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12. The IVSP assay detected 15 of 24 previously identified mutations in *hMSH2*, *hMLH1*, *hPMS1*, or *hPMS2*.
13. The primers and PCR conditions are detailed in B. Liu *et al.* [*Nat. Med.* **1**, 348 (1995)], G. Gyapay *et al.* [*Nat. Genet.* **7**, 246 (1995)], and C. Breukel *et al.* [*Nucleic Acids Res.* **19**, 5804 (1991)].
14. The HCT-15 cell line could not be studied with the techniques in Fig. 3 because normal cells from the patient were not available. However, previous studies have shown that there is less clonal variation in repeated sequences in the HCT-15 line than in other lines with microsatellite instability [D. Shibata *et al.*, *Nat. Genet.* **6**, 273 (1994)] and that mutations in hypoxanthine-guanine phosphoribosyltransferase (HPRT) were different in HCT-15 than in other lines (*19*).
15. Primers used for genomic amplification of *GTBP* sequences were 5′-GTATGAAGAAACTACATACAGC-3′ and 5′-AAGTCCAGTCTTTCGAGCC-3′, yielding a 243-bp product corresponding to codons 777 to 858.
16. F. S. Leach *et al.*, *Cell* **75**, 1215 (1993).
17. A genomic P1 clone for hMSH2 was described previously (*16*). A P1 clone containing GTBP was obtained by similar methods, with the use of the primers described in (*15*). Two-color FISH was done as described in P. Lichter *et al.* [*Science* **247**, 64 (1990)] and C. Lengauer *et al.* [*Cancer Res.* **52**, 2590 (1992)].
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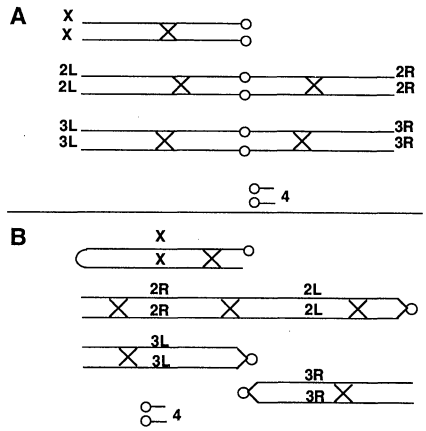

Fig. 1. A schematic diagram of the karyotype of *Drosophila melanogaster* females in wild-type (**A**) and All-Compound females (**B**). With the exception of the obligately achiasmate fourth chromosomes, in the All-Compound females each set of homologs is appended to the same centromere (exchanges are denoted by an "X" between the chromosome arms). The proper designation for such females is *C(1)DX, y f/Y; C(2)EN, c bw/0; C(3)L, h; C(3)R; 4/4*. These females were created by crossing *X/Y; C(2)EN/0; C(3)L; C(3)R; 4/4* males to *C(1)DX, y f/Y; 2/2; 3/3; 4/4* females and recovering the *C(1)DX, y f/X; C(2)EN/2; C(3)L; C(3)R/3; 4/4/4* triploids. Such triploids were then backcrossed to *X/Y; C(2)EN/0; C(3)L; C(3)R; 4/4* males and the All-Compound females were recovered as segregants from this cross that expressed the yellow, forked, curved, brown, and hairy phenotypes. All chromosomes used in this experiment are described in (*14*).

Induction of Metaphase Arrest in *Drosophila* Oocytes by Chiasma-Based Kinetochore Tension

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In normal *Drosophila melanogaster* oocytes, meiosis arrests at metaphase I and resumes after oocyte passage through the oviduct. Thus, metaphase arrest defines a control point in the meiotic cell cycle. Metaphase arrest only occurs in oocytes that have undergone at least one meiotic exchange. Here it is shown that crossovers between homologs attached to the same centromere do not induce metaphase arrest. Hence, exchanges induce metaphase arrest only when they physically conjoin two separate kinetochores. Thus, the signal that mediates metaphase arrest is not the exchange event per se but the resulting tension on homologous kinetochores.

Control of the metaphase-to-anaphase transition is a central component of cell cycle regulation. Meiotic arrest at either metaphase I or II before fertilization is a common component of oogenesis in a wide variety of organisms (*1*). In *Drosophila melanogaster* females, meiotic arrest occurs at metaphase I in stage 13–14 oocytes (*2, 3*). At this point in the meiotic cycle, the exchange bivalents are tightly massed at the metaphase plate. The separation of these bivalents requires the release of sister-chromatid cohesion distal to the site of crossing-over, an event that occurs concomitantly with the initiation of anaphase as the egg passes through the oviduct some 2 hours to a week later (*3, 4*).

Metaphase arrest also halts the precocious poleward movement of nonexchange achiasmate chromosomes, which begins during prometaphase (*5*). During prometaphase, smaller achiasmate chromosomes move away from the main chromosomal mass and by the time of metaphase are positioned between the plate and the poles on long tapered spindles. Larger achiasmate chromosomes frequently remain close to the main chromosomal mass until the onset of anaphase. The release of metaphase arrest is heralded by the completion of the poleward journey of the achiasmate chromosomes. Thus, the triggering of anaphase must allow both the release of sister-chromatid cohesion on exchange bivalents and the inactivation of the antipolar forces acting on nonexchange chromosomes.

Exchange events are required for metaphase arrest, and even a single crossover event is sufficient to induce metaphase arrest (*6*). These conclusions are based on the finding that the vast majority of stage 13–14 oocytes homozygous for any one of four recombination-deficient mutations do not display metaphase arrest. On the basis of these observations, we have proposed that metaphase arrest is the result of tension created on the kinetochores as a consequence of chiasma formation (*6*).

Alternatively, it was possible that recombination events trigger metaphase arrest by means of a chemical signal released by the structures or proteins involved in executing the exchange events (for example, recombination nodules). Distinguishing between these two alternatives required construction of *Drosophila* females in which recombination events occur but tension on the kinetochore does not result. This can be accomplished for an individual pair of chromosome arms by use of a type of chromosome aberration known as a compound chromosome, in which both homologs are attached to the same centromere (*7*). Meiotic recombination events occur between the arms of compound chromosomes at normal frequencies (*7*); however, the resulting crossover events do not conjoin homologous centromeres (Fig. 1).

We constructed females in which all of the arms in the genome that are normally capable of recombination are arranged as compound chromosomes (that is, in which the X, second, and third chromosomes are

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each carried as compound chromosomes) and examined their oocytes for the presence or absence of metaphase arrest. The genetic crosses used to produce such females, denoted here as All-Compound females, are described in the legend to Fig. 1.

Table 1 displays the results of examination of meiotic progression in stage 13–14 oocytes of All-Compound females by confocal microscopy, and representative micrographs are provided in Fig. 2. In wild-type oocytes, post–metaphase I figures are rare (*5*, 6) and meiosis II figures are never observed. In contrast, oocytes from All-Compound females produce anaphase I (Fig. 2B) and meiosis II (Fig. 2C) figures 64% of the time. Thus, most of these oocytes have bypassed metaphase I arrest and are completing the meiotic process, which demonstrates that metaphase arrest does not occur when exchanges do not connect separate kinetochores. Moreover, the All-Compound oocytes scored as metaphase I figures should not be taken as evidence for even a low frequency of metaphase arrest in this genotype. Given the high frequency of anaphase I and meiosis II figures in this genotype, it is possible that many, if not all, of the metaphase I figures in the genotype depict oocytes in transition between prometaphase and anaphase I, rather than oocytes arrested at metaphase I.

The effect of an All-Compound genotype on meiotic progression is similar to what is seen in the absence of crossing-over in homozygous recombination-defective mutants such as *mei-218* (6). On the basis of these data, we conclude that exchanges do not themselves induce metaphase arrest in normal oocytes, but rather arrest is triggered by the tension on homologous kinetochores produced by those exchanges. In the absence of that tension, metaphase arrest does not occur and the oocytes are free to complete meiosis. Early completion of meiosis does not impair the ability of the resulting egg pronucleus to be fertilized and produce a normal offspring, provided that the resulting zygote is euploid (6).

The ability of All-Compound oocytes to void metaphase arrest might have been the result of some unusual property of compound chromosomes rather than of the absence of conjoined kinetochores. We tested this possibility in two ways. First, we examined meiotic progression in triploid females that carried a full set of compound chromosomes plus one haploid set of normal sequence chromosomes. In such females, the normal sequence chromosomes will undergo exchange with one or both of the arms of the compound chromosomes at high frequency, thus connecting the kinetochore on the normal sequence chromosome with the kinetochore on the compound chromosome. Although the sample size was small because oocytes are difficult to recover from such females, no post–metaphase I figures were observed. Thus, the mere presence of compound chromosomes was not sufficient to void metaphase arrest.

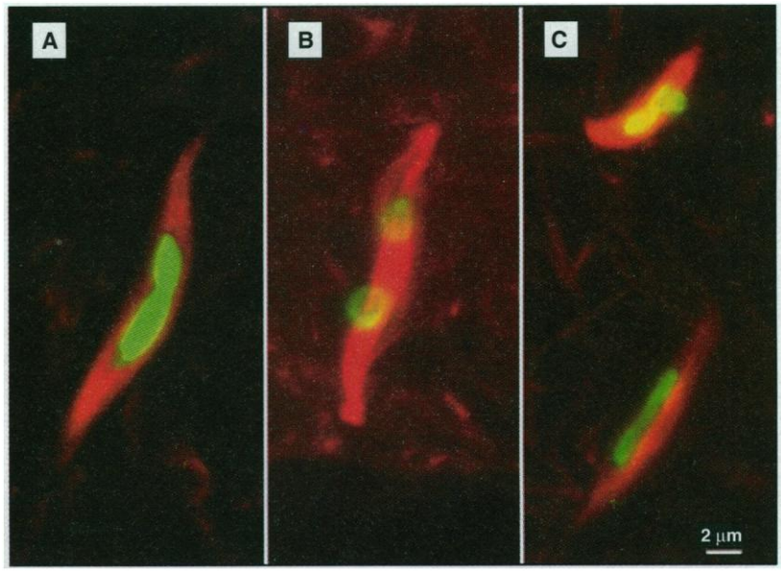

Fig. 2. Confocal images of meiotic figures observed in stage 13 oocytes of All-Compound females. Microtubules (red) labeled with tubulin antibody conjugated to rhodamine, and chromatin (yellow-green) labeled with histone antibody conjugated to fluorescein in All-Compound oocytes showing metaphase (**A**), anaphase I (**B**), and metaphase II (**C**). Oocytes were prepared and examined as previously described (6).

Second, we examined females in which the compound X chromosome was replaced by a pair of normal sequence X chromosomes. In such females, the autosomes are arranged as compound chromosomes, but exchange between two X chromosomes will connect homologous kinetochores. As shown in Table 1 (XX, Compound), anaphase I and meiosis II figures are rare in such females, occurring no more often than would be expected, given that the two X chromosomes will fail to undergo exchange in 6 to 10% of oocytes. Thus, a single pair of conjoined kinetochores is sufficient to ensure metaphase arrest, causing both chiasmate bivalents and the larger achiasmate chromosomes to remain at the metaphase plate.

In summary, our data show that the ability of exchanges to induce metaphase arrest is not a consequence of exchange events themselves or of some chemical signals emitted by the recombination nodule. Rather, exchanges must conjoin two centromeres to ensure metaphase arrest.

Li and Nicklas (*8*) have recently reported that in praying mantid spermatocytes, the

Table 1. Classification of oocyte nuclei. Following McKim *et al.*, meiotic stages were defined as follows. Prometaphase: The karyosome is surrounded either by diffuse spindles or by short untapered spindles. Metaphase I: Elongated and tapered spindles surround stretched chromatin masses at the metaphase plate. Anaphase I: Chromosome masses are clearly separated and positioned between the center of the spindle and the poles. Meiosis II: There are two separate spindles, each encompassing one (metaphase II) or two well-separated (anaphase II) chromatin masses (*6*).

Oocyte type	Total figures	Prometaphase (% of total) (*N*)	Metaphase I (% of total) (*N*)	Anaphase I (% of total) (*N*)	Meiosis II (% of total) (*N*)	Others (*N*)
Oregon-R (wild-type)	194	59.3 (115)	39.7 (77)	1.0 (2)	0 (0)	(0)
All-Compound stock	93*	12.2 (10)	23.2 (19)	30.5 (25)	34.1 (28)	(11)
XX, Compound stock	44	29.5 (13)	63.6 (28)	2.3 (1)	4.5 (2)	(0)
Triploids	31	71.0 (22)	29.0 (9)	0 (0)	0 (0)	(0)
mei-218	85	34.1 (29)	17.6 (15)	21.2 (18)	27.1 (23)	(0)

*Includes 11 examples of multiple chromosome masses on fragmented spindles (classified under others in the table). These were not included in the percent calculations. This phenotype is not observed in oocytes homozygous for recombination-defective mutations and therefore cannot easily be ascribed to a defect in chiasma formation. We propose that this class of oocytes may reflect an important role of kinetochore pairing and alignment. Hawley *et al.* (*13*) have suggested that the alignment of homologous heterochromatic regions surrounding the centromere plays a vital role in holding the chromosomes together during the early stages of spindle formation. Perhaps such alignments are impaired or nonexistent in All-Compound females, resulting in an impairment of spindle formation.

absence of tension on even a single kinetochore was sufficient to signal a meiotic error and trigger a prolongation of metaphase sufficient to result in cell death. Restoration of tension on that kinetochore by micromanipulation relieved that error signal and allowed the cell to proceed into anaphase I. Our studies demonstrate that in *Drosophila* oocytes, it is the presence of kinetochore tension that regulates the proper progression of the meiotic cell cycle (that is, the inclusion of the normal stage of metaphase arrest), and that this tension results from a normal meiotic process, namely recombination. Moreover, the presence of tension on even a single pair of kinetochores is sufficient to trigger metaphase arrest.

In both the mantid spermatocyte and the *Drosophila* oocyte, kinetochore tension is being used to halt the cell cycle at metaphase, albeit toward rather different ends. This dramatic sexual asymmetry reflects the fact that evolution has allowed tension to be used in two very different ways to regulate the meiotic process in males and females. Male meiosis does not normally include pausing stages or arrest points. Indeed, in mantid spermatocytes the prolongation of metaphase is triggered by an absence of tension on one kinetochore, signaling the presence of an unconjoined (univalent) chromosome. Continuing the meiotic process in the presence of a univalent might well lead to the production of aneuploid sperm. The triggering of a terminal metaphase I arrest, which cannot normally be relieved in this organism, acts to prevent these spermatocytes from producing mature, but potentially aneuploid, sperm (*8*). Thus, in these males the function of an arrest at metaphase is to abort an aberrant meiotic process.

Like most female meiotic systems, female meiosis in *Drosophila* includes two normal programmed delays. The first (karyosome formation) allows the building of the egg, and the second (metaphase arrest) adjusts the timing of completion of the two meiotic divisions. Thus, in *Drosophila* females tension-induced metaphase arrest is a normal part of the meiotic process. Metaphase arrest prevents the onset of anaphase until the partial release of sister-chromatid cohesion, which usually occurs during the passage of the egg through the oviduct (*4*). The release of sister-chromatid cohesion distal to the site of exchange allows the chiasma to be resolved, releasing tension and permitting the transition into anaphase I. Entering anaphase before the resolution of chiasmata would result either in the breakage of chromosomes or in whole bivalents being pulled to a single pole. Thus, in *Drosophila* oocytes the function of tension-mediated cell cycle control is not to abort an abnormal process but to prevent the precocious transition of normal oocytes into anaphase until the bivalents can be properly separated.

How might a linkage between two kinetochores control metaphase arrest? Following others, we suggest that tension on a pair of kinetochores alters kinetochore chemistry, which in turn triggers metaphase arrest (*8*, *9*). According to this model, the presence of even a single pair of kinetochores under tension sends a signal to the entire spindle, causing metaphase arrest, and that signal reflects a tension-induced alteration in the chemistry of those kinetochores. This hypothetical signal would serve to reduce the tension on the kinetochores of chiasmate bivalents (perhaps to prevent breakage of kinetochore-microtubule interactions) and to control the precocious poleward movement of achiasmate chromosomes.

Several types of molecules have been implicated in this kinetochore signaling process. For example, a kinetochore protein has been identified whose phosphorylation status is determined by the presence or absence of tension (*10*). Nonchromosomal components of this process might include the *twine* gene, which encodes the *Drosophila* homolog of cdc25 in fission yeast. Mutations in the *twine* gene arrest meiosis in *Drosophila* spermatocytes; however, in oocytes they void metaphase arrest and allow precocious entry into anaphase I (*11*). Indeed, our observations on *twine* oocytes reveal the presence of chiasmate bivalents moving toward the poles on anaphase I spindles (*12*).

In summary, our observations, and those of others, strongly support a model in which tension-induced changes in kinetochore chemistry play a crucial role in controlling the meiotic cell cycle.

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