The *Drosophila* l(1)zw10 Gene Product, Required for Accurate Mitotic Chromosome Segregation, Is Redistributed at Anaphase Onset

Byron C. Williams,* Tim L. Karr,† John M. Montgomery,* and Michael L. Goldberg*

*Department of Biochemistry and the Beckman Institute, University of Illinois, Urbana, Illinois 61801

†section of Genetics and Development, Biotechnology Building, Cornell University, Ithaca, New York 14853-2703; and

‡Department of Biochemistry and the Beckman Institute, University of Illinois, Urbana, Illinois 61801

Abstract. Mutations in the gene l(1)zw10 disrupt the accuracy of chromosome segregation in a variety of cell types during the course of *Drosophila* development. Cytological analysis of mutant larval brain neuroblasts shows very high levels of aneuploid cells. Many anaphase figures are aberrant, the most frequent abnormality being the presence of lagging chromosomes that remain in the vicinity of the metaphase plate when the other chromosomes have migrated toward the spindle poles. Finally, the centromeric connection between sister chromatids in mutant neuroblasts treated with colchicine often appears to be broken, in contrast with similarly treated control neuroblasts. The 85-kD protein encoded by the l(1)zw10 locus displays a dynamic pattern of localization in the course of the embryonic cell cycle. It is excluded from the nuclei during interphase, but migrates into the nuclear zone during prometaphase. At metaphase, the zw10 antigen is found in a novel filamentous structure that may be specifically associated with kinetochore microtubules. Upon anaphase onset, there is an extremely rapid redistribution of the zw10 protein to a location at or near the kinetochores of the separating chromosomes.

Materials and Methods

**Drosophila Stocks**

The zw10 alleles zw101, a spontaneous mutation (Schalet, 1986), and zw10ts1, a mutation generated in a me-9 background (Lindsay and Zimm, 1990), were obtained from A. Schalet (Yale University, New Haven, CT). B. Judd (National Institute of Environmental Health Sciences, Research Triangle Park, NC) kindly provided two additional zw10 alleles (zw10ts20, induced by ethyleneimine and zw10ts21, induced by ethyleneimine and X rays), representative alleles of nearby lethal complementation groups (zw10odi, zw10szu, and zw10ts11), and the rearrangements Df(l)w17, Df(l)64j4, and Dp(l,2)w70k3. These strains have been described in detail by Judd et al. (1972). The allele zw10 (Smith et al., 1985) was received from B. Baker (Stanford University, Palo Alto, CA). Mutations and rearrangements were maintained in females either of the X chromosome balancers FM2a or FM6, 10ka, or in males covered by the Y chromosome derivatives w1Y or Bw1w1Y (see Lindsay and Zimm, 1986, 1987, 1990 for further explanation of chromosomes and genetic symbols used).

To obtain larvae hemizygous for mutant zw10 alleles (zw10o), males of genotype zw10/FM2a w/+ Y were crossed with *C(1)DX, y f/Y r y*506 females (from D. Glover, Dundee University, UK). Male larvae, distinguished by the external morphology of the larval gonad, are thus zw10/Y. Females heterozygous for zw10 mutations and deletions of the zw10 region were generated by crossing zw10/FM2a females to *Df(l)w17'/Bw1w1Y* males. Offspring larvae of interest (zw10'/Df(l)w17') could be differentiated from their siblings by sex and by virtue of their yellow-colored (w1) Malphigian tubules.

Preparations used in germline transformation experiments and subsequent genetic manipulations are described below.

**Cytology**

Metaphase figures were examined using standard cytological procedures.
The preparation of recombinant DNA from plasmid, cosmid, or bacteriophage lambda vectors, or genomic DNA from Drosophila adults, has previously been noted (Gunnarane et al., 1986; Mansukhani et al., 1988a,b). Genomic clones were isolated either from an EMBL4 genomic library (Gunnarane et al., 1986), or from a cosmid library kindly provided by J. Tamkun (University of Colorado, Boulder, CO). Restriction fragments were subcloned into the polylinker of Bluescript KS+ (Stratagene Inc., La Jolla, CA). Transfer of DNA from agarose gels to Gene Screen Plus membranes (New England Nuclear, Boston, MA) was carried out using the alkaline blot method (Sambrook et al., 1989). For radio labeling, primer extensions from either purified DNA or DNA fragments excised from low-melt agarose gels (SeaPlaque, FMC Marine Colloids, Rockland, ME) were performed essentially as described by Feinberg and Vogelstein (1983). In situ hybridization of DNA fragments labeled with biotin-11-UTP to salivary gland polytene chromosomes was performed as previously described (Gunnarane et al. 1986) using the Detek kit (Enzo Biochemistry Inc., New York, NY). Poly(A)+ RNA was isolated from staged wild-type Drosophila according to the procedure of Dombrádi et al. (1989). Electrophoresis of glyoxalated poly(A)+ RNA on agarose gels, transfer to Hybond-N membranes, and hybridization of Northern blots with labeled probes was also carried out as detailed by the same authors. Full length zw10 cDNA clones were isolated from a Drosophila imaginal disc cDNA library supplied by Dr. Nicholas Brown (Harvard University, Cambridge, MA). Since the pB40 vector used allows directional cDNA cloning (Brown and Kafatos, 1988), the orientation of transcription could be readily determined by restriction analysis. A BglII-NoI fragment containing the entire zw10 cDNA was cloned into the BamHI and NotI sites of Bluescript KS+, yielding the construct 20.4KS-. Deletions were constructed using partial EcoRII nuclease digestion as described by Henikoff (1984) using the Erase-a-Base kit (Promega Corp., Madison, WI). 20.4KS- was digested with KpnI and ClaI for deletions starting from the 5' end of the cDNA, while SacI and NotI were used for deletions starting from the 3' end. The resulting cDNA was purified by the dideoxy chain termination method of Sanger et al. (1977) using the T7 DNA polymerase, so that zw10 was in frame with lacZ. The construct zw10 was blunt-ended ligated into the BamHI site of pATH3 (which was made blunt by blunting in with Klenow DNA polymerase), so that zw10 was in frame with trpE sequences. The resulting construct was called pATH3-zw10. Derivatives containing shorter zw10-specific segments were created by digesting pATH3-zw10 with XhoI or HindIII and recircularizing the products in a very dilute ligation mixture. The XhoI derivative (trpE-zw10) lacks the central portion of zw10 (amino acids 177-475) and the HindIII derivative (trpE-zw10H) does not contain the COOH-terminal end (amino acids 528-721). It should be noted that all lacZ-zw10 and trpE-zw10 fusion proteins lack the NH2 terminus of the zw10 protein (amino acids 1-76). The trpE-zw10 fusion proteins were induced as described by Dieckmann and Tzagoloff (1985). Induced trpE-zw10 fusion proteins were isolated in the soluble fraction after the lysis of cells with lysozyme and NP-40 in TEN buffer (50 mM Tris-HCI, pH 7.5, 0.5 mM EDTA, 0.3 M NaCl). Protein was solubilized in cracking buffer (0.01 M sodium phosphate, pH 7.2, 1.2 M-mercaptoethanol, 1% SDS, 8 M urea) and dialyzed overnight in PBS.

For immunoassay purification, the trpE-zw10 fusion protein of interest was resuspended in washing cytoplasmic fraction lysates containing activated Sepharose 4B (Sigma Chemical Co.) for 16 h in coupling buffer (0.1 M NaHCO3, pH 8.3, 0.5 M NaCl) at 4°C. The buffer was removed and the remaining active groups blocked for 1 h at room temperature in 0.2 M glycine, pH 8.0. The fusion protein-coupled resin was poured into a column and washed extensively with PBS. Crude serum diluted 1:10 in PBS was first applied to a column of Sepharose 4B-coupled insoluble protein extract from XLI Blue cells not expressing the fusion protein and prepared according to the same protocol. The resultant preadsorbed crude serum was then recirculated during a period of 12 h through the column containing the immobilized fusion protein. The column was washed extensively with PBS, and the bound antibody was eluted with 0.2 M glycine, pH 2.7. The purified antibody solution (ca. 100 µg/ml) was immediately neutralized by the addition of 1/7 vol. of 1 M Tris-HCl, pH 8.9, and dialyzed for 24 h in three changes of PBS. This procedure was carried out separately for preimmune sera and immune sera obtained from each rabbit; results obtained with both rabbits were qualitatively identical. The preimmune sera failed to identify zw10 fusion proteins on Western blots, and did not detect signals above background in immunofluorescence experiments. Antibodies purified against the derivatives trpE-zw10X and trpE-zw10H generated identical results on Western blots and in embryo staining.

### Germline Transformation

A 4.6 kb BamHI fragment from the cosmids genomic clone cosB was inserted into the BamHI site of pW6, a transformation vector carrying a mini-white+ gene (Klenzuen et al., 1987). The resulting construct was injected into w; Sb e Delta2-3/TM6, Ubx embryos, which express transposase (Robertson et al., 1988); G0 survivors were single-pair mated to w+ and the G1 was screened for pigment eye color. Independently derived transformants were isolated and mated to w+ to determine the linkage of "w+" and to maintain the line. In one line (G1-1) the fragment had integrated into the Delta2-3, Sb chromosome and was subsequently crossed away from Delta2-3 and Sb. In two others (2A and 3A), w+ was linked to Ubx, a marker on TM6. Males containing these autosomal located transduced DNA fragments were mated with w+101/lot Balancer females to test for the presence of non-Bar eyed zw10 male progeny in the next generation. Three different zw10 alleles (zw10p, zw10dm, and zw10(1D)) were rescued by the 1, 2A and 3A transformant lines. In each case, the low viability, sterility, eye morphology, and mitotic (cytological) phenotypes of the zw10 mutations were complemented by the autosomal fragment.
Results

Cytological Characterization of Mutant Larval Brains

The viability of flies homozygous or hemizygous for mutant alleles of zwiO is severely reduced. The majority of zwiO mutant animals die during late larval and pupal stages (Shannon et al., 1972), survival through embryonic and early larval stages is thought to be ensured by maternal wild-type gene product from heterozygous mothers (Gatti and Baker, 1989; see also Discussion below). Division of brain neuroblasts and imaginal disk cells occurs during the third larval instar, and is required for subsequent adult metamorphosis but not for larval life itself. The third instar larvae of mitotic mutants thus contain populations of cells undergoing improper mitosis. In particular, the dividing cells in the neural ganglia (brain neuroblasts) of third instar larvae are well suited to cytological observation which may suggest the origin of the mitotic defect. To elucidate the role played by the zwiO product in ensuring proper chromosome segregation during cell division, we have continued the analysis of mitosis in zwiO mutant larval neuroblasts initiated by Smith et al. (1985).

Aneuploidy. In preparations of wild-type larval ganglia, four pairs of chromosomes are clearly visible in each metaphase figure (Fig. 1 a). However, a high proportion (~50%) of brain cells in animals homozygous or hemizygous for a variety of zwiO alleles are hyperploid (Table I; Smith et al., 1985). This is almost certainly an underestimate of the number of cells with abnormal chromosome complements: because chromosomes may be lost during squashing, hypoploid nuclei have not been scored in these studies. Examples of hyperploid mitotic figures are shown in Fig. 1, b and c. The distribution of karyotypes (Table I) suggests that zwiO mutations cause essentially random mitotic segregation of chromosomes, with all major chromosomes affected to a similar extent.

Abnormal Anaphases. Aneuploidy caused by zwiO mutations clearly involves improper chromosome segregation at anaphase. In wild-type male larval ganglia, most anaphases resemble that shown in Fig. 1 d; only a small percentage of anaphases can be classified as aberrant. Conversely, a high frequency (~40%) of abnormal anaphases are present in neuroblasts of animals hemizygous for any of several zwiO mutant alleles (Table II). The defect most often observed is the presence of one or more chromatids lagging at the metaphase plate when the remainder have already migrated to their respective poles (Fig. 1, e and f). In some cases, some chromatids are clearly pulled at their kinetochores but are nonetheless delayed in their approach to a pole, while other chromatids appear not to be subjected to poleward forces at the time of fixation (Fig. 1 g). It is of interest that lagging chromatids are often found in the near vicinity of their sisters, which thus appear to be similarly affected (Fig. 1, f and g). In a fraction of cases, anaphases are either completely disorganized (Fig. 1 i), or result in obviously unequal chromosome complements at the two spindle poles (Fig. 1 h). Any of these aberrant events at anaphase could potentially produce aneuploidy in daughter cells.
Figure 1. Cytological effects of zw10 mutations in neuroblast cells. (a–c) Colchicine-treated metaphase figures. (a) Oregon R wild-type males showing two pairs of metacentric autosomes, a telocentric X chromosome, a linear, heterochromatic Y chromosome (4A, XY), and two dot-like fourth chromosomes not scored in subsequent figures. (b) Adjacent aneuploid zw10St/Y cells (4A, XXY and 6A, XY). (c) 7A, 4XY zw10a~ cell. (d–i) Anaphase figures from cells not treated with colchicine. (d) Oregon R wild type. (e–f) zw10St/Y Arrows denote lagging chromatid(s). (g) zw10a~ The open arrow shows a lagging sister chromatid pair; the black arrow indicates a chromatid apparently not subjected to poleward forces. (h) zw10St/Y Unequal anaphase. (i) zw10St/Y Disorganized anaphase. (j–l) Colchicine-treated metaphase figures showing precocious sister chromatid separation in zw10a~ (j and l) and zw10a~ (k) neuroblasts. In some cases, the apposition between sister chromatids, particularly at centromeric regions, remains close (l; arrow in k).

levels of aneuploidy observed in zw10 mutant brain cells. They reported that in many colchicine-induced metaphase figures in mutant ganglia, sister chromatids appeared to be physically unconnected with each other. These investigators proposed that prematurely separated chromatids could establish connections at random to microtubules (MTs) emanating from the two spindle poles, and would thus segregate randomly at anaphase.

We have verified the occurrence of this form of precocious sister chromatid separation (PSCS). In the presence of col-
chicine, 30–40% of zw10 mutant nuclei display one or more chromatid pairs with PSCS (Table II). Sister chromatids are often adjacent to each other, yet appear unattached at their centromeres (Fig. 1, j, k, and l). Control ganglia from C(1)DX, y f/Y siblings of hemizygous mutants show PSCS at a frequency of <2%. Particular care was taken to ensure similar treatment of mutant and control brains. Both types of ganglia were dissected at the same time from animals grown in the same bottle; the brains were mixed and exposed to colchicine in the same vessel. Mutant and control brains were squashed together on the same slide; control ganglia can be recognized by the characteristic morphology of the attached X chromosome (see Materials and Methods). The absence of sister chromatid separation in wild-type, colchine-treated Drosophila neuroblasts has also recently been noted by González et al. (1991).

Although the PSCS phenomenon is a characteristic of zw10 mutants, the hypothesis of Smith et al. (1985) in its simplest form (see above) is unlikely to explain elevated levels of aneuploidy. First, zw10 ganglia cells which have been subjected to neither colchicine nor hypotonic treatments do not show PSCS. Sister chromatids in these neuroblasts remain closely apposed to each other (data not shown); however, it is not clear whether or not the connection at the centromere is broken. A stronger argument is that random attachment of the chromatids to the spindle should not result in the lagging chromosomes or chromatids observed in anaphase figures (although figures with unequal numbers of chromosomes at the two poles would be apparent). We thus consider that PSCS is possibly an artefactual colchicine-dependent effect that may nonetheless reveal important differences between the centromeres of chromosomes in wild-type and mutant cells (see Discussion).

Other Mitotic Parameters. It is conceivable that the cytological effects of zw10 mutations result from difficulties in progression through the cell cycle. For example, lagging chromatids appear at high frequencies after release of cells from a metaphase-arrested state (Hsu and Satya-Prakash, 1985). We have thus measured two parameters that provide some indication of the numbers of cells in various stages of mitosis. The mitotic index, an average of the number of cells undergoing mitosis per optic field, is similar in wild-type larval brains and in larval brains from a mutant that appears to represent the null state of the zw10 locus (see below; Table II). In addition, the ratio between the number of cells in anaphase relative to the total number of mitotic figures is also not grossly affected by this mutation in zw10 (Table II).

Tests for the Null Phenotype. To determine whether the phenotypes discussed above mirror the null state of the zw10 gene, we have examined the cytological characteristics of

### Table I. Aneuploidy in l(l)zw10 MutantBrains

| Genotype | Number of brains | Number of figures | 4AXY | 5AXY | 4AXXY | 4AXYY | 6AXY | 5AXXY | 5AXYY | 7AXY | Other | Percent aneuploid |
|-----------|------------------|------------------|------|------|-------|-------|------|-------|-------|------|-------|------------------|
| zw10hso2/ Y | 6 | 240 | 95 | 41 | 12 | 10 | 13 | 6 | 5 | 3 | 55 | 60.4 |
| zw10hsm/Y | 7 | 232 | 104 | 16 | 11 | 8 | 4 | 3 | 2 | 46 | 55.1 |
| zw10P/Y | 6 | 323 | 147 | 53 | 24 | 19 | 9 | 5 | 6 | 1 | 59 | 54.5 |
| X/Y (Oregon R) | 10 | 1000 | 989 | 41 | 12 | 10 | 13 | 6 | 5 | 3 | 55 | 60.4 |

Neural ganglia from males hemizygous for the indicated zw10 alleles or from wild-type Oregon-R males were dissected, incubated with colchicine, treated with hypotonic solution, fixed, stained, and squashed as described in Materials and Methods. Number of brains and Number of figures refer respectively to the total number of brains and the total number of colchicine-induced metaphase figures examined. The number of figures with particular karyotypes are listed. Because of difficulties in recognition of the small fourth chromosome, only the large second and third autosomes (A), which cannot be distinguished from each other, and the X and Y chromosomes were scored. Thus, the normal diploid male karyotype would be 4AX. Similar results were obtained by Smith et al. (1985) for brains from individuals hemizygous for other l(l)zw10 alleles.

This table catalogs the results of three separate experiments. (a) The percentage of anaphases which appear abnormal is scored under Percent aberrant anaphases. This information was obtained from neural ganglia that were fixed and stained in the absence of colchicine or hypotonic treatments (see Materials and Methods). The first of the adjacent numbers, presented in smaller type and within parentheses, indicates the total number of anaphases scored; the second number refers to the number of brains examined. (b) PSCS shows the percentage of colchicine-induced, hypotonic-treated metaphase figures in which the centromeric connection between one or more pairs of sister chromatids appears to be severed. The larger of the adjacent numbers within parentheses indicates the total number of metaphase figures, the smaller, the total number of brains. (c) Brains fixed and stained without pretreatment (no colchicine or hypotonic incubations) were analyzed for mitotic index (MI) and percentage of total mitotic figures that are in anaphase (Percent anaphase). The mitotic index is expressed as the number of nuclei in division per optic field under standard conditions (see Gatti and Baker, 1989). As shown, hemizygosity for the l(l)zw10 mutation has little effect on the mitotic index. For comparison, known mitotic mutants which result in few or no cells in division have mitotic indices two orders of magnitude lower than wild-type controls, while the mitotic index in brains of individuals carrying mutations causing metaphase arrest is 1.5–6 times higher than controls (Gatti and Baker, 1989). In the last column, both normal- and aberrant-appearing anaphases were combined. By visual inspection, ~40% of l(l)zw10P/Y anaphases in this experiment were aberrant, in accord with the results from the independent experiment tabulated in the second column. For comparison, <0.6% of dividing cells are in anaphase in ganglia from known metaphase arrest mutants (Gatti and Baker, 1989). Adjacent numbers here refer to the total number of optic fields and the number of brains examined (see Materials and Methods).

### Table II. Mitotic Parameters of l(l)zw10 MutantBrains

| Genotype | Percent aberrant anaphases | PSCS | MI | Percent anaphase |
|-----------|----------------------------|------|----|------------------|
| zw10hso2/Y | 40.3 (149;9) | 28.3 (956;6) | ND | ND |
| zw10hsm/Y | 38.6 (153;8) | 40.4 (755;5) | ND | ND |
| zw10P/Y | 47.4 (156;9) | 30.0 (1136;7) | 0.60 | 15.4 (2305;7) |
| C(l)DX,yf/Y | ND | 1.1 (3000;15) | 0.55 | 14.1 (2380;9) |
| X/Y (Oregon R) | 3.2 (222;10) | 1.8 (1000;5) | 0.57 | 14.4 (3211;8) |
| zw10P/Df(l)w122 | 40.7 (222;2) | 32.3 (295;2) | ND | ND |

This table catalogs the results of three separate experiments. (a) The percentage of anaphases which appear abnormal is scored under Percent aberrant anaphases. This information was obtained from neural ganglia that were fixed and stained in the absence of colchicine or hypotonic treatments (see Materials and Methods). The first of the adjacent numbers, presented in smaller type and within parentheses, indicates the total number of anaphases scored; the second number refers to the number of brains examined. (b) PSCS shows the percentage of colchicine-induced, hypotonic-treated metaphase figures in which the centromeric connection between one or more pairs of sister chromatids appears to be severed. The larger of the adjacent numbers within parentheses indicates the total number of metaphase figures, the smaller, the total number of brains. (c) Brains fixed and stained without pretreatment (no colchicine or hypotonic incubations) were analyzed for mitotic index (MI) and percentage of total mitotic figures that are in anaphase (Percent anaphase). The mitotic index is expressed as the number of nuclei in division per optic field under standard conditions (see Gatti and Baker, 1989). As shown, hemizygosity for the l(l)zw10 mutation has little effect on the mitotic index. For comparison, known mitotic mutants which result in few or no cells in division have mitotic indices two orders of magnitude lower than wild-type controls, while the mitotic index in brains of individuals carrying mutations causing metaphase arrest is 1.5–6 times higher than controls (Gatti and Baker, 1989). In the last column, both normal- and aberrant-appearing anaphases were combined. By visual inspection, ~40% of l(l)zw10P/Y anaphases in this experiment were aberrant, in accord with the results from the independent experiment tabulated in the second column. For comparison, <0.6% of dividing cells are in anaphase in ganglia from known metaphase arrest mutants (Gatti and Baker, 1989). Adjacent numbers here refer to the total number of optic fields and the number of brains examined (see Materials and Methods).
larvae heterozygous for a deletion (Df(l)w′) that removes zw10 and for a zw10 mutant allele. Levels of hyperploidy (52.1% [74/142 nuclei] for zw10/Df(l)w′ and 49.6% [64/129] for zw10/Df(l)w′) were similar to values observed in zw10 hemizygous animals (compare with Table I). Both frequencies of aberrant anaphases and PSCS in zw10/Df(l)w′ mitotic figures were also close to values seen for the mutant alleles alone (Table II). If the zw10 alleles examined were hypomorphic, it would be expected that deficiency/mutant heterozygotes would display a stronger phenotype than animals homozygous or hemizygous for the mutation alone. These classical genetic tests therefore suggest that the mutations we have analyzed, all of which have similar effects, characterize the null state of the zw10 gene; this hypothesis is supported by analysis of the zw10 protein in mutant animals, as reported below.

**Molecular Mapping of the zw10 Locus**

A portion of the *Drosophila* X chromosome including the zw10 gene was cloned during the course of a chromosomal walk through the *teste-white* interval (Goldberg et al., 1983; Gunaratne et al., 1986; our own unpublished data). Within this region, zw10 must be located distal (relative to the centromere) of the deletion Df(l)64j4, and completely within the duplication Dp(l;2)w +70h31 (Fig. 2). The locations of the breakpoints associated with these two rearrangements were ascertained by whole genomic Southern blots and by in situ hybridization to salivary gland polytene chromosomes from larvae of the appropriate genotype (Fig. 2; data not shown). These results delimit zw10 to a region of 11 kb within the cloned X chromosome interval.

Two observations suggested that part of the zw10 locus must lie near the Dp(l;2)w +70h31 breakpoint at coordinate −0.3 on Fig. 2. First, the genetic map distance between zw10 and the adjacent lethal complementation group zw4 is very small (<0.026 map units, or <7.8 kb based on a conversion of 1 map units = 300 kb in this region of the X chromosome) (Kidd et al., 1983), yet Dp(l;2)w +70h31 encompasses zw10 but not zw4. Second, a spontaneous allele, zw10 s (Schalet, 1986), is associated with a 4.5-kb insertion of DNA at coordinate +1.7. This DNA insertion in zw10 s is most likely a Doc mobile element, based on the similarity of their restriction maps (Driver et al., 1989).

Northern blot analysis of adult poly(A)+ mRNA re-

---

**Figure 2.** Genetic and molecular map of the zw10 region. Lethal complementation groups in the vicinity of l(1)zw10 are shown at the top of the figure, distances between genes are given in centimorgans (Judd et al., 1972). (b) Loci contained within the duplication Dp(l;2)w +70h31; genes deleted in Df(l)64j4 are indicated by the hatched bar. The molecular map is shown only for a 16-kb region immediately adjacent to the zw10 gene; coordinates are given in kb, with 0 defined as the BamHI site at the left end of Fragment A used to rescue zw10 phenotypes (see text). The mobile element in zw10 s is diagrammed to the same scale as the remainder of the restriction map. The direction of transcription producing poly(A)+ RNAs from this region is shown by arrows; the sizes of mature transcripts are indicated below. H, HindIII; X, Xhol; B, BamHI; R, EcoRI; C, SacI; N, NotI; and S, SalI.
revealed a 2.6-kb transcript located just proximal to the Dp(l;2)w77A3 breakpoint and that spanned the site of the zwl0 insertion element (Fig. 2); this was therefore a likely candidate for the zwi0 mRNA. A 4.8-kb fragment of genomic DNA (Fragment A: coordinates 0–4.8 on Fig. 2), encoding the entire 2.6-kb transcript but containing no other complete transcriptional unit, was transformed into Drosophila by P element-mediated germline transformation. This fragment, as present in three independent autosomal transformant lines, is necessary and sufficient to rescue the zwl0 lethal phenotype of several zwi0 alleles (Materials and Methods). Fragment A also repairs the zwi0 mitotic phenotype. For example, in rescued zwi0/Y; P[Fragment A]/+ larval ganglia, the frequency of aneuploidy (1.2%) and PSCS (4.3%) were at wild type levels (compare with Table II), while anaphases were normal (not shown). Thus, the entire zwi0 locus must reside within the 4.8-kb Fragment A depicted in Fig. 2, and appears to be transcribed into a poly(A)+ RNA 2.6 kb in length.

**Molecular Analysis of the zwi0 Locus**

The zwi0 transcript is developmentally regulated. The highest levels of this RNA are found in embryos and in adult females (Fig. 3), consistent with the idea that maternally supplied zwi0 supports the rapid syncytial divisions of early embryogenesis. Levels of zwi0 RNA are substantially decreased during the first and second larval instar, but then increase in third instar larvae and in early pupae. Presumably, this reflects the increased number of proliferating cells in late larval/early pupal imaginal discs. Only a single 2.6-kb RNA band is seen at any stage of development on Northern blots; it nonetheless remains possible that alternatively processed species of similar length, or rare RNAs of different sizes, may be produced from the same transcriptional unit.

Three cDNA clones homologous to the 2.6-kb zwi0 mRNA were isolated from an imaginal disc cDNA library (Brown and Kafatos, 1988). Substantial overlap in the restriction maps of these cDNAs suggests that they all represent the same species (data not shown). The sequence of the longest of these cDNAs (2,576 bp) contains an open reading frame beginning at nucleotide 103 that encodes a protein of 721 amino acids (Fig. 4). However, it should be cautioned that the nucleotides in the vicinity of this putative initiation codon show only partial agreement with the Drosophila translation start consensus (C A C/A C/A AUG; Cavener and Ray, 1991). Better matches are seen in the vicinity of the downstream methionine codons at nucleotide positions 232–235, 343–345, and 400–402. Although it is thus possible that translation of the corresponding mRNA may initiate at any of these positions, the size of the zwi0 protein (see below) is most consistent with use of the codon indicated in Fig. 4.

A computerized search has failed to reveal significant homologies to any protein within the Genbank, EMBL, PIR, and Swiss-Prot databases. Given the cell cycle-dependent changes in the intracellular location of zwi0 protein that will be described below, it is of interest that the zwi0 sequence contains motifs that may be subject to phosphorylation. A potential tyrosine kinase phosphorylation site (consensus sequence R/K [2,3] D/E [2,3] Y) is found beginning at amino acid 48, while sites possibly available for phosphorylation by the cdc2 kinase (TP and SP) start at amino acids 63 and 167 (Shalloway and Shenoy, 1991).

**Antibodies Against the zwi0 Protein**

Polyclonal rabbit antibodies were generated against gel-purified β-galactosidase-zwi0 fusion protein (β-gal–zwi0) that had been produced in E. coli cells. Crude antisera were purified by immunoaffinity chromatography against trpE–zwi0 protein fusions (see Materials and Methods for further information concerning the preparation and characterization of these reagents). The purified antibodies recognize a single band of 85 kD, consistent with the size of the predicted zwi0 protein, in Western blots of 0–16-h old embryos, of third instar larvae, and of Drosophila Schneider Line 2 tissue culture cells (Fig. 5, A and B). When the same Schneider cell line is transfected with a construct containing the complete zwi0 open reading frame under the control of the strong transcriptional unit, the frequency of aneuploidy (1.2%) and PSCS (4.3%) were at wild type levels (compare with Table II), while anaphases were normal (not shown). Thus, the entire zwi0 locus must reside within the 4.8-kb Fragment A depicted in Fig. 2, and appears to be transcribed into a poly(A)+ RNA 2.6 kb in length.

**Figure 3. Developmental regulation of the zwi0 transcript.** The poly(A)+ RNA fractions from various stages (0–24-h embryos, first, second, and third larval instars, light early pupae [E] and dark late pupae [L]), adult males [M] and adult females [F] were isolated (see Materials and Methods). In the blot shown, 10 μg of poly(A)+ RNA was loaded in each lane. The zwi0 probe used was a fragment containing the full-length zwi0 cDNA, and hybridizes to a single 2.6-kb transcript. Hybridization of the same Northern blot with a probe prepared from Dmras64B4 (ras) (Mazer et al., 1985) to control for loading is shown below.
Figure 4. Sequence of the zw10 cDNA. The nucleotide sequence of the coding strand of the longest zw10 cDNA clone, with position 1 assigned to the Y-most base. The ATG at 103 is the most likely site of initiation, but other ATGs (at 232, 343, and 400) are also possible choices (see text). A stop codon (TAG) at position 2,268 has been signified by an asterisk. The sequence is thus postulated to contain a 5'-untranslated region from nucleotides 1-102, and a 3'-untranslated region from nucleotides 2,269-2,576. A single long open reading frame encodes a predicted protein sequence, here represented by the single-letter code, of 721 amino acids in length, having a molecular weight of 82,115. These sequence data are available from EMBL/GenBank/DDBJ under accession number X64390.

actin5C promoter (Bond and Davidson, 1986), this same band is clearly overproduced (Fig. 5 A).

Evidence that the antibody reacts specifically with the zw10 gene product is provided by the analysis of extracts from larvae hemizygous for mutant zw10 alleles. The zw1051 and zw102° mutants have no detectable 85-kd zw10 protein (Fig. 5 B). The zw1051 allele does not appear to encode stable zw10 cross-reactive polypeptides, and is thus likely to represent the null state of the locus. This result is expected, because the zw1051 gene is interrupted by a DNA insertion (Fig. 2), These exogenous sequences are located within an exon near the middle of the coding sequence (see Materials and Methods), between nucleotides 1,220 and 1,375 (Fig. 4). Males of genotype zw102°/Y display a band of ~75 kD that we presume to be a truncated product caused by a nonsense or frameshift mutation; the amount of this 75-kD protein is reduced relative to the amount of 85-kD zw10 protein in wild type (Fig. 5 B).

Immunolocalization of the zw10 Antigen
To determine the location of zw10 protein at different stages of the cell cycle, we chose to examine mitosis in syncytial blastoderm embryos by indirect immunofluorescence using
of these the purified antibodies described above. Distinctive patterns are observed at different stages of mitosis. The progression distribution identical in the mitotic domains of cellularized embryos derm stages 9-13 (data not shown).

**Figure 5.** Detection of the zw10 protein on Western Blots. (A) The zw10 protein is recognized by purified antibodies. Protein extracts from *Drosophila* Schneider Line 2 tissue culture cells (S2), 0-16-h-old embryos, and Schneider cells transfected with a construct placing the zw10 cDNA under the control of the high-level actin5C promoter (S2+pPAC-zw10) were subjected to SDS-PAGE and blotted, followed by detection with an affinity-purified polyclonal antibody to zw10 epitopes (see Materials and Methods). The 84-kd zw10 protein (arrowhead, left) is overexpressed when pPAC-zw10 is transfected into S2 cells. (B) The zw10 protein is altered in zw10 mutants. In wild-type larvae (lane 1) the zw10 protein appears as a single band of 85 kD (arrowhead, left). In zw10^0^ larvae, there is no detectable protein of this size present (lane 2). In zw10^{go^{p}} larvae (lane 3), protein of the wild type size is also not observed, and is replaced by a cross-reacting protein of ∼75 kD present in lower abundance (smaller arrowhead, right). Total protein from two third-instar male larvae of the appropriate genotype was loaded in each lane. Anti-zw10 crude serum was diluted 1/700. (C and D) Reaction of the filters depicted in A and B, respectively, with antibody to a 54-kD *Drosophila* protein (R. Gandhi and M. L. Goldberg, manuscript in preparation) as a loading control.

the purified antibodies described above. Distinctive patterns are observed at different stages of mitosis. The progression of these zw10 staining patterns through the cell cycle is also identical in the mitotic domains of cellularized embryos (Foe, 1989) and in larval neuroblast cells, so the observed distribution of zw10 protein is not specific to syncytial blastoderm stages 9-13 (data not shown).

**Prometaphase to Metaphase.** During interphase and most of prophase, the zw10 protein appears to be excluded from the nucleus (see below). At a time we assume corresponds to the partial breakdown of the nuclear envelope at the beginning of prometaphase (Stafstrom and Staehelin, 1984; Hiraoka et al., 1990), zw10 antigen starts to coalesce in the nuclear domain (Fig. 6, a and f). The staining is mostly amorphous and surrounds the condensing chromosomes, but a few discrete spots appear to be recognized by the antibody.

By metaphase, zw10 protein becomes localized to discrete, filamentous structures residing in the central, longitudinal portion of the spindle (Fig. 6, b and e; Fig. 7, A–C). The strands originate near the centrosomes at opposite poles of the spindle apparatus, but zw10 staining of the centrosomes per se is not observed (Fig. 7 B). Remarkably, and in contrast with the total tubulin pattern, zw10 antigen appears to pass directly through the chromosomal mass at the metaphase plate (Fig. 7, A and C).

**Anaphase.** At the beginning of anaphase, zw10 antigen becomes excluded from the region of the metaphase plate, and the filaments shorten from their ends nearest the centrosomes. As a result, zw10 protein becomes concentrated into punctate structures at the leading edges of the separated chromatids (Fig. 6, c and f; Fig. 7, D–F). At this level of resolution, the number and position of these structures is consistent with localization at the centromere/kinetochore region of individual chromatids. The zw10 protein appears to remain at or near the kinetochores through the remainder of anaphase, although the shortening of kinetochore microtubules (KMTs) at these later stages of anaphase renders resolution in the kinetochore–centrosome interval difficult (Fig. 6, g and j).

The transition between the metaphase and early anaphase states of zw10 is very rapid. Mitosis in syncytial *Drosophila* embryos is metasynchronous: division starts at successively later times in nuclei increasingly closer to the embryonic equator, forming a mitotic wave (Foe and Alberts, 1983). As seen in Fig 8, adjacent nuclei can display the mature metaphase and anaphase zw10 patterns, but intermediate structures are sometimes observed. Although the rate of mitotic wave propagation is quite variable, at an average value of 100 μm/min (Foe and Alberts, 1983), and given the approximate distance between adjacent nuclei (∼20 μm at blastoderm stages 11–12), we estimate that the transition between the metaphase and anaphase states of zw10 may be accomplished in periods as short as 10–12 s.

**Telophase, Interphase, and Prophase.** At the beginning of telophase, the zw10 antigen becomes excluded from the domain of the reforming nucleus (Fig. 6, h and k). Staining is also restricted to the extranuclear cytoplasm during interphase (Fig. 6, i and l) and prophase (not shown).

**Discussion**

**zw10 Mutations Affect Chromosome Segregation**

Several observations indicate that zw10^0^ function is necessary to ensure accurate chromosome segregation during cell division in most, if not all, *Drosophila* tissues. (a) A temperature-sensitive mutation of zw10 (zw10^ts^) caused a 120-fold increase in the incidence of clones of homozygous multiple wing hair (mwh) cells in the wings of zw10^ts^/Y; mwh+/ males raised at semi-restrictive temperature. Additional tests implicated an elevated frequency of mitotic non-
Figure 6. Immunolocalization of the zw10 protein during the cell cycle in wild-type syncytial blastoderm embryos. Embryos were fixed, stained, and processed for indirect immunofluorescence as described (see Materials and Methods). (a–c and g–i) zw10 protein localization is shown; below each of these is shown the corresponding DNA staining (d–f and j–l). (a and c) Prometaphase; zw10 protein moves into the nuclear domain; punctate staining is visible. (b and e) Metaphase; zw10 filamentous strands are completely formed; considerable substructure is apparent. (c and f) Anaphase; zw10 protein is rapidly relocalized to the kinetochore regions of separating sister chromatids (arrows). (g and j) Late anaphase; zw10 protein remains on kinetochores (arrows). (h and k) Telophase; zw10 antigen disappears from kinetochores. Faint cytoplasmic staining is apparent. (i and l) Interphase; no nuclear localization is visible. Bar, 10 μm.

Disjunction in the formation of these somatic clones (Smith et al., 1985). (b) As discussed above, a high proportion of zw10 mutant larval brain neuroblast cells are aneuploid. Many anaphase figures in these cells are obviously aberrant. (c) Upon completion of the second meiotic division in escaper males, spermatid nuclei containing different numbers of chromosomes are produced (our own unpublished observations). It is not presently clear whether missegregation occurs during the first or second meiotic divisions, or during earlier mitoses in the male germline. (d) Maternally supplied zw10 gene product is necessary for embryogenesis, as shown by germline clonal analysis (Perrimon et al.,...
Figure 7. Localization of zw10 protein in comparison with chromosomes, centrosomes, and tubulin. The embryos were processed for indirect immunofluorescence and confocal microscopy as outlined in the Materials and Methods. Superimposed images were obtained from the same focal plane. (A) At metaphase, zw10 protein (orange) filaments extend through the chromosomal mass (blue) and have considerable substructure (particularly apparent in the nucleus at far left). (B) Centrosomes (yellow) and the zw10 protein (red) at metaphase. zw10 protein filaments extend to, but do not overlap with, centrosomes. (C) Tubulin staining (green) and zw10 protein (red) at metaphase. The zw10 protein is localized to only a subsection of the mitotic spindle (arrow; regions of overlap between zw10 and tubulin are yellow.) (D) Regions in or near kinetochores of chromosomes (blue) are the sites of zw10 protein localization (red) during early-mid anaphase. (E) At early anaphase, centrosomes (yellow; solid arrows) are well resolved from zw10 (red; outline arrow). (F) zw10 (red) is restricted to discrete spots at the centrosome-distal portion of each hemispindle at anaphase onset (tubulin staining is green while overlap is yellow). Thus, zw10 is not found along the length of the KMTs at this stage of the cell cycle. Bars, 10 μm.

1989). In addition, syncytial blastoderm embryos derived from zw10 escaper females (see below) have abnormally spaced nuclei of aberrant morphology (our own unpublished results).

These findings may be understood in terms of the varying requirements for cell division at different stages in Drosophila development. Maternally derived gene products within the egg must be used to construct the molecular machinery required for the rapid embryonic mitoses after fertilization, because little transcriptional activity occurs during this period. For many proteins involved in mitosis, the maternal contribution is sufficient to allow development into larval
stages (Gatti and Baker, 1989). Larval growth per se is accomplished through an increase in cell size, accompanied in some tissues by polytenization. Cell division within the larva is in general restricted to tissues that will play a role in subsequent adult morphogenesis: the nervous system, imaginal discs, and abdominal histoblasts.

The phenotype of zw10 mutants is consistent with this picture. Because sufficient zw10 product is supplied by zw10/+ heterozygous mothers, zw10 embryos can survive as larvae. However, zygotic expression of zw10 in the larval tissues enumerated above would be necessary for further development. Mitosis in larval brain neuroblasts and the imaginal disks giving rise to the adult cuticle would therefore be abnormal, resulting in substantial late larval/pupal lethality (Gatti and Baker, 1989). On the other hand, effects of zw10 mutations on embryogenesis would only be observed when zw10 protein within the egg is depleted, as in eggs produced by homozygous escaper females.

**Cytological Effects of zw10 Mutations**

The evidence presented above clearly shows that mutations in the zw10 gene cause aberrant anaphases within larval neuroblasts, in turn generating a high proportion of aneuploid brain cells. We have also verified that the phenomenon of PSCS is an additional consequence of zw10 lesions in colchicine-treated neural ganglia. However, the proposition that the anaphase defects observed are due to PSCS before anaphase onset remains questionable, because of uncertainties about the state of cells exposed to colchicine and because of our lack of knowledge concerning the forces that determine sister chromatid interactions.

The consideration of published precedents for the cytological phenotypes discussed above may be instructive in speculations concerning potential functions of the zw10 protein. Lagging sister chromatids have been postulated to result from damage to the centromere/kinetochore produced by drugs (Brinkley et al., 1985; Hsu and Satya-Prakash, 1985), by injection with anticientromere antibodies (Bernat et al., 1991), or by microirradiation of kinetochores with a laser (McNeill and Berns, 1981). PSCS has been documented in two Drosophila meiotic mutants, orientation disruptor (ord) (Goldstein, 1980; Lin and Church, 1982) and mei-5332 (Davis, 1971; Kerrebrock et al., 1992). Finally, several cytological features of zw10 mutations, including aneuploidy and lagging chromatids at anaphase, have been noted both in Drosophila mutant for the gene rough deal (rod) (Karess and Glover, 1989) and in cell cultures derived from patients with the human genetic disorder Roberts Syndrome (Jabs et al., 1991).

A puzzling phenotypic consequence of mutations in zw10 arises from the survival of a small number of "escaper" adults of both sexes that are either hemizygous or homozygous for all known zw10 mutations. Both male and female escapers are sterile and exhibit a variety of cuticular defects (Shannon et al., 1972). Although the sterility of zw10 escaper females can be explained by embryonic mitotic defects we have observed in their progeny, the fact that mutant hemizygous males contain immotile sperm (Shannon et al., 1972; our own unpublished observations) is more difficult to understand. Examination of the "onion stage" of spermatogenesis in mutant testes shows the presence of variable-sized spermatid nuclei, indicating that chromosomal nondisjunction or chromosome loss has occurred in the male germline. However, this alone does not account for sperm immotility: even sperm that contain only the tiny fourth chromosome are motile and capable of fertilization (Lindsley and Grell, 1969). The zw10 product might therefore play an additional role in spermatogenesis or sperm function that is independent of chromosome segregation.

**zw10 Protein Is Dynamically Localized in Mitotic Structures**

Embryonic zw10 protein undergoes cell-cycle dependent redistribution to different components of the mitotic apparatus. At the prophase–prometaphase transition, zw10 protein becomes localized in the nuclear domain, and becomes associated with, or forms, a filamentous structure which persists through metaphase. A very rapid transition to a region at or near kinetochores is seen coincident with anaphase onset. At telophase, the zw10 protein is excluded from the reforming nuclear domain and becomes dispersed in the cytoplasm. The dynamic nature of zw10 distribution through these cell cycles is most likely because of intracellular movement of the same protein pool, rather than to reflect new protein synthesis, given the rapidity of embryonic nuclear divisions (Foe and Alberts, 1983).

The nature of the structures recognized by anti-zw10 antibodies at metaphase and anaphase is uncertain. It is possible that the zw10 protein filaments form an independent structure not directly associated with MTs. Alternatively, some manner of zw10 association with spindle MTs would seem possible. It is clear that the zw10 antigen could not be associated...
with all MTs within the spindle, but is instead generally confined to a narrower region than the wide barrel-shaped spindle (Fig. 7 C). Thus, zw10 protein may be specifically associated with the KMTs, in accordance with its movement to the kinetochore region at anaphase onset. Moreover, although zw10 protein colocalizes with the leading edges of the chromosomes during anaphase at the resolution of light microscopy, we have no evidence that this site corresponds to the kinetochore per se. The resolution of these issues awaits further ultrastructural and biochemical studies.

The zw10 protein does not appear to correspond to any of the large number of proteins already known to inhabit the mitotic spindle or the kinetochore/centromere. Such proteins have been identified by several protocols: as microtubule-associated proteins (MAPs) based on their copurification with MTs (reviewed by Olmstead, 1986; Kellogg et al., 1989), on the basis of antisera obtained by immunization against nuclei (Frasch et al., 1986) or mitotic chromosome scaffolds (Compton et al., 1991), or as polypeptides recognized by antisera from various patients with autoimmune disease (CREST sera; Moroi et al., 1980). Recently, it has also been observed that cytoplasmic dynein is associated with kinetochores and the spindle (Pfarr et al., 1990; Steuer et al., 1990; Wordeman et al., 1991). To our knowledge, none of these or other components show the same pattern of cell cycle-specific localization as zw10 (see Brinkley, 1990; Pluta et al., 1990; Pankov et al., 1990; Earnshaw and Cooke, 1991).

The structure assumed by the zw10 antigen at metaphase is strongly evocative of the location of an antigen called spoke (Paddy and Chelsky, 1991). Anti-spoke antibodies stain KMTs, revealing a filamentous structure with a regular helical substructure, similar to that seen in Fig. 6, b and e and Fig. 7, A–C. However, we do not believe that zw10 represents the Drosophila homolog of spoke: unlike spoke, the metaphase structures identified by anti-zw10 antibodies extend through the chromosomal mass. Furthermore, spoke is not redistributed onto kinetochores at anaphase, and instead remains associated with KMTs (Paddy and Chelsky, 1991). It nonetheless remains possible that the zw10 metaphase structure is formed in association with a putative Drosophila spoke polypeptide.

The Role of zw10 in Mitotic Chromosome Segregation

Although the cytological evidence clearly shows that the zw10 product is required to ensure the accuracy of mitotic chromosome segregation, several unresolved issues preclude precise determination of its molecular function. Perhaps most importantly, we do not yet understand the significance of the high levels of PSCS observed in colchicine-treated zw10 neuroblastos. It is unclear whether this phenomenon monitors some indirect but nonetheless differential response of zw10 and wild-type brain cells to colchicine, or instead indicates differences in some inherent property of the centromeric attachment between sister chromatids. In addition, because zw10 mutations do not result in cell cycle arrest, zw10 activity cannot be unambiguously ascribed to a particular phase of the cell cycle. In spite of these gaps in our knowledge, the available cytological and immunocytochemical results nonetheless provides some clues to the possible roles played by the zw10 protein.

zw10 protein in the structure seen during metaphase that is roughly coincident with the spindle could be imagined to function in any of several ways. This protein could be of importance for spindle organization, for chromosome attachment or anchorage to the spindle, for ensuring the bipolar connection of sister kinetochores to opposite spindle poles, or for chromosomal movement during congression to the metaphase plate. However, preliminary three-dimensional observations in zw10 mutant brains and in embryos produced by homozygous mutant germline clones show no obvious defects in the structures of the metaphase spindle or of the metaphase plate (our own unpublished results). Of course, we cannot exclude the possibilities that subtle metaphase defects occur, or that zw10 activity during metaphase is required for subsequent anaphase movements. In addition, it should be remembered that one phenotypic effect of zw10 mutations, that of PSCS, presumably occurs during a metaphase-like state induced by colchicine (González et al., 1991).

We believe that hypotheses predicting an activity of zw10 protein at anaphase onset (rather than earlier at metaphase) are more compatible with the apparent normality of metaphase in mutants and with the extremely rapid transition between metaphase and anaphase locations of the zw10 antigen. The nature of the signals governing entry into anaphase remains quite mysterious (Murray et al., 1989), and little is understood of the crucial process that breaks the centromeric connection between sister chromatids at the beginning of anaphase (Murray and Szostak, 1985). We can thus only guess at the manner by which the movement of zw10 to the kinetochore region at anaphase onset could influence the accuracy of sister chromatid disjunction.

zw10 could be imagined to be a partially redundant component of a system positively required to activate sister chromatid separation or chromatid movement to the poles. Alternatively, zw10 protein could act as a feedback control rendering certain events at anaphase onset dependent upon the successful completion of earlier events. For example, zw10 might help ensure that anaphase will not begin if the spindle is not intact or if one or more chromosomes have not yet become properly aligned at the metaphase plate. It has been suggested that kinetochores unattached to the spindle may generate signals blocking anaphase onset (Zirkle, 1970; Ault and Nicklas, 1989; Rieder and Alexander, 1989; Bernat et al., 1991). Recently, "checkpoint" genes that apparently fulfill such a role have been found in yeast by Li and Murray (1991) and by Hoyt et al. (1991). Because we observe no obvious changes in cell cycle progression in zw10 mutants (Table II), delays of anaphase onset caused by zw10 activity would have to be quite short. In a different scenario, zw10 might render sister chromatid separation dependent upon M phase promoting factor (MPF) inactivation, which is normally a precondition for most anaphase events (Murray et al., 1989). Loss of such zw10-mediated feedback inhibition could potentially explain the PSCS phenomenon observed in colchicine-treated mutant neuroblasts that retain high levels of MPF activity (Whitfield et al., 1990).

The unexpected distribution of the zw10 protein as a function of the cell cycle remains difficult to interpret in terms of molecular activities that explain the observed phenotypes. We nonetheless believe that these initial results are sufficiently intriguing that future studies of the zw10 protein and phenotype will provide unique insights into the function of the spindle and of the centromere/kinetochore.
We thank Maurizio Gatti, David Glover, and the members of their respective laboratories for their generosity, advice and assistance. We are indebted to Burke Judd and Abe Schalet for fly stocks, David Glover for anticentromere antibodies, Janis Werner for embryo microinjection, Gerry Chu for providing Northern blot strips, Ingrid Monteleone for supplying fly media, Diane LaPoint and Deborah Whiting for help with antibody production, and James Slattery, Carol Bayles, and Cathy Anderson for assistance with the confocal microscope at Cornell.

This work was supported by National Institutes of Health (NIH) grant GM13935 and an NIH Fogarty Center Senior International Fellowship to M. L. Goldberg. B. C. Williams was supported by NIH training grant GM07617 to the Field of Genetics and Development at Cornell University. Received for publication 3 February 1992 and in revised form 13 May 1992.

References

Ault, J. G., and R. B. Nicklas. 1989. Tension, microtubule rearrangements, and the proper distribution of chromosomes in mitosis. Chromosoma (Berl.). 98:33–39.

Bier, S. D., and A. W. Cleveland. 1989. Mitotic domains reveal early commitment of cells in functional laboratories for their generosity, advice and assistance. We are in-...
Paddy, M. R., and D. Chelsky. 1991. Spoke: a 120-kD protein associated with a novel filamentous structure on or near kinetochore microtubules in the mitotic spindle. *J. Cell Biol.* 113:161–171.

Pankov, R., M. Lemieux, and R. Hancock. 1990. An antigen located in the kinetochore region in metaphase and on polar microtubule ends in the midbody region in anaphase, characterized using a monoclonal antibody. *Chromosoma (Berl.*) 99:95–101.

Perrimon, N., L. Engstrom, and A. P. Mahowald. 1989. Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster*. I. Loci on the X Chromosome. *Genetics* 121:333–352.

Pfarr, C. M., M. Coue, P. M. Grissom, T. S. Hays, M. E. Porter, and J. R. McIntosh. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature (Lond.*) 345:263–265.

Pluta, A. F., C. A. Cooke, and W. C. Earnshaw. 1990. Structure of the human centromere at metaphase. *Trends Biol. Sci.* 15:181–185.

Rieder, C. L., and S. P. Alexander. 1989. The attachment of chromosomes to the mitotic spindle and the production of aneuploidy in newt lung cells. *In Mechanisms of Chromosome Distribution and Aneuploidy. M. A. Resnick and B. K. Vig, editors. Alan R. Liss Inc., New York. 185–194.

Robertson, H. M., C. R. Preston, R. W. Phillips, D. Johnson-Schlitz, W. K. Benz, and W. R. Engels. 1988. A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics* 118:461–470.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual. Second Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Volumes 1–3.*

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Schalet, A. 1986. The distribution of and complementation relationships between spontaneous X-linked recessive lethal mutations recovered from crossing long-term laboratory stocks of *Drosophila melanogaster*. *Mutat. Res.* 163:113–144.

Shalloway, D., and S. Shenoy. 1991. Oncoprotein kinases in mitosis. *Adv. Cancer Res.* 57:185–225.

Shannon, M. F., T. C. Kaufman, M. W. Shen, and B. H. Judd. 1972. Lethality patterns and morphology of selected lethal and semi-lethal mutations in the *zeste-white* region of *Drosophila melanogaster*. *Genetics.* 72:615–638.

Smith, D. A., B. S. Baker, and M. Gatti. 1985. Mutations in genes controlling essential mitotic functions in *Drosophila melanogaster*. *Genetics* 110:647–670.

Stafstrom, J. P., and L. A. Staehelein. 1984. Dynamics of the nuclear envelope and of nuclear pore complexes during mitosis in the *Drosophila* embryo. *Eur. J. Cell Biol.* 34:179–189.

SteuEr, E. R., L. Wordeman, T. A. Schroer, and M. P.Sheetz. 1990. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature (Lond.*) 345:266–268.

Xiao, H., and J. T. Lis. 1989. Heat shock and developmental regulation of the *Drosophila melanogaster hsp83* gene. *Mol. Cell. Biol.* 9:1746–1753.

Warn, R. M., and A. Warn. 1986. Microtubule arrays present during the syncytial and cellular blastoderm stages of the early *Drosophila* embryo. *Exp. Cell Res.* 163:201–210.

Whitfield, W. G. F., C. González, G. Maldonado-Codina, and D. M. Glover. 1990. The A and B type cyclins are accumulated and destroyed in temporally distinct events that define separable phases of the G2/M transition. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2563–2572.

Wordeman, L., E. R. Steuer, M. P. Sheetz, and T. Mitchison. 1991. Chemical subdomains within the kinetochore domain of isolated CHO mitotic chromosomes. *J. Cell Biol.* 114:285–294.

Zirkle, R. E. 1970. Ultraviolet-microbeam irradiation of newt-cell cytoplasm: spindle destruction, false anaphase, and delay of true anaphase. *Radiat. Res.* 41:516–537.