Journal of Cell Science 110, 451-464 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS3456

Monastral bipolar spindles: implications for dynamic centrosome organization

P. G. Wilson[1,2,*], M. T. Fuller[2] and G. G. Borisy[1]

[1]Laboratory of Molecular Biology, University of Wisconsin, Madison WI 53706, USA
[2]Department of Developmental Biology, Stanford University School of Medicine, Stanford CA 94305, USA

*Author for correspondence

SUMMARY

Implicit to all models for mitotic spindle assembly is the view that centrosomes are essentially permanent structures. Yet, immunofluorescence revealed that spindles in larval brains of *urchin* mutants in *Drosophila* were frequently monastral but bipolar; the astral pole contained a centrosome while the opposing anastral pole showed neither gamma tubulin nor a radial array of astral microtubules. Thus, mutations in the *urchin* gene seem to uncouple centrosome organization and spindle bipolarity in mitotic cells. Hypomorphic mutants showed a high frequency of monastral bipolar spindles but low frequencies of polyploidy, suggesting that monastral bipolar spindles might be functional. To test this hypothesis, we performed pedigree analysis of centrosome distribution and spindle structure in the four mitotic divisions of gonial cells. Prophase gonial cells showed two centrosomes, suggesting cells entered mitosis with the normal number of centrosomes and that centrosomes separated during prophase. Despite a high frequency of monastral bipolar spindles, the end products of the four mitotic divisions were equivalent in size and chromatin content. These results indicate that monastral bipolar spindles are functional and that the daughter cell derived from the anastral pole can assemble a functional bipolar spindle in the subsequent cell cycle. Cell proliferation despite high frequencies of monastral bipolar spindles can be explained if centrosome structure in mitotic cells is dynamic, allowing transient and benign disorganization of pericentriolar components. Since *urchin* proved to be allelic to *KLP61F* which encodes a kinesin related motor protein (Heck et al. (1993) *J. Cell Biol.* 123, 665-671), our results suggest that motors influence the dynamic organization of centrosomes.

Key words: Centrosome, Cytoskeleton, Kinesin, Mitosis

INTRODUCTION

Bipolarity of mitotic spindles is fundamental to accurate chromosome transmission at cell division. In animal cells containing a discrete centrosome, assembly of a bipolar spindle is initiated at the onset of mitosis when two centrosomes separate from each other and move toward opposing sides of the nucleus. After breakdown of the nuclear envelope, sister kinetochores of chromosomes attach to microtubules radiating from opposing centrosomes and the chromosomes then congress to form a metaphase plate. Thus, both centrosomes and chromosomes contribute to spindle bipolarity. Anaphase followed by cytokinesis delivers centrosomes as well as chromosomes to each daughter cell. As both centrosomes and chromosomes participate in mitosis, it has been difficult to clearly establish whether centrosome separation is essential for spindle bipolarity or whether a bipolar spindle can be organized by chromosomes alone. Mutants that uncouple centrosome organization from spindle bipolarity should therefore be helpful in unraveling the relative contributions of centrosomes and chromosomes to spindle organization.

Centrosomes in virtually all animal cells consist of a pair of centrioles surrounded by a cloud of pericentriolar material whose size and activity varies as a function of the cell cycle. Microtubules are nucleated within the pericentriolar cloud (Robbins et al., 1968; Gould and Borisy, 1977; Moritz et al., 1995a), their minus ends within or near the pericentriolar material and their plus ends extending into the cytoplasm. Centrioles are not required for microtubule nucleation (Szollosi et al., 1972), but appear to facilitate organization of dispersed pericentriolar components into a focus (Calarco-Gillam et al., 1983). Thus, organization of pericentriolar components at centrioles effectively determines the polarity of microtubule arrays. Gamma tubulin, a highly conserved member of the tubulin superfamily (Oakley and Oakley, 1989; Zheng et al., 1991; Stearns et al., 1991; Horio et al., 1991; Sorbel and Snyder, 1995), is present at the minus end of centrosomal microtubules (Moritz et al., 1995b) and, together with other components in a ring complex, nucleates microtubule assembly in vitro (Zheng et al., 1995). Loss of gamma tubulin function results in loss of microtubules or microtubule organization in *Aspergillus* (Oakley et al., 1990), mouse oocytes (Joshi et al., 1992), and *Xenopus* egg extracts (Felix et al., 1994; Stearns and Kirschner, 1994), larval brains in *Drosophila* (Sunkel et al., 1995) and *Saccharomyces* (Sorbel and Snyder, 1995). Gamma tubulin is probably not sufficient for microtubule nucleation in mammalian cells since immunoinactivation of pericentrin, source of the well known 5051 antigen in sclero-

derma patients, also limits the number of microtubules nucleated at centrosomes in vitro (Doxsey et al., 1994).

Non-canonical spindle structures generated in meiotic cells have raised the question of whether centrosome separation is required for spindle bipolarity. When one centrosome was forced off the nuclear envelope in *Pales* spermatocytes by mechanical intervention during prophase or prometaphase, a monastral but bipolar spindle was observed in metaphase (Dietz, 1966; Bastmeyer et al., 1986; Steffen et al., 1986). Only one pole of these monastral bipolar spindles was associated with a centrosome and a radial array of astral microtubules while the opposing pole was acentrosomal. Monastral bipolar spindles have also been reported in meiotic divisions of two male sterile mutants in *Drosophila*, *ms(1)516* and *ms(1)RD7* (Lifschytz and Meyer, 1977; Lifschytz and Hareven, 1977). In an independent study, bivalents pulled off meiotic spindles and placed in the cytoplasm of *Drosophila* spermatocytes showed microtubules attached to both kinetochores, but free of centrosomes (Church et al., 1986). These studies have been interpreted to indicate that meiotic chromosomes in *Drosophila* and *Pales* are necessary and sufficient to organize microtubules into a bipolar spindle. Similar arguments have been invoked to explain assembly of meiotic spindles in *Drosophila* females, given an apparent absence of centrosomes in female meiosis (Theurkauf and Hawley, 1992). However, as a recent study has identified microtubule organizing centers that contain centrosomal antigens at the poles of meiosis II spindles in females (Riparbelli and Callaini, 1996), the role of microtubule organizing centers in female meiosis in *Drosophila* is not yet clear.

If chromosomes are indeed necessary and sufficient to organize a bipolar spindle, then centrosomes would seem to be dispensable and centriole duplication unnecessary. However, cells that enter mitosis with a single centriole pair assemble a monopolar rather than a bipolar spindle (Sluder and Rieder, 1985; Sluder et al., 1986, 1989). In the general case, the two centrioles of a centriole pair disjoin, usually in early G_1, and each buds a procentriole that elongates throughout interphase (Kuriyama and Borisy, 1981). Treatment of sea urchin eggs with reducing agents can induce premature disjoining of the two centrioles in a centriole pair (Mazia et al., 1960; Sluder and Rieder, 1985). Daughters that receive a single centriole rather than a centriole pair at cytokinesis of such cells assemble a monopolar spindle in the following mitosis and do not form a bipolar spindle until centrioles have had an opportunity to duplicate in an intervening interphase. However, faithful and accurate acentriolar mitoses do proceed in some exceptional cell lines in which centrioles are absent (Brenner et al., 1977; Debec and Abbadie, 1989) and in a *Chlamydomonas* mutant in which centrioles are severely defective (Goodenough and St Claire, 1975). These and other examples of mitoses in the absence of centrioles led Mazia (1984) to interpret the 'phenotypic centrioles' as nonessential indicators for the reproductive units of centrosomes. Sluder and Begg (1985) introduced the concept of 'polar organizers', defined functionally as the reproductive elements of a centrosome. Each centrosome normally has two polar organizers, that may or may not be manifested as centrioles. Thus, in contrast to the work pointing to the sufficiency of chromosomes, the work of Mazia, Sluder and colleagues suggests that duplication and separation of polar organizers are necessary to establish spindle bipolarity. An essential role for separation of polar organizers is also consistent with identification of a number of mutants that generate monopolar spindles in mitotic cells (Gonzales et al., 1988; Sunkel and Glover, 1988; Sullivan et al., 1990; Enos and Morris, 1990; Hagan and Yanagida, 1990; Hoyt et al., 1992; Roof et al., 1991; Heck et al., 1993; Glover et al., 1995).

Zhang and Nicklas (1995a,b) have recently reinvestigated the role of centrosomes and chromosomes in spindle formation in grasshopper spermatocytes. By selectively removing centrosomes and/or chromosomes by microsurgical techniques, they demonstrated that centrosome separation is necessary for assembly of a bipolar spindle in this experimental system. Chromosomes, by themselves or in combination with a single centrosome, were not sufficient to establish spindle bipolarity. However, chromosomes or factors associated with chromosomes contributed to spindle organization by stabilizing centrosomal microtubules. The findings of Zhang and Nicklas (1995a,b) are consistent with studies (Sluder and Rieder, 1985; Sluder et al., 1986, 1989) indicating that separations of polar organizers in prophase is required to establish and maintain bipolar spindle organization, but inconsistent with work (Dietz, 1966; Lifschytz and Meyer, 1977; Lifschytz and Hareven, 1977; Bastmeyer et al., 1986; Steffen et al., 1986) indicating that chromosomes are both necessary and sufficient to organize a bipolar array of microtubules that were first nucleated at centrosomes. Thus, compelling results in the literature point towards apparently mutually incompatible conclusions.

In a screen for mutations that disrupt spindle function in *Drosophila*, we identified mutants, originally designated as *urchin*. This work shows that *urchin* is allelic to *KLP61F* (Heck et al., 1993), a member of the *bimC* family of kinesin related motor proteins. The original description of spindle defects in *KLP61F* mutants described only a monopolar spindle phenotype. However, our examination of *KLP61F* mutants has revealed monopolar spindles as well as monastral bipolar spindles similar to those described by Dietz (1966), Lifschytz and Hareven (1977), and Lifschytz and Meyer (1977). The existence of both monopolar and monastral bipolar spindles in *KLP61F* mutants raises questions not only as to the pathways of their formation and the organization of centrosomes, but also to the role of BimC motor proteins in spindle assembly.

MATERIALS AND METHODS

Fly strains

Flies were raised in humidified incubators at 25°C on standard cornmeal molasses medium unless noted otherwise. Mutations are homozygous unless otherwise noted. Visible mutations, deletions, and balancer chromosomes are described by Lindsley and Zimm (1992). The *TM3* and *TM6C* balancer chromosomes used carried the mutations *Sb* and *cu, ca, Sb, Tb*, respectively. *Df(3L)Ar14-8* (61C3-4; 62A8) and *Df(3L)Ar12-1* (61C1-4; 61E) are gamma ray induced deletions (cytology according to Hillary Ellis and James Posakony, University of California).

Isolation of KLP61F alleles, mapping, and nomenclature

The original *urchin*^{1} mutant was identified in a screen for mitotic mutants that might show defects in spindle structure or function. A collection of lines containing independent insertions of *P-lacW*$^{+}$ (Bier et al., 1989) were generated in a joint effort by members of the research groups of Margaret Fuller and Matthew Scott, Stanford. To identify lethals that survived until third instar, we balanced chromo-

somes bearing a lethal mutation and a *P-lacW*$^{+}$with balancer chromosomes bearing the dominant larval marker *Tb*. Mutants that died late in larval development produced Tb^{+} third instar progeny. We next examined brains of late larval mutants stained with aceto-orcein by phase microscopy for mitotic defects such as polyploidy, hypercondensed chromosomes, and anaphase bridges. The *urchin* mutant was selected as a promising mutant worthy of further study, although the lethal phenotype of *urchin* failed to revert in the presence of transposase expressed by *P(Δ 2-3)* (Robertson et al., 1988).

To map the *urchin* mutation by meiotic recombination, *urchin*^{1}*/ru h th st p*^{p} *cu sr e* virgins were mated to *ru h th st p*^{p} *cu sr e Bsb /TM3* males. The region of recombination event(s) was determined by scoring visible markers carried on recombinant chromosomes of F_1 males. Inheritance of *urchin* was determined by mating individual F_1 males to *urchin*^{1}*/TM6C* virgins and scoring the absence of *Sb*$^{+}$ flies in the F_2 generation. The cytological phenotype of *urchin*^{1} co-segregated with lethality, suggesting that the lethal and mitotic defects were linked. The *urchin* gene lies within polytene interval 61C3;4-62A8, based on complementation of the lethal and mitotic phenotypes of *urchin*^{1} *by Df(3L)Ar12-1* (61C1-4; 61E) but not *Df(3L)Ar14-8* (61C3-4 ; 62A8).

To isolate additional alleles of *urchin*, males bearing isogenic third chromosomes marked with *red* and *e* were mutagenized with methane sulfonic ethyl ester (EMS) as outlined by Lewis and Bacher (1968). Mutagenized (∗) males were mass mated to *red B2t*^{n} *cvc jvl/TM3* virgins from a virginizer stock cultured at 18°C, a temperature where *B2t*^{n} causes dominant male sterility (Regan and Fuller, 1990). Individual F_1 *red e*/TM3* males were mated to *urchin*^{1}*/TM6C* virgins and putative alleles of *urchin* were identified by lack of *red e*/urchin*^{1} F_2 progeny. To establish stocks, F_2 *red e*/TM6C* sibling males and virgins were inbred. Three new alleles of *urchin* were identified in a screen of 1369 chromosomes.

Three P-element induced alleles of *KLP61F* (kindly provided by Margaret Heck and Allan Spradling, Carnegie Institute of Washington) were identified independently (Heck et al., 1993). Allelism between *urchin* and *KLP61F* was established by the failure of the three independent P-element induced mutations in *KLP61F* to complement the lethal and cytological phenotypes generated by mutations in *urchin.* In addition, the deficiency *Df(3L) Ar14-8* failed to complement each of the *KLP61F* insertion mutants. On the basis of the rules for nomenclature (Lindsley and Zimm, 1992) and preceding identification of this locus as one encoding a kinesin related protein (Stewart et al., 1991), we redefine the four alleles of *urchin* listed in Flybase (URL: http://fly.bio.indiana.edu) as *urc*^{1}*, urc*^{2}*, urc*^{3}*, urc*^{4} as *KLP61F*^{4}*, KLP61F*^{5}*, KLP61F*^{6}*, and KLP61F*^{7}*,* respectively.

Cytology of aceto-orcein stained brains

Mitotic figures were examined in third instar larval brains stained with synthetic aceto-orcein (Sigma, St Louis MO) as described by Karess and Glover (1989). To examine chromosome morphology in wild-type brains paused in mitosis, Canton S larval brains were dissected in 0.7% saline and incubated for 30 minutes in 10^{-5} M colchicine (Sigma, St Louis MO). In these experiments with wild-type animals, hypotonic shock was applied by incubation in 0.5% sodium citrate for 10 minutes.

Antibodies

To generate antibodies against gamma tubulin, a translational fusion between gamma tubulin and glutathione S-transferase was generated by ligation of the *Bcl*I-*Sma*I fragment of pPL6 (Zheng et al., 1991) into the *Bam*HI-*Sma*I sites of pGex3X (Smith and Johnson, 1988). Antiserum was prepared against the 402 C-terminal amino acids of gamma tubulin. Fusion protein expression was induced in cultures grown at 37°C to A_{600} = 0.8 with 1 mM IPTG. Inclusion bodies were collected from cell pellets and fractionated by SDS-PAGE. Gel slices were macerated with a mortar and pestle and mixed with Ribis adjuvant. New Zealand white rabbits were immunized and boosted at 4 week intervals. To affinity purify antibodies against the gamma tubulin portion of the fusion protein, the *Bcl*I-*Sph*I fragment of pPL6 was ligated into the *Bam*HI and *Sph*I sites of pQE-32 (Qiagen, Chatsworth, CA). In this construct the C-terminal 402 amino acids of gamma tubulin is fused to an N-terminal 6×-histidine peptide. Tagged fusion proteins were induced, harvested, and recovered from a Ni^{2+}-NTA-agarose column (Qiagen, Chatsworth CA) under denaturing conditions as recommended by the manufacturer. Fusion proteins were released from an Ni^{2+}-NTA-agarose column, solubilized by dialysis against DMSO, and coupled to Affigel 15 (Bio-Rad, Hercules, CA) as recommended by the manufacturer. A protein of approximately 50 kDa in crude extracts of *Drosophila* was recognized by affinity-purified antibodies against gamma tubulin (data not shown). Recognition of gamma tubulin by immunoblot analysis and by immunofluorescence was lost when affinity purified antibodies were preadsorbed to bacterially expressed gamma tubulin protein (data not shown). In some experiments, affinity purified antibodies were used that were generated against synthetic peptide corresponding to the C-terminal 19 amino acids of the gamma tubulin gene in region 23CD of salivary polytene chromosomes (P. G. Wilson et al., unpublished).

Mouse 3A5 or 4A1 monoclonal antibodies against alpha tubulin (Piperno and Fuller, 1985) were used at 1:10 dilution. Rabbit polyclonal antibodies against CP190 were obtained from D. Kellogg and B. Alberts and used at 1:500 dilution. Horse anti-mouse secondary antibodies conjugated to fluorescein and goat anti-rabbit secondary antibodies conjugated to Cy3 were obtained from Vector (Burlingame, CA) and Jackson Immunological Research (West Grove, PA), respectively. Secondary antibodies were used at dilutions determined empirically for each lot.

Immunofluorescence microscopy

Third instar larvae were washed briefly in 0.7% NaCl. Brains or testes were dissected from the carcass in PEM (80 mM Pipes, 10 mM EGTA, 10 mM $MgCl_2$, pH 6.8) and placed in a drop of PEM with 5% (v/v) glycerol (PEMG) on a microscope slide treated with polylysine (1 mg/ml H_2O) and spread gently under a silanized coverslip. The slide was plunged into liquid nitrogen for 5 to 10 seconds, the coverslip was removed with a razor blade, and the tissue immediately fixed for 5 minutes in methanol cooled with dry ice. Slides were placed in 66% (v/v) methanol and PBST (130 mM NaCl, 7 mM Na_2HPO_4 $2H_2O$, 3 mM NaH_2PO_4 $2H_2O$ (pH 7.1), 0.1% (v/v) Triton X-100) for up to 5 minutes, 33% (v/v) methanol and PBST for up to 5 minutes and finally in PBST. In some cases, tissues were post-fixed by a 5 minute incubation in 4% paraformaldehyde in PBS.

To stain with antibodies, tissue samples were washed three times in PBST for 10 minutes. Nonspecific protein binding was blocked with 3% (w/v) BSA (bovine serum albumin, fraction V) and PBST for at least 30 minutes. Tissue was then incubated for 2 hours to overnight at 4°C in primary antiserum diluted in 3% (w/v) BSA and PBST. Following three washes in PBST for 20 minutes, tissue was incubated for 2 hours at room temperature or overnight at 4°C in secondary antibodies diluted in 3% (w/v) BSA and PBST. Following incubation with secondary antibodies, brains or testes were washed once in PBST with 0.1 μg/ml DAPI followed by 3 washes for 20 minutes in PBST, and one final wash in PBS. Stained tissue was mounted in Vectastain (Burlingame, CA), covered with a 22 mm square coverslip, and sealed with nail polish for observation.

Imaging

Tissues were examined with Zeiss Universal, Zeiss Axioplan, and Nikon IM-35 microscopes. Some images were recorded in digital form with a Photometrics CCD camera (Photometrics, Tuscon). Other images were first photographed with either Techpan hypersensitized in N_2 or Kodak film and subsequently digitized with a Polaroid Quickscan35 slide scanner. Digitized files were imported into Photoshop for final image enhancement and presentation.

RESULTS

Allelic series of KLP61F mutants

To illuminate the relationship between monopolar and monastral bipolar spindles in *KLP61F* mutants, we first characterized and placed six independent *KLP61F* lethal mutants in an allelic series on the basis of cell proliferation in larval brains and imaginal discs and on the basis of three mitotic defects observed in orcein stained preparations of larval brains, infrequency of chromosomal anaphase figures, polyploid mitotic figures, and mitotic figures composed of hypercondensed and X-shaped chromosomes (Fig. 1; Table 1). These cytological defects may reflect, in part, assembly of monopolar spindles that cause mitotic delay and preclude cytokinesis.

The extent of cell proliferation in larval brains and imaginal discs, the frequency of chromosomal anaphase figures, the degree of polyploidy, and the proportion of mitotic figures consisting of overcondensed chromosomes (Table 1) suggested the following allelic series from the most to the least severe: $KLP61F^4 > KLP61F^6 > KLP61F^2$, $KLP61F^3 > KLP61F^1 > KLP61F^7$. Assignment of $KLP61F^4$ as the most severe allele is consistent with the essentially identical phenotypes of homozygous animals that contain two copies of $KLP61F^4$ and hemizygous animals that bear a single $KLP61F^4$ gene. Since an additional copy of an $KLP61F^4$ mutant gene does not appear to provide additional *KLP61F* function, $KLP61F^4$ is a severe loss-of-function allele. In contrast to $KLP61F^4$, the frequency of mitotic defects in brains of the P-element insertion mutants was higher in hemizygous animals than in homozygous animals (Table 1, and data not shown). Because two copies of the mutated gene provide more function than one, the P-element insertion mutants $KLP61F^1$, $KLP61F^2$ and $KLP61F^3$

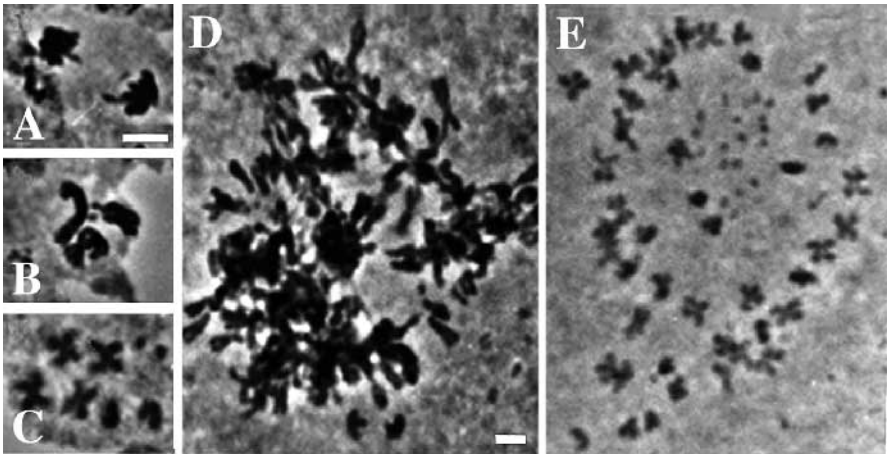

Fig. 1. Mitotic figures in KLP61F larval brains. Orcein stained chromosomes in (A-C) wild-type and (D,E) $KLP61F^4$ larval brains. (A) Anaphase mitotic figure showing chromosomes segregating to opposing spindle poles. (B) Mitotic figure showing typical chromosome condensation and somatic pairing of homologs. (C) Overcondensed chromosomes in wild-type brains treated with colchicine and hypotonic shock. (D) Highly polyploid mitotic figure with normal chromosome condensation in $KLP61F^4$ brain. (E) Overcondensed chromosomes in highly polyploid mitotic figure in $KLP61F^4$ larval brain which was not treated with colchicine. Magnification identical in A-C and in D-E. Bar, 2 μm.

Table 1. Mitotic figures and cell proliferation in KLP61F neuroblasts and imaginal discs

Genotype	No. of brains	No. of figures*	Percentage anaphase†	Percentage polyploid‡	Percentage highly polyploid§	Percentage overcondensed chromosomes¶	Imaginal** discs
wt††	5	336	19.8±4.8	0.0	0.0	0.0	+++
wt/Df‡‡	11	537	22.8±11.7	0.0	0.0	0.0	+++
$KLP61F^4$	5	81	0.0	68.7±12.9	44.0±5.8	77.3±18.1	–
$KLP61F^4$/Df	8	229	0.0	77.3±18.1	49.7±14.1	90.5±3.8	–
$KLP61F^6$/Df	10	200	1.0±2.1	73.5±19.1	50.5±21.9	74.2±20.2	–
$KLP61F^3$	2	165	1.2±0.1	69.1±3.8	45.9±1.5	45.0±2.3	+/–
$KLP61F^1$	3	199	0.9±0.7	49.4±3.8	18.4±4.1	33.4±10.4	+/–
$KLP61F^2$	3	224	4.1±3.6	30.2±11.0	7.2±6.9	14.9±15.5	+
$KLP61F^1$/Df	3	216	1.5±0.4	70.8±11.7	43.7±18.2	76.8±6.6	+/–
$KLP61F^7$/Df	5	366	0.2±0.4	48.8±13.3	26.1±12.3	46.3±16.1	+
$KLP61F^7$/$KLP61F^1$	3	363	9.1±3.7	2.5±1.5	1.0±0.9	15.3±13.9	++

*Mitotic figures with chromosomes sufficiently condensed to be detectable with orcein.

†Bipolar anaphase figures showing chromosomes moving to opposing spindle poles (Fig. 1A). Standard deviations of all entries were calculated as σ_n.

‡Mitotic figures with >4N genetic complement. Because of the difficulty of reliably scoring the very tiny 4th chromosomes, only the two pairs of large autosomes (4) and the sex chromosomes (2) were scored, making 6 and 12 the 2N and 4N complement, respectively. The percentage includes polyploid as well as highly polyploid figures.

§Mitotic figures with > 8N (24) autosomes and sex chromosomes.

¶Mitotic figures with overcondensed chromosomes resembling chromosomes in wild-type cells treated with colchicine (Fig. 1C).

**Size and morphology of brain and imaginal disks: (–) very tiny brains and no recognizable imaginal discs; (+/–) small brains, small imaginal discs in approximately half of the animals; (+) brains and imaginal disks approximately half of the size of those in wild-type animals; imaginal disks were present in more than 50% of animals; (++) all animals examined showed imaginal discs and brains that were sometimes slightly smaller than wild-type but typically similar insize and morphology; (+++) wild-type animals.

††Ore R animals was used as the (wt) wild-type control.

‡‡Df (3L)Ar14-8 was used for all phenotypic analyses of hemizygous animals. Phenotypes of homozygous $KLP61F^6$ and $KLP61F^7$ animals were not examined due to potential secondary mutations on the EMS mutagenized chromosomes.

are hypomorphic or partial loss-of-function mutants. Given that the P elements in these mutants lie in the upstream untranslated region of *KLP61F* (Heck et al., 1993), the mitotic defects in these mutants probably reflect a lowered expression of a wild-type gene product rather than expression of an altered protein. On the basis of viability, the frequency of polyploid figures, and hypercondensed chromosomes, $KLP61F^{7}$ was the least severe mutant as it presented the lowest overall frequencies of these mitotic defects.

Cell proliferation in larval brains and imaginal discs reflects the extent of mitosis and cell division. Mitotic mutants can survive until late in development, probably because mitosis per se is not required for larval development (Gatti and Baker, 1989). The extent of cell proliferation in *KLP61F* mutants varied with the strength of the mutant allele. While animals homozygous or hemizygous for the severe loss-of-function $KLP61F^{4}$ allele had very tiny brains and lacked imaginal discs, animals homozygous for the P-insertion alleles had much larger brains and generated imaginal discs and testes that varied in size with the mutant examined. Transheterozygous $KLP61F^{7}/KLP61F^{1}$ animals were semilethal, showing larval brains and imaginal discs similar in size and morphology to those of wild-type animals. Placement of *KLP61F* alleles in a series on the basis of cell proliferation in imaginal discs and tissues was consistent with their assignment on the basis of mitotic defects. We conclude that increased *KLP61F* function is accompanied by increased normal cell proliferation.

Mutations in KLP61F generate monastral bipolar spindles

Heck et al. (1993) showed that three independent P-element insertions in the 5′ untranslated region of *KLP61F* generated monopolar spindles as did mutations in other *bimC* homologs. However, Heck et al. did not determine whether monopolar spindles were the exclusive spindle type. We found that all of the six *KLP61F* mutants described in this study, including the three mutants examined by Heck et al. *(*1993) also showed monastral bipolar spindles in which only one of the two half-spindles appeared to be organized by a centrosome that contained a focus of gamma tubulin (Fig. 2; Table 2). We designate the pole of these half-spindles as the astral pole. The opposing half-spindle did not appear to be organized by a centrosome as it showed neither a focus of gamma tubulin nor, in separate experiments, a focus of CP190, another centrosomal marker in mitotic cells (Kellogg et al., 1989; Oregema et al., 1995). We designate the half-spindle lacking gamma tubulin as the anastral half-spindle and the corresponding spindle end as the anastral spindle pole. The lack of centrosomal antigens suggested that the anastral half-spindles lacked a canonical microtubule organizing center.

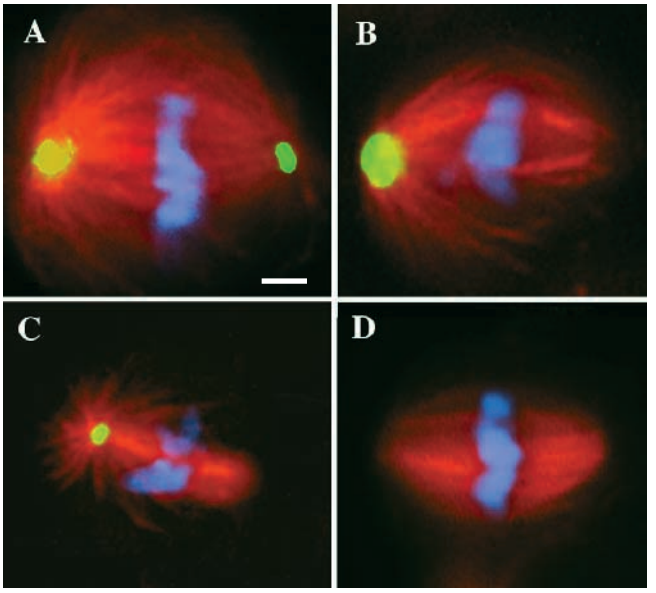

Fig. 2. Monastral bipolar spindles. Larval brains from (A) wild-type and (B-D) $KLP61F^{3}$ mutants were fixed and stained (Materials and Methods) with (red) antibodies against alpha tubulin, (green) antibodies against gamma tubulin, and (blue) DAPI. (A) Bipolar spindle in wild-type brain showing gamma tubulin at each spindle pole and chromosomes aligned in a linear array. The yellow appearance of the left centrosome in a reflects spindle orientation and the additive effects of the red color applied to microtubules to the green color applied to gamma tubulin during image processing. (B,C) Monastral bipolar spindles in which acentrosomal half-spindles lacked a focus of gamma tubulin and microtubule bundles formed (B) a broad or (C) a more focused pole. (D) Rare bipolar anastral spindle in which neither pole showed a focus of gamma tubulin. (B,C) Chromosomes aligned on monastral bipolar spindles and (D) on bipolar anastral spindles in linear array similar to (A) chromosomes in wild-type cell. Bar, 2 μm.

Monastral bipolar spindles typically showed a metaphase-like structure with microtubules extending on either side of chromosomes aligned in a linear array. Microscopic examination through multiple focal planes of these spindles revealed two discrete half-spindles rather than coincidental positioning of chromosomes at the midline of a monopolar array of microtubules. Some spindles showed equal and symmetric anaphase-

Table 2. Spindle organization in KLP61F larval neuroblasts

Genotype	No. of brains	No. of mitotic spindles*	Percentage biastral bipolar†	Percentage monastral bipolar‡	Percentage monopolar§
wt	13	422	99.1±1.4	0.9±1.4	0.0
$KLP61F^{4}$	17	164	0.0	12.2±10.4	87.8±7.3
$KLP61F^{1}$	9	366	6.4±5.0	65.2±11.7	28.4±12.9
$KLP61F^{7}$/Df	4	145	16.0±10.6	39.6±7.9	35.5±25.6
$KLP61F^{1}/KLP61F^{7}$	7	241	60.9±9.5	25.8±8.6	13.3±9.1

*Mitotic spindles had condensed chromosomes by fluorescence with DAPI, visibly distinct centrosomes stained with gamma tubulin, and one or more spindle poles. Prophase cells were not included in this tally.

†Bipolar spindles had two poles, each organized by a centrosome stained with gamma tubulin. Bipolar anastral spindles were not included in this tally.

‡Monastral bipolar spindles were bipolar, but only one pole contained gamma tubulin.

§Monopolar spindles which had a single pole that contained gamma tubulin.

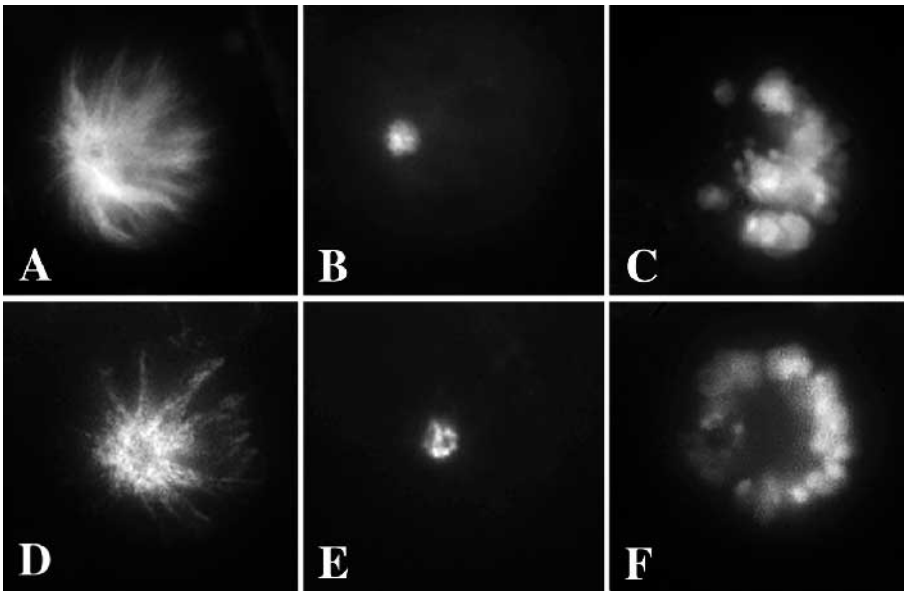

Fig. 3. Monastral bipolar spindles may resemble monopolar spindles. Larval brains from (A-C) *KLP61F*^{4} and (D-F) *KLP61F*^{3} were fixed and stained with antibodies against (A,D) alpha tubulin or (B,E) gamma tubulin, and (C,F) DAPI. (A) Monopolar spindle with microtubules organized at a single pole (B) containing gamma tubulin. (C) Chromosomes form a radial array. (D-F) Ambiguous spindle structure with (D) bundles of microtubules that were distal to (E) gamma tubulin and (F) chromosomes. Bar, 2 μm.

like positions of chromosomes between opposing astral and anastral spindle poles (data not shown). The metaphase alignment and anaphase position of chromosomes suggested that monastral bipolar spindles were probably comprised of two discrete sets of microtubules organized in an antiparallel orientation. The organization of microtubules and/or microtubule bundles forming the poles of the anastral half-spindles varied from broad (data not shown) to focused (Fig. 2B,C). Variation in anastral spindle pole morphology was found in all cell types examined. Larval brains of wild-type animals also showed a low frequency of monastral bipolar structures. As both wild-type and *KLP61F* mutant brains were prepared with identical methods, and in some cases on the same slide, the observed high frequency of monastral bipolar spindles in *KLP61F* mutant brains is due to defects in KLP61F function, rather than mechanical stresses placed on the tissues during tissue preparation.

Using a focus of gamma tubulin as the criterion for the presence of a centrosome and detection of two discrete half-spindles by examination of multiple focal planes of each spindle, all *KLP61F* mutants generate monastral bipolar spindles (Table 2, and data not shown). With the exception of the severe loss-of-function *KLP61F*^{4} mutant, all other *KLP61F* mutants also showed normal biastral spindles with a centrosome containing gamma tubulin located at each spindle end. Spindles in which both ends appeared to be anastral were observed (Fig. 2D), but comprised less than 1% of the total scored. Larval brains of all *KLP61F* mutants also contained monopolar spindles with all microtubules organized about a single pole (Fig. 3; Table 2). Since discrimination between monopolar and monastral bipolar structures required a favorable spindle orientation, oblique or axial spindle orientations such as in Fig. 3D-F prevented clear detection of two discrete half-spindles. Such spindles could not be scored unequivocally as monastral bipolar and were scored as monopolar. The frequency of such ambiguous spindle structures varied with the allele and among samples, but ranged from 10-30% of all monopolar spindles scored. As a result of ambiguities in scoring, the frequency of monopolar spindles may be overestimated in Table 3. These data indicate that larval brains of all *KLP61F* mutants show monopolar as well as monastral bipolar spindles and, in some mutants, monastral bipolar spindles were the predominant type. Thus, our results extend those of Heck et al. (1993), showing defects in KLP61F function result in monastral bipolar as well as monopolar spindles.

Most cells containing a monastral bipolar spindle appeared to be euploid on the basis of the fluorescence of chromatin stained with DAPI, although a portion appeared polyploid. Conversely, most cells containing a monopolar spindle appeared to be polyploid. We noted that, despite the presence of overcondensed chromosomes in larval brains stained with

Table 3. Prophase centrosomes and monastral bipolar spindles in KLP61F gonial cells

Gonial division	Total no. of cysts	No. cysts in prophase	% Prophase cells with two centrosomes	No. cysts with spindles	% Cells with monastral bipolar spindles*
m1	9	1	100.0 (1/1)	8	0.0 (0/8)
m2	10	6	100.0 (12/12)	4	50.0 (4/8)
m3	17	3	100.0 (12/12)	14	33.3 (18/54)†
m4	7	2	100.0 (16/16)	5	63.9 (23/36)
Total	43	12	100.0 (41/41)	31	42.4 (45/106)

*Monastral bipolar spindles in metaphase, anaphase, and telophase cells were pooled.
Parentheses = number/total number scored.
†One m3 cyst from an KLP61F^{1} animal showed a bipolar anastral metaphase spindle that is not included.

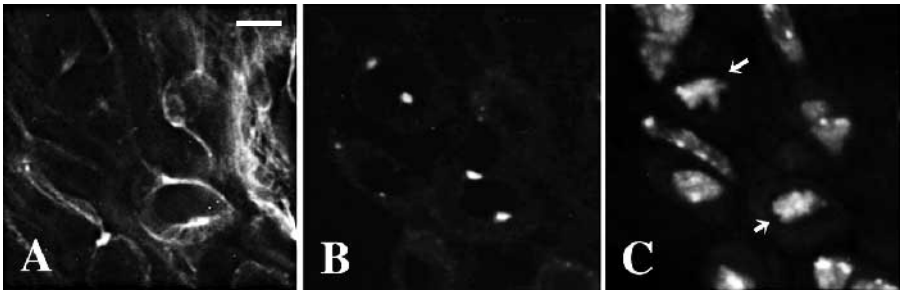

Fig. 4. Gonial cells in prophase showed 2 centrosomal asters containing gamma tubulin Example of m2 gonial cyst in prophase in *KLP61F[1]* testis. (A) Two asters of microtubules on opposing sides of intact nuclear envelope. (B) Each aster was associated with a focus of gamma tubulin. (C) Chromatin condensation was advanced (arrows), but the nuclear envelope was intact and intranuclear microtubules were not observed. Bar, 2 μm.

orcein, chromosomes stained with DAPI in larval brains of all of the *KLP61F* mutants examined were frequently more diffuse than chromosomes in larval brains of wild-type animals fixed and stained in the same way. As this effect on chromatin structure was not allele-specific, defects in KLP61F function may have indirect effects on chromatin structure that are revealed when brains are fixed in cold methanol for immunofluorescence, but not when brains are fixed with acetic acid in preparation for orcein staining.

As centrosomes can release from the nuclear envelope and move freely in the cytoplasm of *Drosophila* embryos (see Glover et al., 1989), we examined multiple focal planes of cells containing monastral bipolar spindles to determine whether a second centrosome was present in the cytoplasm. However, we did not observe a second centrosome in cells containing a monastral bipolar spindle with either antibodies against the centrosomal antigens gamma tubulin and CP190, suggesting that either centrosomes failed to separate or that centrosomes did in fact separate, but one became disorganized subsequent to bipolar spindle assembly. We were unable to apply quantitative immunofluorescence (Glover et al., 1995) to determine the number of centrosomes at the astral pole since the absence of gamma tubulin at the anastral pole raised the possibility that mutations in *KLP61F* could affect the organization of pericentriolar material, thus undermining the validity of the assay.

Sequential mitoses in larval testis show separation of two centrosomes in prophase cells

The trend toward increased cell proliferation and increased frequencies of monastral bipolar spindles in the allelic series of *KLP61F* mutants raised the question of whether monastral bipolar spindles could be functional. If this is the case, analysis of spindle assembly in the subsequent cell cycle might reveal whether the astral pole contained two centrosomes rather than one. To address these questions, we performed pedigree analysis of centrosome distribution and spindle assembly in the male germline where the products of four sequential mitoses are recovered in a cyst of germ cells. Mitosis in a progenitor gonial cell initiates four nearly synchronous rounds of mitotic cell division, designated here as m1, m2, m3, and m4. Cytokinesis between mitotic divisions is incomplete and as a result, a series of cytoplasmic bridges connects members of the cyst. To perform pedigree analysis of spindle defects, we examined the two hypomorphic P-element insertion mutants, *KLP61F[1]* and *KLP61F[3]*, that generate a larval testis and show high frequencies of both monopolar and monastral bipolar spindles in larval brains (Table 2 and data not shown).

We first scored the frequency and distribution of centrosomes during prophase in gonial cells of *KLP61F[1]* and *KLP61F[3]* mutants. Nuclei of interphase and prophase cells appeared as round intact structures outlined by tubulin immunofluorescence. Prophase cells were identified as those with partially condensed chromatin by DAPI fluorescence. Centrosomes were assayed as foci of gamma tubulin from which microtubules radiated. All prophase gonial cells of the *KLP61F* mutants examined showed two discrete centrosomes (Table 3; Fig. 4), indicating that gonial cells in these mutants entered prophase with two, and only two, centrosomes and that these centrosomes can separate in prophase. To determine whether centrosome separation is unique to gonial cells in *KLP61F[1]* and *KLP61F[3]* or whether centrosomes can also separate in larval brains, we examined prophase cells in larval brains of *KLP61F[1].* Similar to larval testis, all of the 100 prophase cells scored in *KLP61F[1]* larval brains contained two foci of microtubules that each contained a focus of gamma tubulin. These data suggested centrosomes typically separate in prophase in larval testis and brains of *KLP61F[1]* mutants.

Monastral bipolar spindles in gonial cell mitoses

Despite the apparent entry into prophase with two centrosomes at each mitotic division, spindles in gonial cell divisions in *KLP61F* mutants frequently showed a single centrosome detected by gamma tubulin (Fig. 5; Table 3). Of the 23 cysts of gonial cells containing ≥2 spindles, 44% (10/23) of cysts showed at least two and 30% (7/23) showed exclusively monastral bipolar spindles. Similar to larval brain cells in *KLP61F* mutants, gonial cells with monastral bipolar spindles showed both metaphase alignment and anaphase position of chromosomes. Some telophase cells showed a constriction

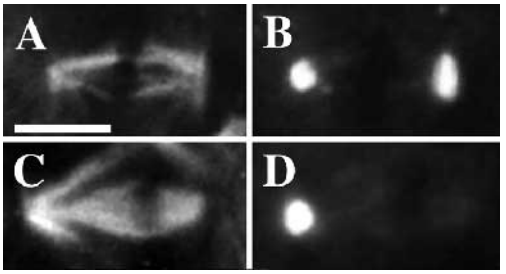

Fig. 5. Gonial cells show monastral bipolar spindles. (A-D) Gonial cells from *KLP61F[3]* animals stained with (A,C) alpha tubulin, (B,D) gamma tubulin. (A) One of eight bipolar spindles in m3 cyst with (B) focus of gamma tubulin at both spindle poles. (C) One of 5 monastral bipolar spindles in m3 cyst that showed a single focus of gamma tubulin and a radial array of astral microtubules at one pole. Opposing pole was anastral and lacked a focus of gamma tubulin. Bar, 2 μm.

furrow and one nucleus with a focus of gamma tubulin while the partner nucleus lacked a focus of gamma tubulin, suggesting that cells with monastral bipolar spindles could segregate chromosomes and at least initiate cytokinesis. Monastral bipolar spindles were observed in the m2, m3, and m4 divisions of all mutants examined. The absence of monastral bipolar spindles in m1 may reflect the small sample size. The observed frequencies of monastral bipolar spindles at m2, m3, and m4 showed no increasing or decreasing trend, suggesting that these spindles were formed with essentially equal probability at each mitotic division. In contrast to *KLP61F* mutants, the morphology of spindles in gonial cells obtained from wild-type animals was normal; spindles were exclusively bipolar and biastral. Taken together with the observed separation of two centrosomes in prophase gonial cells in *KLP61F* mutants, these observations suggest either that monastral bipolar spindles reflect disorganization of one centrosome or that the two centrosomes return to a side-by-side position subsequent to bipolar spindle assembly.

As in larval brains, bipolar anastral spindles in the gonial cell divisions were rarely observed (<1%). In contrast to larval brains, we did not detect clear examples of monopolar spindles in larval testis. The reason for the apparent infrequency of monopolar spindles in larval testis is not clear, but may reflect scoring of ambiguous monastral bipolar spindles as monopolar or possibly differences in usage of KLP61F in testis and brain. Another difference between brain and testis in *KLP61F* mutants is that mitotic cells in the male germline of displayed a focus of gamma tubulin displaced from spindle ends in 5 of 106 gonial cells that contained spindles in metaphase or later stages (data not shown). Cells containing a focus of gamma tubulin at a spindle end and a displaced focus of gamma tubulin were scored as having normal bipolar spindles rather than monastral bipolar spindles. Foci of gamma tubulin were never observed displaced from spindle ends in wild-type animals nor removed from the nuclear envelope in prophase cells in *KLP61F* mutants. The basis for this difference between brains and testis in *KLP61F* mutants is not known.

Primary spermatocytes show equivalence in chromatin, but not centriole content

Virtually all *KLP61F*^{1} and *KLP61F*^{3} spermatocytes were of equal size and contained equivalent amounts of chromatin by DAPI fluorescence. In total, over 500 cells in more than 40 mutant cysts were examined, including at least 8 intact cysts that contained 16 spermatocytes each (Fig. 6). As cysts usually break apart during tissue preparation, partial cysts with less than 16 spermatocytes were more common than complete cysts in testis of both wild-type and *KLP61F* mutant animals. Similar to complete cysts, spermatocytes in partial cysts and free spermatocytes not contained in a cyst showed equivalence in size and chromatin content as assessed by intensity of DAPI fluorescence. The size and ploidy of these spermatocytes in *KLP61F* mutant animals was indistinguishable from the size and ploidy of spermatocytes in wild-type animals. The observed equivalence in size and ploidy suggests that gonial cells in *KLP61F* mutants, as in wild-type animals, can complete 4 sequential rounds of accurate chromosome segregation and equal cell division even though these same mutants showed high frequencies (33-64%) of monastral bipolar spindles in the m2, m3 and m4 gonial cell mitoses. These data suggest that cells derived from the anastral poles of monastral bipolar spindles can assemble either a functional monastral bipolar spindle or a normal bipolar spindle in a subsequent mitosis.

To determine the fate of centrioles in the mitotic divisions of *KLP61F* testis, we scored the number and distribution of centrioles in primary spermatocytes by immunofluorescence microscopy with antibodies to gamma tubulin. In wild-type animals, centrioles undergo a final round of duplication following m4 such that each primary spermatocyte contains two centriole pairs. Centrioles then leave the nuclear envelope and lie at the cell surface during a prolonged growth period. During premeiotic growth, cells grow to 25 times their original volume and each centriole cylinder reaches approximately 2 μm in length (reviewed by Fuller, 1993). Staining with antibodies against gamma tubulin revealed two pairs of orthogonal rods in mature primary spermatocytes. These pairs of orthogonal rods were typically associated with sparse foci of microtubules and located near the cell surface (Fig. 7A). The subcellular localization, size, and shape of these rods suggest they are centrioles. Localization of gamma tubulin at centrioles in primary spermatocytes provided a means to use conventional indirect immunofluorescence to determine the fate of centrioles in cysts of *KLP61F* spermatocytes.

Spermatocytes in *KLP61F*^{1} and *KLP61F*^{3} mutant testis

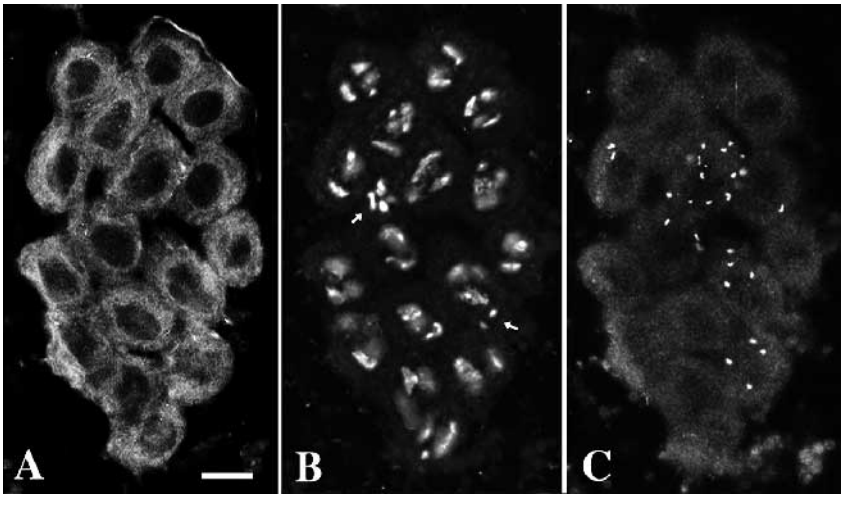

Fig. 6. Cyst of 16 spermatocytes in *KLP61F* mutant testis that are equivalent in size and chromatin content. Example of intact cyst of primary spermatocytes from *KLP61F*^{1} larval testis stained with (A) antibodies against alpha tubulin, (B) DAPI and (C) antibodies against gamma tubulin. (A) Focusing through focal planes in this cyst revealed sixteen spermatocytes with (B) equal chromatin content. This cyst contained close to 32 centriole pairs; one cell contained 14, 3 cells contained four, 2 cells contained two, and 10 cells were acentriolar. (B, arrows) Chromosomes in two somatically derived cyst cells. The cyst cells did not show centriole pairs. Bar, 10 μm.

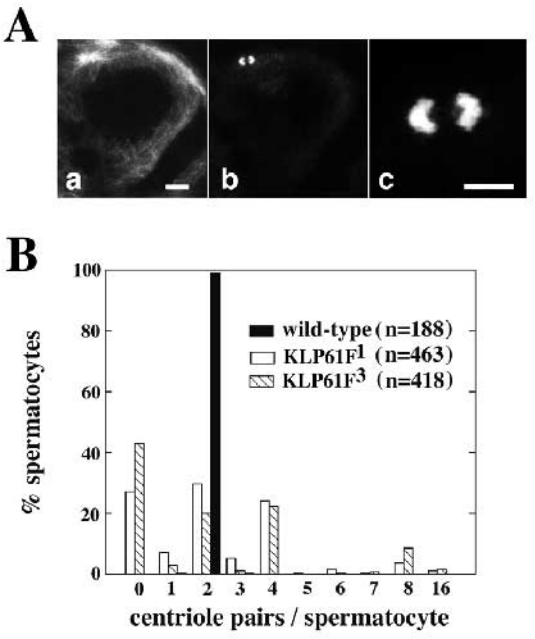

Fig. 7. (A) Centriole pairs in wild-type spermatocyte decorated with gamma tubulin. Testis dissected from wild-type animal stained (Materials and Methods) with antibodies against (a) alpha tubulin and (b,c) gamma tubulin. (a) Primary spermatocyte showing two sparse foci of microtubules at the cell surface organized around (b) orthogonal pairs of centrioles decorated with gamma tubulin. (c) Enlarged view of centriole pairs in b. Magnification in a and b are identical. (a,b) Bars, 2 μm. (c) Bar, 1 μm. (B) Centriole pair distribution in spermatocytes of wild-type and *KLP61F* mutant animals. Spermatocytes were scored for the number of centriole pairs as revealed by antibodies against gamma tubulins. Due to the difficulty in distinguishing overlapping centriole pairs, spermatocytes with 9 to 16 centriole pairs were scored as having 16. Most of the cells pooled in the group containing 9-16 centriole pairs showed 14-16 pairs.

showed a variable number of centriole pairs (Fig. 7B). Cells with 0, 2, and 4 centriole pairs were the most common, being 35%, 25%, and 24% of the spermatocytes scored, respectively. Spermatocytes with odd numbers of centriole pairs (1, 3, 5, etc) comprised 10% of the spermatocytes scored. Two separate centrioles were sometimes found in the same cell, in which case they were scored as a single centriole pair. Spermatocytes with ≥4 centriole pairs comprised 9% of the total, most of which showed at least 8 centriole pairs and some showed close to 16. The ploidy of spermatocytes containing an abnormal number of centriole pairs did not obviously correlate with the number of centriole pairs (Fig. 6). For example, spermatocytes with 4 times the normal centriole pair content, 8 pairs, did not show evidence of 4 times the normal chromatin content.

The total number of centriole pairs within an intact cyst was typically close to the number expected, 32. For example, 4 intact cysts of 16 spermatocytes from 4 different $KLP61F^3$ mutant animals contained 27-32 centriole pairs. These cysts were typical in that each showed a range of centriole pair content per spermatocyte with approximately equal numbers of spermatocytes with 0, 2, and 4 centriole pairs, although one cyst contained a spermatocyte with 8 centriole pairs. A similar distribution of 21-30 centriole pairs was observed in three intact cysts from 3 $KLP61F^1$ animals. One of these three cysts also showed a spermatocyte with 8 centriole pairs. In addition to these three cysts, the centriole pair content of one other cyst of 16 cells was atypical in that the cyst lacked acentriolar spermatocytes but contained close to 60 centriole pairs, distributed primarily in cells with 2, 4, or 8 pairs. This cyst may have entered spermatogenesis with two centrosomes or have actually been two partial cysts inadvertently scored as one. Cysts with too few centriole pairs may reflect scoring errors, defects in centriole duplication or maturation, or poor localization of gamma tubulin at centriole cylinders that precluded their detection. Nevertheless, taken together, our data suggest that sequential rounds of gonial cell mitosis occurred with conservation of centriole number.

DISCUSSION

Even though the size and activity of the centrosome is known to vary during the cell cycle, the centrosome has been regarded as an essentially permanent cell structure. The concept of a permanent centrosome has influenced the interpretation of non-canonical spindle structures and the mechanisms proposed to organize microtubules into bipolar arrays. Mutations in *KLP61F* uncouple spindle bipolarity and centrosome organization in mitotic cells. Monastral bipolar spindles appear to be competent in the functions of canonical bipolar spindles: stable attachment of microtubules to kinetochores, chromosome alignment on the metaphase plate, and anaphase segregation of chromosomes. As these functions require antiparallel microtubules, we infer that monastral bipolar spindles are composed of two discrete sets of microtubules oriented antiparallel to each other. On the basis of gonial cell proliferation in *KLP61F* mutant testis, monastral bipolar spindles can function in mitosis and cell division.

Monastral bipolar spindles: possible origins

Evidence has been presented for the bipolar organization of spindles in *Drosophila* either by centrosomes or by chromosomes (see Introduction). In light of this evidence, we consider three models for assembly of monastral bipolar spindles and the effects predicted by each on subsequent mitoses (Fig. 8). Other possible models are variations on these themes. In the first model which invokes bipolar organization of microtubules by chromosomes (Fig. 8a), two centrosomes either fail to separate or to maintain separation. Bipolar spindles are formed because mono-oriented chromosomes either induce the assembly of correctly oriented microtubules or capture the plus-ends of microtubules released from centrosomal nucleation sites at monopoles. If cytokinesis ensues, one daughter cell should be acentriolar and organize a bipolar anastral spindle in the following mitosis. The other daughter should receive 2 centriole pairs, which would replicate to give 4 centriole pairs by onset of the next mitosis. If centrosomes again fail to separate or to maintain separation, a monastral bipolar structure would be regenerated in the subsequent cell cycle. In the second model which invokes a dynamic centrosome organization, centrosomes separate in prophase and a bipolar spindle is assembled (Fig. 8b). One centrosome becomes disorganized, but without detrimental effects on spindle bipolarity, chromosome segregation or cell division.

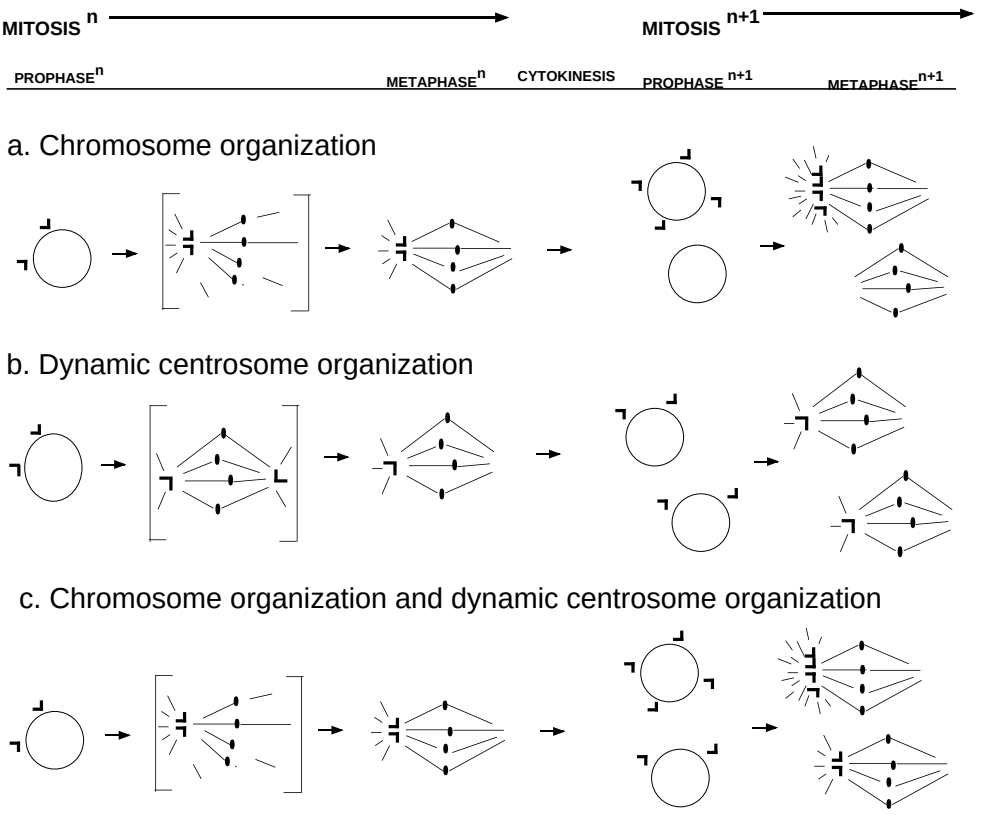

Fig. 8. Models for assembly of monastral bipolar spindles. (a) Assembly of monastral bipolar spindles by chromosome organization. At prophase of mitosis$_n$, centrosomes fail to separate or to maintain separation. The intermediate is a monopolar spindle (bracket). Mono-oriented chromosomes either induce the formation of correctly oriented microtubules or capture microtubules released from centrosomes at the monopole. If cytokinesis proceeds, one daughter will be acentriolar and one daughter will contain two centriole pairs. The acentriolar daughter will assemble a bipolar anastral spindle in mitosis$_{n+1}$. The daughter receiving two centriole pairs will have four centriole pairs due to centriole duplication in interphase. If the centrosomes again fail to separate, a monastral bipolar spindle will be regenerated at the same probability as in the parental cell. (b) Assembly of monastral bipolar spindles reflects dynamic centrosome organization. In prophase of mitosis$_n$, two centrosomes separate across the nuclear envelope. The intermediate is a bipolar structure organized by stable attachment of kinetochores to microtubules nucleated and organized by opposing centrosomes. A monastral bipolar spindle is generated by centrosome disorganization subsequent to nuclear envelope breakdown at the onset of prometaphase. Cytokinesis delivers an equal complement of chromosomes and a disorganized microtubule organizing center to each daughter. Centrosomes reorganize prior to prophase of mitosis$_{n+1}$ and monastral bipolar spindles are assembled with the same probability as the in parental cell. (c) Assembly of monastral bipolar spindles reflects aditive effects of chromosome organization of microtubules and dynamic centrosome organization. Centrosomes fail to separate and chromosomes organize microtubules in the anastral pole. Subsequent to cytokinesis and prior to prophase of mitosis$_{n+1}$, a centrosome is reorganized, either with centrioles assembled de novo as shown or without centrioles as discussed in the text.

Cell proliferation proceeds because a canonical centrosome is reorganized in the daughter cell derived from the anastral pole prior to prophase of the next mitosis. A third model is additive (Fig. 8c), invoking both chromosome organization of microtubules and a dynamic centrosome organization. According to this view, centrosomes fail to separate and chromosomes organize the anastral pole. Following cytokinesis and prior to prophase, a centrosome is reorganized in the daughter derived from the anastral pole.

The chromosome organization model does not account for centrosome distribution and spindle structure in *KLP61F* mutants

According to the chromosome organization model, monastral bipolar spindles are derived from monopolar spindles. One consequence of this pathway is that cytokinesis of cells containing monastral bipolar spindles should generate a daughter cell containing an excess of centrosomes, predicting that four separated centrosomal asters should be visible in the prophase of the subsequent cell cycle. Yet, all prophase cells examined showed only two separated centrosomes. A second consequence of this model is that cytokinesis of gonial cells with monastral bipolar spindles should generate an acentrosomal daughter cell, predicting bipolar anastral spindles in the subsequent cell cycles at a frequency commensurate with the frequency of anastral spindle poles in the preceding cell cycle. Contrary to the prediction of high frequencies of bipolar anastral spindles, these spindle structures were rarely observed. Our data join a significant body of work which indicates that chromosomes are not sufficient to organize a bipolar spindle in mitotic cells (Sluder and Rieder, 1985; Sluder et al., 1986, 1989; Mazia et al., 1960) and in meiotic grasshopper spermatocytes (Zhang and Nicklas, 1995b). Our results are also consistent with the work of Nicklas (1989) showing anaphase chromosome segregation to opposing ends of a monastral bipolar structure in which one pole of a bipolar spindle was removed by microsurgery. It should be noted, however, that a substantial body of data also exists pointing to the sufficiency of chromosomes to organize a bipolar spindle in meiotic cells of *Pales* (Dietz, 1966; Bastmeyer et al., 1986; Steffen et al., 1986) and *Drosophila* (Lifschytz and Hareven, 1977; Lifschytz and Meyer, 1977; Church et al., 1986; Theurkauf and Hawley, 1992).

Dynamic centrosome organization

The pivotal prediction of a model invoking dynamic centrosome organization is that each daughter receives a polar organizing center at cytokinesis, whether or not a canonical centrosome was present at both spindle poles at the preceding metaphase. If a centrosome is reconstituted in a cell derived

from an anastral pole, both daughters should show two centrosomes at the subsequent prophase. Following assembly of a biastral spindle, as in the parental cell cycle, a centrosome may become disorganized in either daughter cell and generate a monastral bipolar spindle. Despite the high frequency of spindle poles lacking a conventional centrosome structure in the gonial divisions, these predictions were borne out; prophase cells showed two centrosomes and virtually all of the spindles were bipolar. The observed equivalence in size and ploidy of spermatocytes is consistent with normal spindle assembly and requires spindle function in sequential mitotic divisions of the precursor gonial cells. Thus, our observations support the dynamic centrosome model.

Can our data be explained by an additive model in which chromosomes organize the anastral spindle pole and a dynamic centrosome organization allows de novo assembly of a centrosome in the corresponding daughter cell? While providing an explanation for the absence of anastral spindles when centrosomes fail to separate, a key prediction of this model, an excess of centrosomes in prophase gonial cells, was not observed. In addition, this view predicts a net gain of a substantial number of centrioles in all cysts by de novo assembly while our data suggested conservation of centriole number. Although not included in Fig. 8c, a variation of this model is one in which only gamma tubulin is organized at the minus ends of microtubules. Such acentriolar centrosomes might be more unstable and prone to disorganization at metaphase. While this variation allows for conservation of centriole number, such instability should generate a substantial number of bipolar anastral spindles in cells derived from anastral poles. However, such bipolar anastral spindles were rare. Thus, the absence of gonial cells with an excess of centrosomes and the virtual absence of bipolar anastral spindles are inconsistent with a model invoking both chromosome organization of microtubules and dynamic centrosome organization. Taken together, our data most closely fit the view that monastral bipolar spindles reflect centrosome separation coupled with transient and benign centrosome disorganization.

We infer from the *KLP61F* mutant phenotype that organization of pericentriolar components at polar organizers in wild-type animals may be reversible in that loss of centrosome structure does not preclude reorganization of a functional centrosome and bipolar spindle assembly in the subsequent cell cycle. Such disorganization and reorganization of pericentriolar components has precedence in female meiosis in the mouse where a broad band of pericentriolar material at acentriolar spindle poles disperses at telophase of meiosis I and reorganizes by metaphase of meiosis II (Szollosi et al., 1972). Similarly, the first few mitotic divisions of the mouse embryo also show disorganization and reorganization at spindle poles until centrioles appear in the third or fourth mitotic division (Calarco-Gillam et al., 1983). Taking these studies together with our data, we propose that centrosomes in wild-type *Drosophila* animals have the capacity to undergo transient and benign disorganization at centrosomes containing centrioles.

The minus-ends of microtubules forming the anastral poles of monastral bipolar spindles lacked a detectable density of gamma tubulin staining, suggesting that the relationship between microtubule minus ends and pericentriolar components may be dynamic. This view is consistent with recent experiments probing the role of gamma tubulin in microtubule organization at centrosomes. Gamma tubulin is located in a large 25 S complex in the cytoplasm of *Xenopus* egg extracts (Stearns and Kirschner, 1994). Ring structures containing gamma tubulin have been demonstrated to nucleate and bind the minus end of microtubules (Zheng et al., 1995; Moritz et al., 1995b) and to cap assembly at the minus end (Zheng et al., 1995). However, the capping was not complete, suggesting that the association of the gamma tubulin complex with the minus end of the microtubule may be reversible. Thus, the apparent absence of gamma tubulin staining at anastral spindles poles may reflect reversible binding of gamma tubulin to microtubule ends as well as disorganization of pericentriolar material.

The extent of centrosome disorganization at anastral poles is not clear. Centrioles are stable structures that persist through at least several cell cycles in vivo (Kochanski and Borisy, 1990). As centriole number appears to be conserved in intact cysts of spermatocytes, the simplest expectation is that centriolar cylinders remain intact at anastral poles even though the organization of pericentriolar components is lost. Alternatively, the centriolar cylinders may be dismantled at the anastral pole and reorganized in the subsequent cell cycle. Regardless of the extent of disorganization, transient disorganization of centrosomes in mitotic cells in *KLP61F* mutants suggests that the association of gamma tubulin and other pericentriolar components at polar organizers may be dynamic.

If centrosome organization is intrinsically dynamic and centrosomes are physically discrete structures, why was the frequency of bipolar anastral spindles in larval brains of $KLP61F^{1}$ mutants (<0.01) less than the frequency predicted for two independent centrosome disorganization events (0.32^{2} or 0.12)? One possible explanation for the observed infrequency of bipolar anastral spindles is that the two centrosomes forming a bipolar spindle are not equivalent. Because one centriole in a cell is at least one generation older than the remaining centrioles, a grandparental centriole may be more (or less) likely to retain pericentriolar components than the centrioles at the opposing pole. If this is the case, the oldest centriole among the two centriole pairs in mitotic cells may afford more (or less) centrosome stability than is provided by the centriole pair in the opposing centrosome. An alternative, but not mutually exclusive, possibility is that loss of pericentriolar components from one centriole pair may allow reassociation of these components with the opposing centriole pair to provide additional stability. It is also possible that cellular asymmetry, either in the cytoskeleton and/or the distribution of cytoplasmic components, impacts centrosome organization in ways that are not yet understood.

Centriole pair distribution in primary spermatocytes

The non-equivalent distribution of centriole pairs in spermatocytes was surprising and is not readily explained by either the dynamic centrosome or the chromosome organization model. Although the total number of centriole pairs contained within an intact cyst of primary spermatocytes was close to the expected value, 32 pairs, the number of centriole pairs per spermatocyte showed a wide distribution. According to a model invoking dynamic centrosome organization, cytokinesis of a cell containing a monastral bipolar spindle should deliver a single centriole pair to each daughter. As centrioles duplicate in the interphase following m4, each spermatocyte should contain 2 centriole pairs rather than the wide distribution

observed. Because neither the alternative chromosome organization model nor the additive model fits the observed distribution of centrosomes and spindle structures in the mitotic gonial divisions, we cannot easily invoke a failure of centrosome separation to explain the observed distribution of centriole pairs in primary spermatocytes. Inability to fit the observed distribution of centriole pairs in spermatocytes to either model points to deficiencies in our understanding of KLP61F function. Possibly, the unequal centriole distribution in *KLP61F* mutant testis may reflect a property of the highly differentiated spermatocyte. The passage of centriole pairs through the cytoplasmic bridges between nurse cells and the oocyte has been well documented in wild-type *Drosophila* females (Spradling, 1993). It is possible, for reasons that we do not understand, that centrioles move between spermatocytes in *KLP61F* mutants as well.

Implications of dynamic centrosome structure for the role of BimC homologs in spindle assembly

Our data indicate that monastral bipolar spindles are functional in mitosis and that centrosome disorganization can be transient and benign in *KLP61F* mutants. We infer that the lethality of mutations in *KLP61F* is due, at least in part, to monopolar spindles and the resulting defects in cell proliferation and polyploidy. KLP61F is a member of the conserved BimC family of kinesin related motor proteins (Heck et al., 1993) that are also present in fungi (Enos and Morris, 1990; Hagan and Yanagida, 1990; Hoyt et al., 1992; Roof et al., 1991) and vertebrates (Le Guellec et al., 1991). Like mutations in *KLP61F*, monopolar spindles are generated in *bimC*-like mutants in fungi and monopolar-like structures are generated by immunodepletion of Eg5 from *Xenopus* egg extracts (Sawin et al., 1992). Primarily on the basis of the monopolar spindles, this family of motor proteins has been proposed to separate centrosomes in prophase and/or separate spindle poles in anaphase B (reviewed by Moore and Endow, 1996; Barton and Goldstein, 1996; Fuller and Wilson, 1992).

Sawin et al. (1992) proposed that monopolar spindles in *bimC* mutants are a downstream consequence of defects in microtubule attachment to centrosomes. Reminiscent of monastral bipolar spindles in *KLP61F* mutants, immunodepletion of Eg5 from *Xenopus* egg extracts results in defects in spindle pole organization, in that microtubules were frequently splayed at spindle ends rather than focused (Sawin et al., 1992). Since Eg5 is a plus-end microtubule motor, as is KLP61F (Barton et al., 1995), Sawin et al. proposed that Eg5 is tethered to centrosomes through an extensible element. The motor domain of Eg5 could bind to and effectively capture microtubules released from nucleation sites, providing a more long-lasting connection between microtubules and centrosomes. According to this model, poles comprised of splayed microtubules reflect loss of microtubule tethering to centrosomes. However, if anastral poles in *KLP61F* mutants reflected only loss of microtubule attachment to centrosomes, cells with monastral bipolar spindles should contain a free centrosome, as revealed by a focus of gamma tubulin and an aster of microtubules. Given that we did not detect free centrosomes in cells with monastral bipolar spindles, our data indicate that loss of spindle pole structure at anastral poles in *KLP61F* mutants reflects loss of centrosome organization. Also, monopolar spindles in severe loss-of-function $KLP61F^4$ mutants are inconsistent with an essential role for KLP61F or other BimC homologs in attachment of microtubules to centrosomes. Thus, failure to achieve spindle bipolarity cannot be attributed simply to a defect in attachment of microtubules to centrosomes.

The presence of monastral bipolar spindles in all *KLP61F* mutants indicates that KLP61F plays a role in centrosome organization. Given that monopolar spindles are the predominant spindle type in the severe loss-of-function $KLP61F^3$ mutant and monastral bipolar spindles are the predominant spindle type in hypomorphic mutants such as $KLP61F^1$, one explanation for the range of spindles defects in *KLP61F* mutants is that more KLP61F function is required for centrosome separation than for centrosome organization. However, this explanation is inconsistent with retention of centrosomes at the pole of monopolar spindles in $KLP61F^1$ mutants as well as other loss-of-function *bimC* mutants (Enos and Morris, 1990; Hagan and Yanagida, 1990; Hoyt et al., 1992; Roof et al., 1991). Another explanation for our data is that KLP61F, directly or indirectly, antagonizes the activity of another protein(s) that can both generate loss of centrosome organization and hold centrosomes together such that they are not free to separate in prophase. It is noteworthy that loss of another kinesin related protein, KAR3, apparently bypasses the requirement for *bimC* function in *S. cerevisiae* (Hoyt et al., 1993). Similarly, loss of *bimC* function in *Aspergillus* is bypassed by loss of the KAR3 homolog, KlpA (O'Connell et al., 1993). Identification of the KAR3 homolog in *Drosophila* may provide insight into the relationship between monopolar and monastral bipolar spindles in *KLP61F* mutants.

We thank R. Karess, Centre de Genetique Moleculaire, CNRS, for training in preparation and analysis of orcein stained larval brains and many helpful discussions. We thank A. Scurbecq and R. Heil, University of Wisconsin, for technical assistance in fly husbandry. We are also grateful to S. Limbach, University of Wisconsin, for the sensitized film and B. Robertson and B. Ganetsky, University of Wisconsin, for preparation of fly food and propagation of the Canton S flies used in most of these studies. This work was supported by (P.G.W.) NIH Post-doctoral Fellowship in Gamete and Embryo Biology HD07432-06, American Cancer Society Post-doctoral Fellowship PF-328, Lucille P. Markey Charitable Trust 86-7, (M.T.F.) American Cancer Society Grant NP-711, and (G.G.B.) NIH Grant GM25062.

REFERENCES

Bier, E., Vaessin, H., Shephard, Lee, S. K., McCall, K., Barbel, S., Ackerman, L., Carretto R., Uemura, T., Grell, E., Yan, L. Y. and Jan, Y. N. (1989). Searching for pattern and mutation in the Drosophila genome with a P-lacZ vector. *Genes Dev.* **3**, 1273-1287.

Barton, N. R., Pereira, A. J., Goldstein, L. S. B. (1995). Motor activity and mitotic spindle localization of the Drosophila kinesin-like protein KLP61F. *Mol. Biol. Cell* **6**, 1563-1574.

Barton, N. and Goldstein, L. S. (1996). Going mobile: Microtubule motors and chromosome segregation. *Proc. Nat. Acad. Sci. USA* **93**, 1735-1742.

Bastmeyer, M., Steffen, W. and Fuge, H. (1986). Immunostaining of spindle components in tipulid spermatocytes using a serum against pericentriolar material. *Eur. J. Cell Biol.* **42**, 305-310.

Brenner, S., Branch, A., Meredith, S. and Berns, M. W. (1977). The absence of centrioles from spindle poles of rat kangaroo (PtK2) cells undergoing meiotic-like reduction division in vitro. *J. Cell Biol*. **72**, 368-379.

Calarco-Gillam, P., Siebert, M. C., Hubble, R., Mitchison, T. and Kirschner, M. (1983). Centrosome development in early mouse development defined by an autoantibody against pericentriolar material. *Cell* **35**, 621-625.

Church, K., Nicklas, R. B. and Lin, H.-P. P. (1986). Micromanipulated bivalents can trigger mini-spindle formation in Drosophila melanogaster spermatocyte cytoplasm. *J. Cell Biol.* **103**, 2765-2773.

Debec, A and Abbadie, C. (1989). The acentriolar state of the Drosophila cell lines 1182. *Biol. Cell* **67**, 307-311.

Dietz, R. (1966). The dispensability of the centrioles in the spermatocyte division of Pales ferruginea (Neatocera). *Chrom. Today* **1**, 161-166.

Doxsey, S. J., Stein, P., Evans, L., Calarco, P. D. and Kirschner, M. (1994). Pericentrin, a highly conserved centrosome protein involved in microtubule organization. *Cell* **76**, 639-650.

Enos, A. P. and Morris, N. R. (1990). Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in A. nidulans. *Cell* **60**, 1019-1027.

Felix, M. A., Antony, C., Wright, M. and Maro, B. (1994). Centrosome assembly in vitro: role of gamma-tubulin in recruitment in Xenopus sperm aster formation. *J. Cell Biol.* **124**, 19-31.

Fuller, M. T. and Wilson, P. G. (1992). Force and counterforce in the mitotic spindle. *Cell* **71**, 547-550.

Fuller, M. T. (1993). *Spermatogenesis.* In *The Development of* Drosophila melanogaster (ed. M. Bate and A. M. Arias), pp. 71-147. Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Gatti, M. and Baker, B. S. (1989). Genes controlling essential cell-cycle functions in Drosophila melanogaster. *Genes Dev.* **3**, 438-453.

Glover, D. M., Alphey, L., Axton, J. M., Cheshire, A., Dalby, B., Freeman, M., Girdham, C., Gonzalez, C., Karess, R. E. and Leibowitz, M. H. (1989). Mitosis in *Drosophila* development. *J Cell Sci Suppl. 12*, 277-291.

Glover, D. M., Leibowitz, M. H., McLean, D. A. and Parry, H. (1995). Mutations in *aurora* prevent centrosome separation leading to the formation of monopolar spindles. *Cell* **81**, 95-105.

Goodenough, W. W. and St Clair, H. S. (1975). Bald-2: a mutation affecting the formation of doublet and triplet sets of microtubules in Chlamydomonas reinhardtii. *J. Cell Biol.* **66**, 480-491.

Gonzales, C., Casal, C. and Ripoll, P. (1988). Functional monopolar spindles caused by mutations in *mgr*, a cell division gene of *Drosophila melanogaster*. *J. Cell Sci.* **89**, 39-47.

Gould, R. R. and Borisy, G. G. (1977). The pericentriolar material in Chinese hamster ovary cells nucleates microtubules formation. *J. Cell Biol.* **73**, 601-615.

Hagan, I. and Yanagida, M. (1990). Novel potential mitotic motor protein encoded by the fission yeast cut7^{+} gene. *Nature* **347**, 563-566.

Heck, M. S., Pereira, A., Pesavento, P., Yannoni, Y., Spradling, A. C. and Goldstein, L. S. B. (1993). The kinesin-like protein KLP61F is essential for mitosis in Drosophila. *J. Cell Biol.* **123**, 665-671.

Horio, T., Uzawa, S., Jung, M. K., Oakley, B. R., Tanaka, K. and Yanagida, M. (1991). The fission yeast gamma-tubulin is essential for mitosis and is localized at microtubule organizing centers. *J. Cell Sci.* **99**, 693-700.

Hoyt, M. A., He, L., Loo, K. K. and Saunders, W. S. (1992). Two Saccharomyces cerevisiae kinesin-related gene products required for mitotic spindle assembly. *J. Cell Biol.* **118**, 109-120.

Hoyt, M. A., He, L. and Saunders, W. S. (1993). Loss of function of Saccharomyces cerevisiae kinesin-related CIN8 and KIP1 is suppressed by KAR3 motor domain mutations.*Genetics* **135**, 35-44.

Joshi, H., Palacios, M. J., McNamara, L. and Cleveland, D. W. (1992). Gamma tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. *Nature* **356**, 80-83.

Karess, R. and Glover, D. (1989). Rough deal: a gene required for proper mitotic segregation in Drosophila. *J. Cell Biol.* **109**, 2951-2961.

Kellogg, D. R., Field, C. M. and Alberts, B. M. (1989). Identification of microtubule-associated proteins in the centrosome, spindle, and kinetochore of the early Drosophila embryo. *J. Cell Biol.* **109**, 2977-2991.

Kochanski, R. S. and Borisy, G. G. (1990). Mode of centriole duplication and distribution. *J. Cell Biol.* **110**, 1599-605.

Kuriyama, R. and Borisy, G. G. (1981). Centriole cycle in Chinese hamster ovary cells as determined by whole mount electron microscopy. *J. Cell Biol.* **91**, 814-821.

Le Guellec, R., Paris, J., Couturier, A., Le Guellec, K., Omilli, F., Camonis, J., S. MacNeill and Philippe, M. (1991). Cloning by differential screening of a Xenopus cDNA coding for a protein highly homologous to cdc2. *Proc. Nat. Acad. Sci. USA* **88**, 1039-1043.

Lewis, E. B. and Bacher, F. (1968). Method for feeding ethyl methane sulfonate (EMS) to Drosophila males. *Drosophila Inform. Serv*. **43**, 193.

Lifschytz, E. and Hareven, D. (1977). Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. *Dev. Biol.* **58**, 276-294.

Lifschytz, E. and Meyer, G. F. (1977). Characterization of male meiotic-sterile mutations in *Drosophila melanogaster*. *Chromosoma* **64**, 371-392.

Lindsley, D. L. and Zimm, R. (1992). *The Genome of* Drosophila melanogaster. New York, Academic Press.

Mazia, D., Harris, P. J. and Bibring, T. (1960). The multiplicity of the mitotic centers and the time course of their duplication and separation. *J. Biophys. Biochem. Cytol.* **7**, 1-20.

Mazia, D. (1984). Centrosomes and mitotic poles. *Exp. Cell Res*. **153**, 1-15.

Mitchison, T. J. (1989). Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* **109**, 637-652.

Moore, J. D. and Endow, S. A. (1996). Kinesin proteins: a phylum of motors for microtubule based motility. *BioEssays* **18**, 207-219.

Moritz, M., Braunfeld, M. B., Sedat, J. W., Alberts, B. and Aagard, D. (1995a). Three-dimensional structural charaterization of centrosomes from early Drosophila embryos. *J. Cell Biol.* **130**, 1149-1150.

Moritz, M., Braunfeld, M. B., Sedat, J. W., Alberts, B. and Aagard, D. (1995b). Microtubule nucleation by γ-tubulin-containing rings in the centrosome. *Nature* **378**, 638-640.

Nicklas, R. B. (1989). The motor for poleward chromosome movement in anaphase is in or near the kinetochore. *J Cell Biol.* **109**, 2245-2255.

Oakley, C. E. and Oakley, B. R. (1989). Identification of gamma-tubulin, a new member of the tubulin superfamily encoded by the mipA gene of Aspergillus nidulans. *Nature* **338**, 662-664.

Oakley, B. R., Oakley, C. E., Yoon, Y. and Yung, M. K. (1990). Gamma-tubulin is a component of the spindle pole body that is essential for microtubule function in Aspergillus nidulans. *Cell* **61**, 1289-1301.

O'Connell M. J., Meluh, P. B., Rose, M. D. and Morris, N. R. (1993). Suppression of the bimC4 mitotic spindle defect by deletion of klpA, a gene encoding a KAR3-related kinesin-like protein in Aspergillus nidulans. *J. Cell Biol*. **120**, 153-162.

Oregema, K., Whitfield, W. G. F. and Alberts, B. (1995). The cell cycle-dependent localization of the CP190 centrosomal protein is determined by the coordinate action of two separable domains. *J. Cell Biol*. **131**, 1261-1273.

Piperno, G. and Fuller, M. T. (1985). Monoclonal antibodies specific for an acetylated form of alpha-tubulin recognize the antigen in cilia and flagella from a variety of organisms. *J. Cell Biol.* **101**, 2085-2094.

Regan, C. and Fuller, M. T. (1990). Interacting genes that affect microtubule function in *Drosophila melanogaster*: Two classes of mutation revert the failure to complement between haync2 and mutations in tubulin genes. *Genetics* **125**, 77-90.

Rieder, C. L. and Borisy, G. G. (1982). The centrosome cycle in PtK2 cells: asymmetric distribution and structural changes in the pericentriolar material. *Biol. Cell* **44**, 117-132.

Ripabelli, M. G. and Callaini, G. (1996). Meiosis spindle organization in fertilized *Drosophila* oocyte: presence of centrosomal components in the meiotic apparatus. *J. Cell Sci.* **109**, 911-918.

Robbins, E. L., Jentzsch, G. and Micall, A. (1968). The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* **36**, 329-339.

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

Roof, D. M., Meluh, P. B. and Rose, M. D. (1991). Kinesin-related proteins required for assembly of the mitotic spindle. *J. Cell Biol.* **118**, 95-108.

Saunders, W. S. and Hoyt, M. A. (1992). Kinesin-related proteins required for structural integrity of the mitotic spindle. *Cell* **70**, 451-458.

Sawin, K. E., LeGuellec, K., Phillipe, M. and Mitchison, T. J. (1992). Mitotic spindle organization by a plus-end-directed microtubule motor. *Nature* **359**, 540-543.

Sluder, G. and Begg, D. A. (1985). Experimental analysis of the reproduction of spindle poles. *J. Cell Sci.* **76**, 35-51.

Sluder, G. and Rieder, C. (1985). Centriole number and the reproductive capacity of spindle poles. *J. Cell Biol.* **100**, 887-896.

Sluder, G., Miller, F. J. and Rieder, C. L. (1986). Experimental separation of pronuclei in fertilized sea urchin eggs. Chromosomes do not organize a spindle in the absence of centrosomes. *J. Cell Biol.* **100**, 897-903.

Sluder, G., Miller, F. J. and Rieder, C. L. (1989). Reproductive capacity of sea urchin centrosomes without centrioles. *Cell Motil. Cytoskel.* **13**, 264-273.

Smith, D. B. and Johnson, K. S. (1988). Single step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. *Gene* **67**, 31-40.

Sorbel, S. G. and Snyder, M. (1995). A highly divergent γ-tubulin gene is

essential for cell growth and proper microtubule organization in *Saccharomyces cerevisiae*. *J. Cell Biol.* **131**, 1775-1788.

Spradling, A. C. (1993). Developmental genetics of oogenesis. In *The Development of* Drosophila melanogaster (ed. M. Bate and A. M. Arias), pp. 1-70. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Stearns, T., Evans, L. and Kirschner, M. (1991). Gamma-tubulin is a highly conserved component of the centrosome. *Cell* **65**, 825-836.

Stearns, T. and Kirschner, M. (1994). In vitro reconstitution of centrosome assembly and function: the central role of γ-tubulin. *Cell* **76**, 623-637.

Steffen, W., Fuge, H., Dietz, R., Bastmeyer, M. and Muller, G. (1986). Aster-free spindle poles in insect spermatocytes: Evidence for chromosome-induced spindle formation. *J. Cell Biol.* **102**, 1679-1687.

Stewart, R. J., Pesavento, P. A., Woerple, D. N. and Goldstein, L. S. B. (1991). Identification and partial characterization of six members of the kinesin superfamily. *Proc. Nat. Acad. Sci. USA* **88**, 8470-8474.

Sullivan, W., Minden, J. S. and Alberts, B. M. (1990). *daughterless-abo-like*, A *Drosophila* maternal effect mutation that exhibits abnormal centrosome separation during the late blastoderm divisions. *Development* **110**, 311-323.

Sunkel, C. and Glover, D. M. (1988). *polo*: a mitotic mutant of *Drosophila* displaying abnormal spindls poles. *J. Cell Sci.* **89**, 25-38.

Sunkel, C. E., Gomes, R., Sampaio, P., Perdigao, J. and Gonzalez, C. (1995). Gamma-tubulin is required for the structure and function of the microtubule organizing centre in Drosophila neuroblasts. *EMBO J.* **14**, 28-36.

Szollosi, D., Calarco, P. and Donohue, R. P. (1972). Absence of centrioles in the first and second meiotic spindles in mouse oocytes. *J. Cell Sci.* **11**, 521-541.

Theurkauf, W. E. and Hawley, R. S. (1992). Meiotic spindle assembly in Drosophila females: Behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* **116**, 1167-1180.

Zhang, D. and Nicklas, R. B. (1995a). The impact of chromosomes and centrosomes on spindle assembly as observed in living cells. *J. Cell Biol.* **129**, 1125-1131.

Zhang, D. and Nicklas, R. B. (1995b). Chromosomes initiate spindle assembly upon experimental dissolution of the nuclear envelope in grasshopper spermatocytes. *J. Cell Biol.* **131**, 1287-1300.

Zheng, Y., Jung, M. K. and Oakley, B. R. (1991). Gamma-tubulin is present in Drosophila melanogaster and Homo sapiens and is associated with the centrosome. *Cell* **65**, 817-823.

Zheng, Y., Wong, M. L., Alberts, B. and Mitchison, T. (1995). Nucleation of microtubule assembly by a γ-tubulin containing ring complex. *Nature* **378**, 578-583.

(Received 7 August 1996 - Accepted 6 December 1996)