Meiosis: how to create a specialized cell cycle

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During the meiotic cell cycle, a single round of DNA replication precedes two nuclear divisions. Recent work has shown that the proteins controlling the mitotic cell cycle are either replaced by homologous proteins only expressed during the meiotic cell cycle or modulated by meiosis-specific factors to bring about this specialized cell cycle.

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Current Opinion in Cell Biology 2001, **13**:770–777

0955-0674/01/$ – see front matter

Abbreviations
APC/C anaphase promoting complex/cyclosome
CDK cyclin-dependent kinase
ORC origin recognition complex
SPB spindle pole body

Introduction

In eukaryotes, a specialized cell cycle, the meiotic cell cycle, allows for the exchange of genetic material between parental chromosomes and the formation of haploid gametes. This generates offspring that are genetically different from their parents, thus maintaining genetic diversity. During meiosis, a single round of DNA replication is followed by two consecutive rounds of nuclear divisions, termed meiosis I and meiosis II. In the first meiotic division, homologous chromosomes segregate to opposite poles; during the second division, which resembles a mitotic division, sister chromatids separate from each other, thereby generating haploid gametes (Figure 1). This modification of the canonical G1-S-G2-M mitotic cell cycle requires retooling of the basic cell cycle machinery to meet meiosis specific requirements.

Although recombination is a key aspect of the meiotic cell cycle, we will not, due to space limitation, discuss this topic but refer to recent reviews by Zickler and Kleckner [1], Smith and Nicolas [2] and van Heemst and Heyting [3]. Instead, this review focuses on the regulation of the meiotic cell cycle, in particular on how pre-meiotic DNA replication and meiotic chromosome segregation differ from their counterparts in the mitotic cell cycle. How the meiotic cell cycle is regulated is best understood in the two yeast model systems, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Recent progress in both systems forms the basis of this review and we will refer to other model organisms when appropriate.

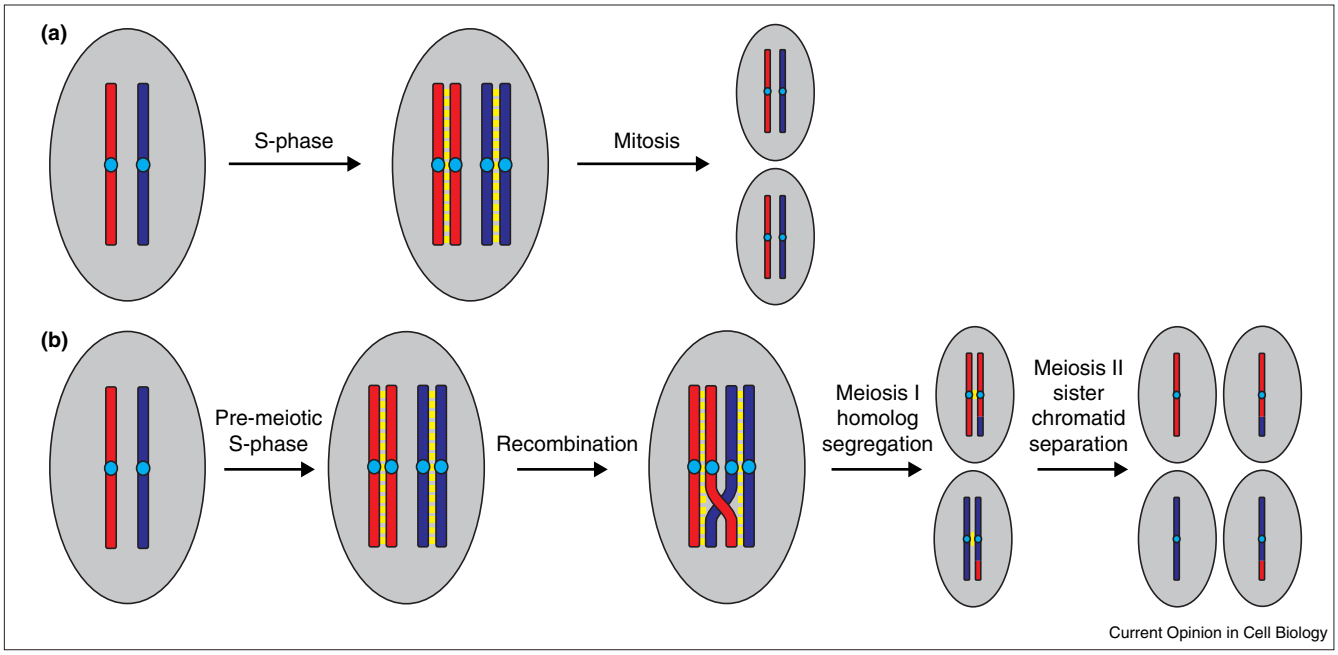
G1: time to get started

Budding yeast enters the mitotic cell cycle when nutrients are in ample supply. The G1 cyclins Cln1, Cln2 and Cln3 associate with the cyclin-dependent kinase (CDK) Cdc28 and trigger bud formation, spindle pole body (SPB) duplication and DNA replication (Figure 2a). How Cln–CDKs promote bud formation and SPB duplication is not yet understood but their role in initiating DNA replication is through phosphorylation of Sic1, a potent inhibitor of S-phase and mitotic CDKs (Clb–CDKs), leading to its degradation. The elimination of Sic1 allows Clb–CDKs to become active to initiate DNA replication [4].

Since cells can enter either the mitotic or meiotic cell cycle during G1, a mechanism is necessary to prevent simultaneous initiation of both cell cycle programs. During vegetative growth, Cln–CDKs prevent entry into the meiotic cell cycle by inhibiting the inducer of the meiotic program, Ime1 [5]. On the other hand, when diploid cells commit themselves to the meiotic cell cycle, which occurs upon nitrogen and carbon source starvation, Cln–CDKs are inactivated by down regulation of *CLN* transcription [5] (Figure 2a, 2b). The lack of nutrients and the a/α cell type signal also lead to activation of the transcription factor Ime1, which activates transcription of early meiotic genes [6] (Figure 2b). The three essential functions of Cln–CDKs during the mitotic cell cycle, bud emergence, SPB duplication and initiation of S-phase, are either dispensable, as in the case of bud formation, or performed by the meiosis-specific protein kinase Ime2, as in the case of S-phase initiation [7] (Figure 2b). Ime2 is thought to initiate pre-meiotic DNA replication by phosphorylating Sic1. Indeed, the requirement for *IME2* to initiate pre-meiotic DNA replication can be bypassed by deleting *SIC1*. Furthermore, Sic1 is stabilized in cells lacking *IME2* (Figure 2b) [7]. The regulation of meiotic SPB duplication remains largely unknown.

When *S. pombe* cells are starved for nitrogen and fermentable carbon sources, a/α diploid cells enter the meiotic cell cycle through inactivation of the protein kinase Pat1 [8]. As in the case of budding yeast, *S. pombe* pre-meiotic DNA replication is initiated in a manner similar to that of pre-mitotic DNA replication, but one of the key activators of DNA replication is replaced by a meiosis-specific factor. Pre-mitotic DNA replication depends upon the activity of the transcriptional activators Cdc10–Res1 and Cdc10–Res2–Rep2. These complexes are also required for pre-meiotic DNA replication, except for Rep2, which is replaced by a meiosis specific factor, Rep1/Rec16 (Figure 2c,d). The *rep1/rec16*$^+$ gene is essential for pre-meiotic DNA replication, whereas *rep2*$^+$ is required for pre-mitotic DNA replication, suggesting that fission yeast also possesses a system to functionally separate pre-mitotic and pre-meiotic S-phase while using the same basic cell cycle machinery [8] (Figure 2c,d).

Figure 1



The mitotic and meiotic cell cycles. **(a)** During pre-mitotic DNA replication, the genetic material is duplicated creating sister chromatids. At the onset of anaphase, sister chromatids are separated. Cytokinesis then generates two genetically identical daughter cells. **(b)** During pre-meiotic DNA replication, sister chromatids are generated. During prophase I, homologous chromosomes (maternal chromosome in red, paternal chromosome in blue) undergo recombination. At the metaphase I to anaphase I transition, homologous chromosomes are segregated. The second meiotic division resembles mitosis; sister chromatids are segregated. Thus, meiosis leads to the production of four cells that are genetically not identical.

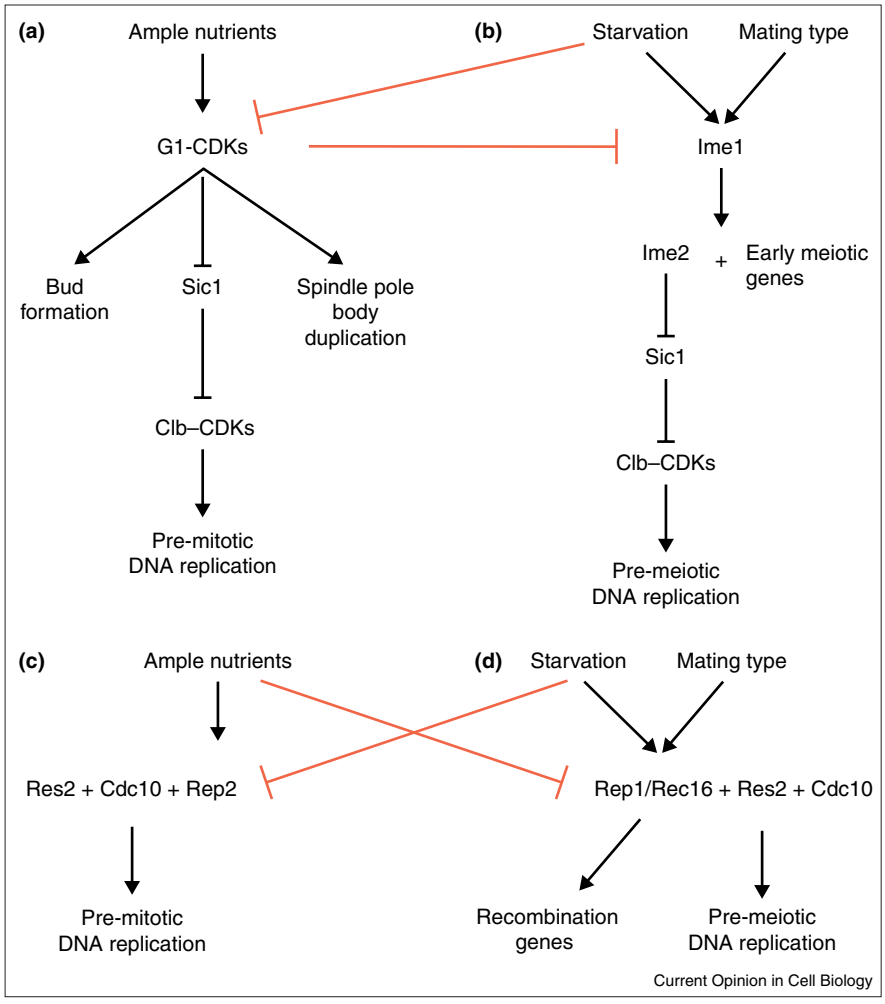
Pre-meiotic S-phase: a time of courtship

In all organisms, pre-meiotic S-phase is substantially longer than pre-mitotic S-phase. An extended S-phase is thought to be required to establish interhomolog interactions required for meiotic recombination and faithful segregation of homologous chromosomes. Indeed, when recombination is abolished in budding yeast, pre-meiotic DNA replication is shortened, suggesting that preparation for recombination is one factor responsible for lengthening pre-meiotic S-phase [9•]. In addition cells deleted for the S-phase cyclins *CLB5* and *CLB6* fail to complete pre-meiotic DNA replication. These cells initiate homologue synapsis and recombination but display defects in both processes [10••]. Further support for the idea that pre-meiotic DNA replication is required for recombination, came from the observation that removal of origins from the left arm of chromosome III delays DNA replication. The formation of recombination-initiating double-stranded breaks (DSBs) in this region was delayed by the same amount of time [11••]. This coupling of recombination to DNA replication is likely to require meiosis-specific factors such as Mum2. *MUM2* genetically interacts with the DNA replication machinery and is required for normal levels of meiotic recombination [12•].

In *S. pombe*, pre-meiotic DNA replication does not appear to be required for the initiation of meiotic recombination. Murakami and Nurse [13••], found that the formation of DSBs is not affected when DNA replication is inhibited or delayed. It is possible that coupling of meiotic recombination to DNA replication is fundamentally different in *S. cerevisiae* and *S.pombe*. It is also possible that differences in methods of assessing the levels of double strand breaks led to these contradictory results. Finally Murakami and Nurse suggest that perhaps in budding yeast DNA replication *per se* is not required for the initiation of recombination, but that S-phase cyclins and origins of replication or factors assembling onto them play a crucial role in meiotic recombination [13••].

Despite the difference in length and added complexity of meiotic DNA replication, the core replication machinery appears to be the same between the mitotic and meiotic cell cycle, at least in *S. cerevisiae* and *S. pombe* [11•,13••,14•]. In addition, regulators of DNA replication, such as S-phase cyclins, are required for both pre-mitotic and pre-meiotic DNA replication [7,10••,15]. Whether the mitotic and meiotic cell cycles use the same replication initiation machinery is, however, controversial. Two reports from fission yeast reach opposite conclusions. Murakami and Nurse found that replication initiation proteins such as the mini chromosome maintenance proteins (MCMs) and Cdc18 are essential for the initiation of pre-meiotic DNA replication [13••], whereas a report by Forsburg and

Figure 2



Control of entry into the mitotic and meiotic cell cycle in budding and fission yeast. **(a,b)** When nutrients are in ample supply, budding yeast reproduces vegetatively. G1-CDKs (Cln–CDKs) promote bud formation, SPB duplication and DNA replication. The initiation of DNA replication requires the activity of another set of CDKs, the Clb–CDKs. Before Cln–CDKs are active, Clb–CDKs are inhibited by the CDK inhibitor Sic1. Cln–CDKs phosphorylate Sic1, thereby targeting it for ubiquitin-mediated degradation. This allows Clb–CDKs to initiate DNA replication. How Cln–CDKs promote bud formation and SPB duplication is poorly understood. While promoting entry into the mitotic cell cycle, Cln–CDKs inhibit cells from entering the meiotic cell cycle. Cln–CDKs inhibit the meiosis-specific transcription factor Ime1 from activating transcription of early meiotic genes, which include *IME2*. The lack of nutrients and the a/α cell type signal lead to activation of the transcription factor Ime1, which activates transcription of early meiotic genes. Nitrogen and carbon source starvation also lead to inactivation of Cln–CDKs by down regulation of *CLN* transcription. The meiosis-specific protein kinase Ime2 replaces Cln–CDKs in promoting DNA replication. **(c,d)** In *S. pombe*, the presence of ample nutrients promotes vegetative growth. A transcription factor complex composed of Cdc10, Res2 and Rep2 induces transcription of genes required for pre-mitotic DNA replication. The lack of nutrition and mating type signals induce the meiotic cell cycle by promoting the accumulation of the meiosis-specific protein Rep1/Rec16. Rep1/Rec16 replaces Rep2 in the Cdc10–Res2–Rep2 transcription factor complex, enabling the complex to promote transcription of genes required for pre-meiotic DNA replication and meiotic recombination. Signals indicating the presence of nutrition inhibit the accumulation of Rep1/Rec16, ensuring that meiosis is not initiated in the presence of ample nutrients. Starvation signals, on the other hand, repress Rep2, ensuring that Rep1/Rec16 can take its place in the Cdc10–Res2 transcription factor complex and promote entry into the meiotic cell cycle.

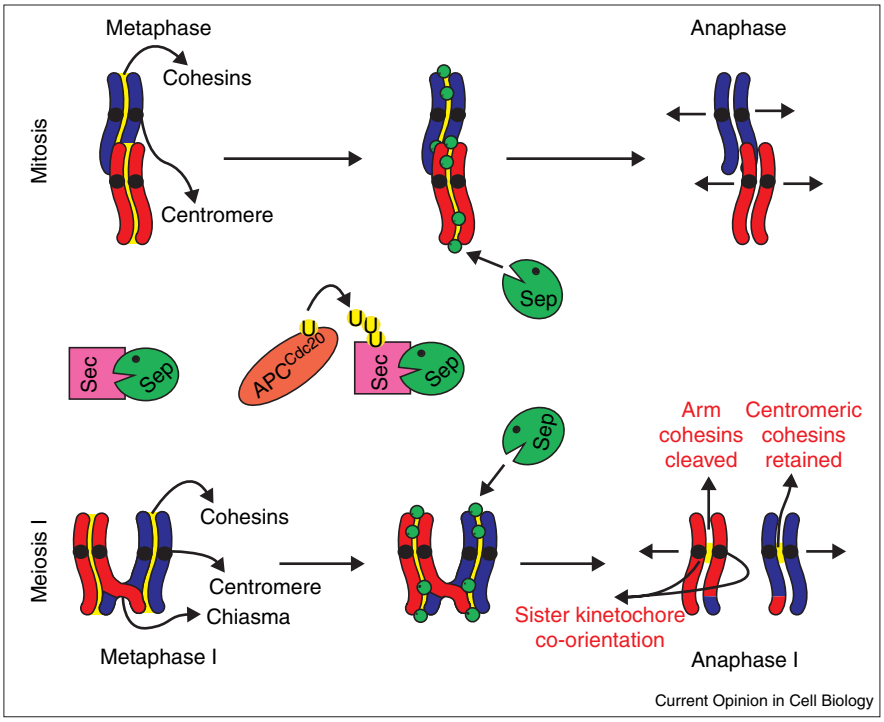
Hodson [14•] suggests that they are not. Both reports agreed, however, that the origin recognition complex (ORC) is required.

Meiosis I: a time of parting

During mitosis, sister chromatids are segregated to each daughter cell (Figure 1). This is accomplished by the pulling force of the mitotic spindle and is resisted by protein complexes called cohesins. Cohesins hold sister chromatids together until they are released at the onset of anaphase (Figure 3). This release is initiated when a ubiquitin ligase called the anaphase promoting complex or cyclosome (APC/C), together with its activator, Cdc20, ubiquitinates the anaphase inhibitor, securin (Pds1 in budding yeast and Cut2 in fission yeast), thereby targeting it for degradation by the proteasome. During S-phase, G2 and metaphase, securin binds to and inhibits a protease known as separase (Esp1 in budding yeast and Cut1 in fission yeast). Upon degradation of securin, separase is free to cleave the cohesin subunit Scc1/Mcd1 in budding yeast and Rad21 in fission yeast, thereby triggering sister-chromatid separation and the onset of anaphase [16] (Figure 3).

During meiosis I, homologous chromosomes and not sister chromatids are segregated. In fact, sister chromatids migrate to the same, rather than opposite, poles of the meiosis I spindle (Figure 1). To accomplish this specialized division, three events need to occur: a physical linkage between homologous chromosomes has to be established to resist the pulling force of the meiosis I spindle; a linkage between sister chromatids has to be maintained beyond meiosis I to prevent premature sister chromatid separation prior to meiosis II; and sister kinetochores have to attach to microtubules emanating from the same pole rather than from opposite poles. Over the past two years, significant progress has been made towards understanding

Figure 3



Control of the metaphase to anaphase transition during mitosis and meiosis. During pre-mitotic DNA replication, cohesins (yellow) are laid down between sister chromatids. At the onset of anaphase, a protease called separase cleaves the cohesin subunit Scc1/Mcd1, thereby initiating sister-chromatid separation. Prior to the onset of anaphase, separase is inhibited by securin. Activation of separase is brought about by a ubiquitin ligase known as the anaphase promoting complex or cyclosome (APC/C), which, together with its specificity factor Cdc20, ubiquitinates securin, thereby targeting it for degradation. Cohesins are also assembled onto chromosomes during pre-meiotic DNA replication. During prophase I of meiosis, homologous chromosomes (blue and red) pair and recombination occurs, leading to the formation of chiasmata. At the onset of anaphase I, separase cleaves the cohesin subunit Rec8 at chromosome arms (Rec8 is a homolog of Scc1/Mcd1 that is specifically expressed during the meiotic cell cycle). Rec8 is spared from cleavage at pericentromeric regions, ensuring that sister chromatids do not separate prematurely. Furthermore, kinetochores of sister chromatids attach to microtubules of the same pole, causing homologous chromosomes to segregate during anaphase I. During the second meiotic division, sister-chromatid kinetochores attach to microtubules from opposite poles, and upon cleavage of the remaining Rec8 around centromeres, sister chromatids separate. Sec, securin; Sep, separase; U, ubiquitin.

the molecular mechanisms controlling these events, leading towards the emergence of the following working model. First, chiasmata, the physical manifestations of recombination events, provide the physical linkage between homologous chromosomes. Second, cohesins at centromeres are protected from cleavage during meiosis I, providing a link between sister chromatids beyond meiosis I. Lastly, specific proteins facilitate co-orientation of sister kinetochores during meiosis I, ensuring that sister chromatids migrate to the same pole during the first meiotic division (Figure 3b).

Chiasma: the tie that binds

In most organisms, chiasmata and cohesion distal to chiasmata link homologs together, allowing them to align on the meiosis I spindle. Indeed, when initiation of recombination is abolished in budding yeast, chromosome segregates randomly during meiosis I and the first meiotic division occurs significantly earlier than in cells that complete recombination successfully [9•,17] The proteins involved in establishing this linkage between the homologs are unknown. Additionally, it is important that each pair of homologous chromosomes experiences at least one reciprocal exchange. The mechanisms that ensure that these exchanges occur are also not understood.

Sister chromatid cohesion: one step at a time

It has long been recognized that cohesion along chromosome arms is lost during meiosis I but that sister chromatids remain associated at centromeres until the onset of anaphase II. It was proposed that this stepwise loss of cohesion is critical for chromosome segregation during both meiotic divisions [18]. Loss of arm cohesion is required for the resolution of chiasmata and thus meiosis I chromosome segregation, whereas maintenance of cohesion at centromeres is important for proper meiosis II segregation [18]. The recent isolation and characterization of a meiosis specific cohesin subunit, Rec8, provides evidence in support of this model. *rec8*$^+$ was isolated in *S. pombe* as a gene affecting meiotic recombination, particularly at the centromeric region [19]. Further studies revealed that Rec8 is a conserved protein with homology to the mitotic cohesin subunit Rad21 and is required for meiotic cohesion [20,21,22]. Studies in *S. cerevisiae* revealed that Rec8 and another cohesin subunit, Smc3, are required for meiotic cohesion since *rec8Δ* and *smc3-73* cells show precocious sister chromatid separation [23]. Rec8 in higher eukaryotes is likely to perform a similar function. Inactivation of REC8 by RNAi in *C. elegans* leads to precocious separation of sister chromatids [24•]. Mammalian REC8 homologs have been isolated but await further characterization [22]. Additional meiosis-specific cohesion subunits are likely to exist in mammalian cells. Stag3/Sa3, a protein with homology to the meiotic cohesion subunit Sa1/Sa2 is only expressed during meiosis and was recently shown to be associated with meiotic chromosomes [25,26].

Immunolocalization of Rec8 in *S. cerevisiae* and *S. pombe* showed that it is lost from chromosomes in a stepwise manner. Rec8 is removed from chromosome arms at the onset of anaphase I but is retained at centromeric regions until sister chromatids separate at the onset of anaphase II [20,23]. In budding yeast, Rec8, like its mitotic counterpart Scc1/Mcd1, is cleaved by separase (Esp1), and this cleavage is essential for progression into anaphase I [27••]. Mutations that render Rec8 uncleavable by Esp1 arrest at metaphase I. When recombination is abolished, cells expressing an uncleavable version of *REC8* progress through anaphase I and arrest at metaphase II [27••]. Thus, in the absence of chiasmata, Rec8 cleavage is dispensable for meiosis I, although it is essential for sister-chromatid separation at the onset of anaphase II. Together, these data strongly support the idea that loss of arm cohesion allows for homolog segregation, and subsequent loss of centromeric cohesion at anaphase II allows for sister-chromatid separation (Figure 3).

The finding that *ESP1* is required for progression into anaphase I suggests that the metaphase I to anaphase I transition may be regulated in a manner similar to that in mitosis (Figure 3). Accordingly, budding yeast securin is present in the nucleus in metaphase I and metaphase II cells, but is absent from anaphase I and anaphase II nuclei [28•]. This pattern of Pds1 localization is consistent with the model that Esp1 is inhibited prior to anaphase I and anaphase II but becomes active during both meiotic divisions when Pds1 is destroyed. In addition, low level expression of a form of Pds1 resistant to ubiquitination by APC/C^{Cdc20} during meiosis causes a delay in metaphase I [29••]. This suggests that APC/C^{Cdc20} is also likely to regulate the metaphase I to anaphase I transition in budding yeast. In support of this model, Cdc20 accumulates in the nucleus just prior to Pds1 destruction during both meiotic divisions [28•]. Evidence that APC/C regulates the onset of anaphase I has also been found in *C. elegans*. Mutations in several APC/C subunits lead to a metaphase I arrest [30•,31•].

In vertebrates, however, APC/C^{Cdc20} does not appear to regulate the metaphase I to anaphase I transition. Immunodepletion of the *Xenopus* Cdc20 homolog Fizzy, or injection of antisense oligonucleotide against *FIZZY* mRNA, cause a metaphase II arrest [32•,33•]. Immunodepletion of the APC/C core subunit, Cdc27 or microinjection of a non-degradable version of securin also fails to arrest cells at metaphase I, suggesting that APC/C and separase activity are not required for entry into anaphase I in *Xenopus* oocytes [32•]. This difference could be explained by the existence of two mechanisms to remove cohesins in vertebrates. An APC/C–separase-independent mechanism preferentially removes cohesins from chromosome arms [34,35] and an APC/C–separase-dependent mechanism removes cohesins from centromeric regions [36]. Perhaps during meiosis I, arm cohesion is removed from chromosomes by the APC/C–separase-independent mechanism and during meiosis II, centromeric cohesin is removed by the APC/C–separase-dependent mechanism.

Meiotic checkpoint

As during the mitotic cell cycle, surveillance mechanisms exist that function to prevent cell cycle progression when meiotic recombination or kinetochore attachment are incomplete or defective. The 'pachytene checkpoint' inhibits cell cycle progression when meiotic recombination is ongoing or when defects in recombination or chromosome synapsis occur [37]. The cell cycle is halted by the phosphorylation of the tyrosine 19 (Y19) residue in Cdc28. Phosphorylation of Y19 inhibits Cdc28 activity leading to cell cycle arrest in prophase I.

Defects in mitotic spindle formation or attachment of chromosomes to the mitotic spindle activate a surveillance mechanism, the spindle checkpoint, that halts cell cycle progression at the metaphase to anaphase transition. Checkpoint proteins such as Mad2 bind to and inhibit APC/C^{Cdc20}, thereby preventing the degradation of securin [38]. Recent work has shown that the spindle checkpoint also functions during meiosis I and is, in fact, essential for meiosis. In *S. cerevisiae*, cells deleted for the spindle checkpoint component *MAD2* show a 10-fold increase in non-disjunction during meiosis I, suggesting that the spindle checkpoint is required to delay homolog segregation until all chromosomes have attached to the meiosis I spindle [29••]. Similar results were obtained in *S. pombe*, where *mad2Δ* and *bub1Δ* cells show moderate non-disjuction during meiosis I [39•].

Centromeric cohesin: linking sisters beyond meiosis I

A key aspect of the regulation of sister-chromatid cohesion during meiosis is that cohesins at pericentromeric regions are protected from cleavage during anaphase I. This is a special property of Rec8-mediated cohesion since expression of *SCC1/MCD1* instead of *REC8* during meiosis results in sister-chromatid cohesion being lost along the entire chromosome during the first meiotic division [40••]. This suggests that either Rec8 itself is refractory to cleavage when bound to pericentromeric regions or, more likely, that factors that specifically bind to Rec8 around centromeres protect Rec8 from being cleaved. Such factors are likely to be or are regulated by Drosphila MEI-S332 and *S. cerevisiae* Spo13 and Slk19. MEI-S332 has been shown to localize to centromeres and mutations in *MEI-S332* lead to premature separation of sister chromatid during meiosis II suggesting that cohesion is lost between the meiotic divisions [41,42]. Slk19 in budding yeast also localizes to meiotic kinetochores prior to meiosis I. Furthermore, in cells lacking *SLK19*, Rec8 staining is greatly reduced around centromeres during anaphase I suggesting that Slk19 plays a role in protecting Rec8 from cleavage at centromeres [43••]. Budding yeast *SPO13* has also been implicated in the regulation of Rec8 cleavage since *spo13Δ* cells loose Rec8 along the entire length of the chromosome during anaphase I [23].

Even though it remains unclear how centromeric Rec8 is protected from cleavage during anaphase I, experiments in grasshopper spermatocytes show that whether or not cohesion is lost at centromeres is a chromosome intrinsic property suggesting that the protector of centromeric cohesin is likely to be chromosome associated. When a meiosis I chromosome is transferred onto a meiosis II spindle, this chromosome segregates in a reductional (meiosis I-like) manner even though the other chromosomes on the same spindle segregate in an equational (meiosis II-like) manner. The reciprocal result is obtained when a meiosis II chromosome is transferred onto a meiosis I spindle; while the meiosis II chromosome segregates equationally, the other chromosomes on the meiosis I spindle segregate reductionally [44••]. Further characterization and isolation of factors like Spo13, Slk19 and MEI-S332 will be critical for determining the exact mechanisms by which linkage between sister chromatids is retained at centromeres until anaphase II and for understanding the meiotic pattern of chromosome segregation.

Co-orientation of kinetochores: sister chromatids unite

To ensure that sister chromatids segregate as a single unit during the first meiotic division, the kinetochores of sister chromatids have to attach to microtubules emanating from the same centrosome/SPB. Recently, a mutant, *mam1*, has been isolated in budding yeast that is defective in co-orientation of sister kinetochores. In *mam1Δ* cells, sister chromatids attach to the meiosis I spindle as if it were a mitotic spindle, with kinetochores attaching to microtubules emanating from opposite rather than the same pole [40••]. The stepwise loss of Rec8, however, is not affected in these cells, as Rec8 disappears from chromosome arms but not from centromeres during this division. This result suggests that co-orientation of kinetochores is genetically separable from the protection of Rec8 at centromeres. The regulation of co-orientation of kinetochores and protection of Rec8 at centromeres are likely, however, to be intimately linked as mutations in *SPO13* or *SLK19* affect both processes. Cells lacking *SPO13* or *SLK19* undergo a single meiotic division in which chromosomes are segregated largely in an equational rather than a reductional manner. This indicates that Rec8 is not protected at centromeres and that a significant percentage of sister kinetochores attaches to microtubules emanating from opposite poles in these mutants [43••,45•,46].

Evidence that protection of Rec8 at the centromeres and co-orientation of kinetochores are intimately linked processes is also found in *S. pombe*. In fission yeast, *rec8Δ* mutants undergo an equational instead of a reductional division at meiosis I, indicating that in the absence of Rec8, sister kinetochores are oriented to opposite poles [20]. In addition, cells lacking the spindle checkpoint component Bub1, which localizes to meiotic kinetochores, are defective in sister kinetochore co-orientation and protection of Rec8 at centromeres in *S. pombe* [39•]. In *bub1Δ* cells, a high percentage of chromosomes undergoes an equational division at meiosis I.

In summary, these findings suggest that proteins at the kinetochore, such as Bub1, Slk19, and Mam1, are required to force sister kinetochores to face the same pole in meiosis I. They also suggest that sister kinetochore co-orientation and protection of Rec8 are likely to be coordinately regulated since many mutants affect both processes. How one influences the other, however, is at present unclear and awaits characterization of protein complexes bound to meiosis I kinetochores.

Conclusion

In the past two years, it has become clear that the cell cycle machinery controlling progression through the mitotic cell cycle is also employed to regulate progression through the meiotic cell cycle with meiosis-specific modifications. The exact mechanism by which cells modulate the mitotic cell cycle machinery to bring about a meiotic cell cycle and the factors involved in this process are still largely uncharacterized. It is clear, however, that the field is progressing at a rapid pace. New tools, such as genome-wide expression analysis and functional genomic approaches [47,48] has and will lead to the identification of meiotic cell cycle regulators [49] making it likely that we will soon know 'how meiosis can work' [50].

Acknowledgements

We are grateful to members of the Amon lab for their critical reading of this manuscript.

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- •• of outstanding interest

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