most things in biology. The fact that rheological data cannot completely determine molecular mechanisms is no different from the fact that aspirin or insulin were useful drugs decades before there was a hint of the molecules they affected.

Why should a biologist care about rheology? Actually, biologists used to care a lot more about rheology than they have lately. A prominent physiology text from the 1950s (*An outline of general physiology* by L. Heilbrunn) stated that "In any attempt to interpret the machinery of a living cell, it is essential to know something about the mechanical properties of the protoplasm in the cell that is being investigated." This point of view was largely eclipsed by the emphasis and power of chemical and genetic regulation of cell function. But cells are mechanical as well as chemical and electrical devices, and understanding their biology requires knowledge of all these aspects. Numerous recent studies show that application of external forces or challenging a cell's internal force generation by adhesion to substrates of different stiffnesses generates signals that can augment or override chemical stimuli. Understanding how forces affect cell growth, division, differentiation and activation requires defining the rheological properties of cells in the same way that understanding chemical signaling requires knowledge of rate constants and affinity constants and signal transduction pathways.

Where can I find out more?

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[1]Institute for Medicine and Engineering, University of Pennsylvania, 1010 Vagelos Laboratories, 3340 Smith Walk, Philadelphia, Pennsylvania 19104, USA.
[2]Institute for Cell Biology and Center for Integrated Protein Science, University of Munich, Schillerstr. 42, 80336 Munich, Germany.
E-mail: janmey@mail.med.upenn.edu, schliwa@med.uni-muenchen.de.

Primer

Meiosis

Andreas Hochwagen

In the process of sexual reproduction, two gametes fuse and combine their genomes to form the next generation. To avoid the otherwise inevitable doubling in genetic material with every new generation, genome copy number must be reduced by half before the next round of gametes is formed. This reduction in ploidy is achieved by an unusual type of cell division — meiosis.

The main difference between meiosis and the mitotic cell division pattern is the number of chromosome separation steps that follow chromosome duplication; mitotic cells separate chromosomes in a single step, whereas meiosis is characterized by two sequential separation steps — meiosis I and meiosis II. Meiosis II is an equational division very similar to mitosis (Figure 1). It leads to the equal segregation of duplicated sister chromatids into the future daughter cells. The innovation of the meiotic division pattern is meiosis I. During meiosis I, homologous chromosomes, the near-identical chromosome copies originally contributed by mom and dad, are separated. As a result, ploidy is halved, and thus meiosis I often is referred to as the reductional division. Because the two chromosomal divisions occur without an intervening chromosome duplication phase, meiosis results in four products, each with half the ploidy of the starting cell. Depending on the organism, all four products may become gametes, or, as occurs during metazoan oogenesis, only one meiotic product continues as the female pronucleus of the egg, whereas the other three are discarded.

Separating homologous chromosomes

Meiosis relies on the same basic mechanics of chromosome segregation as mitosis. In mitosis, DNA replication leads to duplicated sister chromatids that are connected by sister-chromatid cohesion. Cohesion is mediated by cohesin protein rings that are thought to encircle the sister chromatids. Cohesins resist the pulling forces when microtubule fibers from opposite spindle poles attach to the microtubule-binding surfaces (the kinetochores) of the two sister chromatids. As a result of this resistance, sister chromatids come under mechanical tension on the spindle, which is required for their proper alignment in the division plane. Once all sister chromatid pairs are aligned, cohesins are destroyed and chromatids are pulled to opposite sides, into the future daughter cells.

Meiotic cells also use the establishment of tension as a mechanism to align and separate chromosomes. However, the need to separate homologous chromosomes in addition to sisters adds a number of mechanistic challenges. First, similar to sister chromatids, pairs of homologous chromosomes must also be connected to allow establishment of tension between them. Second, unlike in mitosis, sister chromatids must move to the same spindle pole during the reductional division. Third, sister chromatids have to remain linked until meiosis II. These modifications of the mitotic pattern are achieved by three meiosis-specific processes: (1) pairing and recombination of homologous chromosomes, (2) monopolar attachment of sister kinetochores on the meiosis I spindle, and (3) step-wise loss of sister-chromatid cohesion.

Pairing and recombination

In contrast to the cohesins that connect sister chromatids from the moment of their synthesis, no such *a priori* linkages exist for homologous chromosomes. Thus, to establish tension between homologous chromosomes, these linkages must be newly created. Linkage of homologous chromosomes occurs after meiotic DNA replication and typically involves two steps. First, homologous chromosomes are paired on the basis of sequence similarity. Then, in a process called crossover recombination, physical connections are established by exchanging DNA strands between

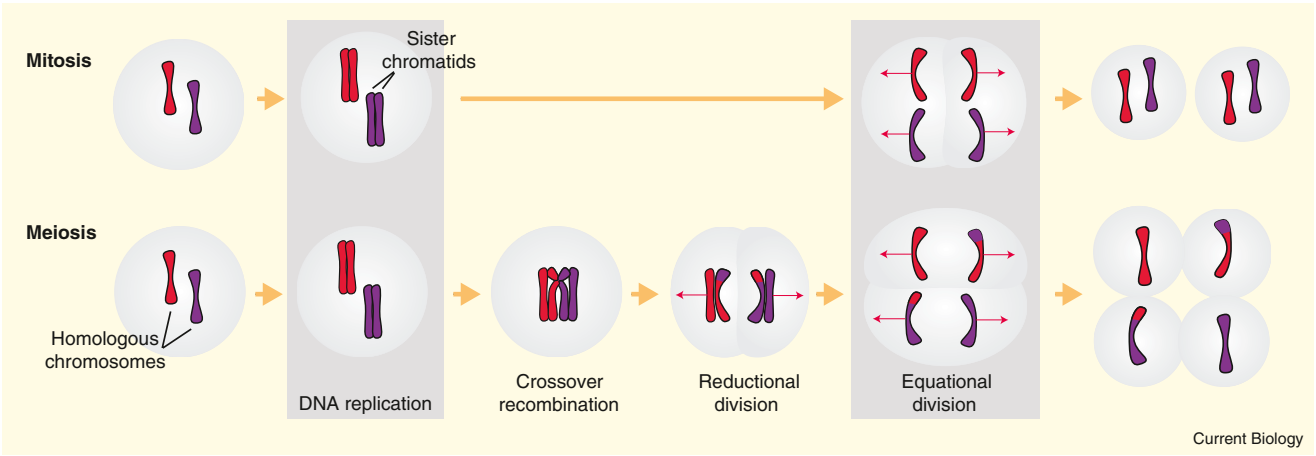

Figure 1. Mitotic and meiotic division patterns.

Depicted are cells with one pair of homologous chromosomes (red and purple) undergoing mitosis or meiosis. Pulling forces created by microtubule attachment to chromosomes are indicated by red arrows. (Figure by Tom DiCesare.)

homologous chromosomes. These DNA exchanges are a truly ingenious way to link homologous chromosomes. By reconnecting individual chromatids, the cell takes maximal advantage of the cohesion that already exists between sister chromatids. As shown in Figure 2, as a result of the exchange, sister cohesion distal to the site of crossing-over now physically connects homologous chromosomes and allows the establishment of tension between them on the meiosis I spindle. Thus, to successfully separate homologous chromosomes during meiosis I, each pair of homologous chromosomes has to cross over at least once.

The first step in connecting homologous chromosomes is the identification of matching chromosome pairs based on sequence homology. Scanning the entire genome for homology could be likened to searching for a needle in a haystack. In a number of organisms, chromosome-scale pairing mechanisms have been identified that may reduce the amount of time necessary to scan the genome, by moving homologous chromosomes into proximity of each other. For example, in fission yeast, microtubules attach to chromosome ends and align chromosomes by pulling them in a mop-like movement across the cell. Similar microtubule- or actin-dependent motor functions have also been implicated as mediators of chromosome alignment in other organisms.

A different pairing mechanism has been identified in the nematode *Caenorhabditis elegans*. In this organism, unique sequence elements identify individual chromosomes. These so-called 'pairing centers' are recognized by chromosome-specific zinc-finger transcription factors that greatly enhance alignment of homologous chromosomes. Interestingly, in humans, allelic differences in a related zinc-finger transcription factor are associated with differences in recombination rate, suggesting that this process may be conserved. An extreme example of chromosome pairing exists in the fruit fly *Drosophila melanogaster*. In this organism, homologous chromosomes are paired even in mitotically proliferating cells. Possibly as a consequence of this efficient pairing, *Drosophila* males have evolved a mechanism to segregate homologous chromosomes without crossover recombination. Instead, a cohesin-related protein glue serves to connect homologous chromosomes and establish the necessary tension during meiosis.

Concomitant with the alignment of entire chromosomes, homology searches continue at individual genomic loci to identify sites for DNA exchange and crossover recombination. This process initiates with the introduction of hundreds of DNA double-strand breaks (DSBs) across the genome (Figure 3). Resection of such breaks exposes stretches of single-stranded DNA (ssDNA) that are bound by recombinase enzymes. Recombinase–ssDNA filaments then scan the genome for homology by randomly invading double-stranded DNA. If initial base-pairing interactions are stabilized, extended single-end invasion intermediates can form. This is followed by capture of the second break end and the formation of double Holliday junctions, which are then resolved to form crossovers (Figure 3). If a single-end invasion intermediate fails to be stabilized, breaks can also be repaired as non-crossovers. In this case, the invading end is expelled from the double-stranded DNA and is then free to anneal with the second break end to restore the original chromosome. Remarkably, during meiosis, sister chromatids are largely excluded from the homology search by the invading ssDNA. In this manner, the majority of DSB repair events occur between homologous chromosomes, increasing the chances of producing an inter-homologue crossover.

As crossover recombination proceeds, homologous chromosomes become progressively more tightly aligned and in many organisms a protein lattice called the synaptonemal complex (SC) transiently assembles along the entire length of paired chromosomes. Cytologically, SC morphogenesis occurs in distinct stages called leptotene, zygotene, and pachytene, characterized by increasingly complete SC structures.

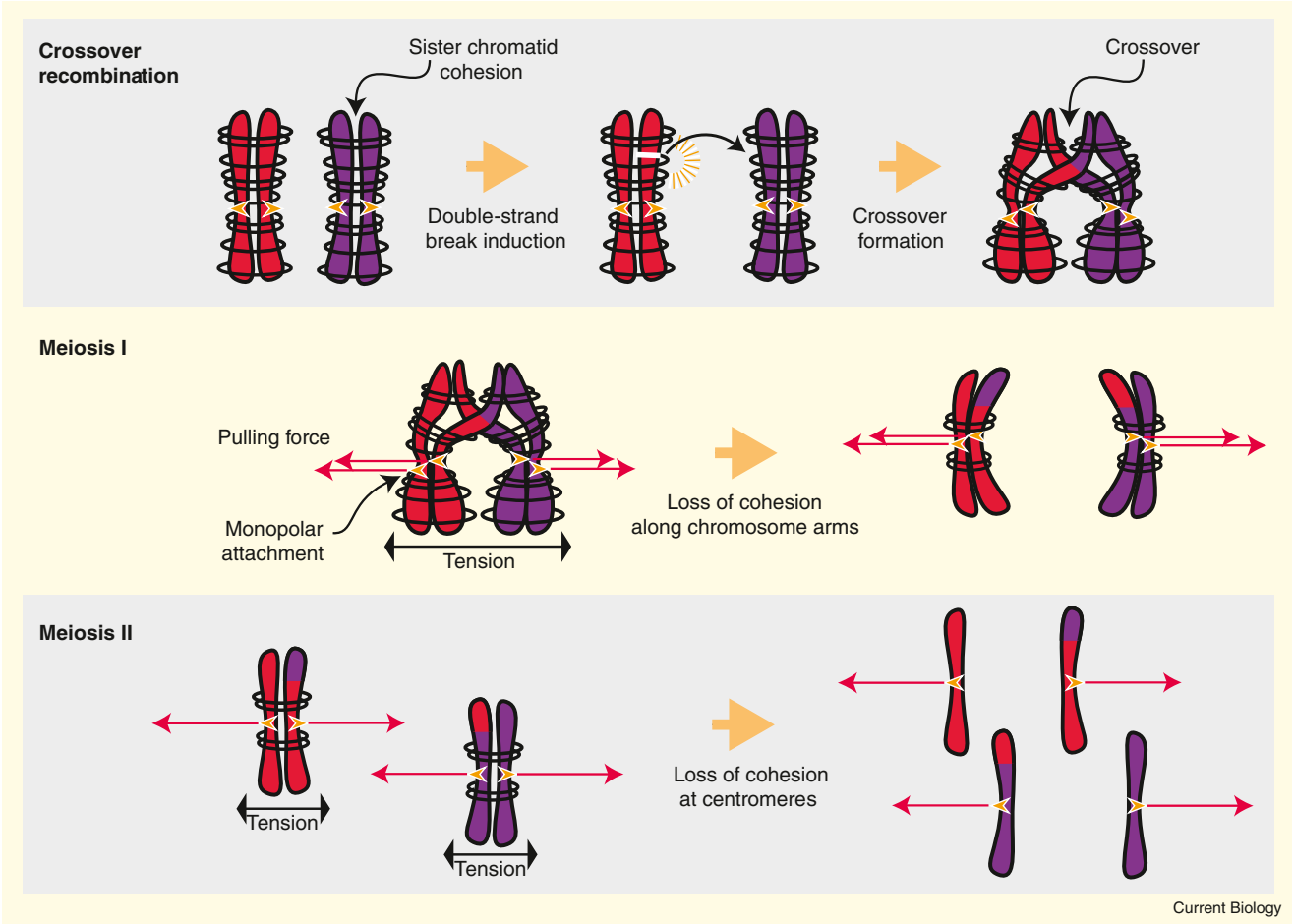

Figure 2. Crossovers, monopolar attachment, and step-wise loss of cohesion.

Shown is a duplicated pair of homologous chromosomes, proceeding through crossover recombination and the two meiotic divisions. Cohesin complexes are indicated as black rings, kinetochores are depicted as orange arrowheads oriented in the direction of microtubule attachment. (Figure by Tom DiCesare.)

This is followed by diplotene, when SCs disassemble. In many organisms, including mammals and budding yeast, SC formation is coupled to meiotic recombination and thus serves as a cytological mark to stage meiotic recombination. However, the actual role of the SC during meiosis has remained elusive. One proposed role for the SC is to ensure that crossovers do not occur in close proximity to each other, a phenomenon called crossover interference. Consistent with this idea, organisms such as fission yeast that do not exhibit crossover interference also do not have SCs. However, recent experiments indicate that at least the early events of crossover interference occur independently of SC formation. Thus, although the SC is a prominent feature of meiotic chromosomes, its precise function remains unclear.

Finally, it should be noted that although the primary role of meiotic crossovers is to ensure proper separation of homologous chromosomes, the creation of new allele combinations during meiotic recombination also has beneficial evolutionary consequences. Indeed, it is likely that the increased diversity created by meiotic recombination has contributed to the enduring evolutionary success of sexual reproduction (and hence the need for meiosis).

Monopolar attachment

Once homologous chromosome pairs are connected to each other, they have to be oriented on the meiosis I spindle. A pair of homologous chromosomes consists of two pairs of sister chromatids, each of which has the potential to bind microtubules through its kinetochore. To establish tension, the kinetochores of one sister pair need to bind to microtubules from one spindle pole, whereas the kinetochores of the other sister pair need to attach to microtubules from the opposite pole. Because sister kinetochores are not normally arranged in a configuration that supports such monopolar attachment, this mode of attachment requires specific modification of sister kinetochores prior to meiosis I.

Early cytological observations in *Drosophila* and grasshopper indicated that sister kinetochores undergo a maturation process prior to meiosis I, whereby sister kinetochores initially appear as a single joint structure. Prior to meiosis I, this structure separates into two distinct kinetochores, both of which bind microtubules emanating from the same pole.

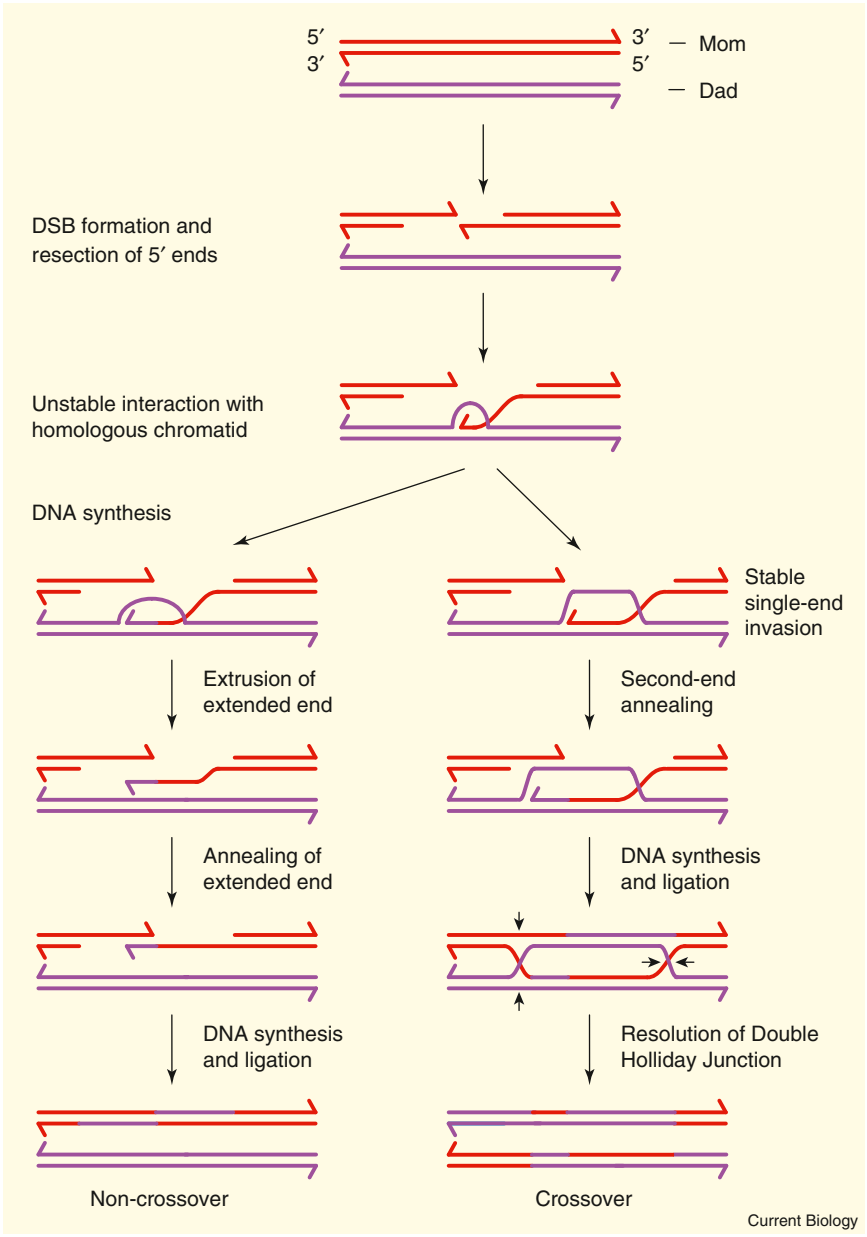

Figure 3. Crossover recombination.

For clarity, only one sister chromatid of each homologous chromosome (mom and dad) is depicted.

It is possible that the initial joint state sterically favors monopolar attachment of the sister kinetochores. A similar side-by-side arrangement of kinetochores facing the same pole is also observed in fission yeast and maize. On the molecular level, monopolar attachment in these organisms requires a meiosis-specific cohesin complex that localizes to centromeres before meiosis I. Although an additional kinetochore-associated regulator of monopolar attachment called Moa1 was recently identified in fission yeast, how these proteins interact to ensure proper attachment of sister kinetochores on the meiosis I spindle remains poorly understood.

Unlike the kinetochores of fission yeast and most other eukaryotes that bind multiple microtubules, the kinetochores of budding yeast each only bind a single microtubule. Interestingly, this organism also has a different mechanism of monopolar attachment. Ultrastructural studies of meiotic microtubule numbers indicate that budding yeast achieves monopolar attachment by allowing only one kinetochore per sister chromatid pair to attach to microtubules. Mechanistically, this type of monopolar attachment does not require cohesin function. Rather, in budding yeast, the relocalization of a multi-protein complex called monopolin from the nucleolus to centromeres is both necessary and sufficient to establish monopolar attachment. Remarkably, a complex homologous to monopolin is also present in fission yeast, although in this organism it is not required for monopolar attachment during meiosis I. Instead, it performs a related function in mitosis, ensuring that all microtubule-binding surfaces of a single kinetochore attach to microtubules emanating from the same spindle pole.

Step-wise loss of cohesion

In addition to the directionality provided by kinetochore orientation, loss of sister-chromatid cohesion has to be modified to support the meiotic division pattern. During mitosis, cohesion is destroyed in one step to allow separation of sister chromatids. This is not possible in meiosis because, as a result of crossover recombination, sister chromatid cohesion also connects homologous chromosomes (Figure 2). If cohesion were completely lost during meiosis I, sister chromatids would separate prematurely because no new cohesin complexes are loaded between meiosis I and meiosis II. As shown in Figure 2, only the sister chromatid cohesion distal to the site of crossing over is responsible for connecting homologous chromosomes, whereas cohesion close to centromeres still links sister chromatids. Consequently, to establish the meiotic division pattern, sister chromatid cohesion must be lost in a step-wise manner, first along chromosome arms to separate homologous chromosomes, then at centromeres to separate sister chromatids.

Sister chromatid cohesion is destroyed by proteolytic cleavage of one of the cohesin subunits. To achieve step-wise loss of cohesion, meiotic cells incorporate a meiosis-specific cohesin subunit that is

largely refractory to cleavage unless it is phosphorylated by polo-like kinase (PLK). PLK activation prior to meiosis I results in abundant cohesin phosphorylation only along chromosome arms. Cohesins at centromeres remain unphosphorylated due to the centromere-specific recruitment of protein phosphatase 2A, which is thought to counteract PLK. As a result, only cohesion along chromosome arms is destroyed during meiosis I, and homologous chromosomes are able to separate. Cohesin complexes at centromeres remain intact until sister chromatids are under tension on the meiosis II spindle. At that point, these cohesin complexes are also cleaved and sister chromatids undergo an equational division. Notably, in metazoans, PLK activity also leads to an early wave of cohesin dissociation from chromosomes during mitosis. However, in contrast to meiosis, PLK-dependent loss of mitotic cohesins does not require cohesin cleavage, and thus occurs by a different mechanism.

The timing of meiosis

The meiotic divisions are embedded within the larger program of gametogenesis and are often coordinately regulated with gamete differentiation. For example, in mammals, meiosis arrests twice in the course of oocyte differentiation: first after meiotic recombination at the diplotene stage of meiosis, then a second time in metaphase II immediately prior to meiosis II chromosome segregation. During the diplotene arrest the oocyte recruits supporting cells and begins to grow and differentiate. Oocytes remain in this first meiotic arrest for a long time. The diplotene block initiates in the embryo and persists until the oocyte is ovulated. Thus, in humans, an oocyte can remain in diplotene for over forty years. Upon ovulation, the oocyte completes meiosis I, and then arrests again in metaphase II with chromosomes aligned on the meiosis II spindle. The metaphase II arrest is characteristic of mature eggs and is maintained until fertilization. For both arrests, the timing of eventual meiotic progression depends on external inputs. Exit from diplotene into meiosis I occurs in response to hormonal cues, which stimulate individual oocytes to activate the key cell-cycle motor Cdk1-cyclin B. Conversely, exit from metaphase II is triggered by sperm entry, which signals the destruction of cyclin B, thereby initiating the second meiotic division.

External signals are also required for germ cells to enter the meiotic program. For example, meiosis is initiated in response to nutrient limitation in yeast, whereas mouse germ cells require retinoic acid signaling from the surrounding tissue to trigger meiotic entry. In fact, the differential exposure to retinoic acid is thought to be the key mechanism allowing female mouse germ cells to enter meiosis in the embryonic ovary, whereas male germ cells first initiate meiosis in the adult testis. Thus, although meiosis is a differentiation program of individual cells, ample opportunities exist to coordinate the meiotic program with external stimuli.

Meiosis and human disease

The findings from model organisms have greatly improved our understanding of meiosis and provided important clues about the regulation of meiosis in humans. Compared with most model organisms, the fidelity of meiotic chromosome segregation in humans is surprisingly poor, resulting in high rates of spontaneous abortions and birth defects such as Down syndrome (trisomy 21). The reasons for the high levels of aberrant human meioses are still largely unclear. Analysis of non-disjoined chromosomes 21 from Down syndrome patients has revealed that several specific recombination patterns are associated with meiotic mis-segregation. In many cases, non-disjoined chromosomes fail to undergo crossover recombination entirely and thus segregate randomly during meiosis I. In addition, non-disjoined chromosomes 21 from Down syndrome patients frequently exhibit crossovers in immediate proximity to the centromere, where crossover recombination is normally strongly suppressed. In both fruit flies and budding yeast, such centromere-proximal crossovers can cause premature loss of sister-chromatid cohesion and chromosome mis-segregation. The high incidence of centromere-proximal crossovers in Down syndrome patients suggests that such crossovers may have similar deleterious effects on sister-chromatid cohesion in humans.

It is a well-established phenomenon that in humans the incidence of oocytes with an aberrant chromosome number increases with maternal age. Attention has focused in particular on the diplotene arrest because the decades-long arrest times may exaggerate even subtle defects in chromosome stability. Indeed, work in mice has shown that several proteins required to align homologous chromosomes on the meiotic spindle appear to be present at lower levels in older oocytes and has revealed a correlation between such defects and an age-dependent increase in meiotic errors. Moreover, the elimination of a meiosis-specific cohesin subunit in mouse oocytes also causes an age-dependent increase in meiotic chromosome mis-segregation, raising the possibility that defective sister-chromatid cohesion may contribute to the increased error rate in humans. Thus, studies in model organisms are providing a first glimpse at the possible mechanisms underlying human meiotic defects. As these studies provide a more detailed mechanistic picture of meiosis, we will be able to get a better understanding of the complexities of human meiosis and hopefully identify some of the causes and possible treatments of infertility and birth defects.

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Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA.
E-mail: andi@wi.mit.edu