Bipolar Spindle Attachments Affect Redistributions of ZW10, a *Drosophila* Centromere/Kinetochore Component Required for Accurate Chromosome Segregation

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Abstract. Previous efforts have shown that mutations in the *Drosophila zw10* gene cause massive chromosome missegregation during mitotic divisions in several tissues. Here we demonstrate that mutations in *zw10* also disrupt chromosome behavior in male meiosis I and meiosis II, indicating that ZW10 function is common to both equational and reductional divisions. Divisions are apparently normal before anaphase onset, but ZW10 mutants exhibit lagging chromosomes and irregular chromosome segregation at anaphase. Chromosome missegregation during meiosis I of these mutants is not caused by precocious separation of sister chromatids, but rather the nondisjunction of homologs. ZW10 is first visible during prometaphase, where it localizes to the kinetochores of the bivalent chromosomes (during meiosis I) or to the sister kinetochores of dyads (during meiosis II). During metaphase of both divisions, ZW10 appears to move from the kinetochores and to spread toward the poles along what appear to be kinetochore microtubules. Redistributions of ZW10 at metaphase require bipolar attachments of individual chromosomes or paired bivalents to the spindle. At the onset of anaphase I or anaphase II, ZW10 rapidly relocalizes to the kinetochore regions of the separating chromosomes. In other mutant backgrounds in which chromosomes lag during anaphase, the presence or absence of ZW10 at a particular kinetochore predicts whether or not the chromosome moves appropriately to the spindle poles. We propose that ZW10 acts as part of, or immediately downstream of, a tension-sensing mechanism that regulates chromosome separation or movement at anaphase onset.

A variety of interactions between the kinetochores and kinetochore microtubules (kMTs)[1] is essential for ensuring the accuracy of chromosome segregation during cell division. (*a*) Kinetochores can capture microtubules, initially binding to the lateral surface of a single microtubule and later to the plus ends of several microtubules (Rieder and Alexander, 1990; Pawletz et al., 1994). (*b*) Microtubule-based motors at the kinetochore participate in chromosome movements during prometaphase, metaphase, and anaphase. Certain motors move the chromosome along the lateral surface of the first captured microtubule toward the corresponding pole (Rieder and Alexander, 1990). The eventual congression of bioriented chromosomes to the metaphase plate appears to involve the ability of kinetochores to switch abruptly between phases of poleward and away-from-the-pole movements ("kinetochore directional instability"; for review see Rieder and Salmon, 1994). Finally, the poleward migration of chromosomes during anaphase A depends upon forces generated at the kinetochore. Congression and anaphase A movements are probably based upon the ability of microtubule motors at the kinetochore to couple chromosomes to polymerizing or depolymerizing kMTs (Hyman and Mitchison, 1991; Lombillo et al., 1995; Desai and Mitchison, 1995). (*c*) Kinetochores sense tension applied through kMTs across chromosomes to control at least two processes. First, tension stabilizes kinetochore/kMT interactions during prometaphase to ensure that sister kinetochores are connected to microtubules from opposite spindle poles (Nicklas, 1985). Second, tension at the kinetochore makes certain that anaphase onset does not occur until the spindle is properly assembled (Li and Nicklas, 1995).

Consistent with this complexity of function, a variety of proteins has been found to be associated with kinetochores (for review see Pluta et al., 1995). These include proteins that are present at kinetochores throughout the cell cycle, as well as other molecules that associate with kinetochores only transiently during discrete phases of the

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1. *Abbreviations used in this paper*: FISH, fluorescent in situ hybridization; kMT, kinetochore microtubule; PSCS, precocious sister chromatid separation.

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cell cycle. In general, little is known about the biochemical activities of these proteins. Notable exceptions include several yeast centromere-binding proteins (Hyman et al., 1992; Meluh and Koshland, 1995), and in mammalian cells, the microtubule motors cytoplasmic dynein (Pfarr et al., 1990; Steuer et al., 1990) and CENP-E (a kinesin-like protein) (Yen et al., 1992; Lombillo et al., 1995). In contrast, to our knowledge, only a single spindle component that is unique to kMTs has been identified. This is the "spoke" antigen, whose molecular properties remain unknown (Paddy and Chelsky, 1991).

We have recently found a novel protein in *Drosophila* called ZW10 that appears to migrate from kMTs, where it is localized during metaphase, to kinetochores, where it is found during anaphase of mitosis. This protein is the product of the *l(1)zw10* gene (hereafter *zw10*). ZW10 is essential for accurate mitotic chromosome segregation, as null mutations in the *zw10* gene result in very high levels of aneuploidy in a variety of *Drosophila* tissues (Smith et al., 1985; Williams et al., 1992; Williams and Goldberg, 1994). In mutant cells, mitosis seems to proceed properly through metaphase: the metaphase spindle is normal in morphology, and all chromosomes congress to the metaphase plate. However, many anaphases are aberrant, with segregating chromosomes that are misoriented or lag at the metaphase plate. In addition, mutations in *zw10* produce precocious sister chromatid separation in colchicine-arrested mitotic cells. Overall, these phenotypes have suggested to us that the movement of ZW10 from kMTs to the kinetochores helps ensure the synchrony of sister chromatid disjunction at mitotic anaphase onset.

In meiosis I, sister chromatids together elaborate a single kinetochore (Goldstein, 1981) and fail to separate at anaphase. This prompted us to explore whether *zw10* function is also needed during the first meiotic division. Examination of meiosis in *zw10* mutants would allow us to determine whether ZW10 is specifically involved in sister chromatid cohesion, or instead participates in some aspect of chromosome disjunction common to meiosis I and mitosis. We chose to perform these experiments in *Drosophila* spermatocytes, which offer an ideal system for examining meiosis in higher eukaryotes, due to their large size and well-characterized series of nuclear movements and spindle transformations during meiosis (Cenci et al., 1994). We have also exploited these advantages of spermatocytes to examine ZW10 localization during meiosis, both in wild-type cells and in cells in which chromosomal behavior can be manipulated genetically.

In this paper, we show that ZW10 is required for proper chromosome segregation during both meiotic divisions. Missegregation in *zw10* mutants during meiosis I is not dependent upon precocious separation of sister chromatids, but instead involves improper movement of dyads. Thus, ZW10's function in chromosome segregation must affect both the separation of sister chromatids (during mitosis and meiosis II) and the separation of homologous chromosomes (during meiosis I). In addition, the superior cytology of spermatocytes has altered our understanding of ZW10 distribution. It now appears that ZW10 is localized on the kinetochores of prometaphase chromosomes, moves to kMTs during metaphase, and then moves back to the kinetochores at anaphase. Remarkably, we have also found that ZW10 association with individual chromosomes is both correlated with proper chromosome segregation and influenced by the presence or absence of spindle tension. We discuss these results in light of the hypothesis that ZW10 is involved in a tension-sensing mechanism that coordinates chromosome separation or movement at the beginning of anaphase.

Materials and Methods

Drosophila Stocks

Descriptions of *zw10* mutant alleles and the means by which *zw10* mutant males were generated can be found in Williams et al. (1992). Male meiotic mutants and their alleles used to study ZW10 localization were ord^5 (Miyazaki and Orr-Weaver, 1992), $mei\text{-}S332^7$ (Kerrebrock et al., 1992), and *Dub* (Moore et al., 1995) (all the kind gift of T. Orr-Weaver, Whitehead Institute, Boston, MA). Univalent chromosomes were studied in $Df(1)X\text{-}1/B^sw^+y^+\cdot Y$ testes (McKee and Karpen, 1990) and in a stock carrying an attached X-Y and a compound fourth chromosome ($Y^sX\cdot YL$, $In[1]EN$; $C[4]RM$, $ci\ ey^R$; Lindsley and Zimm, 1987). Dicentric chromosomes were examined for ZW10 localization in a stock containing $Y^s\cdot Y^L2Rh4\cdot CB25\text{-}1$ (Ault and Lyttle, 1988; obtained from T. Lyttle, University of Hawaii, Manoa). Oregon-R was used as the wild-type strain. See Lindsley and Zimm (1992) for further explanation of chromosomes and genetic symbols used.

Orcein Squashes

Cytological analysis of meiotic figures in orcein squashes was carried out according to Lifschytz and Meyer (1977). Testes were dissected in saline solution (0.7% NaCl), transferred to aceto-orcein, and squashed gently.

Immunostaining

For simultaneous visualization of microtubules and chromosomes, testes from *zw10* mutants were fixed with methanol/acetone according to previously published protocols (Cenci et al., 1994; Williams et al., 1995) and stained to visualize microtubules and DNA. Testes were incubated with a monoclonal anti-α-tubulin antibody raised against native chick brain microtubules (code N356; Amersham Corp., Arlington Heights, IL), which was used at a dilution of 1:50. After overnight incubation, slides were washed in three changes of PBT (2.6 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, 8.1 mM Na_2HPO_4, 0.02% NaN_3, 0.1% Triton X-100) for a total of 15 min. The slides were then incubated with TRITC-conjugated anti-mouse IgG (The Jackson Laboratory, Bar Harbor, ME) overnight at 4°C to visualize tubulin localization. The slides were then rinsed in PBT for 10 min at room temperature, stained with Hoechst 33258 at a concentration of 0.5 μg/ml in Hoechst buffer, and mounted in Hoechst citrate buffer as described by Cenci et al. (1994). Specimens were examined with a Zeiss Axioskop equipped for epifluorescence with an HBO 100 W/2 mercury short arc lamp (Zeiss, Oberkochen, Germany), using the 09 filter sets (BP 450-490, FT 510, LP 420). Photographs were recorded on TMAX-100 film (Eastman-Kodak Co., Rochester, NY).

To visualize the ZW10 antigen, testes were fixed in formaldehyde and stained exactly according to Williams and Goldberg (1994) as described for mitotic figures in larval brains. Affinity-purified anti-ZW10 antibodies were used at 1:50–1:100 dilution and incubated with testes overnight at 4°C. Laser-scanning confocal microscopy was primarily carried out using a Zeiss Axiovert 10 attached to a Bio-Rad MRC600 confocal imaging system equipped with a Krypton/Argon laser (Bio-Rad Laboratories, Cambridge, MA). Image collection was performed using a No. 2 neutral density filter to attenuate photobleaching, and assisted by Kalman averaging to improve the signal/noise ratio. Double fluorescence images were collected simultaneously as two full-screen images and merged in pseudocolor using COMOS software (Bio-Rad Laboratories). Immunostained testes preparations were also observed using an ImagePointR charge-coupled device (Photometrics Ltd., Tucson, AZ) connected to a Zeiss Axioskop microscope. Exposure times ranged from 0.2–1.0 s. Hoechst, FITC, and TRITC signals were collected separately using IP Lab Spectrum software and programs written by E. Marchetti (University of Rome, Italy). Images were then converted to Photoshop format (Adobe Systems Inc.,

Mountain View, CA) and merged in pseudocolor. Final images were printed using a dye sublimation process.

Fluorescent In Situ Hybridization

In situ hybridization to methanol/acetone-fixed testes (Cenci et al., 1994) was used to determine the X and Y chromosome constitutions of meiotic and postmeiotic nuclei. We used probes based on the X chromosome 359-bp satellite repeat sequence (Hsieh and Brutlag, 1979; Lohe et al., 1993), which were amplified by PCR from whole genomic DNA (parameters from A. Dernberg, University of California at San Francisco), and AATAC repeat satellite sequences specific for the Y chromosome (Bonaccorsi and Lohe, 1991; Lohe et al., 1993). Probes were labeled by primer extension (Feinberg and Vogelstein, 1984) incorporating either digoxygenin-11-d-uridine 5'-triphosphate (for the Y chromosome) using the Genius 2 DNA labeling kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) or biotin-11-d-uridine 5'-triphosphate (for the X chromosome). After methanol/acetone fixation (Cenci et al., 1994), testes were air dried, denatured in 0.2 M HCl for 30 min at 37°C, and then dehydrated in ethanol at room temperature and air dried (Lohe et al., 1993). The X and Y probes were mixed together with hybridization solution (Gunaratne et al., 1986), added to the slides, and processed according to standard in situ hybridization protocols (Gunaratne et al., 1986). Hybridization and washes were carried out at room temperature (25°C). After the initial wash, probes were detected with rhodamine-conjugated anti-digoxygenin mAb (3 μg/ml final concentration; Boehringer Mannheim Biochemicals) and with avidin-FITC (4 μg/ml final concentration; Vector Laboratories, Burlingame, CA) diluted in PBT, and incubated overnight at 4°C. Slides were stained with Hoechst 33258, mounted in anti-bleaching media containing *n*-propyl gallate, and photographed under epifluorescence using TMAX-100 film. X and Y constitutions of nuclei were scored from enlarged photographs.

Results

Mutations in zw10 Cause Nondisjunction during Anaphase of Both Meiotic Divisions

Animals hemizygous or homozygous for mutations in *zw10* almost all die in the pupal stages, although rare escaper adults that display reduced viability and sterility are occasionally found (Shannon et al., 1972). Adult *zw10* escaper males are sterile because their testes are small and contain immotile sperm. As a result, the detection of meiotic aberrations in *zw10* mutant males cannot be accomplished by genetic testing, but instead requires direct cytological observation of meiotic divisions and postmeiotic stages of spermatogenesis.

Defects in meiotic chromosome segregation associated with mutations in *zw10* are suggested by examination of the so-called "onion stage" spermatids produced at the conclusion of meiosis. During spermatogenesis, the premeiotic spermatogonial divisions and the two meiotic divisions in the spermatocytes yield a cyst of 64 spermatids, each containing a single nucleus associated with a mitochondrial derivative, called the nebenkern (Fig. 1 *A*). Within a wild-type cyst, these onion-stage spermatid nuclei are all the same size. This regularity indicates that chromosome segregation has occurred properly, since nuclear size is proportional to chromatin content (González et al., 1990). In contrast, the nuclei in *zw10* mutant onion-stage spermatids vary considerably in size, indicative of chromosome missegregation during the meiotic divisions (Fig. 1 *B*). Cytokinesis defects are also observed; these will be discussed in a subsequent section.

Mistakes in chromosome behavior occur during both meiotic divisions in *zw10* mutant testes obtained from third instar larvae, pupae, and escaper adults. Observations of both squashed living preparations (Fuller, 1993) and of fixed specimens (Lifschytz and Meyer, 1977; Cenci et al., 1994) show that the two daughter nuclei in a large proportion of *zw10* mutant telophases in both meiotic divisions are of unequal size (Figs. 2 and 3; Table I). Both first and second division ana/telophases exhibit lagging chromosomes or apparent chromatin bridges (Figs. 2 and 3; Table I). Although lagging chromosomes are sometimes found at the center of the spindle, they are most often found adjacent to one of the daughter nuclei, suggesting that their movements during anaphase were delayed (Fig. 3). Overall, ana/telophase aberrations are quite common, but they are more often observed during the first meiotic division than during the second division (Table I).

Lagging chromosomes and chromatin bridges could be the indirect consequence of difficulties encountered earlier in the cell cycle, or instead might reflect the direct effect of a problem occurring during either anaphase onset

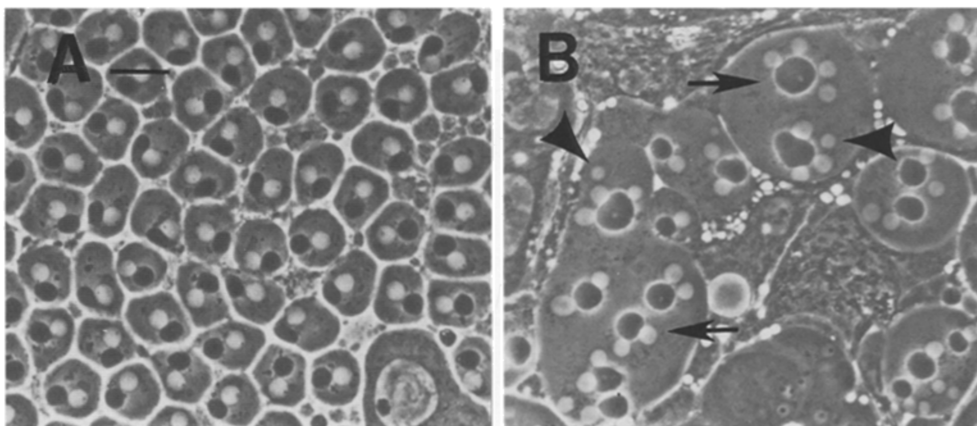

Figure 1. Aneuploid postmeiotic nuclei in *zw10* mutants. Live testes were viewed by phase-contrast microscopy to examine spermatid defects in onion-stage cysts. (*A*) Wild-type cyst showing spermatids, each of which contain a phase-dark mitochondrial derivative (the nebenkern) and a phase-clear nucleus. Note the one-to-one association between the nebenkern and nucleus, and the uniform size of both organelles. (*B*) A partial onion-stage cyst from $zw10^{S1}/Y$ testis with variably sized spermatid nuclei (see *arrowheads* for examples of smaller nuclei), indicating that meiotic chromosome segregation has been defective (see text). Micronuclei (nuclei containing only one or two chromosomes) are also present. Furthermore, many nebenkerns are abnormally large and are each associated with two or four nuclei (*bottom* and *top arrows*, respectively). These aberrations indicate that cytokinesis has failed during the meiotic divisions (Fuller, 1993). Bar, 10 μm.

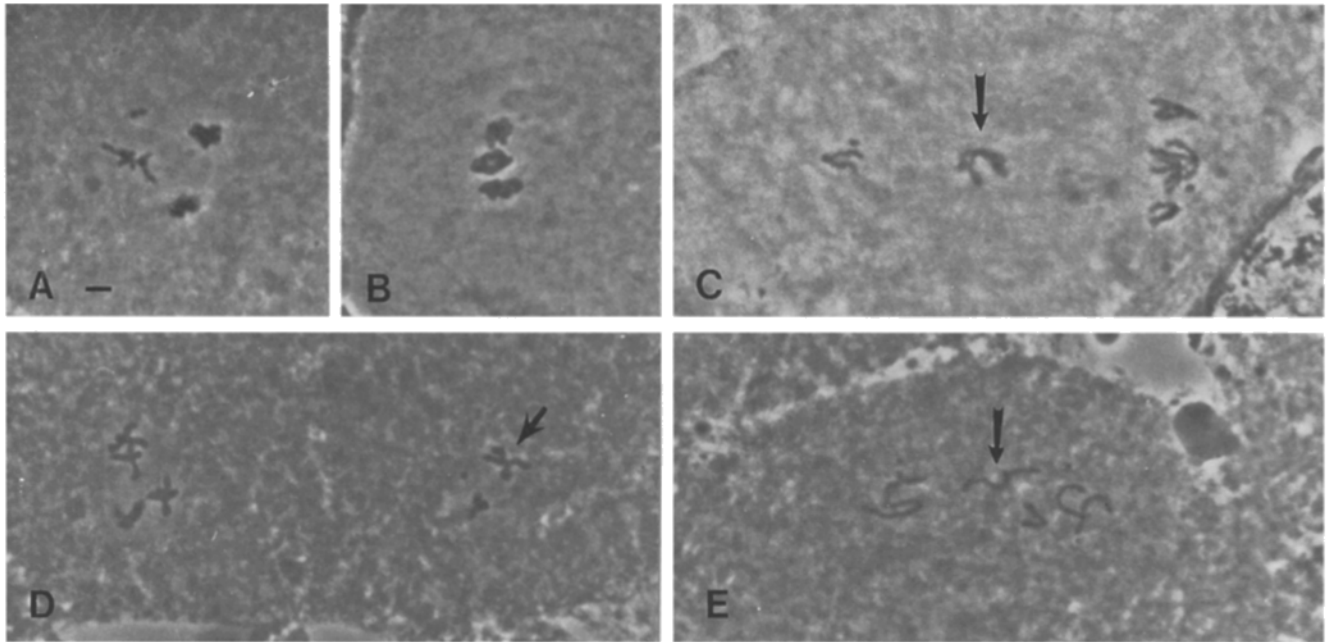

Figure 2. Meiotic chromosome segregation in *zw10* mutants. $zw10^{S1}/Y$ testes were squashed in acetic acid–orcein solution (Lifschytz and Meyer, 1977) to visualize chromosomes. (*A*) Prometaphase I. The bivalents are of normal appearance. (*B*) Metaphase I. The chromosomes are properly aligned at the metaphase plate. (*C*) Anaphase I. Abnormal segregation of chromosomes (3 autosomes, Y, and 4 to right; X and 4 to left pole), as well as a lagging autosome remaining at the equator with no apparent poleward movement (*arrow*). (*D*) Prometaphase II. Two aneuploid daughter cells from a single defective meiosis I division. The cell on the left has more chromosomes than the one on the right. Note in *C* and *D* that sister chromatids remain attached to each other at the centromeres through metaphase II (*arrows*), indicating that *zw10* mutations do not cause precocious sister chromatid separation. (*E*) Anaphase II. A disorganized anaphase II with a lagging chromatid (*arrow*). Bar, 1 μm.

or anaphase itself. Our results are much more consistent with the latter possibility. The stages before anaphase in both meiotic divisions in *zw10* mutants appear to be normal: chromosomes condense properly and migrate to the metaphase plate in a fashion comparable to that seen in wild type (Figs. 2 and 3). Our cytological assays are sufficiently sensitive that they would have detected problems in congression had they occurred (see below). Moreover, all aspects of spindle morphology in *zw10* mutants are indistinguishable from normal throughout all stages of meiosis (Fig. 3; Cenci et al., 1994; Williams et al., 1995).

It is possible that some of the meiosis I defects observed are the indirect consequence of missegregation during the prior mitotic divisions in the germline. However, we believe that chromosome misbehavior during spermatogonial divisions has little if any influence on the meiosis I aberrations documented in Figs. 2 and 3. All chromosomes in prometaphase I primary spermatocytes appeared to be paired as bivalents; we would anticipate that unpaired chromosomes would be visible if the chromosome complement of the spermatocytes was abnormal. Furthermore, using probes for fluorescent in situ hybridization (FISH) that are specific to the X and Y chromosomes (see Materials and Methods), we found that all primary spermatocytes had a single X and a single Y, consistent with the absence of prior chromosome loss. Nonetheless, ~17% of mutant anaphase and telophase I figures showed a failure of sex chromosome disjunction by FISH analysis (data not shown). Together, these findings indicate that most meiosis I aberrations are specific to that division and do not reflect abnormal chromosome complements inherited by primary spermatocytes. Surprisingly, it appears that mutations in *zw10* do not significantly disrupt chromosome segregation during spermatogonial divisions; alternatively, cells generated by spermatogonial missegregation fail to develop into recognizable spermatocytes.

The testes of *zw10* mutant males were also examined for possible perturbations in cell cycle progression that might indirectly result in lagging chromosomes and chromatin bridges (Brinkley et al., 1985). We have found no significant differences in cell cycle progression parameters between wild-type and mutant spermatogenesis (Table II). These results are consistent with previous measurements showing that *zw10* mutations do not grossly disrupt the cell cycle in mitosis (Williams et al., 1992).

In summary, our results taken together strongly suggest that the meiotic as well as mitotic defects associated with mutations in *zw10* are specific for events occurring either at anaphase onset or during anaphase proper.

Mutations in zw10 Do Not Cause Precocious Sister Chromatid Separation during the First Meiotic Division

The phenomenon of precocious sister chromatid separation (PSCS) seen in *zw10* mutant cells arrested in mitosis with microtubule poisons (Smith et al., 1985; Williams et al., 1994) suggested the possibility that ZW10 protein functions specifically in the machinery controlling the separation of sister chromatids at anaphase onset. If this were true, we would anticipate that meiosis I nondisjunction in

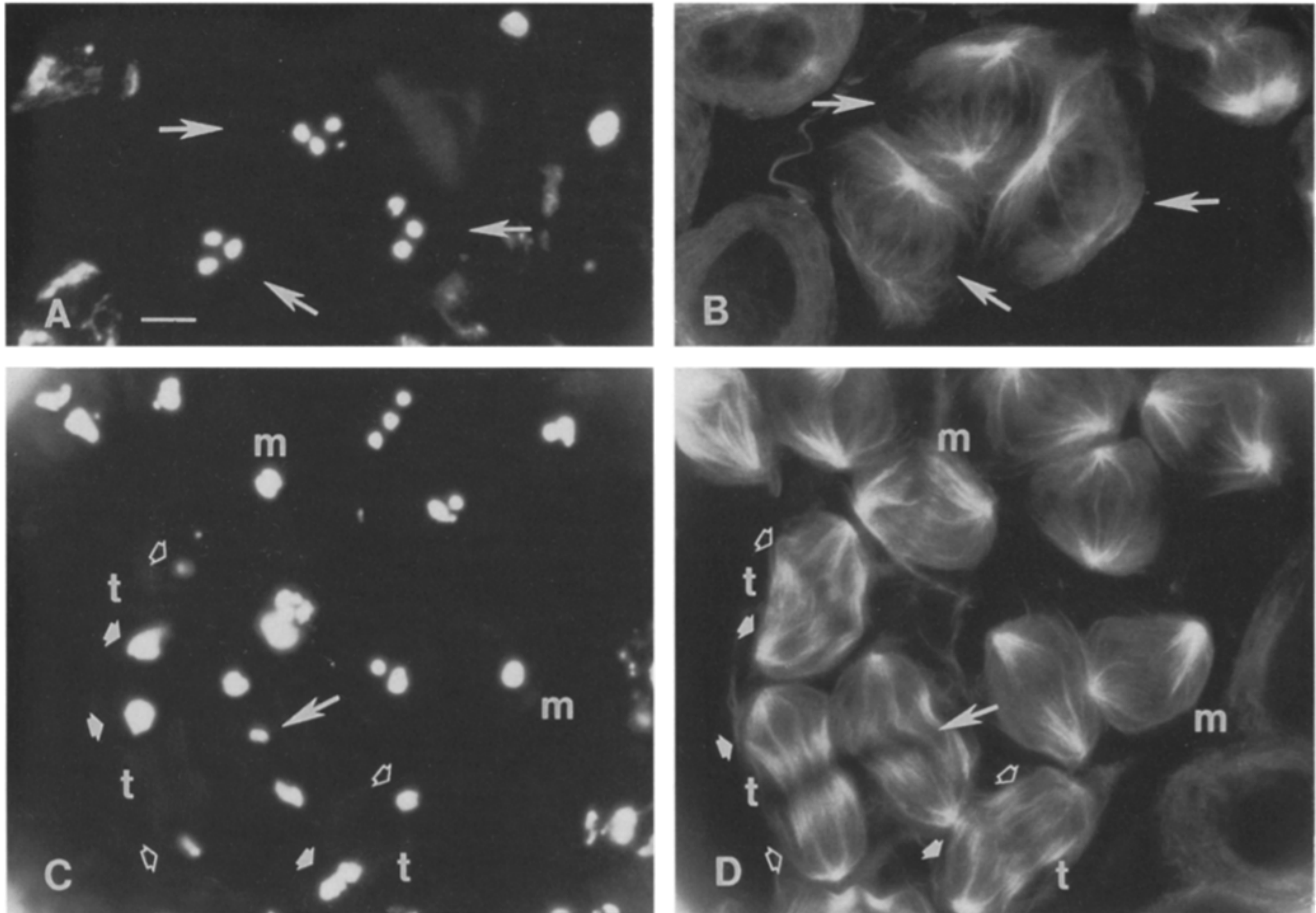

Figure 3. Normal spindle morphology in *zw10* mutant meiosis. *zw10* mutant testes were squashed, fixed, and stained to visualize chromosomes with Hoechst 33258 (*A* and *C*) and microtubules with anti–α-tubulin antibody (*B* and *D*) by indirect immunofluorescence (see Materials and Methods). For comparison with wild type, refer to Cenci et al. (1994). (*A* and *B*) Prometaphase I (stage M2). Three cells (*arrows*) with normal bivalent formation (*A*) and normal appearance of the meiotic spindle (*B*). (*C* and *D*) Spermatocytes in the same field undergoing the stages from metaphase (*m*) to late anaphase/telophase (*t*) of the first meiotic division (stages M3, M4c/M5). At metaphase, the chromosomes align at the metaphase plate properly, and their spindles have normal morphology. However, the ana/telophase figures in the same cyst show unequal distributions of chromosomes (see also Fig. 2, *C* and *D*), as visualized by the unequal Hoechst-bright masses at the cell poles, with more chromosomes going to one pole (*small solid arrows*) than to the other (*small outline arrows*). A lagging chromosome in one ana/telophase cell is visible (*large arrow*). The morphology of late anaphase/telophase spindles is normal, with formation of the central spindle comparable to that of wild type (Cenci et al., 1994). Bar, 5 μm.

zw10 mutant spermatocytes would be the consequence of the separation of sister chromatids during this division, rather than during the second division as in wild type. A precedent for this situation is seen during anaphase I in testes mutant for the gene *orientation disruptor* (*ord*; Goldstein, 1980; Miyazaki and Orr-Weaver, 1992), where PSCS during prometaphase I results in high levels of nondisjunction.

Analysis of orcein squashes failed to show the separation of sister chromatids at any stage before anaphase II in *zw10* mutants (Fig. 2). Furthermore, we have used FISH analysis with probes specific for the X and Y chromosomes to verify that PSCS is not the cause of chromosome missegregation in *zw10* mutant meiosis I divisions. In all of the 200 telophase I figures scored, sex chromosome dyads remained intact, even in cases where the aberrant movements of these dyads were apparent (Fig. 4). In contrast, *ord* first divisions commonly contained sister chromatids migrating to opposite poles (data not shown). Thus, *zw10* mutations can affect not only the segregation of individual chromatids (during mitosis and the second meiotic division) but also the separation of homologous chromosomes, each consisting of attached sister chromatids (during the first meiotic division). We conclude that the ZW10 protein is not part of a mechanism specific to the direct control of sister chromatid attachment and separation, but is rather involved in events required for proper chromosome segregation that are common to both reductional and equational divisions.

Lesions in zw10 Also Affect Cytokinesis

Surprisingly, *zw10* mutants show high frequencies (40–50%) of spermatids containing more than one nucleus per nebenkern (Fig. 1; Table III). These unusual spermatids are the result of defects in cytokinesis after the meiotic di-

Table I. Frequency of Anaphase Defects in zw10 Mutants

	Figures	Normal	Uneven	Laggards	Bridges	Abnormal
	Percentage of figures					
	n					%
MEIOSIS I						
Oregon-R	185	99.0	1.0	0.0	0.0	1.0
$zw10^{S1}$	444	52.5	39.9	7.2	0.4	47.5
$zw10^{i20}$	93	56.9	36.6	5.4	1.1	43.1
$zw10^{S2}$	116	56.1	37.9	4.3	1.7	43.9
MEIOSIS II						
Oregon-R	149	99.8	0.2	0.0	0.0	0.2
$zw10^{S1}$	455	83.3	16.3	0.2	0.2	16.7
$zw10^{i20}$	104	81.7	13.5	4.8	0.0	18.3
$zzw10^{S2}$	103	83.5	14.6	1.9	0.0	16.5

Anaphase and telophase figures (stages M4b-M5 and M10b-M22) were examined in testes stained with Hoechst as in Fig. 3. Defects were scored in testes from wild-type and three *zw10* null mutations. Divisions were marked as "uneven" if the intensity of Hoechst fluroescence differed between the two daughter nuclei.

visions. During meiosis I and meiosis II in males, the mitochondria line up along the spindle, and then are partitioned into daughter cells at cytokinesis (Fuller, 1993). If cytokinesis does not occur at the end of both meiotic divisions, the result is the generation of spermatids with a single large nebenkern and four nuclei. A failure of cytokinesis at only one of the two meiotic divisions would yield cells with intermediate-sized mitochondrial derivatives and two nuclei. Both types of abnormal spermatids are observed in *zw10* mutants (Fig. 1 *B*). Similar defective spermatids have been seen in the testes of flies bearing a variety of other mutations affecting cytokinesis (Fuller et al., 1987; Castrillon et al., 1993; Williams et al., 1995; Gunsalus et al., 1995).

In Wild-type Spermatocytes, the Intracellular Distribution of ZW10 Protein Changes Several Times during the Cell Cycle

Previously, the intracellular distribution of ZW10 during the cell cycle was studied in mitotic divisions occurring in the early embryo (Williams et al., 1992) and in larval brain neuroblasts (Williams and Goldberg, 1994). In this section, we describe the localization of ZW10 in spermatocytes. These data add significantly to our previous findings, primarily because of the much-improved cytology provided by spermatocytes undergoing meiosis.

Table II. Meiotic Parameters in zw10 Mutants

Stage	Oregon-R	$zw10^{S1}$
	Percentage of cells	
Late Prophase - Early Prometaphase I	9.6	13.5
Prometaphase I - Metaphase I	11.2	10.3
Anaphase I - Telophase I	15.3	18.8
Interphase II - Early Prometaphase II	27.5	22.8
Prometaphase II - Metaphase II	8.4	7.7
Anaphase II - Telophase II	28.0	26.9
Number of testes	25	56
Number of cells	1504	1929

Testes from wild-type and $zw10^{S1}$ were fixed with methanol/acetone, stained to visualize DNA and microtubules, and scored for the presence of the various meiotic stages according to the criteria of Cenci et al. (1994). Percentage of cells = number of cells at a specific stage/total number of cells in meiosis.

Most of ZW10 is located inside the nucleus throughout the growth of the primary spermatocyte to its maturity (stages S0 to S5, according to the stage terminology of Cenci et al. [1994]; Fig. 5 *a*). This intranuclear distribution during meiotic prophase is uniform and diffuse, with no apparent concentration in discrete intranuclear structures (Fig. 5 *a*). The pattern remains essentially unchanged through late prophase and early prometaphase of the first meiotic division (stages M1a and M1b), periods that can be recognized by the presence of meiotic asters and by the condensation of chromosomal bivalents.

By late prometaphase I (stage M2), ZW10 begins to localize to specific positions associated with the condensed bivalents (Fig. 5 *b*). On the basis of several criteria, we postulate that these sites of ZW10 staining are the kinetochores. (*a*) There are two ZW10-containing spots per bivalent, which are usually located far apart from each other, oriented toward opposite spindle poles. This is precisely the pattern that would be predicted for a protein associated with the kinetochores of *Drosophila* chromosomes during prometaphase I in males, as each kinetochore at this stage is shared by the two sister centromeres in each dyad comprising the bivalent (Goldstein, 1981; Church and Lin, 1982). In contrast, during prometaphase II, two spots of ZW10 staining are observed per dyad (see below). (*b*) ZW10 localizes to prometaphase I minichromosomes (Williams, B.C., T. Murphy, G. Karpen, and M.L. Goldberg, unpublished results) containing very little more than centromere sequences (Murphy and Karpen, 1995). (*c*) Antibodies directed against a human protein with considerable homology to *Drosophila* ZW10 colocalize with human anti-kinetochore CREST antisera (Starr, D., Z. Li, B.C. Williams, and M.L. Goldberg, unpublished results).

Metaphase I (stage M3) reveals a dramatic change in ZW10 distribution. When the bivalents are situated at the metaphase plate, ZW10 extends in filament-like structures outward from the kinetochores along the long axis of the spindle (Fig. 5, *c* and *d*). The staining colocalizes with a small subset of spindle microtubules (Fig. 5 *d*). We believe that these ZW10-containing metaphase structures are most likely the kMTs, the bundles of microtubules that span the distance from the pole to each kinetochore (Church and Lin, 1985). We assume that the ZW10 on these filaments is recruited from that previously at the kinetochore, but this remains to be proven.

From the onset of anaphase through midanaphase I (stages M4a–b), ZW10 again becomes associated with the kinetochore regions of each dyad as it separates from its homolog (Fig. 5, *e* and *f*). As late anaphase progresses into telophase (stages M4c to M5), the meiotic spindle becomes "pinched" at the midbody during cytokinesis (Cenci et al., 1994). During telophase, only a small amount of ZW10 is still found at the kinetochores, while some ZW10 staining is also observed at the spindle midbody (Fig. 5 *g*).

After completion of the first meiotic division, ZW10 becomes dispersed so that interphase secondary spermatocytes (stages M6a-M6b) show a diffuse pattern of ZW10 staining throughout the cell. In contrast with interphase primary spermatocytes, ZW10 antigen does not appear to be constrained to the nuclear domain in interphase/prophase secondary spermatocytes (not shown).

The distribution of ZW10 during the second meiotic di-

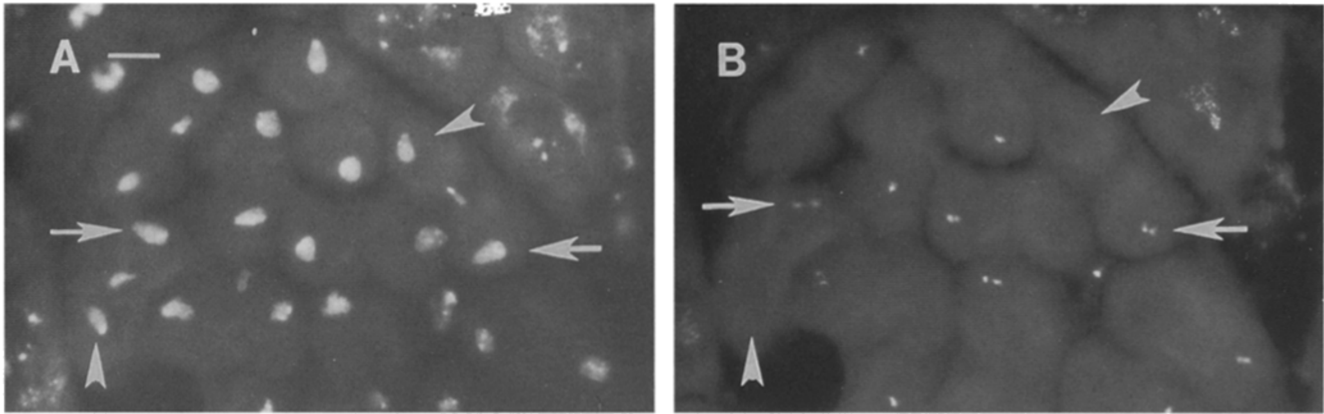

Figure 4. Sister chromatids remain attached during the first meiotic division in *zw10* mutants. Testes were fixed and subjected to FISH analysis in which the chromosomes were stained with Hoechst 33258 (*A*) and probed with a Y chromosome–specific probe (*B*). (*A*) Cyst of telophase I figures in which some divisions are uneven and lagging chromosomes are present (cells marked with *arrows*). (*B*) The Y chromosome dyad remains intact and migrates to only one pole (*arrows*), not to both poles (*arrowheads*). If precocious separation occurred, the two Y chromatids would often be expected to migrate to both poles. This is never the case (*A* and *B*). The two fluorescent dots in some cells in *B* result from the presence of the two Y sister chromatids. This bipartite signal is also seen in wild type and does not indicate the precocious separation of chromatids during the first meiotic ana/telophase. Bar, 5 μm.

vision appears quite similar to that seen during the first meiotic division. ZW10 is localized to kinetochore regions of prometaphase II chromosomes; here, however, two regions of staining per dyad are visible, consistent with the individualization of the sister kinetochores (Fig. 5 *h*). During metaphase II, ZW10 is again found on discrete filaments composing a subset of the spindle, consistent with localization on kMTs (Fig. 5, *i* and *j*). ZW10 staining becomes reassociated with the kinetochore regions during anaphase II (stages M10a–c; Fig. 5 *k*). In late telophase II (stage M11), ZW10 appears to concentrate at the spindle midbody (Fig. 5 *l*).

In postmeiotic cells, ZW10 does not accumulate in specific cellular compartments and progressively disappears as spermiogenesis proceeds. In particular, some weak ZW10 staining of spermatid nuclei is visible during the onion stage that occurs immediately after completion of meiosis II, but this quickly disappears during the subsequent elongation phases of spermatogenesis (not shown).

In summary, during both meiotic divisions, the ZW10 protein acts stereotypically as follows: it is first found at the kinetochore regions of chromosomes at prometaphase, moves outwards along what we presume are kinetochore microtubules at metaphase, and then returns to the kinetochores at anaphase onset. In addition, some ZW10 protein accumulates at the spindle midbody during telophase.

Table III. Cytokinesis Failure in zw10 Mutants

	Spermatids	Percentage of spermatids					Abnormal
		1	2	3	4	5–6	
	n						%
Oregon-R	875	99.5	0.5	0.0	0.0	0.0	0.5
zw10^{S1}	1646	51.2	26.9	8.0	13.4	0.5	48.8
zw10^{i20}	569	66.0	28.0	3.0	3.0	0.0	34.0
zw10^{S2M}	568	50.7	30.8	9.9	8.5	0.1	49.3

The postmeiotic onion-stage spermatid cysts from wild-type and designated *zw10* mutant alleles were examined, and the number of spermatids containing one through six nuclei associated with a single nebenkern was determined (see examples in Fig. 1).

Univalents and Bivalents in the Same Cell Differ in Their Pattern of ZW10 Staining

To better understand how the cell directs the various redistributions of ZW10 documented above, we have introduced several alterations into the genetic background to determine their effects on ZW10 distribution. Because the changes we observed in ZW10 localization during the cell cycle suggested the possibility of a role in measuring bipolar tension across chromosomes at the metaphase plate (see below), we first examined the consequences of perturbing the bipolarity of chromosome attachments to the spindle. During meiosis I, tension across chromosomes at metaphase is ensured by the pairing of homologous chromosomes, which stabilizes the bipolar orientation of kinetochores to opposite poles of the spindle (Nicklas, 1985). Chromosomes that do not possess a pairing partner, called univalents, arise in special circumstances. For example, sex chromosomes whose pairing sites (within the ribosomal RNA genes) have been deleted behave as univalents (Ault, 1984; Church and Lin, 1988; McKee and Karpen, 1990). The same is true of compound chromosomes in which the X and the Y chromosomes or homologous autosomes are attached to a single centromere (Yamamoto, 1979). Univalents can attach to only a single pole during prometaphase I and metaphase I, and are thus not subject to normal forces of bipolar tension (Ault, 1984; Ault and Nicklas, 1989). Because they cannot attain a stable metaphase orientation, univalents oscillate along the spindle from one pole to the other and are incorporated randomly into daughter nuclei (Church and Lin, 1988). Thus, the examination of cells containing univalents allows us to determine how bipolar tension and kinetochore orientation influence ZW10 distribution.

We have found that ZW10 localizes to the kinetochores

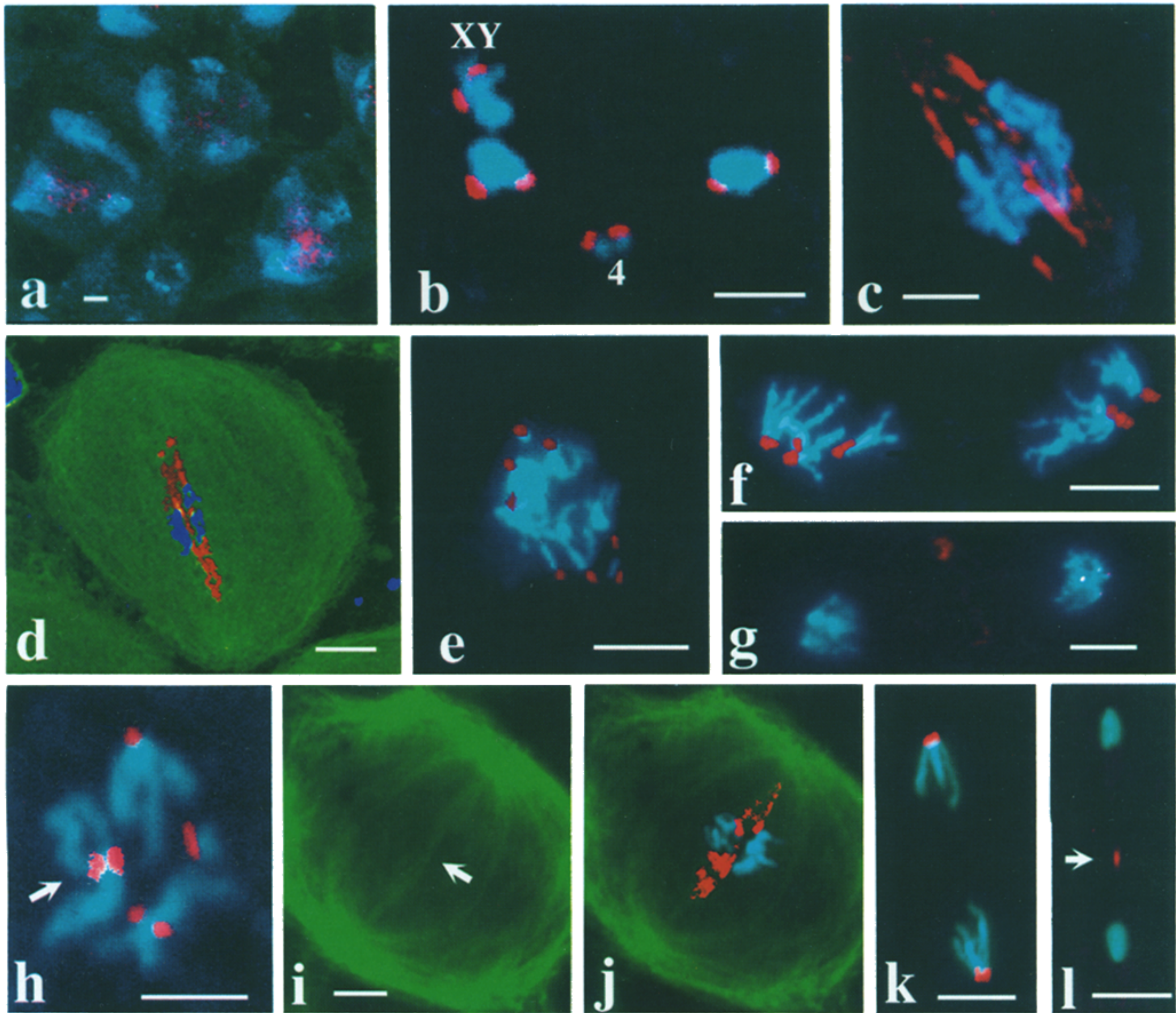

Figure 5. Localization of ZW10 protein in wild-type spermatocytes. Wild-type testes were fixed and stained for indirect immunofluorescence to visualize ZW10 protein (*red*) and chromosomes (*blue*) during the two meiotic divisions. In some panels (*d*, *i*, and *j*), spermatocytes were additionally stained to visualize microtubules (*green*). (*a*) Prophase I (S5 stage). ZW10 is intranuclear but not localized to discrete structures. (*b*) Prometaphase I (M2 stage). ZW10 localizes to the kinetochore regions of the bivalents (XY, the two autosomal, and the fourth chromosome [4] bivalents). (*c*) Metaphase I (M3 stage). The chromosomes are aligned at the metaphase plate, with poles at upper left and bottom right. ZW10 is found streaming outward toward the poles onto the presumptive kMTs. (*d*) Metaphase I (M3 stage). ZW10 localizes to only a small region of the meiotic spindle (*green*). (*e* and *f*) Anaphase I. Exclusive localization of ZW10 to the kinetochores of both very early (*e*) and early (*f*; M4a/b stage) anaphases. (*g*) Telophase I (stage M5). While a small amount of ZW10 remains to the kinetochores, some protein becomes visible at the midbody region. (*h*) Prometaphase II (M7 stage). Localization at the sister kinetochores (*arrow*). (*i* and *j*) Metaphase II (M9 stage). Two panels showing the meiotic spindle (*i*) and the merged image of ZW10, chromosomes, and spindle (*j*) of the same cell. As in *d*, ZW10 appears to localize to a very specific subset of microtubules (presumably the kMTs; *arrow* in *i*). (*k*) Anaphase II (M10a/b stage). Kinetochore-region staining. (*l*) Telophase II (M11 stage). Some ZW10 becomes localized to the midbody region. Bar, 5 μm.

of both univalents and bivalents during prometaphase I (Fig. 6 *a*), even though the bivalents at this stage are bioriented while the univalents, which contain only a single kinetochore (shared by two sister centromeres), are monooriented. In contrast, at metaphase I, the localization of ZW10 relative to univalents and bivalents is quite different. In most metaphase I cells containing both bivalents and univalents, ZW10 appears to stream out from the kinetochores of the bivalents toward the kMTs, while ZW10 is found only as a single spot of kinetochore staining on the univalents (Fig. 6, *b* and *c*). It should be noted that in a substantial fraction of cases (~30%), ZW10 does appear to stretch from univalent kinetochores, but this only occurs when the univalents are situated at the metaphase plate with the bivalents. In this light, previous studies have shown that sex chromosome univalents can sometimes achieve bipolar orientation at metaphase (Ault, 1984; Church and Lin, 1988). Finally, during anaphase I, when

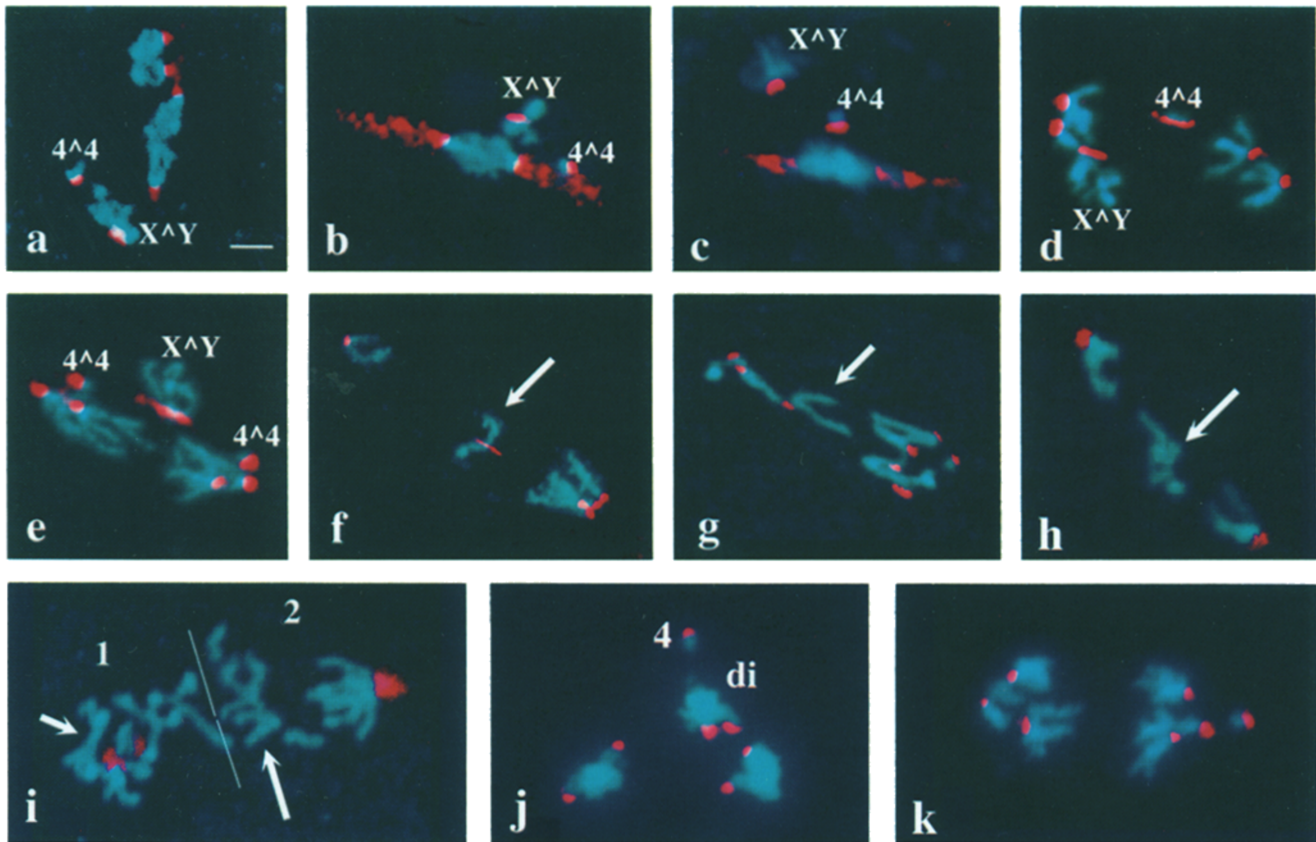

Figure 6. Experimental perturbation of ZW10 protein distribution. Cells were stained as described in Fig. 5 (ZW10, *red*; chromosomes, *blue*). (*a–e*) Univalents: the compound chromosomes X^Y and 4^4. (*a*) Prometaphase I. The ZW10 protein is present at kinetochores of all chromosomes equally as in wild type (see Fig. 5 *B*). (*b* and *c*) Metaphase I. The distribution of ZW10 on the bivalents and the univalents differs. The stretching of ZW10 along the presumptive kMTs is present on the normally orienting bivalents but not the maloriented univalents. (*d* and *e*) Anaphase I. Lagging univalents ([4^4 and X^Y] in *d*, X^Y in *e*) are associated with stretching of ZW10 to the poles, as opposed to the limited kinetochore staining of normally segregating dyads. The cell in *e* contains two 4^4 chromosomes, which can pair with each other and thus segregate normally, and on which no stretching of ZW10 is apparent. (*f*) *ord* mutant anaphase II showing stretching of ZW10 on a lagging chromatid (*arrow*); (*g*) *mei-S332* mutant anaphase II with lagging chromatid and unequal division. All chromatids regardless of their position contain the ZW10 protein at their kinetochores. (*h* and *i*) *Dub* figures. The cell in *h* is in the process of a bipolar anaphase II; lagging chromosomes lack ZW10, whereas those that migrate properly localize ZW10 normally. In *i*, cell 1 is undergoing prometaphase II; some kinetochores lack ZW10 (*short arrow*). In *i*, cell 2 exhibits a unipolar anaphase II; laggards do not stain with ZW10 antibodies (*long arrow*). (*j* and *k*) Stable dicentric chromosomes composed of the Y and the fourth chromosome. In *j*, the dicentric (*di*) pairs with the free fourth (*4*) and the X chromosome to form a trivalent during prometaphase I containing four centromeres. However, only three spots of ZW10 staining are observed associated with the trivalent. In the large majority of anaphase I figures (*k*), ZW10-containing kinetochores are distributed four to three, indicating the dicentric chromosome has only a single spot of ZW10 staining (presumably at the Y kinetochore). Bar, 5 μm.

ZW10 stains the kinetochore regions of the separated bivalent chromosomes, ZW10 in >80% of the cases is associated with fibers that extend from the univalent in both directions toward the poles (Fig. 6, *d* and *e*). Thus, the behavior of ZW10 relative to univalents and bivalents is similar during prometaphase I, but is quite different during metaphase I and anaphase I. These results indicate that ZW10 appears on kMTs only when the corresponding chromosomes or bivalents are attached in bipolar fashion to microtubules emanating from both spindle poles.

Precocious Sister Chromatid Separation Prevents Streaming of ZW10 from Kinetochores to the Spindle

To further understand the relationship between bipolar tension and ZW10 localization, we have examined testes in animals carrying mutations in the genes *orientation disruptor* (*ord*) and *mei-S332*. These mutations cause PSCS during the first meiotic division. Mutations in *ord* cause PSCS during prometaphase I, resulting in first- and second-division nondisjunction (Goldstein, 1980; Miyazaki and Orr-Weaver, 1992), whereas mutations in *mei-S332* cause PSCS during anaphase I, resulting almost exclusively in second-division missegregation (Goldstein, 1980; Kerrebrock et al., 1992). Note that in both types of mutant, sister chromatids have separated before meiosis II. As a result, second-division aberrations are common because single chromatids not subjected to bipolar forces do not orient properly. Congression of chromosomes to well-defined metaphase II plates is not observed in these mutants (Goldstein, 1980). Although anaphase II–like figures are readily observable, chromosomes either move ran-

domly to either pole or lag in the middle of the spindle.

We have found that in both *ord* and *mei-S332* mutant testes, ZW10 localization is normal during meiosis I with kinetochore staining on all chromosomes at prometaphase I and anaphase I. Throughout the second division, all chromosomes contain ZW10 at their kinetochores, including those that are lagging behind during anaphase (Fig. 6, *f* and *g*). Importantly, we have found no second-division figures in either mutant in which ZW10 staining of the spindle is well defined as in wild-type metaphase II. However, in many cases, lagging anaphase II chromosomes exhibit bidirectional streaks of ZW10 staining similar to those associated with anaphase I univalents (Fig. 6 *f*). Again, it appears that ZW10 staining of the spindle requires the presence of bioriented chromosomes connected to both spindle poles. The chromosomes in *ord* and *mei-S332* mutant meiosis II figures are monooriented; accordingly, ZW10 protein is mostly absent from kMTs.

Defective Centromeres Fail to Associate with ZW10

Two observations suggest that the presence of ZW10 at the centromere/kinetochore is an indicator of proper centromere function. First, we examined animals homozygous for mutations in the gene *Double-or-Nothing* (*Dub*; Moore et al., 1995) for possible defects in ZW10 localization during the male meiotic divisions. In these animals (which die at the late larval/pupal stage), high levels of nondisjunction occur in both divisions. Most notably, anaphases are uneven or unipolar, and many figures show lagging chromosomes. In such anaphases, ZW10 localizes to the kinetochores of chromosomes that have moved to the poles, but not to those that lag behind at the position of the metaphase plate (Fig. 6, *h* and *i*). Thus, on a chromosome-by-chromosome basis, there is a clear correlation between accuracy in chromosome behavior and the presence of ZW10. We believe that this relationship is established as early as prometaphase, because only a subset of prometaphase chromosomes in *Dub/Dub* spermatocytes stains with ZW10 (Fig. 6 *i*). The simplest explanation would be that those chromosomes that fail to localize ZW10 at prometaphase are those that missegregate during anaphase, but this remains to be proven. It should be noted that these effects of *Dub* mutants are reminiscent of those caused by *aberrant anaphase resolution* (*aar*) mutations in mitosis, where lagging chromatids similarly do not contain ZW10 (Gomes et al., 1993; Williams and Goldberg, 1994).

Additional support for the notion that the presence of ZW10 is correlated with centromere function comes from our examination of the distribution of this protein relative to a dicentric chromosome. Such chromosomes are normally unstable because chromatid bridging and breakage can occur in anaphase if the two centromeres are oriented towards opposite poles (Bajer, 1963). Exceptions to this rule occur if one of the centromeres is "stronger" than the other, meaning that only one of the two centromeres is efficient in establishing spindle connections (Therman et al., 1974; Zinkowki et al., 1986; Page et al., 1995). In *Drosophila*, a transmissible dicentric chromosome has been described in which the fourth chromosome has been attached to the long arm of the Y (Ault and Lyttle, 1988). Here, the Y chromosome centromere is reported to be dominant to that of the fourth chromosome. We have observed that in the large majority of prometaphase I figures, the trivalent formed by the T(Y;4) dicentric chromosome, the X chromosome, and the free fourth chromosome displays only three spots of ZW10 staining (Fig. 6 *j*). In theory, this trivalent should contain four separate centromeric regions (one on the X, one on the free fourth, and two on the T[Y;4]), so this result suggests that one of the centromeres on the dicentric is inactive. This relationship also appears to hold during anaphase I in the large majority of figures, where only seven spots of ZW10 staining are observed on the separating chromosomes: four at one pole, and three at the other (Fig. 6 *k*). This again suggests that ZW10 recognizes only a single site on the T(Y;4) dicentric chromosome.

Discussion

The Function of ZW10 Is Common to Both Equational and Reductional Divisions

Our phenotypic analysis of testes in *zw10* mutant males demonstrates that ZW10 is critical for accurate chromosome segregation not only in mitosis (Williams et al., 1992; Williams and Goldberg, 1994) but also in both meiotic divisions. The effects of *zw10* mutations are similar in all three cases, giving rise to anaphases with lagging chromosomes and/or unequal numbers of chromosomes at the two poles. Additionally, it appears that the localization of ZW10 is similar during mitosis, meiosis I, and meiosis II. This is clearly the case during metaphase and anaphase, but is less certain during prometaphase. Due to the poor resolution of this phase in mitotic cells, we could not definitively determine ZW10's position during mitotic prometaphase. However, several considerations make it very likely that ZW10 is in fact present at the kinetochore region of prometaphase chromosomes during mitosis as well as during meiosis. ZW10 associates with kinetochores both in mitotic cells blocked in a prometaphase-like state by colchicine or taxol (Smith et al., 1985; Williams and Goldberg, 1994) and in normal, untreated secondary spermatocytes during prometaphase of the equational meiosis II division. In addition, we recently have found that a human protein closely related to ZW10 binds to the kinetochore region of human prometaphase chromosomes (Starr, D., Z. Li, B.C. Williams, and M.L. Goldberg, unpublished results).

The use of ZW10 in both equational and reductional divisions suggests that this protein is unlikely to be involved in the adhesion or separation of sister chromatids. Other observations lead to the same conclusion: (*a*) unlike *ord* and *mei-S332* mutations, *zw10* mutations do not cause precocious sister chromatid separation during the first meiotic division. (*b*) ZW10 is localized to the kinetochore during the first division, when sister centromeres remain attached, as well as during the second division, when sister centromeres separate. (*c*) Mutations in *ord* and *mei-S332* do not affect the ability of ZW10 to localize to the kinetochores, even those of chromatids that were separated precociously during the previous meiotic division. These data together strongly indicate that ZW10 does not play a di-

rect role in maintaining the cohesion of sister chromatids before metaphase onset, or a specific role in severing the centromeric connection between sister chromatids at anaphase onset.

We emphasize that in both meiotic divisions as well as in mitosis, spindle and chromosome behavior appears to be unaffected by *zw10* mutations before anaphase onset. This suggests that ZW10 function is first required at a time very close to anaphase onset, when major changes in the protein's intracellular localization occur.

The Distribution of ZW10 Is Subject to the Presence or Absence of Tension across Individual Chromosomes

In wild-type cells, ZW10 protein is found at the centromere/kinetochore during prometaphase, on the spindle at metaphase, and at the kinetochore during anaphase. For the remainder of this discussion, we adopt the most simple explanation that these alterations in the pattern of staining reflect movements of the same ZW10 molecules. A possible precedent for such movements is the poleward stretching of the fibrous corona of the kinetochore, which has been observed in electron micrographs of metaphase mammalian cells (Rieder and Alexander, 1990). However, it should be remembered that presently we are unable to exclude the possibility that these apparent ZW10 movements actually reflect recruitment of different molecules from cytoplasmic pools.

We interpret our findings on changes in the pattern of ZW10 staining as evidence that ZW10 associates with the kinetochore in the absence of bipolar spindle tension, and then moves to the spindle, presumably along kMTs, only when chromosomes are stably bioriented and thus subject to spindle tension. This model is based on several observations. (*a*) The initial localization of ZW10 to the kinetochore regions at prometaphase is clearly independent of tension across chromosomes. This is most obvious during prometaphase of the second meiotic division in *ord* and *mei-S332* mutants, where ZW10 associates with the prometaphase kinetochores of sister chromatids that were previously disconnected during the preceding division. (*b*) Univalents and bivalents differ in their association with ZW10 during metaphase I. The protein appears on spindle fibers emanating from bioriented bivalents, but it remains at the kinetochore region of monooriented univalents. (*c*) Streaming of ZW10 onto the spindle is generally not observed in secondary spermatocytes mutant for *ord* or *mei-S332*. No metaphase plate forms in these mutant cells (Goldstein, 1980), consistent with the inability of previously separated chromatids to forge balanced connections to opposite spindle poles. (*d*) Early in anaphase, when spindle tension is relieved because of the separation of paired bivalents (during meiosis I) or of sister chromatids (during meiosis II or mitosis), ZW10 protein is almost always found exclusively at the centromere/kinetochore. The picture that emerges is that bipolar tension is required for the movement of ZW10 from the chromosomes to the spindle at metaphase, while the removal of tension is necessary for subsequent movements back to the kinetochores at anaphase.

It should be cautioned that acceptance of this hypothesis requires a particular interpretation of certain meiotic figures that, at first glance, may appear to contradict it. We have observed ZW10 staining stretching in both directions from lagging univalents during anaphase I (Fig. 6, *d* and *e*). We believe these represent chromosomes that have established delayed bipolar connections with the anaphase spindle. This idea is consistent with the electron micrographs of Lin et al. (1984) showing a Y chromosome lagging at anaphase I from which kinetochore material stretches towards opposite poles. Such putative anomalous bipolar orientations of anaphase univalents could, in theory, result if sister kinetochores in the univalent split from each other and established connections to opposite spindle poles. This is conceivable because it is known that sister kinetochores normally separate during metaphase I (Goldstein, 1981). We have also seen ZW10-containing fibers extending in both directions from rare lagging chromatids during anaphase II in *ord* or *mei-S332* mutants (Fig. 6 *f*). We presume that the kinetochores of these chromatids are attached to kMTs from both spindle poles, consistent with the central position they occupy on the spindle. Although there is precedent for this supposition (Christy et al., 1995), we have not directly demonstrated its validity in these cases.

The Association of ZW10 with the Kinetochore Region Is Correlated with Centromere/kinetochore Function

The relationship between ZW10 staining and kinetochore function is seen most clearly in *Dub* mutant meioses (this paper) and in *aar* mutant mitoses (Williams and Goldberg, 1994). In animals carrying mutations in either gene, chromosomes that move to the poles stain with ZW10, while laggards show no ZW10 staining. The behavior of individual chromosomes in these mutants during anaphase is thus predicted by whether or not they associate with ZW10. Additional evidence for this correlation is that ZW10 appears, in almost all cases, to bind to only a single region of what has been reported to be a transmissible dicentric chromosome (Ault and Lyttle, 1988). The genesis of this T(Y;4) chromosome is complicated, and it is not understood which DNA sequences from the Y or fourth chromosome centromeres it contains. However, we support the contention of Ault and Lyttle (1988) that this chromosome indeed includes two centromeres, one of which functions efficiently, while the other is activated only in a small minority of cells. We have observed very rare anaphase figures revealing two sites of ZW10 association with the dicentric chromosome; in these cases, the T(Y;4) appears to be stretched toward both poles, suggesting that this chromosome indeed contains two potentially functional centromeres (data not shown).

In spite of the correlation between ZW10 staining and centromere function, it should be stressed that the presence of ZW10 at the kinetochore alone is not sufficient to ensure proper chromosome segregation. For example, in *ord* and *mei-S332* mutant secondary spermatocytes, chromosomes that are associated with ZW10 nonetheless behave aberrantly during anaphase. Factors other than ZW10 are clearly responsible for missegregation in these cases.

ZW10 Function May Be Partially Redundant

Null mutations in the *zw10* gene do not block or signifi-

cantly delay the progression of mitosis or meiosis. Moreover, ZW10 is not absolutely required either for separation of sister chromatids (mitosis and meiosis II) or homologous chromosomes (meiosis I) at anaphase onset, or for poleward anaphase chromosome movements, since some chromosomes undergo these events in *zw10* null mutant cells. One possibility is that ZW10 function is exclusively that of coordinating chromosome behavior. Alternatively, ZW10 could act directly in these processes, but the lack of its function could be partially circumvented by the activity of related proteins or of subsequent cellular "bailout" mechanisms.

Possible Roles of ZW10 at the Metaphase–Anaphase Transition

The fact that absence of ZW10 does not completely prevent the completion of any aspect of cell division makes it difficult to define the function of this protein with certitude. However, the findings discussed above narrow the possibilities, particularly if it is accepted that chromosome and spindle behavior before anaphase onset is unaffected by *zw10* mutations. This would argue, for example, that ZW10 is not an integral part of kinetochore structure since previous studies have established that destruction of kinetochores in prometaphase inhibits their motion to the metaphase plate (Bajer, 1972; McNeill and Berns, 1981; for review see Rieder and Salmon, 1994).

We also do not favor hypotheses predicting an involvement of ZW10 in poleward chromosome movements during anaphase, for example, by stabilizing the attachment of the kinetochore to microtubules that are disassembling during anaphase (Mitchison et al., 1986; Gorbsky et al., 1987; Lombillo et al., 1995), or by regulating microtubule disassembly at the anaphase kinetochore. We believe that a function for ZW10 in anaphase chromosome movements is unlikely because we have observed at least one effect of *zw10* mutations—i.e., precocious sister chromatid separation—in cells that are arrested before anaphase by microtubule poisons (Williams et al., 1992). These cells do not contain any obvious spindle microtubules upon which ZW10 could influence chromosomal movements. The ZW10 protein would thus need to exhibit two very dissimilar functions if it also played a role in anaphase chromosome migration.

We suggest instead that the most plausible role for ZW10 is as a component of a feedback control mechanism that renders entrance into anaphase dependent upon bipolar tension exerted across all chromosomes (Li and Nicklas, 1995; Rieder et al., 1995). In many organisms, checkpoints delay anaphase until all kinetochores are attached to the spindle (Rieder et al., 1994; Nicklas et al., 1995). Kinetochores are thought to produce an inhibitor of anaphase onset until stable metaphase configurations are attained (Nicklas et al., 1995; Rieder et al., 1995). We envision that ZW10 may either be part of the mechanism that senses tension, or part of the immediate downstream response to this mechanism specifically leading to anaphase chromatid or chromosome disjunction. It should be noted that in *Drosophila* spermatocytes, lack of tension does not completely prevent anaphase onset (which occurs even in cells carrying univalents, or in *ord* or *mei-S332* meiosis II). We believe nonetheless that lack of tension may delay or disrupt the synchrony of the beginning of anaphase in these cells.

This hypothesis explains several aspects of the *zw10* mutant phenotype and of the distribution of its gene product. (*a*) At least in a formal sense, the *zw10* gene in fact acts precisely as part of a checkpoint that normally prevents sister chromatid separation in the absence of a spindle. In colchicine-treated wild-type mutant neuroblasts, sister chromatids stay together, but a large percentage separate in colchicine-treated *zw10* mutant neuroblasts (this is the PSCS phenomenon described above). (*b*) In the absence of colchicine, chromosome missegregation in *zw10* mutant cells would occur if anaphase began abnormally before all kinetochore–spindle attachments were made; the resultant small change in the timing of anaphase onset might not have been detected by our rather crude measurements. (*c*) Chromosomes that are or are not under tension display different distributions of ZW10 at metaphase and anaphase. (*d*) The movements of ZW10 to what we believe are kMTs during metaphase and back to the kinetochore region at anaphase onset might provide a physical basis for the measurement of tension.

With regard to the latter point, we speculate that the apparent movements of ZW10 between kinetochores and kMTs might reflect the conveyance of signals between the chromosomes and the spindle. We envision that a complex containing ZW10, perhaps including the entire fibrous corona of the kinetochore (Rieder and Alexander, 1990), moves from kinetochores to kMTs when the kinetochore is under tension. On the kMTs, this complex would contact and interact with molecules on the spindle involved in directing the metaphase to anaphase transition. One hypothesis is that such interactions might alter the ZW10-containing complex so that its return to the kinetochore region at anaphase onset would stimulate chromosome separation or movement. Alternatively, because we cannot exclude that anaphase onset might begin while ZW10 is on the kMTs, it could be imagined that the movement of ZW10 to the kMTs may turn off a function rather than turn it on. For example, ZW10 on the prometaphase kinetochore could be part of a "prevent anaphase" signal. Movement of ZW10 onto kMTs in response to spindle tension would disable the signal, allowing anaphase to begin. Regardless of the exact mechanism of ZW10 action, the possibility that kMTs serve as signaling conduits is, in our view, very attractive. Previous observations have indicated that the fibrous corona of mammalian kinetochores stretches onto kMTs from chromosomes under tension (Rieder and Alexander, 1990); our findings with ZW10 show that movements of molecules along kMTs may have important implications for regulating the cell cycle and chromosome segregation.

ZW10 and Cytokinesis

The cytokinesis defects observed in *zw10* mutant spermatid cysts are the likely cause of the sterility of *zw10* mutant male escapers (Shannon et al., 1972). This sterility cannot be explained by problems in chromosome segregation alone, as even *Drosophila* sperm lacking all chromosomes but the very small fourth are capable of fertilization (Lind-

sley and Grell, 1969). We do not at present understand why *zw10* mutations disrupt cytokinesis during meiosis; we have never detected such cytokinesis defects in mutant mitotic divisions. This might be related to the fact that cytokinesis in *Drosophila* spermatocytes is different from that in neuroblasts, or simply reflect a particular sensitivity of meiotic cytokinesis to various perturbations. It is of interest in this light to note that a proportion of ZW10 protein localizes to the spindle midbody in late anaphase/telophase of both meiotic divisions (Fig. 5, *g* and *k*), but similar staining of the midbody has not been observed during mitosis. We have previously shown that defects in other midbody components may lead to cytokinesis aberrations (Williams et al., 1995). Recent results with the mammalian INCENP proteins, which move from the centromeres to the ana/telophase spindle midbody (Earnshaw and Cooke, 1991), suggest that these proteins also play a role in cytokinesis (W. Earnshaw, personal communication).

It remains unclear exactly what aspect of cytokinesis in *zw10* mutant meioses fails to occur properly. The structure of late anaphase/telophase mutant spermatocytes appears normal; several antigens indicative of spindle midbody or contractile ring formation, including actin (Gunsalus et al., 1995), PEANUT (Neufeld and Rubin, 1994), and KLP3A (Williams et al., 1995), are localized to these structures in *zw10* mutant telophases (not shown). We presume that the contractile ring probably forms and then regresses, but this supposition, as other aspects of the relationship between ZW10 and cytokinesis, will require further analysis.

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