Chromosomal Control of Meiotic Cell Division

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Chromosomes have multiple roles both in controlling the cell assembly and structure of the spindle and in determining chromosomal position on the spindle in many meiotic cells and in some types of mitotic cells. Moreover, functionally significant chromosome-microtubule interactions are not limited to the kinetochore but are also mediated by proteins localized along the arms of chromosomes. Finally, chromosomes also play a crucial role in control of the cell cycle.

Chromosomes have long been viewed as simply the cargo of the meiotic and mitotic processes. They were thought to be attached to spindles organized by centrosomes and then dragged back and forth by external forces. Over the last decade, this perception has changed markedly with the realization that chromosomes control much of the cell division process in meiotic cells and sometimes even in mitotic cells. This change of view reflects four recent sets of findings. (i) Molecular motors attached to the kinetochores, and in some cases the chromosome arms, move the chromosomes during mitosis and meiosis. (ii) In many meiotic cells, the divisions take place on acentriolar spindles organized by the chromosomes. (iii) The chromosomes function in error recognition in some meiotic and mitotic cell cycles; that is, cell cycle checkpoints exist that are triggered by abnormalities in chromosome structure or position. (iv) At least in *Drosophila* oocytes, the chromosomal consequences of meiotic crossing-over are crucial for the initiation of a normal arrest in the meiotic cell cycle. These examples may be just the first of many processes in which chromosomes and their substructures control the cell cycle.

Chromosomal Control of Meiotic Spindle Formation

As pointed out by Rieder *et al.* (*1*, p. 187), "the route by which the spindle forms can differ considerably between mitosis and meiosis." Although in most mitotic cells spindle formation is mediated by centrosomes, in some oocytes the spindle appears to be acentriolar and is organized by the chromosomes themselves. A well-studied example of this phenomenon occurs in the oocytes of the fruit fly *Drosophila melanogaster*.

Before metaphase, the chromosomes of *Drosophila* oocytes are located in a single mass known as the karyosome. Meiotic spindle formation begins with the establishment of an array of microtubules lacking a defined pole that emanate from the major chromosomes (Fig. 1) (*2*). As prometaphase continues, these bundles of microtubules are sculpted together on each side of the metaphase plate to form a bipolar spindle. It is often possible to observe that the early spindle is comprised of four sets of microtubule bundles, presumably corresponding to each of the four pairs of homologous chromosomes. The spindle then lengthens and tapers, a mechanism that requires the kinesin-like protein NCD (*3*). The structures that form the poles of these spindles share little in common with mitotic centrosomes, as antibodies that recognize the centrosomal antigens DMAP60, DMAP190, and γ-tubulin do not bind to epitopes within these structures (*3*).

The ability to organize bundles of microtubules into polar spindles is intrinsic to all of the chromosomes in the *Drosophila* oocyte. Individual chromosomes or meiotic bivalents (and, perhaps more specifically, their kinetochores) that have been expelled from the karyosome by a variety of genetic means can assemble small minispindles. In the case of mutations that cause the loss or nondisjunction only of univalent chromosomes (univalents), such as mutations in the *nod* gene, these supernumerary spindles are usually monopolar (*2*). In contrast, in meiotic mutants such as nod^{DTW}, which cause high levels of nondisjunction of chiasmate bivalents (*4*), bipolar arrays are often observed. Although individual chromosomes can organize their own spindles, the formation of either a monopolar or a bipolar spindle depends on whether they possess one (univalent) or two (bivalent) kinetochores.

The ability of chromosomes to organize a functional spindle is not limited to *Drosophila* oocytes. Chromosomes have been shown to organize the spindle in the absence of centrosomes in several meiotic systems (*1*, *5*, *6*). There are also more canonical meiotic systems in which the ability of the chromosomes to organize their own spindle appears to be suppressed by functional centrosomes or in which the chromosomes interact with the centrosomes to form the spindle. For example, in *Drosophila* spermatocytes, a chromosome detached from the canonically centriolar meiotic spindle quickly forms a miniature but functional spindle around itself (*7*). The poles of

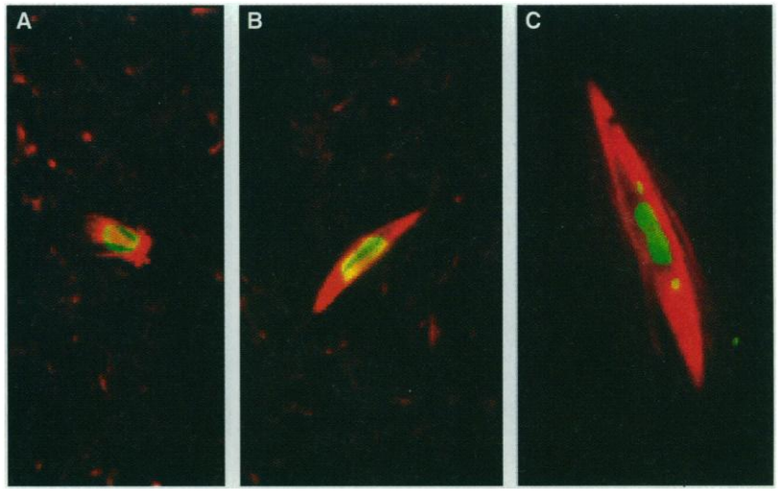

Fig. 1. The normal pathway of spindle assembly in wild-type (+/+) *Drosophila* females. Chromosomes (green) are stained with histone antibody conjugated to fluorescein. Spindles (red) are stained with tubulin antibody conjugated to rhodamine. (**A**) Prometaphase begins with short microtubules that lack a defined pole forming around the karyosome. Pre-assembled spindle microtubules are presumably captured by the chromosomes, stabilizing their plus ends and allowing microtubule elongation. (**B**) The microtubules are then sculpted into a short spindle with defined poles. (**C**) Finally, the metaphase spindle is long and tapered with achiasmate chromosomes precociously moving toward the poles. Meiosis remains arrested at this stage until passage through the oviduct, at which time activation occurs and meiosis is completed.

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these spindles are unrelated to those of the spindle from which the chromosome was detached. To quote Nicklas, "the presence of a chromosome in the cytoplasm not only enhances microtubule assembly but also triggers the formation of a functionally normal spindle, complete with two poles, at a site where a spindle never occurs normally" (8, p. 61). Thus, in this case the chromosomes appear to possess the capability to form a spindle on their own, an ability that is actively suppressed in the vicinity of functional centrosomes. Finally, although the chromosomes cannot organize their own spindles without centrosomes in grasshopper spermatocytes, the chromosomes exhibit a size-dependent effect on the structure of the spindle (9). That is, the addition of one chromosome near one pole of a newly formed spindle increases the microtubule density in the entire half-spindle fourfold relative to the other half-spindle.

The relation of chromosomes to spindle assembly in mitotic cells is equally complex (9). In some cases, such as in echinoderm embryos, the chromosomes cannot organize a spindle in the absence of centrosomes (*10*). There are also systems in which, although the chromosomes cannot organize spindles, they are nonetheless necessary for spindle formation. That is, in echinoderm embryos a pair of centrosomes with microtubule asters cannot produce a spindle in the absence of chromosomes (*9*, *11*, *12*). Spindle formation in these systems may require specific interactions between the kinetochore and the centrosome. In Chinese hamster ovary cells, kinetochores detached from their chromosomal arms interact with microtubules and together with the centrosomes form a functional spindle (*13*). There are also cases in which chromosomes can organize a spindle when the existing centrioles are unable to do so (*14*), again suggesting that the ability of chromosomes to form spindles is actively suppressed or outcompeted by the presence of functional centrosomes.

Microtubule attachments not limited to kinetochores. The ability of kinetochores to capture microtubule fibers is well documented. This ability presumably extends to diffuse centromeres, to neocentromeres (knobs) in corn, and to facultative centromeres in yeast and humans (*15*). However, at least in meiotic cells, the ability to form stable and functional interactions with microtubules may be a property of chromatin per se and not just of kinetochores or kinetochore-like structures. In *Xenopus* oocytes, chromatin from many sources, including bacteriophage DNA, promotes microtubule assembly. When sperm nuclei with their membranes removed are incubated in extracts of *Xenopus* oocytes arrested at metaphase II, microtubule fibers attach to chromosomes most frequently in regions that lack kinetochores (*12*), which suggests that the ability of chromatin to promote microtubule assembly in meiotic cells is not specific to kinetochores.

The functional significance of such nonkinetochore microtubule attachments along the lengths of chromosome arms is attested to by two sets of findings. First, as indicated above, the movement of chromosomes within a grasshopper spermatocyte spindle alters the distribution of microtubules in a fashion that is proportional to the mass of chromatin and not to the number of kinetochores present (9). A spindle was manipulated to have a large bivalent with two kinetochores at one pole and three smaller bivalents with six kinetochores at the other pole. The half-spindle with fewer kinetochores but more chromatin had 30% greater microtubule density than the half-spindle with more kinetochore but less chromatin. Thus, the presence of chromatin had a dramatic effect on spindle structure.

Second, a new class of proteins, the so-called "chromokinesins," have been discovered that localize along the length of chromosome arms and that have important functions in chromosome position, spindle assembly, or both (*16*). The NOD kinesin-like protein, which is found along the length of chromosome arms in *Drosophila* oocytes, positions and holds chromosomes on the developing prometaphase spindle (*17*). In the absence of NOD protein, achiasmate chromosomes are displaced from the developing spindle, apparently by precociously migrating off one of the two ends of the spindle (2).

Thus, the NOD protein appears to provide a plateward force that counterbalances the poleward forces acting on the kinetochore; in doing so, NOD substitutes for chiasmata by holding pairs of achiasmate chromosomes at the metaphase plate during spindle assembly. It remains to be determined if NOD performs this function by acting as a plus end–directed motor that actively pushes chromosomes to the plate or, more simply, as a brake that opposes the poleward forces exerted by the kinetochore. A second such chromokinesin, Xklp1, has been identified in *Xenopus* oocytes, which suggests that nonkinetochore microtubule-chromosome interactions may be a feature of oogenesis in a variety of organisms (*18*). This protein appears to be required for maintaining spindle assembly in vitro and in vivo. In oocyte extracts immunologically depleted of Xklp1, bipolar spindles were formed at reduced frequency, and the microtubule density was reduced compared to that in controls. Furthermore, the chromosomes became delocalized and some were released from the spindle, which suggests that Xklp1 may be required for chromosomes to congress at the metaphase plate. Thus, spindle stability may require interactions between nonkinetochore microtubules and Xklp1 on chromosome arms (*12*).

Building a spindle in Drosophila *oocytes may require chromosome opposition.* In *Drosophila* oocytes, spindle assembly requires the proper pairing and alignment of chromosomes, as mediated by either chiasmata or by the NOD kinesin-like protein. That

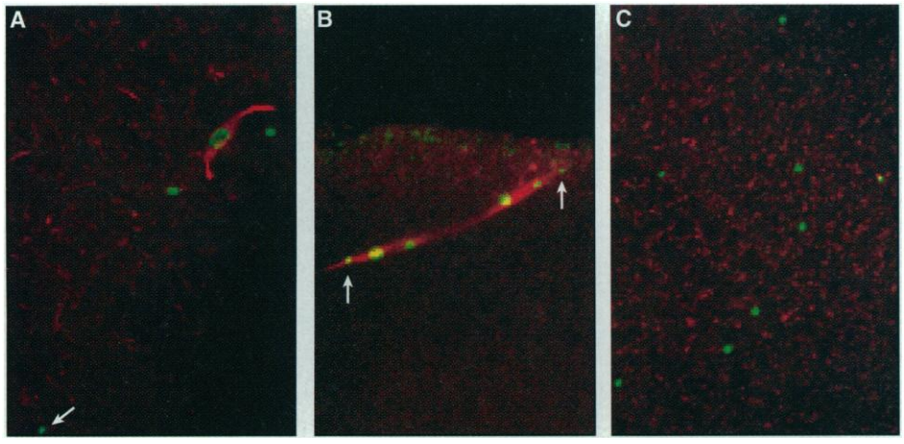

Fig. 2. Spindle formation in *nod* and *mei-218* mutants (*52*). (**A**) In *nod* mutants, only the achiasmate chromosomes are affected. In this oocyte, both the X and fourth chromosomes were achiasmate and, as a result, were ejected from the karyosome. The X chromosome univalents are near the spindle. Shown with an arrow is the single visible fourth chromosome univalent. (**B**) Spindle formation occurs normally in the crossover-defective mutant *mei-218*. In *mei-218* females, the reduction in crossing-over (to less than 10% of that in the wild-type) causes a bypass of metaphase arrest, but this has no effect on spindle assembly or viability (*24*). The two arrows show the small fourth chromosomes leading the larger chromosomes to the poles. (**C**) Spindle formation is severely compromised in the double mutant *nod mei-218*. In these oocytes, both chiasmata and NOD protein are absent. The chromosomes are spread throughout the oocyte, and karyosomes with a mature bipolar spindle are never observed.

is, *Drosophila* oocytes bearing *nod* mutations can assemble a normal spindle in the presence of at least one pair of chiasmate chromosomes (Fig. 2A), and oocytes homozygous for recombination-deficient mutations such as *mei-218* can assemble a normal spindle in the presence of NOD protein (Fig. 2B). However, in double mutant oocytes lacking both chiasmata and NOD protein (*nod mei-218*), mature bipolar spindles are not observed and, instead, the chromosomes are found dispersed throughout the oocyte (Fig. 2C). Bipolar spindle assembly appears defective in these oocytes from the earliest stages of prometaphase; in the double mutant, only the initial stages of bipolar spindle formation are observed.

The requirement for either NOD or at least one chiasma may reflect a need to maintain at least one pair of chromosomes with their kinetochores oriented in opposite directions to provide a central axis for spindle formation. Before nuclear envelope breakdown, homologous chromosomes remain tightly paired, at least in their heterochromatic regions (*19*). Like others, we propose that the kinetochores of each pair of homologous centromeres are usually oriented in opposite directions and thus capture microtubules in such a way as to form a bipolar bundle (*20*). We imagine this kinetochore positioning can be stabilized either by chiasmata or by the NOD kinesin-like protein and that the microtubules captured by the paired kinetochores are sculpted into a bipolar array by the actions of proteins such as the NCD kinesin-like protein. With the spindle formed and the two kinetochores oriented toward opposite poles, the progression of the two chromosomes toward the poles is halted at the metaphase plate by the chiasma, or by the NOD protein in the case of achiasmate chromosomes. This represents a stable position in which the bivalent will remain during metaphase arrest and that provides a framework for spindle formation.

Chromosomal Control of Prometaphase Movement

In centriolar meiotic or mitotic systems, chromosome attachment to the spindle is an error-prone process in which tension on the kinetochore functions as an error-correction mechanism. Because attachment is a stochastic process, at first the chromosomes are usually mono-oriented, such that one or both of the kinetochores are attached to the same pole (Fig. 3). The correction mechanism relies on the fact that these initial kinetochore-spindle attachments are unstable and prone to detachment at the pole. If both kinetochores capture microtubules from the same pole, destabilization and release of an attachment allow for bipolar reattachment. Once the second functional kinetochore captures microtubules from the opposite pole, the microtubule-kinetochore attachment is stabilized and the centromeres congress to the equator (*21*, *22*). Thus, kinetochore-microtubule contacts are tension-sensitive and are stabilized only with bipolar attachment. Moreover, tension on the kinetochore may also control the switching of chromosome movement between poleward and anti-poleward states (*23*). An interesting consequence of having chromosomes organize spindles in meiotic cells is that attaining bipolar attachment is not a problem. The mechanism of spindle formation ensures that the chromosomes are at the metaphase plate with bipolar spindle attachments.

Motors determinining the position of chromosomes. In acentriolar meiotic systems chromosomes begin at the metaphase plate, and thus congression is unnecessary. Nonetheless, multiple mechanisms exist to control the position of chromosomes on these spindles. As the spindle of the *Drosophila* oocyte becomes more elongated, the achiasmate chromosomes move precociously toward the poles, whereas the exchange chromosomes remain locked by their chiasmata at the metaphase plate. The movement of achiasmate chromosomes toward the pole is size-dependent; smaller chromosomes leave the metaphase plate earlier and move farther toward the pole than do larger chromosomes. The timing of movement is clearly a property of size and not a difference between centromeres (*2*). Similarly, in anaphase the smaller chromosomes precede the larger ones to the poles (*24*). This process of size-dependent precocious movement can be explained by the observation that NOD is bound along the entire length of the meiotic chromosomes.

We propose that each chromosome produces a plateward force in proportion to the number of NOD molecules bound along its length, by acting either as a plus end–directed motor that actively pushes chromosomes to the plate or as a brake that opposes the poleward forces exerted by the kinetochore. Because larger chromosomes bind more NOD protein, they might be expected to exert a larger plateward force than smaller chromosomes, thus explaining the fact that larger chromosomes remain closer to the metaphase plate than smaller chromosomes. The process of size-dependent positioning of chromosomes on spindles is not limited to meiotic cells. Severing the arms off of newt mitotic chromosomes results in the kinetochore-bearing fragment moving closer to the pole and the severed arm moving to a position farther from the pole (*22*). Thus, the "chromokinesins" may be a component of the polar ejection force.

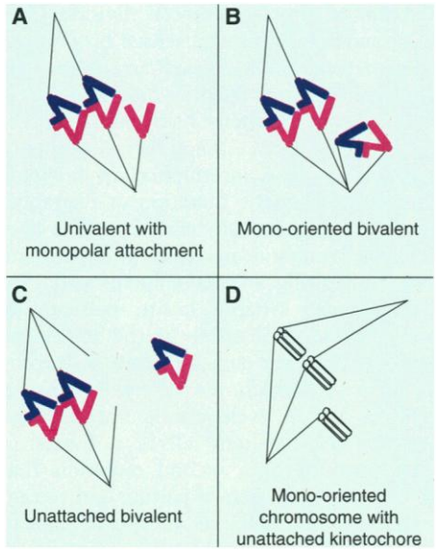

Fig. 3. Four different situations in which meiotic or mitotic chromosomes are misaligned on the spindle. In all four cases, the kinetochores of the misattached chromosomes are not under tension because of the absence of opposing poleward forces. (**A**) A univalent has only one kinetochore and can attach to only one pole. (**B**) Early in spindle attachment a bivalent may have both kinetochores attached to the same pole (mono-oriented). (**C**) A chromosome can become completely detached from the spindle either through micromanipulation or through a failure to attach. (**D**) A mono-oriented chromosome has one kinetochore unattached to spindle fibers. This can occur because the initial attachment of the chromosome to the spindle is often at only one kinetochore. Bipolar orientation is achieved when the other kinetochore makes a spindle attachment.

Kinetochore-Mediated Control of Anaphase Chromosome Movement

For many years, the prevailing model for anaphase chromosome movement was known as the "traction hypothesis" in which chromosomes were reeled in to the poles at anaphase by forces acting at the poles or along the kinetochore microtubules. More recently, it has been found that molecules within the kinetochores provide forces for chromosome movement to the poles. These experiments have been reviewed extensively elsewhere (*8*, *25*). Here, we focus on the degree to which structures within the kinetochore control its meiotic behavior.

Determination of reductional or equational segregation. During meiosis in *Drosophila*, chromosomes conduct two different types of division. In meiosis I (reductional), the sister kinetochores stay together while the homologs segregate. In meiosis II (equational), the sister kinetochores segregate like in a mitotic division. The determinants of

whether a given chromosome will segregate reductionally or equationally reside within the kinetochores rather than on the spindle. This conclusion is derived from an elegant experiment in which two spermatocytes from the grasshopper *Dissosteira carolina* were fused, one at the first meiotic metaphase, the other in the second meiotic metaphase (*26*) (Fig. 4). A bivalent was detached from the meiosis I spindle and allowed to reattach to the adjacent meiosis II spindle. At anaphase II, the displaced bivalent still segregated reductionally (that is, the two homologs comprising this bivalent moved to opposite poles), despite its location on a spindle where all the other chromosomes segregated equationally. Thus, the instructions for proper behavior at meiosis I reside within the chromosome, not in the spindle.

That these instructions lie within the kinetochores and are not general properties of the chromosomes is suggested by work on mutations that produce an apomictic meiosis (meiosis with a single division) in the yeast *Saccharomyces cerevisiae* (*27*). During this division, the segregation behavior of individual chromosomes is autonomous and is determined by the centromeres they carry; some centromeres show a preference for reductional instead of equational segregation, or vice versa. The propensity for reductional or equational segregation is maintained by a given centromere even when it is moved to a different chromosome (replacing the existing centromere). The differential behavior of centromeres within a single meiotic division also occurs in several insect species, when during the first meiotic division the sex chromosomes segregate equationally, whereas the remainder of the complement segregate reductionally (*28*).

Differences in centromere sequences might also be expected to result in different force-generating characteristics at the kinetochore. For example, the amount of force pulling a chromosome to the poles could be modulated by microtubule-kinetochore interactions. This is suggested by cytological analysis of trivalents in praying mantid and grasshopper spermatocytes (*29*), where one kinetochore is oriented toward one pole and the other two kinetochores are oriented to the opposite pole. When trivalents are newly made with radiation, their positions on the spindle are consistent with the effects of having two kinetochores exert a stronger poleward force than a single kinetochore applying force in the opposite direction. That is, their stable position at the metaphase plate is closer to the pole to which the two kinetochores are attached.

In contrast, "natural" trivalents take a more central position at the metaphase plate, despite the fact that the force of two kinetochores is opposed to a single kinetochore pulling in the opposite direction. This suggests that together the two kinetochores moving in the same direction pull with a force that is equal to the one kinetochore pulling in the opposite direction. This behavior might reflect the ability of motors at one kinetochore to pull with only enough force to oppose the forces pulling in the opposite direction. Alternatively, different centromeres, by virtue of their DNA sequences, might build kinetochores of different strength. The possibility that different centromeric regions might differ in their ability to exert force was also suggested in studies on the meiotic behavior of dicentric chromosomes in *Drosophila* oocytes (*30*). The segregational behavior of these dicentrics differed substantially depending on the heterochromatic content of the two chromosomes involved in the dicentric chromosome.

Chromosome-Mediated Cell Cycle Control

In most cases, chromosome damage or aberrant chromosome behavior signals an arrest in the cell cycle in order to allow the correction of errors before cell division (*31*). Chromosome breaks are sensed by a system that detects DNA damage and arrests the cell until they are fixed, or the cell is killed. A variant in this theme has been found in *S. cerevisiae* meiotic cells and mouse spermatocytes (*32*). The interruption of homolog synapsis by genetic mutation or rearrangement results in a pachytene arrest. The *zip-1* or *dmc-1* mutants in yeast, which initiate meiotic pairing and recombination but do not complete synapsis, fail to sporulate because these cells arrest in the pachytene stage (*33*). These data are best explained by a model in which the defect in synapsis triggers a cell cycle checkpoint. Because *zip-1* or *dmc-1* mutant cells do not arrest in the presence of a second mutation that blocks the initiation of pairing and recombination, the pachytene arrest is thought to result from the accumulation of specific recombination intermediates that trigger the checkpoint. Alternatively, the checkpoint might be triggered by a meiosis-specific mechanism that senses the failure or deterioration of synapsis.

Triggering of meiotic or mitotic arrest by single, unattached chromosomes. Unrepaired DNA breaks are not the only chromosomal anomalies that can signal a problem in the cell cycle. Checkpoints monitor the completion of chromosome alignment during metaphase in meiotic and mitotic cells (*34*) (Fig. 3). In mitotic Ptk cells (*35*), the time between nuclear envelope breakdown and anaphase is longer in cells with as few as one kinetochore unattached to microtubules. The delay in metaphase allows time for all kinetochores to attach to microtubules. The failure to prevent division before such errors are corrected would result in nondisjunction and aneuploidy. Similarly, in praying mantid spermatocytes a single univalent with a mono-oriented kinetochore can signal a fatal prolongation of metaphase (*36*). In this situation, the delay leads to the degeneration of cells that otherwise would produce chromosomally abnormal sperm. The studies summarized below reveal that the location of the signal that triggers this checkpoint is at the kinetochore, that the stimulus of the signal is tension, and that the chemical signal involves the phosphorylation of kinetochore proteins.

The arrest signal originates at the kinetochore. Three lines of evidence suggest that bipolar kinetochore attachment to the spindle may be crucial for the metaphase-anaphase transition in some meiotic and mitotic cells. (i) Mutation of the yeast centromere leads to a prolongation of mitosis (*37*). (ii) The injection of centromere protein antibodies into G_2 cells does not disrupt spindle assembly but causes metaphase arrest (*38*); this suggests either that centromeric proteins are involved in signaling the metaphase-to-anaphase transition or that proper kinetochore function is monitored by a checkpoint. (iii) Most directly, when

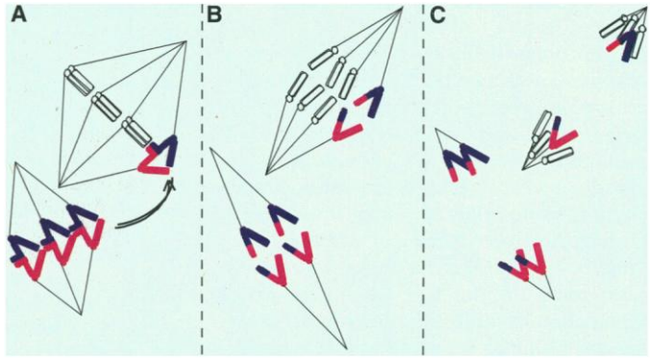

Fig. 4. The results of moving a meiosis I chromosome onto a meiosis II spindle. A schematic diagram showing two spindles; on the left is a meiosis I spindle, and on the right is a meiosis II spindle. (**A**) A bivalent is moved by micromanipulation from the meiosis I spindle onto the adjacent meiosis II spindle. (**B** and **C**) This chromosome orients and segregates reductionally, even though all the other chromosomes on the spindle segregate equationally.

the unattached kinetochore of a mono-oriented chromosome in mitotic Ptk cells was destroyed by laser ablation, it was no longer able to delay the metaphase-to-anaphase transition (39). Thus, the unattached kinetochore of a mono-oriented chromosome inhibits the transition into anaphase.

Transition delay by absence of tension on the kinetochore. Tension arises when a properly oriented chromosome possesses microtubule attachments to opposing poles. During meiosis, for example, each kinetochore of the bivalent applies a poleward force as it moves along kinetochore microtubules. In a normal bivalent, these poleward forces are opposed and balanced by the chiasmata, resulting in tension on both kinetochores. The failure to maintain tension can lead to cell death in some cell types. In praying mantid spermatocytes a single mono-oriented univalent signals a prolongation of metaphase, leading to apoptosis (36). In these cells, three sex chromosomes must remain connected as a trivalent to orient at meiosis I. In some cases, one sex chromosome becomes separated from its two partners and finds itself connected to only one pole. In these cells, the metaphase-to-anaphase transition is delayed and eventually leads to cell death (36). The role of tension in this system was tested by pulling on the mono-oriented sex chromosome with a micromanipulation needle to simulate the bipolar tension experienced by this chromosome when it is part of a trivalent at the metaphase plate (36). When tension was applied, anaphase initiated on schedule. Thus, the lack of tension on a kinetochore signaled the delay in the metaphase-to-anaphase transition.

Absence of tension and change in protein phosphorylation. Tension on the kinetochores can generate a checkpoint signal, but the chemical nature of this signal is not known. A candidate kinetochore signaling protein was detected with an antibody, 3F3, that reacts strongly with some kinetochore proteins when they are phosphorylated. 3F3 reacts strongly with kinetochores before they are attached to the spindle, but the reaction weakens as the chromosomes attach to the spindle and move to the metaphase equator (40). Early in grasshopper spermatocyte spindle formation, the kinetochores of mono-oriented bivalents are intensely stained by the 3F3 antibody. Because both kinetochores of the bivalents are attached to the same pole, the kinetochores are not under tension. Thus, some kinetochore proteins are phosphorylated when not under tension (41). Individual chromosomes were detached from the meiotic spindle with micromanipulation techniques (41). Both kinetochores of these bivalents lacked tension for as long as they were held off the spindle by micromanipulation. When a chromosome was detached for only 5 min, no increase in 3F3 staining was observed. Longer detachment, however, resulted in twofold greater staining of the kinetochore with 3F3. The time required for phosphorylation of the kinetochore in response to "relaxation" of kinetochore forces was between 5 and 10 min (41).

These principles can be illustrated by analysis of bivalents positioned near one pole that had been manipulated to have both kinetochores attached to it (41) (Fig. 3). Without force exerted toward the opposite pole, there was no tension on either kinetochore of the bivalent, and both fluoresced brightly with 3F3. When tension was applied to only one of the two kinetochores of this bivalent (by inserting a microneedle into the bivalent and pulling one of the two kinetochores toward the equator), the kinetochore placed under tension quickly exhibited reduced staining to a level similar to that seen in the other properly oriented chromosomes in the same cell. The other kinetochore of the same bivalent, which lacked tension, retained its bright 3F3 staining, clearly showing that the tension signal is localized to a specific kinetochore.

Although there is no direct link between the phosphorylation state of the kinetochore and the cell cycle arrest, the correlations are strong (41). First, misattached chromosomes have phosphorylated kinetochores and trigger a metaphase checkpoint. Second, both the triggers of the checkpoint and the changes in kinetochore phosphorylation respond to tension and operate at the kinetochore. It is unknown what proteins are recognized by the 3F3 antibody and if they directly mediate the tension signal. Finding these proteins will provide insight into how a physical stimulus such as tension can change kinetochore chemistry and how the signal is transduced from the kinetochore proteins to the cell cycle controls. The immunolocalization of 3F3 (42) shows that the tension-sensitive proteins are found in the middle layer of the kinetochore, among the struts between the inner and outer plates. This is a logical location for proteins that detect tension or stretching of the kinetochore.

Not all kinetochores that lack tension produce a checkpoint signal. In several organisms, univalent X chromosomes can congress to the metaphase plate in the absence of a homolog (43). In *Caenorhabditis elegans* X/O males, univalent X chromosomes also congress to the metaphase I plate in the absence of a homolog (6). This is a specific property of the X chromosome and not of univalents in general. During meiosis, autosomal univalents behave abnormal-

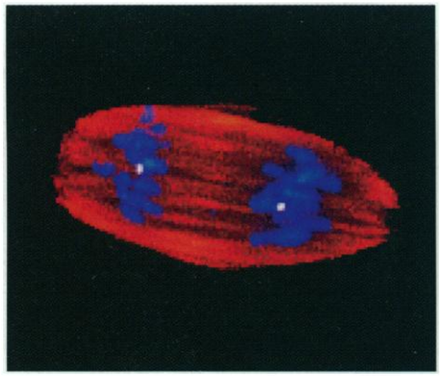

Fig. 5. Anaphase I mouse oocyte showing equational segregation of the univalent X chromosome. The spindle, in red, was detected by rhodamine-labeled antibody. The chromosomes (blue) are stained with TO-PRO, and the X chromosome (white) is detected by a fluoroscein isothiocyanate–labeled probe.

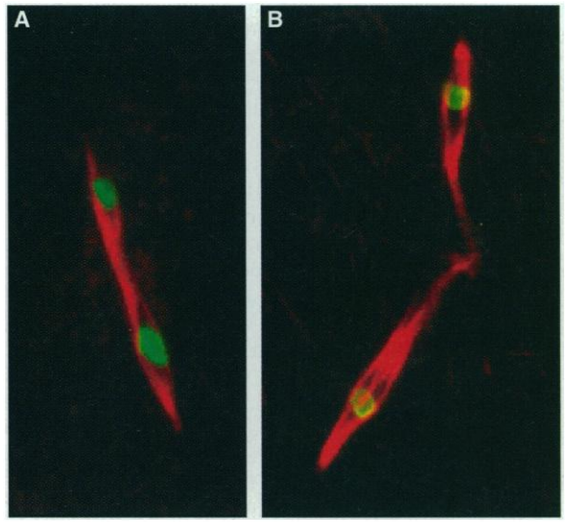

Fig. 6. Mature (stage 14) oocytes in the recombination-defective mutant *mei-W68* (52, 53). Meiosis normally arrests at metaphase I in stage 14 oocytes (see Fig. 1). However, in *mei-W68* mutants, crossing-over and thus chiasmata are eliminated. The result is a loss of kinetochore tension and a bypass of metaphase arrest. Shown here are two stage 14 oocytes with post-metaphase I stages: (**A**) anaphase I and (**B**) metaphase II. Similar results were seen with other meiotic mutants (such as *mei-218*) (Fig. 2).

ly, resulting in chromosome misdivision, loss, or equational segregation (*44*) (Fig. 5). Similarly, in the grasshopper spermatocytes described above, the univalent X chromosome does not delay anaphase—otherwise, no sperm could be made. Nicklas *et al.* (*41*) suggested that these cells must have evolved a mechanism to suppress checkpoint signals from the naturally univalent X chromosome. Consistent with this hypothesis, the kinetochore of the grasshopper X chromosome never stains strongly with 3F3. Thus, the kinetochores of these chromosomes are prevented from signaling metaphase arrest, allowing them to congress and segregate normally.

Kinetochore tension in Drosophila *oocytes.* Tension can be used for different purposes in different cell types. Tension on the kinetochore is a signal in *Drosophila* females to arrest meiosis at metaphase I. Anaphase I does not normally begin until the oocyte passes through the oviduct, at which time sister chromatid cohesion distal to the chiasmata is released (Fig. 1). In oocytes with a drastic reduction in crossing-over as a result of recombination-defective mutations, metaphase I arrest was abolished and anaphase and meiosis II figures were observed (Fig. 6) (*24*). Thus, crossing-over and the resulting chiasmata are required for metaphase arrest. Without chiasmata, all chromosomes would have microtubule attachments to only one kinetochore, which suggests that the balancing of kinetochore forces by chiasmata was the signal for metaphase arrest.

This hypothesis was tested with female fruit flies whose normal karyotype consisted entirely of "compound" chromosomes, in which each pair of homologous chromosome arms is attached to a single centromere (*45*). These chromosomes allow crossing-over to occur, but they do not induce tension on the kinetochores because the chiasmata do not connect centromeres on different chromosomes. As in the recombination-defective mutants, metaphase I arrest was abolished in these females. That the anaphase I and meiosis II figures observed in these two experiments represent the normal continuation of the meiotic process is shown by the fact that eggs produced by these females are fully capable of producing viable offspring upon fertilization by sperm of the correct genotype.

In a separate experiment, females were created in which all but one pair of chromosome arms were arranged as compound chromosomes (*45*). In these females, the one normal pair of chromosomes was able to form chiasmata that joined two centromeres and metaphase arrest was virtually always observed. Similarly, some recombination-defective mutants have a low level of crossing-over, such that approximately 25% of the mutant oocytes possess at least one chiasma. In these mutants, a similar frequency of oocytes that were arrested at metaphase I was observed, which suggests that tension from even a single chiasmate bivalent could induce metaphase arrest (*24*).

There is a crucial difference between the situation in *Drosophila* oocytes and the other two systems mentioned here (mitotic Ptk cells and insect spermatocytes). In *Drosophila* females, tension is a signal that causes metaphase arrest. In the other two systems, it is the lack of tension that causes metaphase arrest. Thus, the transduction of the tension signal has been modified in these systems for specific needs. In the spermatocytes and Ptk cells, the lack of tension is a signal that there is a problem in aligning the chromosomes on the spindle and that anaphase needs to be delayed until the problem is fixed (*39*) or until the cell degenerates (*36*). In *Drosophila* females, metaphase I arrest is the normal course of events, and thus chromosome misalignment may not be a problem. In both systems, the signal from a single chromosome or bivalent can induce cell cycle arrest. It is probably easier for the cell to respond to one misaligned chromosome (or to one chiasmate bivalent in *Drosophila* oocytes) than it is to detect small changes in the number of aligned chromosomes (*39*, *46*).

The absence of a chromosome misalignment checkpoint during *Drosophila* female meiosis (*47*) is striking. Meiosis proceeds without an arrest with any number of univalent chromosomes or disorganization of the spindle (Fig. 2), which demonstrates that there is no checkpoint for mono-oriented or misaligned chromosomes in *Drosophila* female meiosis. Some embryonic systems also lack checkpoints, which is perhaps a compromise to allow rapid cell division (*31*). For example, sea urchin zygotes also proceed into anaphase with unattached chromosomes present (*48*). A similar argument can apparently be made for oocytes in X/O mouse females. In this case, oocytes enter anaphase with a single unpaired X chromosome and without signaling a metaphase I checkpoint. Furthermore, the univalent segregates either equationally or reductionally at the first division, which shows that the nature of the spindle attachment (bipolar or monopolar) does not effect the metaphase-to-anaphase transition (*47*) (Fig. 5). Conceivably, most or all oocyte systems lack a system to detect mono-oriented chromosomes on the meiotic spindle. This may be related to the fact that, unlike spermatocytes and yeast cells, most oocytes naturally arrest at some point during meiosis I or II. The lack of a metaphase checkpoint in human oocytes may cause the very high frequency of meiotic errors in human oocytes as compared to those in sperm (*47*).

Summary

The studies described above point to the existence of multiple roles for chromosomes in the processes of spindle assembly and maintenance and in positioning chromosomes on the meiotic metaphase spindle. We have also pointed out the role of chromosomes, and more specifically that of kinetochores, both in triggering pre-programmed cell cycle arrests and in signaling unplanned arrest in response to mitotic or meiotic errors. Taken together, these studies indicate that chromosomes are not passive gene carriers of the cell division process, but complex organelles that possess both motor activities and the capacity to initiate and respond to cell cycle controls.

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15. In holo-kinetic organisms (*28*), any DNA sequence appears to promote kinetochore assembly, and thus spindle attachment occurs along the entire arm of the chromosomes in mitotic cells. That is, the kinetochores of these organisms are built along the entire length of the chromosomes. In such organisms [*C. elegans* is one of the best studied examples (*49*)], any piece of DNA introduced into the genome possesses the ability to form microtubule attachments that ensure proper segregation. Moreover, some chromosomes contain regions that act like conditional centromeres; under some conditions, they will bind microtubules and function like kinetochores. A classical example is the knob10 (K10) element in maize (*50*). In the presence of a crossover between K10 and the centromere, K10 will bind microtubules and lead the half-bivalent to the pole. Because the leading chromatids at anaphase end up in the gamete, the conditional use of K10 as a microtubule-binding region increases

the chance that K10 will be passed on to the next generation. More recent examples have been found with *Schizosaccharomyces pombe* plasmids carrying portions of a canonical centromere and human marker chromosomes (*51*). In all three cases, epigenetic modifications may control the centromeric activity of these regions.
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Telomeres: Beginning to Understand the End

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Telomeres are the protein-DNA structures at the ends of eukaryotic chromosomes. In yeast, and probably most other eukaryotes, telomeres are essential. They allow the cell to distinguish intact from broken chromosomes, protect chromosomes from degradation, and are substrates for novel replication mechanisms. Telomeres are usually replicated by telomerase, a telomere-specific reverse transcriptase, although telomerase-independent mechanisms of telomere maintenance exist. Telomere replication is both cell cycle– and developmentally regulated, and its control is likely to be complex. Because telomere loss causes the kinds of chromosomal changes associated with cancer and aging, an understanding of telomere biology has medical relevance.

Eukaryotes have linear chromosomes, and the ends of these linear chromosomes are composed of protein-DNA structures called telomeres. Telomeres were first characterized in ciliated protozoans such as *Tetrahymena* (*1*) and *Oxytricha* (*2*, *3*). After meiosis, in a developmentally regulated process, ciliate chromosomes are broken up into subchromosomal sized fragments. These fragments are replicated to generate a polyploid nucleus, the macronucleus, which can contain literally millions of telomeres (reviewed in *4*). Thus, compared to the modest number of chromosomes, and hence telomeres, in most organisms, the ciliate macronucleus is a rich source of both telomeric DNA and the structural proteins and enzymes that protect and replicate this DNA. In spite of the structural novelty of the ciliate macronucleus, many features of telomeres first discovered in ciliates are also true of telomeres in organisms like *Saccharomyces* and humans, both of which have conventional chromosomes. Indeed, telomeres display considerable conservation of both structure and function from single-celled organisms to higher plants and animals, as well as some intriguing interspecies differences.

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Telomeric DNA

In most organisms, telomeric DNA consists of a tandem array of very simple sequence DNA (Table 1) (*1–3*, *5–28*). Most telomeric repeat sequences are short and precise. For example, telomeric DNA in *Tetrahymena* is comprised of the 6-bp (base pair) sequence C_4A_2/T_2G_4. However, some telomeric sequences are heterogeneous (for example, $C_{1\text{-}3}A/TG_{1\text{-}3}$ in *Saccharomyces*) and in some, the repeat unit is considerably longer (for example, 25 bp in *Kluyveromyces lactis*). Moreover, *Drosophila* has a completely different and so far novel telomere structure. Rather than simple repeats, the DNA at the ends of *Drosophila* chromosomes is composed of a transposable element (*29–31*).

In most organisms, the subtelomeric regions immediately internal to the simple repeats consist of middle repetitive sequences, called telomere-associated (TA) DNA, which bear a superficial similarity to the transposons at the ends of *Drosophila* chromosomes. In *Saccharomyces*, there are two classes of TA elements, X and Y′ (*32*), one or both of which are found on most or all telomeres (Fig. 1). The array of TA DNA at a given chromosome end can expand and contract. However, in those or-