). This invasion step and homology search is dependent on the RecA-like Rad51 and Dmc1 proteins that form nucleoprotein filaments on single stranded DNA (Bishop *et al.* 1992; Shinohara *et al.* 1992). Dmc1 is meiosis specific and Rad51's strand exchange activity is inhibited by the meiosis-specific protein, Hed1, and Rad51 (Tsubouchi and Roeder 2006). Rather than promote strand exchange directly, Rad51 acts as an accessory factor for Dmc1 in strand exchange (Cloud *et al.* 2012). The meiosis-specific employment of Dmc1 in strand exchange may contribute to ensuring that repair occurs from the homologous chromosome [thereby enabling CO generation], rather than from the sister chromatid, the predominant mode of repair in vegetative cells (Bishop 2012). The organization of meiotic chromosomes into loops secured on an axis is especially important in imposing a bias toward homologous repair as disruption of the axis or sister chromatid cohesion biases repair toward the sister chromatid (Kim *et al.* 2010).

After strand invasion, there are many possible modes of repair resulting in different genetic outcomes (Serrentino and Borde 2012). The only outcome that generates a linkage between the homologs is a CO event, in which exchanges of homologous chromosome arms occur. The number of COs is far fewer that the number of DSBs and this number is maintained even when fewer DSBs are produced (Martini *et al.* 2006). This "CO homeostasis" ensures that sufficient linkages between the homologs are generated. Meiotic recombination is also subject to a phenomenon known as "CO interference," which prevents additional COs close to sites which have already been designated as COs. The molecular basis for CO interference is unknown but a current hypothesis invokes a stress along the chromatin fiber that prevents further COs in the vicinity (Kleckner *et al.* 2004). A large fraction of DSBs that become COs are processed by a pathway requiring a group of proteins known as the "ZMMs," through a Holliday junction intermediate (Lynn *et al.* 2007). A few COs are also produced through a Mus81-dependent pathway (Hollingsworth and Brill 2004).The remainder of DSBs are processed as noncrossovers (NCOs) through several different mechanisms (Serrentino and Borde 2012). The excess DSBs, which eventually lead to NCOs, have been suggested to be functionally important in chromosome pairing by increasing interhomolog interactions (Tessé *et al.* 2003).

Surveillance mechanisms, or checkpoints, monitor the progression of meiotic recombination to ensure that meiosis does not progress in the presence of DNA lesions (MacQueen and Hochwagen 2011). Once all DSBs have been repaired, the Ndt80 transcription factor becomes active and drives the expression of genes required for pachytene exit and the meiotic divisions (Chu and Herskowitz 1998; Sourirajan and Lichten 2008).

Monoorientation of sister chromatids during meiosis I

A defining feature of meiosis I is the segregation of homologs to opposite poles rather than sister chromatids, which move toward the same pole. That is, sister kinetochores must be uniquely monooriented during meiosis I. Pioneering experiments in grasshopper spermatocytes demonstrated that this is a property of the kinetochore rather than the meiotic spindle (Paliulis and Nicklas 2000). We know little about how this is achieved, except in budding yeast where factors specifically required for kinetochore monoorientation have been identified. Monopolar attachment depends on a meiosis-specific protein, Mam1, two nucleolar proteins, Lrs4 and Csm1, that together with the casein kinase, Hrr25, form a complex called monopolin (Toth *et al.* 2000; Rabitsch *et al.* 2003; Petronczki *et al.* 2006). Cells lacking monopolin fail to monoorient sister kinetochores and biorient sister kinetochores instead, which leads to a failure to undergo the first meiotic division after arm cohesion is lost due to the persistence of centromere cohesion (Toth *et al.* 2000; Rabitsch *et al.* 2003; Petronczki *et al.* 2006). Polo kinase (Cdc5) is also required for monoorientation, in part due to a requirement for Cdc5 in release of Lrs4 and Csm1 from the nucleolus (Clyne *et al.* 2003; Lee and Amon 2003). Cdc5 additionally collaborates with the meiosis-specific regulator, Spo13 and Dbf4-dependent kinase, Cdc7 (DDK) to bring about Lrs4 phosphorylation, enabling monopolin recruitment to kinetochores (Matos *et al.* 2008).

Homologs of Lrs4 and Csm1 exist in fission yeast, but rather than bring about monopolar attachment during meiosis I, they prevent merotely during mitosis (Gregan *et al.* 2007). Instead, Rec8-containing cohesin is important for monoorientation in fission yeast (Watanabe and Nurse 1999). Unlike mitotic Rad21 containing cohesin (the equivalent of Mcd1/Scc1-containing cohesin in budding yeast), Rec8 cohesin is enriched within the core centromere in fission yeast (Yokobayashi and Watanabe 2005). In budding yeast, monopolin is sufficient to link sister kinetochores during meiosis I, even in the absence of cohesin (Monje-Casas

et al. 2007). However, condensin contributes to monoorientation (Brito *et al.* 2010). This has led to the proposal that the essential feature of monooriented kinetochores is that sister centromeres are closely linked either by monopolin in the case of budding yeast or by cohesion in the case of fission yeast (Watanabe 2006).

How does monopolin physically link sister kinetochores? The possibility that two sister kinetochores could be fused into a single microtubule-binding unit was suggested by images of insect kinetochores in meiosis I (Goldstein 1981; Suja *et al.* 1991) and because budding yeast kinetochores were observed to bind a single microtubule by electron microscopy in meiosis I (Winey *et al.* 2005). Alternatively one kinetochore might be "silenced" so that only one kinetochore between the two sisters is competent to bind microtubules. In budding yeast, structural studies of monopolin have led to a model that is most consistent with the kinetochore fusion model. A complex of Lrs4/Csm1 forms a V-shaped structure, the apices of which form contacts with the Dsn1 kinetochore subunits, suggesting that monopolin could crosslink two sister kinetochores (Corbett *et al.* 2010; Corbett and Harrison 2012). Mam1 and Hrr25 associate with Csm1 close to the site of Dsn1 interaction and somehow modulate association of monopolin with the kinetochore (Corbett and Harrison 2012). A major challenge for the future will be to understand how monopolin alters the interface between microtubules and the kinetochore.

Stepwise loss of cohesion

The introduction of at least one CO per pair of homologs generates a physical link between homologs due to sister chromatid cohesion distal to the CO This provides the tension that allows the homologs to align on the meiosis I spindle, ready for their segregation to opposite poles during meiosis I. Release of cohesion on chromosome arms triggers the segregation of homologs to opposite poles. However, cohesion in centromeric regions must be preserved during meiosis I to ensure the accurate segregation of sister chromatids during meiosis II. During meiosis, the Scc1 kleisin subunit of cohesin is replaced by its meiosis-specific homolog, Rec8 (Klein *et al.* 1999). Rec8 on chromosome arms is cleaved by separase during meiosis I, but Rec8 in centromeric regions is maintained until meiosis II (Klein *et al.* 1999; Buonomo *et al.* 2000). The ability of pericentromeric cohesin to resist separase activity during meiosis I is a unique property of Rec8-containing cohesin and cannot be fulfilled by Scc1 (Toth *et al.* 2000). Similarly, Rec8 performs functions in meiotic pairing, recombination, and chromosome axis formation that Scc1 cannot support (Klein *et al.* 1999; Brar *et al.* 2009). For its cleavage during meiosis I, Rec8 needs to be phosphorylated (Brar *et al.* 2006; Katis *et al.* 2010; Attner *et al.* 2013). The Dbf4-dependent Cdc7 kinase (DDK), casein kinase 1δ/ε (Hrr25) and Polo kinase, Cdc5, all phosphorylate Rec8 during meiosis I and promote its cleavage to some extent (Brar *et al.* 2006; Katis *et al.* 2010; Attner *et al.* 2013). Protection of pericentromeric cohesin during meiosis I requires the conserved Shugoshin (Sgo1) protein that is localized in the pericentromere (Katis *et al.* 2004a; Kitajima *et al.* 2004; Marston *et al.* 2004; Kiburz *et al.* 2005; Clift and Marston 2011). Sgo1 recruits a particular form of the protein phosphatase 2A, that contains its Rts1 regulatory subunit, to the pericentromere (Kitajima *et al.* 2006; Riedel *et al.* 2006; Xu *et al.* 2009). In the absence of the alternative PP2A regulatory subunit, Cdc55, excess PP2A–Rts1 complexes form and are highly elevated on chromosomes, preventing Rec8 phosphorylation and cleavage (Bizzari and Marston 2011). Altogether, this leads to a model whereby PP2A–Rts1 recruitment by Sgo1 renders pericentromeric Rec8 resistant to separase activity by maintaining it in the unphosphorylated state.

Other factors that are important for the protection of pericentromeric cohesion during meiosis I include the meiosis I-specific protein, Spo13 (Katis *et al.* 2004b; Lee *et al.* 2004), which is required for proper localization of Sgo1 at centromeres (Kiburz *et al.* 2005). Exactly how Spo13 contributes to cohesion protection is unknown but it is interesting to note that Spo13 binds to Polo kinase Cdc5 during meiosis I (Matos *et al.* 2008), given that Cdc5 is also important for the protection of cohesion during meiosis I under certain conditions (Katis *et al.* 2010). The Aurora kinase Ipl1 additionally contributes to cohesion protection, apparently by maintaining PP2A–Rts1 at centromeres (Yu and Koshland 2007). How these additional protective factors work together to regulate Sgo1–PP2A–Rts1 is an important question to address in the future. The question of how cohesin is "deprotected" during meiosis II is also a priority for future study.

Biorientation of homologs

During meiosis I, sister chromatids are monooriented and instead it is the homologous chromosomes that must achieve biorientation. The arm cohesion distal to chiasmata provides the tension that enables homologs to biorient on the meiosis I spindle. As in mitosis, the spindle checkpoint together with Ipl1 and Mps1 play an important role in this process. As in mitosis, Ipl1 is important to trigger the release of kinetochores from microtubules early in meiosis (Monje-Casas *et al.* 2007; Meyer *et al.* 2013). This provides an opportunity for chromosomes to pair. Following recombination and prophase exit, homologs initially tend to make incorrect attachments to the meiosis I spindle that are destabilized by Ipl1 to provide a further opportunity for the correct attachments to be made (Meyer *et al.* 2013). Mps1 kinase is required for the conversion of lateral kinetochore–microtubule attachments into stable end-on attachments and is therefore also critical for homolog biorientation (Meyer *et al.* 2013). The spindle checkpoint protein, Mad2, also contributes to proper homolog biorientation (Shonn *et al.* 2003). Interestingly, chromosomes with COs far from the centromere are particularly reliant on Mad2 function, suggesting that their alignment presents a particular challenge (Lacefield and Murray 2007). Mad2 plays a further role, together with Mad3, in

delaying the cell cycle in response to kinetochore–microtubule attachment defects during meiosis I to increase the possibility that biorientation will be achieved (Shonn *et al.* 2000, 2003).

How tension at meiosis I kinetochores is sensed is not clear. However, Sgo1 protein, which responds to a lack of tension between sister kinetochores during mitosis, plays only a minor role in sensing tension between homologous chromosomes during meiosis I, suggesting that a distinct mechanism is at work during meiosis I (Kiburz *et al.* 2008). Intriguingly, Zip1, a major component of the SC complex, persists at centromeres after SC disassembly (Gladstone *et al.* 2009; Newnham *et al.* 2010). Centromere-associated Zip1 may aid homolog biorientation during meiosis I by coupling homologous kinetochores to provide a favorable geometry for their capture by microtubules from opposite poles.

Alteration of cell cycle controls in meiosis

The extended prophase in which recombination takes place and the existence of two consecutive chromosome segregation phases without an intervening S phase are defining features of meiosis that require a specialization of cell cycle controls. As in mitosis, cell cycle progression is controlled by a single CDK, Cdc28, in complex with cyclins (reviewed in Marston and Amon 2004). There are six B-type cyclins in budding yeast, Clb1–6. The S phase cyclins Clb5 and Clb6 drive DNA replication, as in mitosis (Dirick *et al.* 1998; Stuart and Wittenberg 1998), and the initiation of recombination (Henderson *et al.* 2006). Clb1, -3, and -4 are important for the meiotic divisions, but the major mitotic cyclin, Clb2, is not produced during meiosis (Grandin and Reed 1993). CDK activity during meiosis is tightly controlled through transcriptional, translational, and post-translational mechanisms (Grandin and Reed 1993; Carlile and Amon 2008). Clb1–CDK activity is restricted to meiosis I, whereas Clb3–CDK activity is restricted to meiosis II (Carlile and Amon 2008). The meiosis-specific CDK-related kinase, Ime2, is also important for meiotic entry and progression (Benjamin *et al.* 2003; Irniger 2011). Ime2 activity peaks during prophase I, declines during meiosis I, and peaks again during meiosis II (Benjamin *et al.* 2003; Irniger 2011; Berchowitz *et al.* 2013). An elegant study showed that this pattern of Ime2 activity is important to restrict the translation of a subset of mRNA, including *CLB3*, to meiosis II (Berchowitz *et al.* 2013). At the meiosis I to meiosis II transition, Ime2-dependent downregulation of the RNA-binding protein, Rim4, relieves the repression on translation of these genes, thereby coordinating the meiotic program (Berchowitz *et al.* 2013).

Meiotic prophase I to meiosis I transition: As recombination takes place, exit from prophase is prevented because the recombination checkpoint represses the Ndt80 transcription factor that is required for the expression of M phase regulators, including cyclins and Polo kinase (Chu and Herskowitz 1998; Hochwagen and Amon 2006; Sourirajan and Lichten 2008). The APC/C additionally prevents the accumulation of M phase regulators during meiotic prophase by targeting them for destruction (Okaz *et al.* 2012). Budding yeast carry a meiosis-specific APC activator, Ama1, that appears in S phase (Cooper *et al.* 2000) and targets M phase regulators for destruction to prevent exit from prophase (Okaz *et al.* 2012). However, remarkably, securin (Pds1) and Sgo1 are spared from APC–Ama1-dependent destruction in prophase by the Mnd2/Apc15 APC subunit, which behaves as a substrate-specific inhibitor of the APC in this context (Oelschlaegel *et al.* 2005; Penkner *et al.* 2005). Note that, in contrast, Mnd2/Apc15 was found to stimulate Cdc20 autoubiquitin (see above; Foster and Morgan 2012), suggesting that regulation of the APC by Mnd2/Apc15 might be complex. The spindle checkpoint protein, Mad2, additionally prevents premature APC–Ama1 activity to ensure proper chromosome segregation in meiosis I, probably indirectly through APC–Cdc20 inhibition (Tsuchiya *et al.* 2011). Simultaneous activation and inhibition of APC–Ama1 toward distinct substrates is therefore critical for faithful chromosome segregation at meiosis I, yet how this is achieved is so far unknown.

The importance of restricting CDK activity until after prophase I exit was demonstrated by finding that production of the meiotic cyclins Clb1 and Clb3 during premeiotic S phase and prophase interferes with the program of meiosis I chromosome segregation (Carlile and Amon 2008; Miller *et al.* 2012). In these cells, kinetochore microtubule attachments are established prematurely so that monoorientation of kinetochores and the protection of centromeric cohesion is precluded (Miller *et al.* 2012). This demonstrates that kinetochore–microtubule interactions must be prevented during prophase I for the key features of meiosis I chromosomes to be established.

Meiosis I to meiosis II transition: After chromosomes segregate during mitosis, CDKs are inactivated, which allows for spindle disassembly and return to G1. This state of low CDK activity upon exit from mitosis is permissive for the resetting of origins of DNA replication, in preparation for the next S phase. However, homolog segregation during meiosis I is followed not by S phase, but by another "M" phase, meiosis II. This means that at meiosis I exit, spindles must disassemble but replication origins must not be reset. This predicts that specialized controls regulate the meiosis I to meiosis II transition.

As during exit from mitosis, the Cdc14 phosphatase is critical for the meiosis I to meiosis II transition (Buonomo *et al.* 2003; Marston *et al.* 2003). Cdc14 is released from sequestration in the nucleolus at meiosis I exit due to the activity of the FEAR network, however the MEN functions only in meiosis II (Buonomo *et al.* 2003; Marston *et al.* 2003; Kamieniecki *et al.* 2005; Attner and Amon 2012). Cells with impaired Cdc14 activity undergo only a single meiotic division in which some chromosomes segregate in a meiosis I-like manner, whereas others segregate in a meiosis II-like manner (Buonomo *et al.* 2003; Marston *et al.* 2003). The

important function of Cdc14 in meiosis appears to be to allow reduplication of the spindle to ensure that meiosis I and meiosis II segregation occur on consecutively built spindles (Marston *et al.* 2003; Bizzari and Marston 2011). As in mitosis, PP2A, together with its Cdc55 regulatory subunit, plays a critical role in keeping Cdc14 sequestered in the nucleolus. In the absence of Cdc55, Cdc14 is released prematurely and this prevents spindle assembly during meiosis I (Bizzari and Marston 2011; Kerr *et al.* 2011). How Cdc14 impinges on spindle assembly and duplication is unclear. It is thought that Cdc14 effects must be restricted during meiosis I exit to prevent events such as the resetting of replication origins occurring. Interestingly, Ime2-dependent phosphorylation events appear to be resistant to Cdc14 activity, providing a potential mechanism to limit its activity toward certain substrates (Holt *et al.* 2007). Indeed, the Mcm2–7 replicative helicase is excluded from the nucleus, which contributes to the prevention of replication origin relicensing, from premeiotic S phase onwards, due to Ime2 and CDK-dependent phosphorylation (Holt *et al.* 2007).

Perspectives

Remarkable progress in understanding chromosome segregation mechanisms in eukaryotes has been gained from studies in budding yeast. However, many features of this process and its regulation remain elusive. Although the central players are all known, understanding how they function mechanistically and cooperate with each other in the context of the cell cycle are key challenges. Structural and biochemical studies as well as systems level analysis, all combined with the powerful genetics that the yeast system offers, will be pivotal in driving forward the next era of chromosome segregation research.

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