Cell, Vol. 112, 423–440, February 21, 2003, Copyright ©2003 by Cell Press

Review

Un Ménage à Quatre: The Molecular Biology of Chromosome Segregation in Meiosis

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Sexually reproducing organisms rely on the precise reduction of chromosome number during a specialized cell division called meiosis. Whereas mitosis produces diploid daughter cells from diploid cells, meiosis generates haploid gametes from diploid precursors. The molecular mechanisms controlling chromosome transmission during both divisions have started to be delineated. This review focuses on the four fundamental differences between mitotic and meiotic chromosome segregation that allow the ordered reduction of chromosome number in meiosis: (1) reciprocal recombination and formation of chiasmata between homologous chromosomes, (2) suppression of sister kinetochore biorientation, (3) protection of centromeric cohesion, and (4) inhibition of DNA replication between the two meiotic divisions.

Heredity: A Historical Introduction

Why children resemble, but are not identical to, their parents is a question that has preoccupied mankind for at least 2500 years. The handing down of certain traits or characteristics from parents to progeny is termed heredity, and the earliest written records on the subject are those of the ancient Greek philosopher Hippocrates (460 to 350 B.C.). Hippocrates believed that "sperm" was produced by both men and women and was derived from all parts of the body, with the healthy parts of the body giving rise to healthy sperm and the unhealthy parts to unhealthy sperm–a theory called "pangenesis."

The classical view of heredity, pangenesis, reigned essentially unchanged until the end of the 19th century. During the latter half of the 19th century, two major threads of inquiry, namely genetic and cytological, culminated in the "chromosome theory of inheritance." In 1865, Gregor Mendel, a Moravian friar who today is heralded as the father of genetics, proposed a revolutionary theory of heredity (Mendel, 1866), which remained in obscurity until the rediscovery of Mendel's work by Carl Correns, Hugo De Vries, and Erich Tschermak at the beginning of the 20th century. Mendel had carefully quantified the ratios of different characters of the progeny resulting from defined crosses, using the garden pea species *Pisum sativum*. This allowed him to build the hypothesis that discreet determinants of unknown nature, one maternal and one paternal, are transmitted from one generation to the next and that the combination of these determinants controls particular traits or characters.

The recognition of the nature of the hereditary determinants postulated by Mendel was a gradual process. As early as 1766, Joseph Kölreuter had found that each parent contributes equally to the characteristics of the offspring by crossing various species of the tobacco plant *Nicotiana*. In the 1870s, it was shown by Oskar Hertwig and Hermann Fol that the parental sperm and egg nuclei fuse at fertilization. A further key observation made by Edouard van Beneden in 1883 was that the nuclei of the gametes of the parasitic nematode *Ascaris* contain only two chromosomes, while the nucleus of the zygote created following fertilization contains four chromosomes, each of which segregates longitudinally during the first cleavage division of the embryo. These and other observations paved the way for Eduard Strasburger, Oskar Hertwig, and August Weismann to recognize that the hereditary information must be contained within the nuclei of the gametes, a conclusion addressed experimentally by Theodore Boveri in 1889. Boveri found that eggs of the sea urchin species *Sphaerechinus granularis*, when fertilized with sperm from the species *Psammechinus microtuberculatos,* developed into larvae intermediate in character between the two species. When enucleated *S. granularis* eggs were fertilized with *P. microtuberculatos* sperm, however, the resulting larvae resembled only *P. microtuberculatos*, leading to the conclusion that it was the sperm nucleus and not the maternal cytoplasm that influenced heredity. Due to the inefficiency with which such "merogenic" hybrids were formed, this conclusion was never established beyond all doubt. Nevertheless, this knowledge, in addition to the cytological characterization of chromosomes during cell division, allowed Walter Sutton and Boveri himself, in 1903, following the rediscovery of Mendel's work, to deduce that the carriers of the discreet hereditary determinants described by Mendel were none other than the chromosomes and, thus, the chromosome theory of Mendelian heredity was borne. By analyzing sea urchin eggs fertilized by two sperm (and hence containing two separate mitotic organizing centers) in which massive chromosome missegregation ensued, Boveri also demonstrated that correct development required a copy of each chromosome (Figure 1). Until this point, it had been assumed by many that all chromosomes carried the same information. Boveri's experiments showed that each chromosome of a haploid set carried unique developmental information and it is, therefore, to Boveri that we owe the discovery of the genome (for details on Boveri's and other historic experiments mentioned above, see Edmund B. Wilson's "The Cell in Development and Heredity," 1925, and references therein).

Errors in the transmission of chromosomes during either meiosis or mitosis can lead to aneuploidy; that is, an aberrant number of chromosomes. As long ago as 1914, Boveri proposed that chromosome abnormalities might cause malignancy in cells. Proof of this proposal would, however, have to await the second half of the 20th

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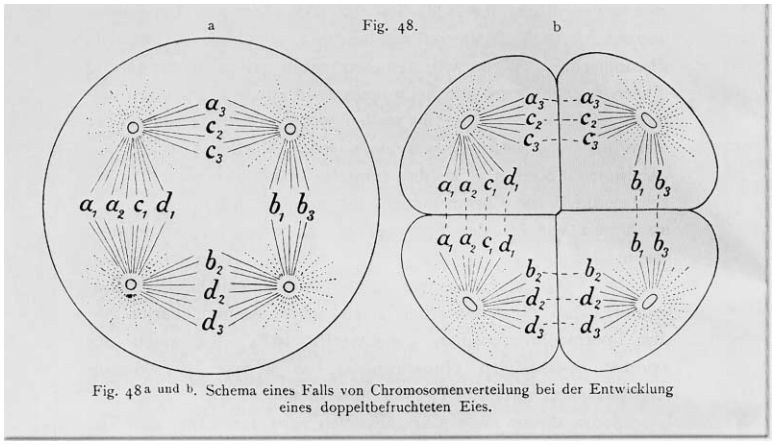


Figure 1. Boveri's Drawing (1904) of Chromosome Segregation in a Sea Urchin Egg Fertilized by Two Sperm

(A) The fertilization of the oocyte by two sperm results in an egg containing four spindle poles (tetrapolar) and three copies of each chromosome (e.g., a_1, a_2, a_3). Boveri used dispermic and, hence, tetrapolar eggs as an experimental means of generating errors in chromosome segregation.

(B) The irregular and simultaneous distribution of the three copies of each chromosome to the four daughter cells allowed Boveri to study the effect of aneuploidy on development. The daughter cell on the top left, for example, lacks chromosome "b," whereas the cell on the lower right lacks chromosomes "a" and "c." Boveri concluded that chromosomes, contrary to contemporary belief, were not identical and that each chromosome of a haploid set carried unique developmental information.

century, when it was discovered that the Philadelphia chromosome (a chromosomal translocation between chromosomes 9 and 22) was the genetic cause of chronic myelogenous leukemia (Nowell and Hungerford, 1960; Rowley, 1975). It is now known that aneuploidy is associated with many forms of cancer (Jallepalli and Lengauer, 2001). Meanwhile, errors in meiotic chromosome segregation are the leading cause of miscarriage and are also responsible for genetic disorders such as Down's Syndrome, which is caused by an extra copy of chromosome 21 (Hassold and Hunt, 2001). The faithful transmission of chromosomes to daughter cells during cell division is, therefore, fundamental to the survival and reproduction of all living organisms.

Meiosis and Mitosis

A question of central importance to heredity in sexually reproducing eukaryotes is how haploid gametes are generated from diploid somatic cells. The answer lies in a specialized form of cell division during which the number of chromosomes is reduced. In 1905, Farmer and Moore termed this specialized form of cell division meiosis (derived from the Greek word μειωσις, meaning "reduction"). The reduction in chromosome number in the gametes is achieved by one round of DNA replication being followed by two rounds of chromosome segregation with no intervening round of DNA replication. Fusion of two gametes during sexual reproduction restores the diploid complement of chromosomes in the zygote that gives rise to a new individual (Figure 2). To understand how chromosomes are segregated during meiosis, which is the focus of this review, it is necessary to first understand the principles of chromosome segregation during mitosis.

The term mitosis (derived from the Greek word μιτος, meaning "thread," which refers to the thread-like appearance of condensed chromosomes) was introduced by Walther Flemming in 1882, who made detailed observations of chromosomes during cell division in *Salamandra maculosa*. During mitosis, one round of DNA replication is followed by a single round of chromosome segregation, thus generating two genetically identical daughter cells. Shortly before cells divide, sister DNA molecules (sister chromatids) are dragged to opposite sides of the cell by microtubules, which are attached to chromatids via specialized structures called kinetochores (Figure 3).

Kinetochore-Microtubule Attachments and Aurora B Kinase

The region of the chromosome containing the kinetochore is known as the centromere. How microtubules attach to kinetochores and pull on them is poorly understood despite the discovery of an increasing number of kinetochore proteins (reviewed in Kitagawa and Hieter, 2001). Both microtubule depolymerization, occurring while the microtubules are still attached to kinetochores, as well as motor proteins are thought to be involved (reviewed in Mitchison and Salmon, 2001).

The crucial question is how mitotic cells ensure that sister kinetochores attach to microtubules with opposite orientations, which is known as amphitelic attachment, and allow their traction to opposite poles (Figures 3D and 4A). Attachment of sisters to microtubules with the same orientation, known as syntelic attachment (Figure 3A), is not only possible, but might also actually occur quite frequently. Syntelic attachment is invariably eliminated in normal cells. A failure to eliminate such events results in gain or loss of chromosomes and, hence, aneuploidy. A yet further complication is that kinetochores in most eukaryotes, the budding yeast *Saccharomyces cerevisiae* being a notable exception, contain multiple microtubule attachment sites, and cells must avoid microtubules with opposing orientations from attaching to binding sites on the same chromatid, which is known as merotelic attachment (Figure 3B). This situation hinders or prevents a chromatid's traction to one pole during anaphase, which can also lead to aneuploidy. It has, in fact, been suggested that merotelic attachment is the prime cause of aneuploidy in human primary tissue culture cells (Cimini et al., 2001).

The conserved Ipl1/Aurora B kinase (Chan and

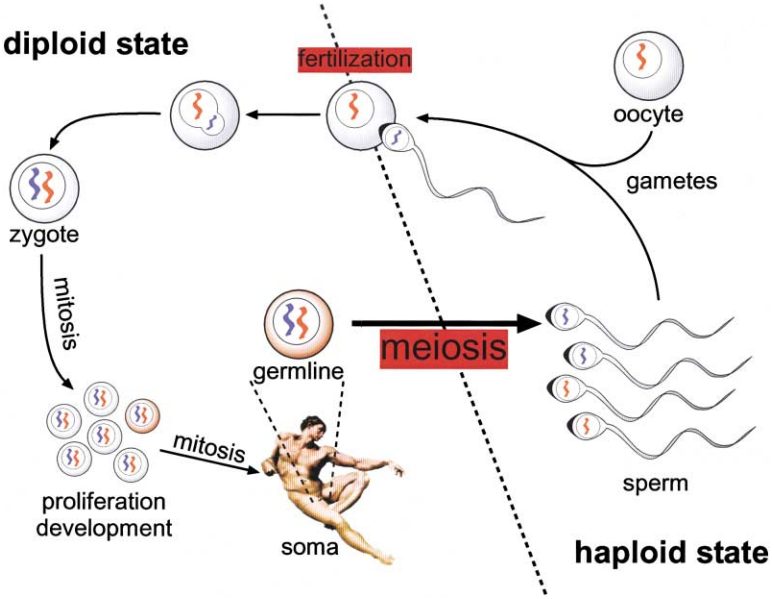


Figure 2. The Metazoan Life Cycle

Chromosome number and genome size are reduced during a specialized cell division called meiosis in order to keep the genome size constant over successive generations. Crossover events occurring during meiosis have been omitted from this figure.

Botstein, 1993; Francisco et al., 1994; Schumacher et al., 1998) appears to lie at the heart of the cellular mechanism that resolves syntelic and promotes amphitelic attachments in budding yeast, the nematode *Caenorhabditis elegans*, and mammalian cells (Biggins et al., 1999; Kaitna et al., 2002; Kallio et al., 2002; Tanaka et al., 2002) (Figure 3). In the absence of this protein kinase, syntelic attachments are not corrected and persist (Tanaka et al., 2002). The kinase is not required for attachment per se but rather for eliminating syntelic attachments when they occur (Figure 3). Ipl1 in *S. cerevisiae* is thought to cause detachment of microtubules from kinetochores by phosphorylating the Dam1 subunit of the Dam-Duo kinetochore complex (Cheeseman et al., 2002). The relevant substrates of Ipl1/Aurora B in higher organisms remain to be identified. Ipl1/Aurora B kinase acts in a conserved complex with two other proteins, INCENP and Survivin, which are required for the proper localization of the kinase and might regulate its activity (reviewed in Adams et al., 2001). The failure of chromosome segregation in Aurora-B-depleted nematode embryos has been attributed to the accumulation of merotelic attachments (Kaitna et al., 2002). Thus, amphitelic attachment may be ensured by destabilizing syntelic and merotelic attachments rather than by preventing their creation in the first place (Figure 3). How the Ipl1/Aurora B kinase distinguishes deleterious syntelic and merotelic from correct amphitelic attachments, destabilizing the former but not the latter, is of pivotal importance for future research.

Recognizing and Biorienting Sisters through Cohesion and Tension

It would be extremely difficult to understand how cells made the crucial distinction between syntelic and amphitelic attachment, were sister chromatids not inter-

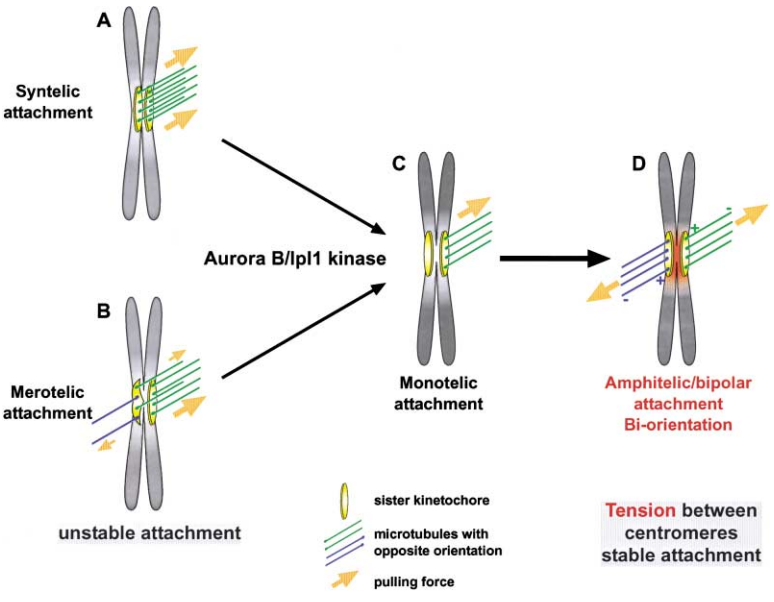


Figure 3. Kinetochore-Microtubule Attachments during Mitosis

During mitosis, kinetochores of replicated sister chromatids can attach to microtubules by different modes, but only amphitelic attachment (D) permits the proper segregation of sister chromatids to opposite poles during cell division. The Aurora B/Ipl1 kinase is thought to destabilize and eliminate syntelic (A) and merotelic (B) attachments, thereby producing monotelic attachments (C). This allows chromosomes to reattach and create stable amphitelic attachments (D), which lead to biorientation of all chromosomes on the metaphase plate.

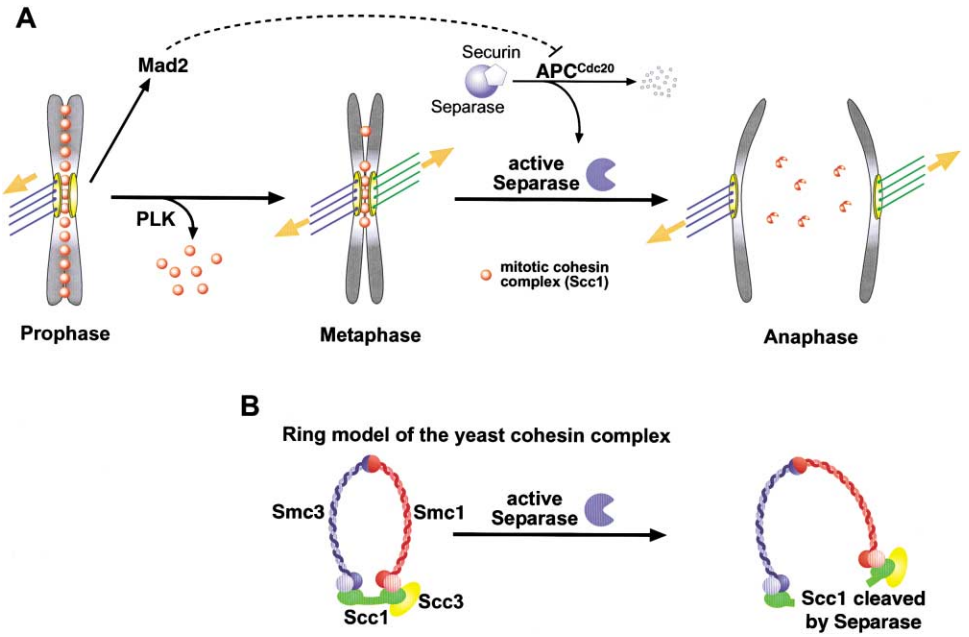


Figure 4. Chromosome Segregation during Vertebrate Mitosis and the Cohesin Complex

(A) Sister chromatids are held together along their entire length by the cohesin complex from their generation during replication until mitosis. During prophase, most cohesin complexes along chromosome arms are removed from chromosomes through their phosphorylation by Polo-like kinase (PLK). The remaining cohesin complexes at centromeric regions hold the resolved sister chromatids together at metaphase, allowing their biorientation on the mitotic spindle. Once all chromosomes have bioriented, the APC/C bound to Cdc20 is activated and induces the degradation of securin, thus liberating separase from its inhibitor. Activated separase proteolytically cleaves the Scc1 subunit of the cohesin complex, leading to cohesin's dissociation from chromosomes, the loss of sister chromatid cohesion, segregation of chromatids to opposite poles, and the initiation of anaphase. Lagging chromosomes with unattached kinetochores, which have not yet bioriented on the mitotic spindle, block the initiation of anaphase by inhibiting the APC/C. This surveillance mechanism is called the spindle checkpoint and requires the Mad2 protein.

(B) The architecture of the budding yeast cohesin complex resembles a ring consisting of Smc3, Smc1, and Scc1. Smc3 and Smc1 form long, antiparallel, intra-molecular coiled-coil stretches and dimerize through a central globular domain. The two ends of the V shaped Smc3/Smc1 heterodimer are linked by Scc1, which thereby closes the ring. It has been suggested that cohesin may hold sister chromatid DNA strands together by entrapping them in the center of the ring. Cleavage of Scc1 by separase leads to opening of the ring and dissociation of cohesin from chromosomes, thus allowing them to be pulled to opposite poles in anaphase.

connected. It has been clear since chromosomes were first detected by early cytologists that sister chromatids remain associated until they are pulled toward opposite poles at the onset of anaphase (Figure 4A). This association is known as sister chromatid cohesion. Cohesion is strongest in the vicinity of centromeres, hence creating the chromosome's central constriction. Connections, nevertheless, also exist along the entire interchromatid axis of each chromosome until sisters disjoin fully and are pulled toward opposite poles of the cell. The degree of cohesion varies between organisms. In yeast, most sister DNA sequences are so closely associated that they appear as a single spot by fluorescence in situ hybridization (FISH) analysis until the onset of anaphase (Guacci et al., 1994), while in most animal and plant cells, there is so little cohesion along chromosome arms that most sister DNA sequences are resolved as distinct twin spots by FISH analysis (Ried et al., 1992).

Due to sister chromatid cohesion, amphitelic, but not syntelic, attachment results in an equilibrium in which the tendency of spindles to pull sisters apart is resisted by cohesion holding them together (Figures 3 and 4A). This generates tension in centromeric chromatin, which is thought to cause kinetochore-microtubule attachments to become refractory to the effects of the Ipl1/Aurora B kinase. This stage of mitosis is, therefore, similar to a "dance" in which the partners not only move together, but also do so under a state of tension between them, similar to dancing a Viennese waltz or rock and roll. The role of tension in stabilizing kinetochore-microtubule attachments has, so far, only been investigated in meiotic cells, where the two dancing partners are not sister chromatids but homologous (maternal and paternal) chromosomes held together by chiasmata (see below). By pulling on one homolog with a glass needle, it is possible to stabilize its partner homolog's connection to microtubules (Ault and Nicklas, 1989), possibly by inactivating the Ipl1/Aurora B kinase.

The state of tension generated by sister chromatid cohesion and amphitelic attachment is known as biorientation. During this process, polar winds, defined as pressure exerted on chromosomes by unattached or polar microtubules growing out from the poles, cause bioriented chromosomes to migrate to the midpoint between poles, creating the metaphase plate (reviewed in Mitchison and Salmon, 2001). When the last chromosome has bioriented at metaphase, destruction of sister chromatid cohesion triggers the migration of chromosomes to the poles during anaphase (Uhlmann et al., 2000) (Figure 4A). The metaphase to anaphase transition

is the point of no return during mitosis. If cohesion between chromatids is destroyed before all chromosomes have bioriented on the mitotic spindle, the cell has no mechanism to ensure that sister chromatids move to opposite poles. It is, therefore, one of the most highly regulated events during the mitotic cell cycle.

Sister Chromatid Cohesion

The nature of sister chromatid cohesion remained elusive long after the discovery of microtubules and the sites of their attachment to chromosomes. It was suggested that inter-catenation of sister DNA molecules produced by the collision of converging replication forks (Sundin and Varshavsky, 1980) might be responsible for holding sisters together until the metaphase to anaphase transition (Murray and Szostak, 1985). This intertwining of chromatids undoubtedly exists, and the cell requires a special enzyme, namely topoisomerase II, to decatenate sisters after replication and during mitosis (DiNardo et al., 1984; Downes et al., 1991). Topoisomerase II is an essential enzyme without which chromatids fail to disengage properly either during the process of chromatid individualization in prophase (Gimenez-Abian et al., 2000) or during chromatid disjunction in anaphase (DiNardo et al., 1984). However, there is no evidence that a delay in decatenating sister DNA molecules is responsible for creating the cohesion needed to resist microtubule-induced splitting during mitosis. Instead, it seems increasingly likely that chromatids are held together by a multisubunit complex called cohesin (Figure 4B), whose constituents were first identified in the yeast *S. cerevisiae* by the isolation of mutants incapable of holding sisters together during metaphase (Guacci et al., 1997; Michaelis et al., 1997; Toth et al., 1999).

The cohesin complex consists of four proteins (Figure 4B): Scc1 (also known as Mcd1 and Rad21), Scc3 (also known as SA1 and SA2), Smc1, and Smc3 (Darwiche et al., 1999; Guacci et al., 1997; Losada et al., 1998; Michaelis et al., 1997; Sumara et al., 2000; Toth et al., 1999). A fifth protein called Pds5 is more loosely associated but is also important for cohesion (Hartman et al., 2000; Panizza et al., 2000). The genomes of all eukaryotic cells encode homologs of all five of these proteins. In both yeast and mammalian cells lacking cohesin, sister chromatids separate precociously, leading to inefficient biorientation, massive errors in chromosome segregation, and hence, to aneuploidy (Hoque and Ishikawa, 2002; Sonoda et al., 2001).

Cohesin's localization both spatially and temporally makes it a good candidate to be directly involved in holding sister DNA molecules together. In budding yeast, where chromatids are very closely held together along their entire length until the onset of anaphase, cohesin is tightly associated with chromosomes until the metaphase to anaphase transition (Michaelis et al., 1997; Toth et al., 1999), whereupon proteolytic cleavage of its Scc1 subunit by a cysteine protease called separase triggers its dissociation from chromatin and the destruction of cohesion (Uhlmann et al., 2000). In mammalian cells, the bulk of cohesin associated with chromosome arms dissociates at the time of their individualization during prophase (Losada et al., 1998; Sumara et al., 2000) (Figure 4A), but much remains at centromeres, where cohesion between chromatids is tightest. Dissociation of cohesin, initiated during prophase, is thought to be driven by its phosphorylation by Polo-like kinases (Sumara et al., 2002) and is required for sister chromatid resolution (Losada et al., 2002) while that of centromeric cohesin at the onset of anaphase is mediated by cleavage of Scc1 by separase (Waizenegger et al., 2000) (Figure 4A).

Whether cohesin really bridges sister DNAs and if so, how, is not fully understood. There is, nevertheless, mounting evidence that cohesin forms a proteinaceous loop within which DNA strands might be entrapped (Haering et al., 2002; S. Gruber et al., submitted) (Figure 4B). Indeed, purified human cohesin forms ring-like structures detectable by electron microscopy (Anderson et al., 2002). Smc1 and Smc3 form long, rod-shaped proteins whose N- and C-terminal halves fold back on themselves to form long, up to 50 nm stretches of intramolecular and antiparallel coiled coils (Haering et al., 2002; Melby et al., 1998) (Figure 4B). One Smc1 and one Smc3 molecule are joined together by globular domains at the center of their folding axes. In both soluble and chromatin bound forms of cohesin, its Scc1 subunit bridges the Smc1 and Smc3 heads, which much of the time are not otherwise stably connected, and thereby closes the ring (S. Gruber et al., submitted) (Figure 4B). Scc1's N- and C-terminal domains bind to the heads of Smc3 and Smc1, respectively. Significantly, separase cleaves Scc1 in the region that connects its N- and C-terminal domains (Uhlmann et al., 2000) (Figure 4B), which opens the ring and causes cohesin to fall off chromosomes. These observations have led to the notion that cohesin acts like a karabiner; that is, a ring with a gate, which traps DNA strands. Trapping of DNA inside the ring would require opening of its Scc1 gate, passage of DNA inside the ring, and closure of the gate after it. How cohesin's gate can first open and then close before being cleaved is an important question for future research.

Cohesin binds to chromosomes prior to DNA replication, but it builds connections between chromatids only during DNA replication (Uhlmann and Nasmyth, 1998). If cohesin is expressed after DNA replication, it still associates with chromatin but is unable to hold sisters together. The close physical proximity of newly born double-stranded sister DNAs, a situation occurring exclusively following the passage of the replication fork, presumably facilitates the establishment of cohesion.

Dissolution of Sister Chromatid Cohesion and Anaphase

The "tug of war" between cohesin and microtubules during metaphase is finally resolved by the cleavage of cohesin's Scc1 subunit by separase (Uhlmann et al., 2000; Waizenegger et al., 2000), which is a cysteine protease distantly related to the caspases involved in programmed cell death (Aravind and Koonin, 2002). Proteolysis of Scc1 by separase triggers the dissociation of cohesin from chromosomes, the destruction of cohesion, and the sudden segregation of sister chromatids to opposite poles of the cell in anaphase (Uhlmann et al., 1999) (Figure 4A). Separase is kept inactive for most of the cell cycle by binding to an inhibitory chaperone

called securin (Ciosk et al., 1998; Uhlmann et al., 1999; Waizenegger et al., 2002; Zou et al., 1999). Securin is only removed at the metaphase to anaphase transition through proteolysis (Cohen-Fix et al., 1996; Funabiki et al., 1996) mediated by an ubiquitin protein ligase called the anaphase-promoting complex or cyclosome (APC/C) (reviewed in Zachariae, 1999) (Figure 4A). Activation of the APC/C by a WD40 protein called Cdc20 (Visintin et al., 1997) causes the ubiquitination and, hence, destruction not only of securin, but also of mitotic cyclins, which, when complexed with the protein kinase Cdk1 (cyclin-dependent protein kinase), drive G2 cells into mitosis.

Because separase appears to act globally within the cell, it must not be activated while chromosomes still exist that have not yet attached to microtubules in an amphitelic manner. Kinetochores of such "lagging" chromosomes activate a mitotic checkpoint, called the spindle checkpoint, and thereby one of its key players, Mad2 (reviewed in Musacchio and Hardwick, 2002). Mad2 inhibits the APC/C and, thereby, exerts a veto on the destruction of securin, and hence, also on separase activation (Figure 4A). Only once every chromosome has bioriented is this inhibitory signal shut off. In budding yeast, lagging chromosomes halt separase activation solely through the control of securin proteolysis by the APC/C (Alexandru et al., 1999). Additional mechanisms controlling separase activity and, hence, anaphase must exist in mammals as cell lines lacking securin still arrest the onset of anaphase in response to spindle poisons (Jallepalli et al., 2001). Cyclin destruction as well as that of securin by the APC/C may be important for separase activation because in vertebrate cells, separase is inhibited through its phosphorylation by Cdk1 (Stemmann et al., 2001). Phosphorylation of Scc1 by Cdc5/Polo kinase, which enhances Scc1's ability to act as a separase substrate, also regulates the Scc1 cleavage reaction, at least in budding yeast (Alexandru et al., 2001).

Coupling DNA Replication to Chromosome Segregation

Re-replication of the genome does not usually occur until chromatids produced at the previous round of replication have already been segregated to daughter cells. This is achieved by coupling preparations for a new round of replication with the execution of anaphase (reviewed in Diffley and Labib, 2002). Initiation of DNA replication in eukaryotic cells is a two-step process. The first step is the assembly at origins, during a period of low Cdk activity, of prereplication complexes containing, amongst other proteins, the Mcm helicase needed to melt origins. The second is activation of the Mcm helicase, which promotes the binding of the single-strand binding protein RP-A and DNA primase, leading to firing of origins and the initiation of DNA replication. The assembly of prereplication complexes is inhibited both by Cdk1 and geminin, a protein whose destruction, like that of mitotic cyclins, is mediated by Cdc20 and the APC/C. Prereplication complex assembly cannot, therefore, occur during G2 or mitosis and only commences upon the destruction of cyclins and geminin, which takes place alongside that of securin at the onset of anaphase. The firing of origins that have previously assembled prereplication complexes is, on the other hand, triggered by the reactivation of Cdk1 (or its close relative Cdk2) along with Cdc7-like protein kinases. As the same protein kinase inhibits the formation of prereplication complexes as that which triggers their activation, each round of chromosome replication depends on a cycle of low and high Cdk activity. S phase is, therefore, linked to the completion of M phase.

The Logic of Meiosis

The purpose of meiosis is to generate haploid gametes from diploid cells. Meiosis, like mitosis, begins with the replication of DNA, thus producing a cell with four chromatids of each type of chromosome—two maternal and two paternal (Figure 5B). These four chromatids have to be distributed to four different nuclei. This is executed simultaneously for all chromosomes and is achieved by two rounds of chromosome segregation without any intervening DNA replication (Figure 6). In the case of spores in fungi, pollen tetrads in plants, or spermatozoa in animals, all four haploid nuclei give rise to four different cells, while in the female meioses of animals, one nucleus from each meiotic division is discarded as a polar body and only one haploid nucleus ends up in the oocyte.

In reducing the chromosome number, it is not merely sufficient to end up, in a random manner, with a haploid number of chromosomes in the gamete. The haploid gametes produced by meiosis must contain one copy of each unique chromosome. The only way of both reducing chromosome number and ensuring that gametes inherit a complete copy of the genome is to segregate maternal and paternal versions of each chromosome, known as homologous chromosomes, in opposite directions at the first of the two meiotic divisions (known as meiosis I and meiosis II) (Figure 6). Sister chromatids can then be segregated during meiosis II. Replicated maternal and paternal chromosomes and not sister chromatids are, therefore, the dancing partners during meiosis I. For this to occur, homologs must be joined prior to their segregation. Reciprocal recombination and the resulting chiasmata between homologous non-sister chromatids usually play a key role in this linkage.

The name chiasma (from the Greek word $\chi\iota\alpha\sigma\mu\omega\varsigma$, meaning "x shaped cross") was given by Janssens (1909) to the cross-shaped structures observed between chromosomes in the diplotene and diakinesis stages of meiotic prophase (Figure 5). Janssens rightly believed that each chiasma results from an exchange between a maternal and a paternal chromatid. In effect, maternal and paternal chromatids are broken at the equivalent position, and the left fragment of one is joined to the right fragment of the other and vice versa. It is important to appreciate that chiasmata only hold homologs together by virtue of cohesion between sister chromatids, which is maintained throughout those regions of the chromosome that have not undergone exchanges (Figure 5). In addition to facilitating chromosome segregation during meiosis I, reciprocal recombination generates new combinations of alleles and genetic variation between the progeny of a given set of parents.

Connecting homologs via chiasmata is not, however, sufficient to ensure that they are segregated from one another during the first division. In stark contrast to

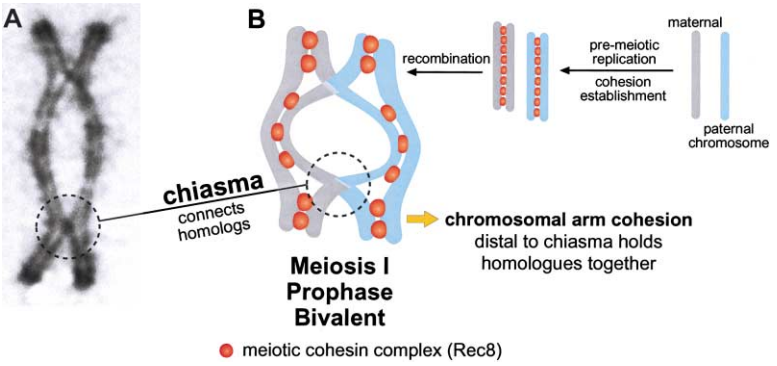


Figure 5. Chiasmata and Cohesin Physically Link Maternal and Paternal Chromosomes in Meiosis

(A) Image of a bivalent at the diplotene stage from grasshopper spermatocytes stained with Feulgen (courtesy of Dr. Jasna Puizina, Institute of Botany, University of Vienna, Austria). Two chiasmata are visible as connections between sister chromatids of the maternal and paternal chromosomes. One chiasma is highlighted by a dashed circle. A schematic representation is shown in (B).

(B) During premeiotic DNA replication, cohesion between sister chromatids is established by a meiosis-specific variant of the cohesin complex containing Rec8. Following replication, meiotic recombination between homologous maternal (gray) and paternal (light blue) chromosomes generates reciprocal exchanges/crossovers. These exchanges lead to the formation of chiasmata, which connect the two chromosomes of a bivalent and can be visualized by microscopy (A). Cohesion between sister chromatids distal to the chiasma serves as the glue that holds maternal and paternal chromosomes together.

mitosis, meiosis I sister kinetochores must attach to microtubules with the same polarity (syntelic attachment of sister kinetochores), a phenomenon called mono-orientation of sister kinetochores (Figures 6 and 9). Because chiasmata link homologous chromosomes, syntelic attachment, which in mitosis is not capable of generating tension, is now able to do so. A new form of equilibrium is created during metaphase I—one in which maternal centromeres are pulled away from paternal ones but are prevented from disjoining by chiasmata and cohesion between sister chromatids holding them together (Figure 6).

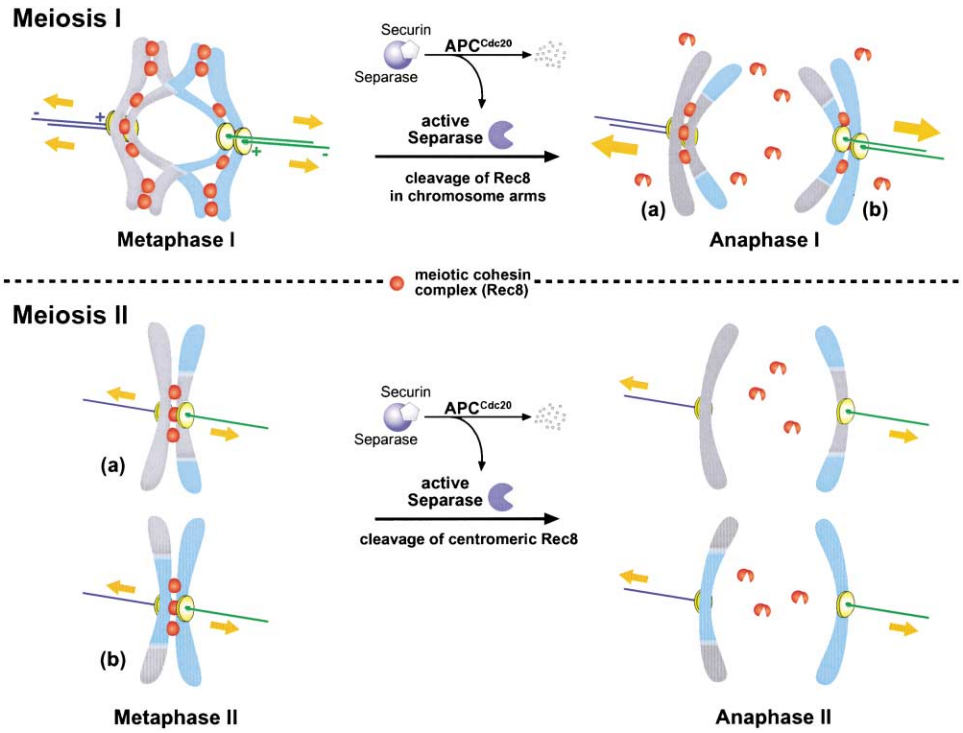


Figure 6. Chromosome Segregation and Rec8 Cleavage in Budding Yeast Meiosis

(Meiosis I) Maternal and paternal chromosomes are connected by chiasmata and are aligned on the spindle in metaphase I. Segregation of the maternal and paternal chromosomes to opposite poles during meiosis I is triggered by cleavage of Rec8 by separase along chromosomal arms distal to chiasmata. Prior mono-orientation of sister kinetochores allows maternal (a) and paternal (b) sister centromere pairs to be pulled apart from each other in anaphase I. Cohesin present at centromeres is protected from cleavage by separase and continues to hold sister centromeres together in order to allow biorientation and segregation in meiosis II.

(Meiosis II) Amphitelic attachment of sister centromeres of maternal and paternal chromosomes and, presumably, cleavage of de-protected centromeric cohesin by separase induces anaphase II. This division strongly resembles conventional mitosis and sister centromeres are segregated to opposite poles. The net result of both meiotic divisions is the generation of recombinant chromosomes, the reduction of chromosome number by half, and the production of haploid gametes.

As in mitosis, the equilibrium achieved by metaphase I is broken by the destruction of sister chromatid cohesion, which permits microtubules to drag maternal and paternal centromere pairs to opposite poles of the cell. However, if sister chromatids were split completely during the first meiotic division as they are during mitosis, meiotic cells would have no means of ensuring that chromatids segregated correctly during the second division. This, then, is one of the great challenges for meiotic cells. How are two rounds of chromosome segregation achieved after only one round of DNA replication and cohesion establishment? This conundrum is solved by using sister chromatid cohesion along chromosome arms for chromosome alignment during the first meiotic division, while using cohesion between sisters in the vicinity of centromeres during the second division (Figure 6). To make this possible, centromeric cohesion must be refractory to the process that destroys cohesion along arms and triggers the first meiotic division so that it can be used during the second division.

Unlike mitosis and the first meiotic division, the second meiotic division is not preceded by a round of DNA replication. Sister kinetochores attach to microtubules in an amphitelic manner and sister chromatids (note that by now most are recombinant) are segregated to opposite poles at the metaphase to anaphase transition due to destruction of the residual cohesion holding sister centromeres together (Figure 6).

The first and second meiotic divisions are often called the reductional and equational division, respectively. These terms are misleading as the arm regions of the chromosomes segregate both partially reductionally and partially equationally at both divisions, depending on where recombination between maternal and paternal chromatids has occurred (Figure 6).

In summary, the behavior of chromosomes in meiosis is much more complex than in mitosis. Additional demands such as chiasmata formation, mono-orientation of sister kinetochores, protection of centromeric cohesion, and prevention of DNA replication between the two divisions are imposed upon the chromosome segregation machinery. These processes are discussed in detail in the following sections. Despite its greater complexity, there is no clear evidence that meiosis evolved later than mitosis. There are, for example, no extant lineages that appear to have split off the eukaryotic tree before the evolution of meiosis (Cavalier-Smith, 2002).

Cohesion in Meiosis

Cohesion between sister chromatids in meiosis is established during premeiotic DNA replication and is mediated by a meiosis-specific form of the cohesin complex (Figure 5). Meiotic cohesin differs from its mitotic equivalent, partly because it has to participate in the recombination process (Klein et al., 1999) and partly because cohesin holding sister centromeres together must survive the first meiotic division so that it can direct the second one (see below). In budding yeast, it appears that meiotic and mitotic cohesin differ only by the replacement of Scc1 by the meiosis-specific variant Rec8 (Klein et al., 1999). In mammalian cells, however, Smc1 is at least partially replaced by a meiosis-specific variant called Smc1β (Revenkova et al., 2001), the Scc3 subunits SA1 and SA2 by a meiosis-specific variant called STAG3 (Prieto et al., 2002; 2001), and Scc1 by meiosis-specific Rec8 (N. Kudo, personal communication). If the *REC8* gene is deleted in *S. cerevisiae*, sister chromatids lose cohesion following premeiotic DNA replication, leading to the formation of aneuploid gametes (Klein et al., 1999). Depletion of the Rec8 ortholog in *C. elegans* oocytes by RNA interference (RNAi) causes sister chromatids to separate almost completely by the end of first meiotic prophase (Pasierbek et al., 2001). In meiosis of the fission yeast, *Schizosaccharomyces pombe*, deletion of *rec8* leads to a loss of cohesion only at centromeres because the mitotic cohesin Scc1/Rad21 is expressed and provides cohesion along arms (Watanabe and Nurse, 1999). As in mitosis, meiotic cohesin can be loaded on chromosomes both before and after DNA replication, but it only appears capable of building cohesion if present during replication. If meiosis is induced after mitotic DNA replication in fission yeast, the meiotic cohesin Rec8 is loaded onto chromosomes but is unable to perform its function, recombination is defective, and the first meiotic division is mitotic in nature (Watanabe et al., 2001). In other words, sister chromatids, as opposed to homologous chromosomes, are separated from one another. It is, therefore, of fundamental importance that the meiotic program is activated before DNA replication, which allows cells to undergo a special premeiotic S phase during which cohesion using the meiosis-specific cohesin complex including Rec8 can be established.

One Cohesin Cycle, Two Divisions

The resolution of chiasmata needed to permit homologous chromosome segregation during meiosis I requires only that cohesion between sister chromatids be destroyed distal to, but not proximal to, chiasmata (Figures 5 and 6). Cohesion in the vicinity of centromeres is preserved until the second meiotic division and is used to biorient and segregate chromatids on meiosis II spindles (Klein et al., 1999; Toth et al., 2000; Watanabe and Nurse, 1999) (Figure 6). This remarkable phenomenon has an important implication. All cohesion distal to the most proximal chiasma must be destroyed at the first meiotic division and, as a consequence, exchanges must not occur too close to kinetochores; as otherwise, insufficient cohesion would remain for meiosis II. Centromeres therefore suppress the formation of exchanges in their vicinity (Lambie and Roeder, 1988), which permits the accumulation of repetitive sequences and "junk" DNA. A corollary is that a single exchange close to the telomere may provide insufficient cohesion to hold homologs together during meiosis I. For example, human trisomies of chromosome 16, which originate in oocytes, appear to be associated with recombination events occurring close to the ends of chromosomes (Hassold et al., 1995).

By preserving cohesion at centromeres, the second meiotic division can take place using exactly the same mechanisms employed during mitosis. Sister kinetochores attach to microtubules with opposite orientations, and the destruction of centromeric sister chromatid cohesion triggers their disjunction and segregation to opposite poles of the cell, thereby creating haploid

cells containing a single chromatid of each chromosome (Figure 6).

A key question is whether destruction of cohesion distal to chiasmata during meiosis I is mediated by the same mechanism that triggers the disjunction of sister chromatids during mitosis, namely cleavage of cohesin's Scc1 subunit by separase. The evidence from yeast is affirmative. Scc1's meiotic counterpart, Rec8, is both present along the inter-chromatid axis of metaphase I cells and disappears from chromosome arms at the onset of anaphase I in both budding yeast (Klein et al., 1999; Watanabe and Nurse, 1999) and in mice (N. Kudo, personal communication). In budding yeast, both resolution of chiasmata and Rec8's disappearance from chromosome arms depends on its cleavage by separase (Buonomo et al., 2000), which is activated through securin's sudden destruction by Cdc20 working in consort with the APC/C (Figure 6). Thus, the segregation of homologs in meiosis I is triggered by cleavage of cohesin on chromosome arms distal to chiasmata. Because *mad2* mutants missegregate chromosomes during meiosis I (Shonn et al., 2000), a meiotic checkpoint monitoring chromosome attachment most likely has an important role in determining when precisely separase is activated.

Rec8 cleavage might be a common trigger for meiosis I, as *C. elegans* mutants defective in separase (Siomos et al., 2001) or the APC/C (Davis et al., 2002; Furuta et al., 2000; Golden et al., 2000) also fail to segregate chromosomes during meiosis I. Furthermore, in grasshopper spermatocytes, the sister chromatids of meiosis II chromosomes, when transferred to meiosis I spindles, disjoin at exactly the same time as chiasmata of meiosis I bivalents are resolved (Paliulis and Nicklas, 2000). This implies that the anaphase trigger for meiosis I and II is the same and presumably identical to that for mitosis. It is, therefore, surprising that two studies have reported that neither the APC/C nor securin destruction is required for meiosis I in *Xenopus* oocytes (Peter et al., 2001; Taieb et al., 2001). The role of Rec8 cleavage in resolving chiasmata in vertebrates clearly needs further investigation.

Protection of Centromeric Cohesion

Cleavage of both Scc1 and Rec8 accompanied by cohesin's dissociation from chromosomes. The finding that Rec8 persists in the vicinity of budding yeast centromeres until the onset of anaphase II (Klein et al., 1999) (Figure 6) suggests that centromeric Rec8 is somehow refractory to separase activity at the onset of anaphase I. This fraction of Rec8 is responsible for maintaining cohesion between sister centromeres during their alignment on meiosis II spindles. A similar phenomenon has been observed in *S. pombe* (Watanabe and Nurse, 1999), *C. elegans* (Pasierbek et al., 2001), and in mouse spermatocytes (N. Kudo, A. Peters, and M. Tarsounas, personal communication), suggesting that most eukaryotic organisms might retain sufficient cohesion for meiosis II by protecting centromeric Rec8 from separase during meiosis I.

The molecular mechanism responsible for protecting centromeric Rec8 is not at all understood. In *S. cerevisiae*, centromeric cohesin's ability to resist attack by separase is specified by Rec8 itself; that is, Scc1 does not have this property. When expressed from Rec8's promoter, Scc1 can both promote cohesion and sustain cosegregation of sister centromeres (monopolin function; see below) at meiosis I, but it neither persists at centromeres after anaphase I nor manages to hold sisters together after this point (Toth et al., 2000). The finding that phosphorylation of Scc1 by the Polo-like kinase Cdc5 promotes its cleavage in mitosis (Alexandru et al., 2001) raises the possibility that phosphorylation of Rec8 might be a prerequisite for its cleavage during meiosis I, in which case differential phosphorylation might distinguish Rec8 on chromosome arms and centromeres.

There are hitherto few, if any, candidates for meiosis-I-specific factors needed for protecting centromeric cohesion. The meiosis-specific protein Spo13 might have a role in budding yeast (Klein et al., 1999; Lee et al., 2002; Shonn et al., 2002), but it does not appear to be essential because at least some Rec8 persists at centromeres after anaphase I has been initiated in *spo13* mutants (Klein et al., 1999). The MeiS332 protein in *Drosophila* is necessary for protecting centromeric cohesion (Moore et al., 1998), as is the Bub1 protein kinase in *S. pombe* (Bernard et al., 2001a). Both proteins are found in the vicinity of centromeres and could have direct roles in protection. However, neither of these proteins are specific to meiosis I cells. Though necessary for protection, they are unlikely to be the factors that distinguish meiosis I centromeres from mitotic or meiosis II ones.

Centromeres in most organisms are organized into a heterochromatic domain containing repetitive sequences, the histone H3 variant CENP-A, heterochromatin protein 1 (HP1), and special histone modifications (Choo, 2001). Recent work has uncovered a connection between cohesion and centromeric heterochromatin in fission yeast, where recruitment of cohesin to centromeres, but not to chromosome arms, is dependent on Swi6, the *S. pombe* homolog of HP1 (Bernard et al., 2001b; Nonaka et al., 2002). An involvement of the highly specialized heterochromatin domain at centromeres in the protection of centromeric cohesion during meiosis I would not be surprising.

Protection must be dismantled after the onset of anaphase I because centromeric Rec8 disappears from chromosomes upon reactivation of separase at the onset of anaphase II. One presumes that the process of dismantling protection is tightly coupled to the inactivation of separase following meiosis I. Dismantling protection prior to the inactivation of separase would cause premature disjunction of sister centromeres, which would clearly be deleterious to meiosis II chromosome segregation. In budding yeast, securin rapidly reaccumulates after its destruction by the APC/C at the onset of anaphase I (Salah and Nasmyth, 2000), presumably inactivating separase. As a consequence, cells can dismantle Rec8's protection, laying it open to attack by the next round of separase activity.

Recombination and Chiasmata—Creating Exchanges and Connecting Homologs

Janssens's (1909) chiasma-type theory was so revolutionary that it took nearly 20 years to be accepted. It

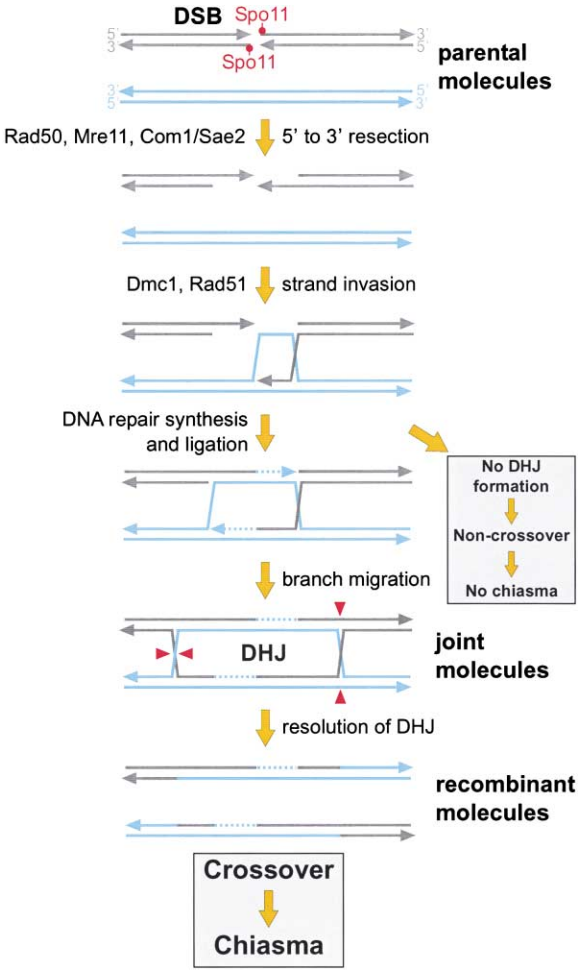


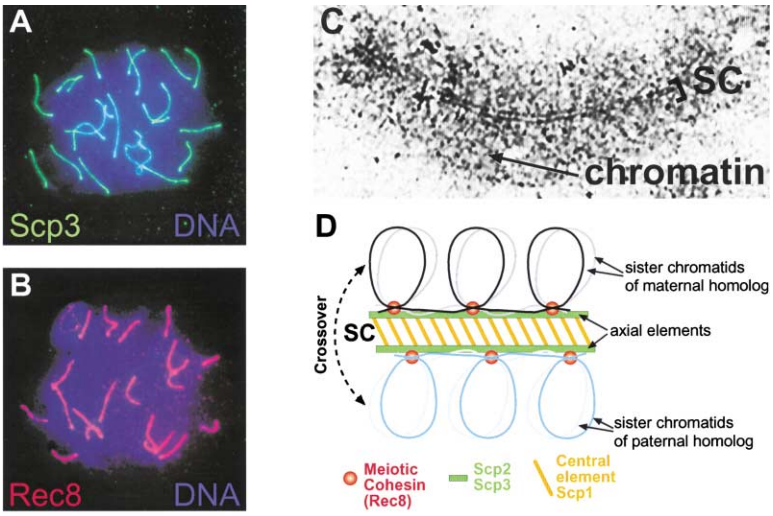
Figure 7. The Double-Strand Break Repair Model of Meiotic Recombination and the Creation of Reciprocal Exchanges

The Spo11 endonuclease generates a double-strand break (DSB) in one of the parental chromatids. 5′ to 3′ resection of the break requires Rad50, Mre11, and Com1/Sae2. Invasion of one protruding 3′ end is catalyzed by the strand exchange proteins Dmc1 and Rad51. DNA repair synthesis and ligation create a double Holliday junction (DHJ) and a joint molecule, which are resolved by an as yet unknown resolvase (red arrowheads) in an asymmetric manner, producing recombinant molecules and later chiasmata. Most noncrossover events are thought to arise without the production of a DHJ. Only the two DNA double strands of a bivalent (which consists of four chromatids) participating in the recombination process are shown. (For details of proteins involved in the recombination pathway see Roeder, 1997, and references therein).

not only provided an explanation for the crossing over between markers on the same chromosome, observed by Morgan in 1911, but it also provided the mechanism by which homologous chromosomes are joined together prior to their biorientation on the first meiotic spindle (Figure 5).

Chiasmata are generated by recombination, leading to reciprocal exchanges between sister chromatids of homologous chromosomes. The recombination process is initiated by the production of double-strand breaks (DSBs) (Sun et al., 1989, 1991; Szostak et al., 1983) (Figure 7). Even before breaks are formed, pairing of replicated maternal and paternal chromosomes occurs (reviewed in Walker and Hawley, 2000). Though the mechanisms are largely obscure, there is general consensus that the pairing process is usually independent of recombination. It, nevertheless, brings DNA sequences that must exchange strands during recombination into much closer proximity than they would otherwise be.

The DSBs that initiate the recombination process (Sun et al., 1989) are generated by the Spo11 endonuclease (Keeney et al., 1997) early during meiotic prophase at several points along each of the four chromatids (two maternal and two paternal) (Baudat and Nicolas, 1997) (Figure 7). In budding yeast, these breaks are not situated at random along chromosomes but occur almost exclusively in intergenic promoter regions and preferentially in GC-rich chromosomal domains (Baudat and Nicolas, 1997; Blat et al., 2002). Spo11 is related to archeal and plant type II-like topoisomerases (Bergerat et al., 1997) and, like these, forms a tyrosine phosphodiester linkage with both 5′ ends created by cleavage (Keeney et al., 1997) (Figure 7). After hydrolytic or nucleolytic removal of Spo11, the 5′ ends are resected by a 5′ to 3′ exonuclease, creating single strand 3′ protruding overhangs on either side of the break. One 3′ protruding overhang invades a homologous non-sister chromatid (first end capture) (Hunter and Kleckner, 2001) (Figure 7). What causes maternal breaks to invade paternal chromatids and vice versa (Collins and Newlon, 1994; Schwacha and Kleckner, 1994) rather than a sister chromatid is poorly understood but requires several meiosis-specific proteins (Schwacha and Kleckner, 1997). The invasion of a non-sister chromatid is crucial for generating exchanges as opposed to merely repairing the break using sister sequences, as occurs in mitotic cells (Paques and Haber, 1999). The invading 3′ end from the paternal chromatid becomes paired with a complementary strand from a maternal chromatid, creating a template for repair synthesis (Figure 7). The same fate eventually befalls the 3′ overhang at the other side of the break (second end capture) and continued repair synthesis presumably causes migration of the heteroduplex branch, which eventually exposes the 5′ ends left over from resection of the original DSB. These, in turn, invade and are eventually ligated to the newly synthesized DNA, creating a joint molecule (Collins and Newlon, 1994; Schwacha and Kleckner, 1994). The net effect of these enzymatic fireworks (see Figure 7 for key proteins involved in the recombination pathway) is the exchange, over a short section of DNA, of a strand from a maternal chromatid by that of a strand from a paternal one. This creates a structure called a double Holliday junction (DHJ) in mid prophase (Holliday, 1964; Schwacha and Kleckner, 1995), whose two joints are separated by heteroduplex DNA and short stretches of DNA synthesized during the repair of the DSB (Figure 7). Recent work has actually detected heteroduplex DNA between the two Holliday junctions (Allers and Lichten, 2001a), as predicted by the original double-strand break repair model (Sun et al., 1991; Szostak et al., 1983). Surprisingly, many joint molecules contained heteroduplex DNA exclusively on one side of the DHJ. Explaining this phenomenon requires a modification of the current DSBR (double-



E

stages of meiotic prophase	leptotene	zygotene	pachytene	diplotene	diakinesis
SC/chromosomes	axial elements appear	SC initiated synapsis starts	SC fully developed full synapsis	SC disassembled chromosome condensation	further chromosome condensation
DSB repair	DSB formed	DSB disappear	DHJ appear	recombinant molecules	

Figure 8. The Synaptonemal Complex, Cohesin, and Chromatin in Pachytene

(A) Chromosome spreads of mouse spermatocytes in pachytene stained with antibodies against Scp3 (green). Chromatin was stained with DAPI (blue). The synaptonemal complex (SC) component Scp3 lines the axes of the synapsed bivalents (courtesy of Dr. Antoine H.F.M. Peters, Research Institute of Molecular Pathology, Vienna, Austria).

(B) Chromosome spreads of the pachytene stage of mouse spermatocytes expressing a Myc-tagged version of Rec8 stained with anti-Myc antibodies (red). Chromatin was stained with DAPI (blue). The meiosis-specific cohesin Rec8 is tightly associated with the axes of the synapsed bivalents (courtesy of Dr. Nobuaki Kudo, Research Institute of Molecular Pathology, Vienna, Austria).

(C) Chromosome spreads of mouse spermatocytes in pachytene were stained with silver nitrate to visualize the SC and chromatin and observed under the electron microscope. The SC is resolved as two parallel linear elements surrounded by a mass of adherent chromatin (courtesy of Dr. Antoine H.F.M. Peters).

(D) Schematic model of the organization of SC proteins, meiotic cohesin, and chromatin in pachytene.

(E) Timeline of stages and events in meiotic prophase. Leptotene is preceded by premeiotic DNA-replication and diakinesis is followed by the two meiotic divisions.

strand break repair) model (see Allers and Lichten, 2001a, for details).

The final step in the recombination process is the resolution of DHJs, which is essential both for disjunction of the maternal from the paternal chromosome as well as for production of the exchanges that will hold them together until the onset of anaphase I. The enzymology of this step is poorly understood, as the enzyme responsible has not yet been identified. The geneticists' failure to identify the resolvase could be explained by the fact that the same enzyme is involved during an earlier stage of the recombination process (e.g., Spo11). Resolution requires cleavage of a pair of strands at each end of the junction and their reciprocal ligation (Figure 7). The cleavages can either be horizontal or vertical. Crucially, reciprocal exchanges (crossovers) are only generated when one junction is resolved horizontally and the other vertically. Such asymmetric resolution seems to be another specialty of meiotic cells, and how it is achieved remains elusive. It seems that most DHJs are resolved in this manner and, therefore, give rise to reciprocal exchanges (Allers and Lichten, 2001b).

The consequences of defects in recombination illustrate the fundamental importance of chiasmata for chromosome segregation during meiosis I. If the Spo11 endonuclease is inactivated in *S. cerevisiae* and *C. elegans* (Cao et al., 1990; Dernburg et al., 1998; Keeney et al., 1997; Klein et al., 1999), chiasmata are not formed and homologous chromosomes cannot be held together. As a consequence, they are segregated at random in meiosis I. This leads to massive aneuploidy and inviability of progeny. Following deletion of Spo11 in mice, neither males nor females manage to produce functional gametes (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000).

An exchange at one site greatly reduces the probability of exchanges being generated in its vicinity. This phenomenon is known as crossover interference (Muller, 1916). It is thought that the purpose of interference is to ensure that each bivalent produces at least one exchange, irrespective of chromosome size. Though most organisms create several exchanges per chromosome, only one chiasma is actually needed to hold homologous chromosomes together. Some organisms, such as *S. pombe*, have such high rates of reciprocal recombination that there is no need for interference (Bahler et al., 1993), whereas others such as *C. elegans* restrict the number of exchanges per chromosome to one (Barnes et al., 1995). Interference in this case is so extreme that a single exchange inhibits the formation of a second exchange on the same chromosome.

Interference is thought not to arise due to the inhibition of DSB formation but, instead, from a reduction in the probability of a break being converted into a DHJ and crossover. What, then, happens to breaks that are not converted to DHJs? It is thought that the one end of the DSB, which invades a homologous non-sister chromatid, is extended by repair synthesis, but the second end fails to be captured (Hunter and Kleckner, 2001). The extended first strand then returns to its parental chromatid without the formation of a DHJ and crossover (Allers and Lichten, 2001b) (Figure 7). Neither stable junctions nor exchanges between homologous chromosomes are ever produced through this outcome. Interference de-

pends on the production of many more potential crossover events than actual ones. Some of the non-crossover recombination events are the "casualties" necessary for interference to function. Others presumably serve to facilitate synapsis (see below) between homologs.

The Synaptonemal Complex and Chromosome Topology in Pachytene

The stages of meiotic prophase have been assigned specific names (Figure 8E) based on cytological landmarks. The most important of these is called the synaptonemal complex (SC) (Figure 8), which results from the close association of homologous chromosomes.

DSBs are created in early meiotic prophase (leptotene) and are processed to create DHJs by pachytene. At this point, chromosomal DNA is organized around a central axis containing meiotic cohesin and the Red1 protein in budding yeast (Klein et al., 1999; Smith and Roeder, 1997). Both cohesin and Red1 are required for the formation of chromosomal axes (Klein et al., 1999; Rockmill and Roeder, 1990). The bulk of chromosomal DNA is found in large parallel loops or coils that emanate back and forth from the axes (Figures 8C and 8D). Both chromatids of each homolog are bound together by cohesin (Figure 8D). The meiotic cohesin complex situated along chromosomal axes is likely to play a structural role during the recombination process as deletion of *REC8* or mutation of *SMC3* causes a failure to process DSBs (Klein et al., 1999).

During pachytene, the axes of homologous chromosomes are so closely associated that cohesin appears to form a single line using immunofluorescence microscopy (Figure 8B), which is presumably composed of two parallel axes that are closely juxtaposed. The close association between maternal and paternal axes along the entire length of the bivalent (a maternal and paternal pair) is called synapsis and is achieved by the SC (Moses, 1958) (Figure 8). Proteins such as Zip1 (yeast) (Sym et al., 1993) or Scp1 (mammals) (Meuwissen et al., 1992; Schmekel et al., 1996) form the center of the SC. In mammalian cells, two further proteins, Scp2 and Scp3 (Schalk et al., 1998), create a bipartite polymer along the bivalent's axes (Figures 8A and 8D). Deletion of *Scp3* in male mice leads to defects in axial element formation, chromosome synapsis, and SC assembly (Yuan et al., 2000). However, mouse oocytes lacking Scp3 display much weaker phenotypes and manage to produce viable offspring (Yuan et al., 2002). Remarkably, the formation of fully synapsed bivalents and SC in some organisms, such as *C. elegans* (Dernburg et al., 1998) and *Drosophila* (McKim et al., 1998), take place in the absence of recombination whereas in others, such as yeast and mice (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000), it cannot.

Toward the end of pachytene, DHJs are resolved and exchanges created. Soon thereafter, the SC is disassembled, homologs desynapse, and chiasmata can be seen cytologically (Figure 5). This stage, known as diplotene, is when many oocytes arrest and undergo the growth needed to produce eggs capable of embryonic development upon fertilization. Following chromosome condensation in diplotene, bivalents are aligned on the metaphase I spindle (Figure 6). Though characteristic of most meiotic cells, the SC remains one of the mysteries of meiosis and its function is still poorly understood. Organisms such as *S. pombe* produce plenty of exchanges without producing SC (Bahler et al., 1993). *S. pombe* also lacks interference, raising the possibility that the intimate synapsis of maternal and paternal chromosomes during pachytene aids interference. Consistent with this hypothesis, *zip1* mutants, which fail to form SC, are defective in interference (Sym and Roeder, 1994).

Cohesin remains associated with the inter-chromatid axes of chromosomes until the first meiotic division (Klein et al., 1999; Prieto et al., 2001; Watanabe and Nurse, 1999); that is, long after they have desynapsed. Because of the connections between sister DNA molecules mediated by cohesin, exchanges produced during pachytene ensure that homologous chromosomes remain connected despite dissolution of the SC (Figure 5). An example of the importance of chiasmata is found in *C. elegans*, where inactivation of Spo11 abolishes recombination and chiasma formation (Dernburg et al., 1998) but, unlike in yeast or mice (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000), does not prevent the formation of SC. Upon dissolution of the SC in the absence of chiasmata, however, homologous chromosomes drift apart and lack the connections needed for them to "dance together" on the meiosis I spindle.

Eukaryotic cells that undergo recombination during meiosis make a virtue of necessity and actually use the exchanges generated by recombination to drive the segregation process. Why they do this is an interesting, but not easily answerable, question because some organisms are capable of producing gametes through a meiosis-like process without any recombination between homologous chromatids. Such organisms include the heterogametic sex of many diptera; for example, male *Drosophila* or lepidoptera. During these recombination-free meioses, maternal and paternal homologs pair with each other after DNA replication and are subsequently pulled in opposite directions by the meiosis I spindle apparatus (Hawley, 2002).

Attachments in Meiosis—Breaking the Cardinal Rule of Mitosis

In mitosis and meiosis II, sister kinetochores are attached to opposite poles (amphitelic) (Figures 4 and 6). This attachment mode would not support the segregation of homologs in meiosis I. Therefore, meiosis I cells must invariably attach both sister kinetochores to microtubules emanating from the same pole (syntelic) (Figures 6 and 9C). This fundamentally different mode of attachment is a prerequisite both for the segregation of homologous chromosomes in meiosis I and the successful reduction of genome size by half (Figure 6).

By holding maternal and paternal chromatids together, chiasmata greatly expand the possibilities for creating tension when microtubules attach to kinetochores. Biorientation of sisters (amphitelic attachment) will of course still generate tension and, hence, potentially stable microtubule-kinetochore connections (Figure 9A). However, syntelic attachment (the attachment of sister centromeres to spindles with the same orienta-

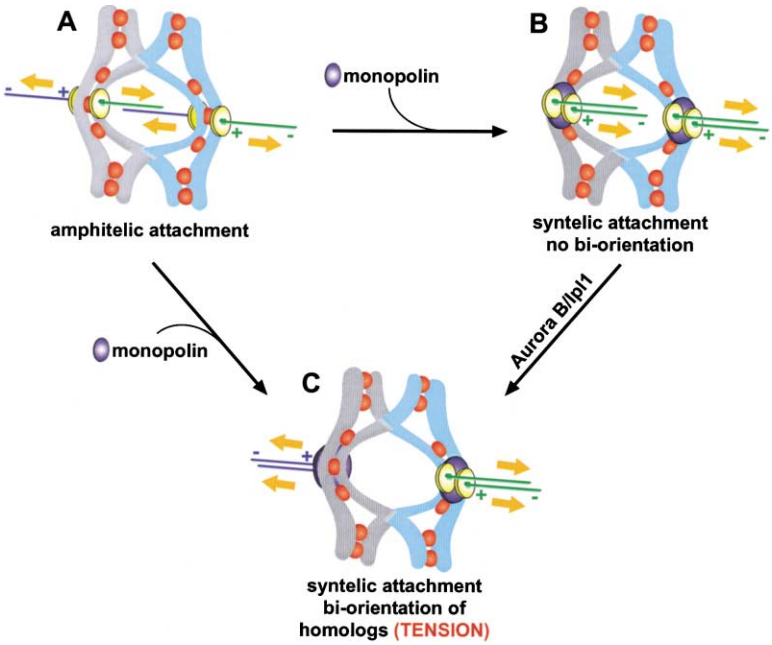


Figure 9. A Model for Microtubule-Kinetochore Attachments in Meiosis I

Monopolin proteins suppress amphitelic attachment of sister kinetochores in meiosis I (A) and promote their syntelic attachment (mono-orientation) (B and C). This could allow the Aurora B/Ipl1 kinase to eliminate syntelic attachments in which the maternal and paternal centromere pairs are attached to microtubules with the same orientation and hence fail to generate tension (B). The action of monopolins and Aurora B/Ipl1 would ultimately lead to the stable biorientation of bivalents under tension in metaphase I of meiosis (C). In this situation, mono-oriented maternal and paternal sister centromere pairs are attached to microtubules with the opposite orientation. The ability of monopolins to mono-orient sister kinetochores might rely on the prior destabilization of amphitelic attachments by Aurora B/Ipl1. These intermediate steps have been omitted in the model for reasons of clarity.

tion), which cannot generate tension during mitosis, is now also capable of doing so (Figure 9C). Chiasmata ensure that tension will be generated if both maternal centromeres attach to microtubules with one orientation while both paternal ones attach to microtubules with the opposite orientation. That both amphitelic and syntelic attachments are possible in meiosis could create terrible confusion and massive aneuploidy if, for instance, a maternal centromere pair attached in an amphitelic manner while its paternal homologs attached in a syntelic manner. To avoid this, cells systematically suppress amphitelic and promote syntelic attachment of sisters during the first meiotic division. Meiosis I cells must, therefore, break the cardinal rule of mitosis.

The ability of meiotic chromosomes to attach syntelically appears to be a property of the chromosomes rather than the meiosis I spindle apparatus or cytoplasm. If grasshopper spermatocytes in meiosis I are fused to spermatocytes in meiosis II and single meiosis I or II, chromosomes are transferred from their respective spindles onto meiosis II or meiosis I spindles, respectively, the chromosomes always segregate as they would have done on their native spindle (Paliulis and Nicklas, 2000). In other words, a meiosis I chromosome (pre-anaphase I) transferred to a meiosis II spindle maintains the mono-orientation of sister kinetochores and homologous chromosomes are segregated from each other, while a meiosis II chromosome transferred to a meiosis I spindle biorients sister kinetochores, leading to the segregation of sister chromatids. Chromosomes gain the ability to biorient sister kinetochores around the time of anaphase I, which is crucial for the correct segregation of sister chromatids in meiosis II.

We still have no idea how meiosis I cells suppress biorientation of sister kinetochores while promoting biorientation of homologs during meiosis I. An important step forward has been the recent identification of specific kinetochore proteins called monopolins (Figure 9), which are essential for suppressing biorientation of sister kinetochores during meiosis I in *S. cerevisiae*. Mutants lacking the Mam1, Csm1, or Lrs4 proteins biorient sister kinetochores during meiosis I, leading to both a failure to separate homologs and to severe aneuploidy of gametes (Toth et al., 2000) (M. Petronczki and K. Rabitsch, personal communication). The mechanistic basis of monopolin's function has yet to be elucidated. It is not even known whether it inhibits binding of one of the sister kinetochores to microtubules or whether it coordinates their attachment to microtubules with the same orientation by clamping or fusing them together into a single structure that is only capable of attaching to spindles with the same orientation. It is possible that such clamping could enable the Ipl1/Aurora B kinase to selectively destabilize attachments, which do not generate tension and cause maternal and paternal centromeres to be pulled toward the same pole (Figure 9B) and, thereby, select for ones in which they are pulled in opposite directions (Figure 9C). Clamping or fusing seems likely because electron microscopic studies in male *Drosophila* suggest that sister kinetochores in prometaphase meiosis I cells are indeed fused and cannot be distinguished from each other (Goldstein, 1981).

In *S. pombe*, meiotic cohesin containing Rec8 could be involved in controlling orientation of sister kinetochores during meiosis I. Both Scc1 (Rad21) and Rec8 are expressed during meiosis I in this organism and deletion of Rec8 only abolishes cohesion in the vicinity of centromeres (Watanabe and Nurse, 1999). This is accompanied by biorientation of sister kinetochores and the disjunction of sister chromatids and not homologs at meiosis I. It is unclear whether *S. pombe* Rec8 has a direct role in suppressing sister kinetochore biorientation as does monopolin in *S. cerevisiae* or whether it is needed to hold sister kinetochores sufficiently close together to permit their clamping together by a yet unidentified *S. pombe* monopolin complex. Unlike mono-

polin in *S. cerevisiae*, which is only present at kinetochores during meiosis I, Rec8 is present during both meiosis I and meiosis II. Its presence at kinetochores cannot, therefore, be sufficient for suppressing sister kinetochore biorientation. No proteins have yet been implicated in meiosis-I-specific kinetochore behavior in animal or plant cells.

Preventing DNA Replication between the Two Meiotic Divisions

Proliferating mitotic cells rely on alternating rounds of DNA replication during S phase and segregation of the duplicated chromatids during mitosis. This rule has to be violated between the two meiotic divisions to allow the formation of haploid nuclei. Meiotic cells, therefore, suppress S phase after meiosis I.

Most insights into how this suppression is brought about come from work in *Xenopus* oocytes. To ensure cells move straight into meiosis II from meiosis I, high levels of the CDK activator and APC substrate cyclin B have to be maintained, which prevents initiation of an intervening S phase. The levels of cyclin B, which are normally completely destroyed in late mitosis, only appear to be reduced to half during the transition from meiosis I to II. This is ensured by two mechanisms, increased synthesis and partial inhibition of cyclin B destruction. The proto-oncogene Mos, an activator of the mitogen-activated protein kinase (MAPK), was identified as a key regulator in both processes. Mos is thought to suppress S phase through activation of MAPK and the downstream Rsk kinase (Furuno et al., 1994; Gross et al., 2000). In addition to promoting cyclin B synthesis (Taieb et al., 2001), Rsk partially inhibits the activity of the APC/C and, hence, cyclin B destruction, potentially by regulating phosphorylation of the APC/C subunit Cdc27 (Gross et al., 2000). This leads to increased levels of cyclin B complexed with CDK and prevents replication. Oocytes in which Mos function or MAPK activity is blocked do not enter meiosis II but, instead, undergo a new round of DNA replication (Furuno et al., 1994; Gross et al., 2000). Similar phenotypes were recently obtained by removing the chromokinesin XKid, which surprisingly appears to play a role in the meiosis I to II transition that is independent of its function in metaphase chromosome alignment (Perez et al., 2002).

Xenopus oocytes temporarily lose the ability to replicate their chromosomes but regain it before undergoing the meiosis I division. Recent work has demonstrated that the absence of the essential prereplication complex component Cdc6 and the cytoplasmic delocalization of Orc proteins and Cdc7 kinase (see before) are responsible for the loss of replication competence (Lemaitre et al., 2002; Whitmire et al., 2002). The key challenge for the future will be to determine how Mos and high CDK-cyclin B levels inhibit replication at the meiosis I to II transition, a time when Cdc6 is present and the other proteins in place. Whether the pathways discovered in *Xenopus* apply to a broader range of organisms and, if not, which other mechanisms suppress replication between meiosis I and II remain to be established.

Might Meiosis Have Worked Otherwise?

Given that the process of producing haploid gametes from diploid germ cells can vary considerably, it is perhaps surprising that meiosis appears to function in fundamentally the same way in such a wide variety of eukaryotic cells. There must be some profound advantages to the conventional form of meiosis. What might these be? Recombination has the advantage of creating new combinations of alleles but has the disadvantage of breaking up favorable allelic combinations. Nevertheless, why when recombination accompanies gamete formation is it invariably also an intrinsic part of the mechanism used to segregate chromosomes during meiosis I? Why does recombination take place only after premeiotic DNA replication? Why go to such lengths to reduce chromosome numbers over two divisions instead of recombining unreplicated homologs and then undergoing a single division?

There are two kinds of answers to this question. Meiosis must function strategically as well as physiologically. It has been suggested (Haig and Grafen, 1991) that recombination prior to DNA replication would unnecessarily expose gametes to "sister killers" and other forms of meiotic drive. By creating the possibility of eliminating your own genes, recombination at the four-strand stage mitigates this form of parasitism. Meanwhile, chiasmata may just be a far more effective way of joining homologs together than simple pairing mechanisms, and the former cannot be produced through recombination between unreplicated chromatids. It is clear, therefore, that recombination at the four-strand stage and the disjunction of maternal and paternal centromeres at meiosis I, while preserving cohesion at centromeres solves both strategic and physiological constraints in an extraordinarily elegant manner. Furthermore, by making recombination compulsory for gamete formation, cells ensure that that they cannot quickly abandon an activity that is healthy in the long run but is dispensable and possibly even disadvantageous in the short run.

Concluding Remarks

There are four major processes that are specific to meiotic chromosome segregation: formation of exchanges that join homologous maternal and paternal chromosomes together, cosegregation of sister centromeres at meiosis I, preservation of centromeric sister chromatid cohesion, and lack of DNA replication at meiosis II. Though the fundamental logic of meiosis was first recognized by Janssens in 1909, we still remain horribly ignorant about the details of the processes described above. One of the reasons for this ignorance is that cytology alone, which has been the workhorse of meiotic studies, cannot unearth molecular mechanisms. This requires genetics to identify the key players, careful physiological measurements of molecules and their states as cells progress through meiosis, and ultimately, biochemistry that illuminates their mechanical properties. For these reasons, a great deal of our knowledge has been gleaned in the past decade or two from the study of yeast meiosis. Much of this will prove useful in understanding other eukaryotes, including humans. The elucidation of the molecular mechanisms underlying meiosis may eventually shed insight into what goes awry in aging oocytes, which is one of the major medical and social issues of Western society (Hassold and Hunt, 2001).

Acknowledgments

We would like to thank Professor Dieter Schweizer for his kind help in providing us with the material for Figure 1 and for critical reading of the manuscript. We are also very grateful to Dr. Jasna Puizina, Dr. Antoine H.F.M. Peters, and Dr. Nobuaki Kudo for providing us with microscopy images. We apologize to those researchers whose work was not cited or discussed due to the breadth of the subject matter.

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