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Mutation of a gene for a *Drosophila* kinesin-like protein, *Klp38B,* leads to failure of cytokinesis

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SUMMARY

Mutations in a gene (*Klp38B*) encoding a novel kinesin-like protein in *Drosophila melanogaster* lead to the formation of polyploid cells in the larval central nervous system and in the follicle cells of adult egg chambers. Some homozygous mutants survive to adulthood and also exhibit morphological defects indicative of abnormal cell cycle progression, including rough eyes, missing bristles, and abnormal abdominal cuticles. In larval brains, there is no accumulation of mitotic cells and the frequency of anaphase figures is comparable to wild type, suggesting that nuclear division is not affected. Such brains contain polyploid cells with metaphase and anaphase chromosomes associated with bipolar spindles. Such spindles have a number of unseparated centrosomes at their poles reflecting the degree of polyploidy of the cell. Follicle cells frequently contain two nuclei of roughly equal size. Taken together, we conclude that these *Klp38B* mutations lead to a failure of cytokinesis resulting in polyploidy, and discuss whether or not this is a direct effect of the mutation.

Key words: *Drosophila*, Cytokinesis, Kinesin, Klp38B

INTRODUCTION

Successful cell division requires accurate segregation of replicated chromosomes in mitosis, spatially and temporally coordinated with the subsequent cytokinesis. *Drosophila melanogaster* is a good model metazoan in which to apply both classical and molecular genetic techniques to study this problem. It provides the means for studying the integration of cell cycle regulatory mechanisms with those of development. In embryonic development, the process of cytokinesis is only introduced after 13 rounds of nuclear division within a syncytium, and at least one gene, *pebble* (Hime and Saint, 1992; Lehner, 1992) is specifically required to be zygotically expressed immediately following this developmental stage to permit cytokinesis to occur.

In general, however, the sets of proteins identified as being required for cytokinesis in *Drosophila* are homologous to those in other eukaryotes. As might be expected, many of these are needed to set up the contractile actin ring at the position of the cleavage furrow. *twinstar*, a gene required for cytokinesis in larval neuroblasts and in the meiotic divisions in testis, was found to encode a homologue of cofilin, a 17 kDa actin severing protein (Gunsalus et al., 1995). *twinstar* mutations lead to the accumulation of abnormal actin aggregates. Similarly, a role for non-muscle myosin is well demonstrated by mutations in *spaghetti-squash*, which encodes the regulatory light chain of this heteromultimeric protein (Karess et al., 1991). Cytokinesis is blocked in these mutants leading to high levels of polyploidy in larval neuroblasts. Most striking is the conserved function of the septins, first identified as products of a set of *cdc* (*cell division cycle*) genes required for bud formation in *Saccharomyces cerevisiae*, and which constitute filaments in the yeast bud neck. The complex of the three septin polypeptides, Sep1, Sep2, and Pnut, has been purified from *Drosophila* embryos and shown to bind and hydrolyse GTP (Field et al., 1996). Mutations in the gene for one of these subunits, *peanut*, lead to large polyploid cells. Its product was localised to the cleavage furrow (Neufeld and Rubin, 1994). Moreover another *Drosophila* gene, *diaphanous*, required for cytokinesis at a number of developmental stages encodes a protein homologous to yeast *BNI1*, identified by genetic interactions with genes encoding the bud neck filament proteins (Castrillon and Wasserman, 1994).

Thus a picture is being built of the structural components required for cytokinesis; but what of the mechanisms that regulate the timing of cytokinesis and the positioning of the cleavage furrow? Cell cleavage should not take place before chromosome separation, and the position of the cleavage plane must lie between each of two daughter nuclei. A body of evidence suggests that in animal cell division the position of the cleavage plane can be determined either by the positions of the asters or by the central spindle itself (reviewed by Rappaport, 1986). This is in contrast to fission yeast, in which the division plane is correctly positioned in the absence of the mitotic

spindle upon overexpression of the *plo1*$^+$ gene (Ohkura et al., 1995). Although the mechanisms that spatially coordinate nuclear division and cytokinesis in metazoans remain unknown, some clues are beginning to emerge from the findings of mutants like *Klp3A* in *Drosophila*, in which the structure of the central spindle is disrupted, preventing subsequent cytokinesis (Williams et al., 1995). The finding by these authors that the KLP3A protein is localised to the central spindle in late anaphase and telophase is a pointer towards the spatial linkage between the processes of mitosis and cytokinesis. Symmetry of division has recently been shown to be under the control of the gene *inscuteable* whose product localizes to the apical cell cortex before mitosis, to establish polarity (Kraut et al., 1996).

Klp3A encodes one of about 30 *Drosophila* kinesin-like proteins, a family of motor proteins that utilise ATP to translocate along microtubules (Endow and Hatsumi, 1991). Of the other *Drosophila* kinesin-like proteins for which there are mutations, one is required for neuronal function and has no mitotic function (*DmKHCI*, Saxton et al., 1991); one is a DNA binding molecule essential for function of the meiotic spindle (*nod*, Afshar et al., 1995); one appears to be required to focus microtubules at the poles of the meiotic spindle (*ncd*, Endow et al., 1990); and one is essential for the process of centrosome separation to form a bipolar spindle (*Klp61F*, Heck et al., 1993). Such a large family of proteins offer much opportunity for functional redundancy. Indeed Williams and colleagues (1995) noted that although the KLP3A protein was present at many developmental stages, the major mutant defect was in cytokinesis during male meiosis, suggesting that other KLPs can provide overlapping functions.

We have been searching for mitotic mutants in the collection of 2,700 lethal and semi-lethal mutants on the 2nd chromosome generated by mobilisation of a modified P-element (*P-lacW*) (Török et al., 1993). As P-element insertions often cause hypomorphic mutations, one set of mutants that we chose to examine result in poor adult survival. Adult escapers from 'semi-lethal' alleles of mitotic mutants typically show morphological defects in the adult body, such as rough eyes and missing bristles, considered to be caused by a low frequency of abnormal cell division during the formation of adult structures (White-Cooper et al., 1996). We focused on some 70 or so mutants out of over 400 semi-lethal mutants in the collection of Török et al. (1993) that showed such adult morphological defects. In this report, we describe the molecular and functional study of a gene represented by two allelic mutants, 63/34 and 57/2, identified from this screen. We show that insertion of the P-elements in these two strains affects the function of a gene encoding a kinesin-like protein, resulting in a failure of cytokinesis with no apparent defects in chromosome segregation.

MATERIALS AND METHODS

Cytological analysis

In order to screen for mitotic defects within the collection of semi-lethal flies (Török et al., 1993), mutated chromosomes were rebalanced against *T(2,3)TSTL, Tb*, a euchromatic translocation between *SM5* and *TM6B*. Non-Tb larvae were selected and their brains were dissected for cytological examination. Squashed preparations of larval brains were examined as previously described (González and Glover, 1993) using a Nikon Microphot microscope with a ×60 phase objective. Photographs were taken using Kodak Pan 100. Ill-squashed regions in which the shapes of cells are not visible were excluded from the quantitative analyses. The mitotic index was determined by counting the average number of mitotic cells in one microscope field which roughly corresponds to 0.02 mm^2.

Whole mounts of the larval central nervous system were prepared for immunofluorescence as previously described (González and Glover, 1993). After fixation with formaldehyde, microtubules were detected using a rat monoclonal antibody against α-tubulin, YL1/2 (SeraLab), and FITC-conjugated anti-rat IgG antibody (Jackson Laboratories) as the first and second antibodies, respectively. DNA was stained with propidium iodide. These preparations were examined using a Nikon Optiphot microscope in conjunction with a Bio-Rad MRC600 confocal scanning head.

Identical procedures were used for immunostaining of follicle cells, except that a rabbit polyclonal anti-spectrin antiserum (Dubreuil et al., 1987; a kind gift from Dr Dan Branton, Harvard University) and FITC-conjugated anti-rabbit antibody (Jackson Laboratories) were used as the first and second antibodies, respectively. Follicle cells were examined at oogenesis stages 5-7 (King, 1970), just before or at the time that mitosis of these cells ceases.

In situ hybridisation

In situ hybridisation to polytene chromosomes from the salivary glands of third instar larvae was performed as previously described (Saunders et al., 1989). A *Bam*HI fragment extending from within the P-element to a position 3.3 kb proximal to the insertion site was used as a probe to localise the rescued fragment flanking the *P-lacW* element on wild-type polytene chromosomes. The pP{CaSpeR} vector (Pirrotta, 1988) was used for localising the *P-lacW* insertion site on polytene chromosomes from heterozygotes between *Klp38B* and wild type.

Nucleic acid techniques

Standard DNA or RNA manipulation techniques (Sambrook et al., 1989) were followed. Poly(A)$^+$ RNAs from wild type, mutants, revertants and mutants containing the HN237 transgene were isolated from 10 or 15 adult females using the QuickPrep Micro mRNA purification Kit (Pharmacia). Poly(A)$^+$ RNAs from various stages of the wild type were kindly provided by Drs Lisa Frenz and Mike Goldberg. Electrophoresis of RNA was carried out on formaldehyde gels. Hybridisation was carried out in the buffer described by Church and Gilbert (1984) at 65°C. The sizes of transcripts were estimated by comparison to RNA molecular size standards (Boehringer). The hybridisation probes were the 1.2 kb *Hin*dIII/*Not*I fragment from the *Klp38B* cDNA, a 1.2 kb *Hin*dIII/*Not*I fragment from the *fok* cDNA, a 0.6 kb *Sal*I/*Hin*dIII fragment from *Dmras64B* (Mozer et al., 1985), and a 0.6 kb *Eco*RI/*Hin*dIII fragment from cDNA encoding the ribosomal protein L17A (Noselli and Vincent, 1992).

A fragment flanking the *P-lacW* insertion in strain 63/34 was cloned by plasmid rescue following digestion of the genomic DNA with *Bam*HI or *Bgl*II, religation, and transformation into *E. coli* (XL1-Blue). It was confirmed that this fragment was derived from the 38B region by in situ hybridisation to wild-type polytene chromosomes, whereupon it was used for screening a wild-type cosmid library (Sidén-Kiamos, 1990). Six cosmids were isolated that together cover a region of about 40 kb around the *P-lacW* insertion site. A 9 kb *Bgl*II/*Not*I fragment from the wild-type genomic DNA extending from a position 2.1 kb distal to 7.4 kb proximal to the *P-lacW* insertion site was used to screen a 0-8 hour embryonic cDNA library constructed by Brown and Kafatos (1988).

Sequences of wild-type genomic DNA, cDNA and genomic DNA flanking the *P-lacW* element were determined by the dideoxy method using modified T7 polymerase (Pharmacia). Clones for sequencing were prepared by construction of deletion series (using the Nested Deletion kit from Pharmacia) or subcloning and sequenced by using appropriate primers. Approximately 10 kb of genomic DNA from 4

kb distal to 6.5 kb proximal to the *P-lacW* was sequenced. This includes the region between *fok* and the 3′ exon of *Klp38B*. No open reading frames of significant length were found except for *fok* and *Klp38B*. Nucleic acid sequences have been deposited in the EMBL Nucleotide Sequence Database and have the following accession numbers: *D. melanogaster* mRNA for kinesin-like protein KLP38B, Y10667; *D. melanogaster* fok mRNA, Y10668.

Reversion analysis

To determine whether the *P-lacW* at 38B is responsible for the defects in $Klp38B^{63/34}$, reversion analyses were carried out as follows: $yw;Klp38B^{63/34}/y^{+}CyO$ males were crossed with *w;Sp/CyO; Δ2-3 e Sb/TM6* females to obtain jump starter males, $w;\ Klp38B^{63/34}/CyO;$ *Δ2-3 e Sb/+*. Single males of this genotype were backcrossed with $yw;\ Klp38B^{63/34}/y^{+}CyO$ females. From 10 independent crosses, 7 crosses gave a total of 14 non-Cy revertants which do not have adult morphological defects. After establishing stocks homozygous for the revertant chromosome, 6 independent lines were examined for deletion of the *P-lacW* at 38B by genomic Southern hybridisation. *Bam*HI and *Bam*HI/*Not*I digested genomic DNAs from the homozygous revertants were analysed by using P-insertion flanking fragments extending together from 2.1 kb distal to 3.3 kb proximal of the *P-lacW* insertion as separate probes. This indicates that all 6 lines have deletions between 1.7 kb and 13 kb in the *P-lacW* sequence.

Introduction of wild-type *fok* gene into the genome

The 10 kb *Bam*HI genomic fragment which spans the intron of *Klp38B* containing the *fok* transcription unit was cloned into a P-element transformation vector pW8 (Klemenz et al., 1987) which has the w^{+} gene as a marker. The plasmid (pHN237) was used for germline transformation of a fly stock which has the *Δ2-3* element at 68B. Stocks of w^{+} transformants were established once *Δ2-3* had been out crossed. Two independent transformant lines with the transgene inserted on the X chromosome gave the same results in the rescue experiment. $P[w^{+},\ fok^{+}]$/Y; $Klp38B^{63/34}$ *sp px*/+ males were crossed with $Klp38B^{63/34}$ *sp px*/*CyO* females. Among the progeny, homozygotes of $Klp38B^{63/34}$ were identified by recessive markers *sp px* and the presence of the transforming wild-type *fok* gene was dictated by sex. The presence of the wild-type *fok* gene did not rescue either adult morphological defects or cytokinesis defects in follicle cells in $Klp38B^{63/34}$ homozygotes.

RESULTS

P-lacW insertions at 38B lead to polyploidy

The mutant strains 63/34 and 57/2 both show a low level of survival of adults that have a range of morphological defects. Squashed preparations of larval neuroblasts revealed polyploid mitotic figures in both strains, which carry allelic mutations resulting from independent P-element insertion events. Although most of the analysis presented here was carried out with homozygotes of the 63/34 strain, 57/2 homozygotes and trans-heterozygotes between two strains show identical defective phenotypes (see below).

To identify the gene responsible for the defects in these mutants, we first located the position of the *P-lacW* element on the polytene chromosomes. In situ hybridisation using the *P-lacW* sequence as a probe localised a single insertion site to 38B on the salivary gland chromosomes in the two strains. We then carried out complementation tests with second chromosomes carrying defined deficiencies in this region. This allowed us to independently position the mutations to the interval 38A6-38C1, consistent with the physical localisation of the P-element (Fig. 1A).

To confirm that the *P-lacW* inserted at 38B is responsible for the mutant defects, we remobilised the *P-lacW* element in strain 63/34 by introduction of *Δ2-3* as a transposase source. After exposure to *Δ2-3*, the mutant chromosomes are tested over the original mutant chromosome for the reversion of adult morphological defects. We obtained a high frequency of such revertants, from which we established six independent lines (see Materials and Methods). Moreover, full fertility and viability were restored and there were no cell division defects in these revertants (see below). Southern hybridisation experiments showed that the 6 revertants examined had all undergone deletions in the *P-lacW* sequences at 38B (data not shown). This indicates that both the adult and cell division defects are caused by the *P-lacW* at 38B, not by other mutations or undetectable P-elements on the chromosome.

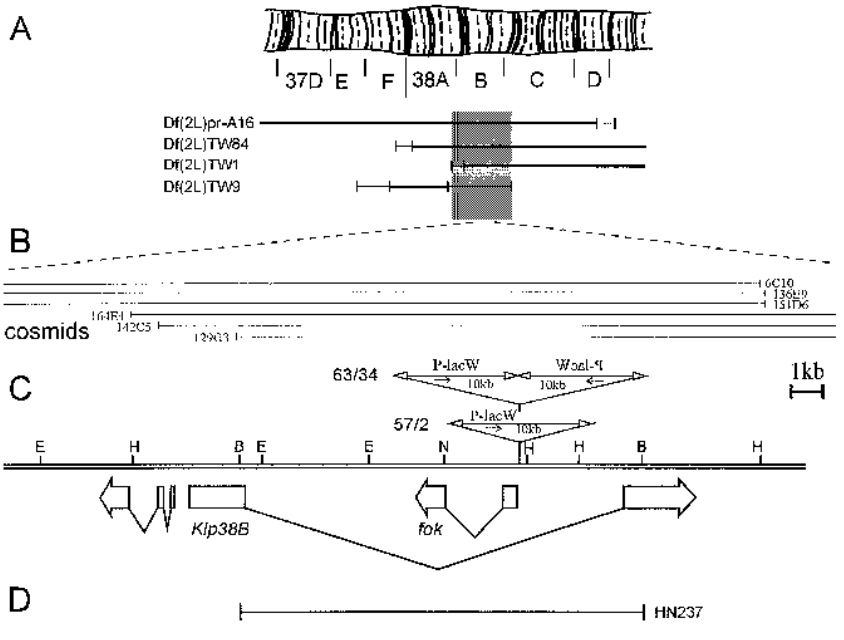

Fig. 1. Cytogenetic and molecular maps around the *P-lacW* insertion site in *Klp38B*. (A) This panel depicts the extent of deficiencies which uncover the *Klp38B* mutations: *Df(2L)pr-A16*, *Df(2L)TW84*, *Df(2L)TW1* and *Df(2L)TW9*. Deficiencies that complement the mutations are *Df(2L)TW50*, *Df(2L)VA17*, *Df(2L)E55* and *Df(2L)pr76* (not shown). This locates the mutation between the distal breakpoint (38A7-B1) of *Df(2L)TW1* and the proximal breakpoint (38A6-C1) of *Df(2L)TW9*. (B) The region covered by each of six cosmids is indicated as a series of horizontal lines. (C) The restriction map of a segment of genomic DNA reconstructed from maps of the cosmids. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Not*I. The insertion sites of the single *P-lacW* element in the strains 57/2, and the inverted elements in the strain 63/34 are indicated. Open arrows indicate transcription units identified by a combination of cDNA isolation, northern hybridisation and genomic sequencing. The introns are indicated by kinked lines. Sequencing of 10 kb genomic DNA surrounding the *P-lacW* indicates there are no other long open reading frames. (D) The horizontal line represents the region used for restoration of wild-type *fok* gene expression in germline transformants.

Reduced expression of a gene (*Klp38B*) encoding a kinesin-like protein leads to the mutant defects

Molecular analysis of the genomic DNA of the 63/34 mutant reveals that two *P-lacW* sequences are inserted at 38B as inverted repeats. Molecular analysis of the 57/2 allele shows a single *P-lacW* element to be inserted at exactly the same site as the insertion in 63/34. As these two alleles arose from independent events, this confirms that insertion of *P-lacW* at this site is responsible for the defects seen in the mutants.

The fragment of genomic DNA flanking *P-lacW,* cloned by plasmid rescue, was used to isolate overlapping cosmid clones from a genomic library (Sidén-Kiamos, 1990). In this way, a 40 kb region of wild-type genomic DNA was identified, from which fragments were used to screen for cDNA clones. A combination of cDNA isolation, northern hybridisation and sequencing of both cDNA and genomic DNA revealed two transcription units whose expression might be expected to be affected by the *P-lacW* elements (Fig. 1C). One transcription unit (3.5 kb) encodes a kinesin-like protein (KLP; see below). We call this gene *Klp38B* reflecting its cytological localisation. It has a 10 kb long intron which interrupts the sequence encoding motor domain. We refer to the second transcription unit as *fledgeling of Klp38B (fok)* since it is nested within the intron. Its direction of transcription is opposite to that of *Klp38B*. In addition there is a third transcription unit divergent to *Klp38B* that is more distant from the P-element insertion.

To determine which gene might be responsible for the cell division defects seen in the mutants, we first examined the expression pattern of both genes (Fig. 2). Cell division takes place only in limited tissues and at restricted developmental stages. Consequently, as a general rule, gene products required for cell division are expressed in gametogenesis in adults, are highly abundant in embryos, and are much less abundant at other stages. The transcript of *Klp38B* follows this pattern, and shows a developmental profile of expression similar to other cell cycle genes (e.g. cyclins A and B, Whitfield et al., 1989; *cdc2* and *string*, Jimenez et al., 1990). In contrast, *fok* transcripts are abundant in 0-24 hour embryos and adults, but much less so in 0-4 hour embryos. Our detailed analysis of the mutant phenotype indicates that the mutants show defects in cytokinesis. As cytokinesis begins at around 70 minutes of embryonic development, this suggests that the *fok* transcript is not needed for this process.

Although there appears to be a correlation between periods of cell division and the developmental time of expression of the *Klp38B* gene rather than the *fok* gene, this does not exclude the possibility that either of these two genes may be functionally redundant, and that related proteins may substitute for their function at any given stage. Comparison of transcript levels between wild-type and mutant flies and their revertants resolved this problem. When we carried out northern hybridisation against poly(A)$^+$ RNAs isolated from adult female flies of both wild-type and homozygous mutant, we found that *Klp38B* transcripts were below the level of detection, and that *fok* transcript levels were also reduced in the mutant (Fig. 3). Thus insertion of the P-element affects the expression of both genes. We next determined whether either transcript is restored in those lines showing full reversion to the wild-type phenotype, and in which part of the *P-lacW* sequences at 38B have been deleted from the chromosome. We found that *Klp38B* transcripts were restored in these lines, whereas *fok* transcripts were not (Fig. 3). This indicates that regaining the *Klp38B* transcript is sufficient to revert the defective phenotypes in 63/34 to wild type.

Finally, to exclude the possibility that a reduced transcript level of *fok* is responsible for the defects in the mutants, we introduced a 10 kb segment of wild-type genomic DNA (HN237, Fig. 1D) containing the *fok* gene and flanking sequences into the genome by P-element mediated germline transformation. We then constructed strains homozygous for the 63/34 insertion, but with one extra copy of wild-type *fok* gene on the X chromosome. Despite the presence of the wild-

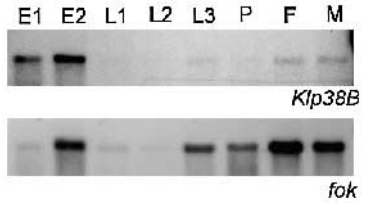

Fig. 2. Expression pattern of *Klp38B* and *fok* during development. *Klp38B* and *fok* transcripts examined by northern hybridisation to 2 μg of wild-type poly(A)$^+$ RNAs from the following developmental stages: E1, 0-4 hour embryos; E2, 0-24 hour embryos; L1, first instar larvae; L2, second instar larvae; L3, third instar larvae; P, late pupae; F, adult females; M, adult males. The same filter was re-used to detect the indicated transcripts by successive hybridisation and melting.

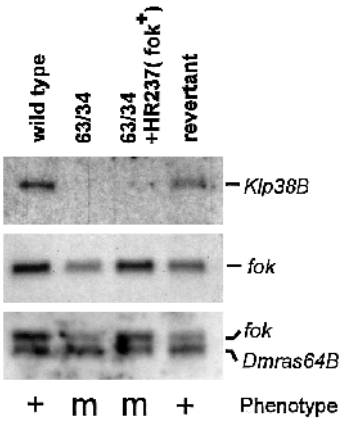

Fig. 3. Reduction in the *Klp38B* transcript is associated with the mutant phenotype. *Klp38B* and *fok* transcripts in 63/34 mutants were examined by northern hybridisation. Poly(A)$^+$ RNA was isolated from adult females of wild type (the 1st lane); homozygotes of strain 63/34 (the 2nd lane); 63/34 homozygotes also carrying the HN237 transforming segment containing the wild-type *fok* gene (the 3rd lane); and a revertant (1-4) of strain 63/34 obtained following P-element remobilisation (the 4th lane). The *Klp38B* transcript was detected by a probe which hybridises to a region downstream of the intron. *Dmras64B* was used for a loading control and its transcripts were detected at the same time as the *fok* transcripts for direct comparison. In the bottom row marked 'Phenotype', + indicates strains that appear wild type, and m indicates strains that show defects typical of 63/34, including reduced fertility and viability, adult morphological defects and cytokinesis defects. The results indicate that *Klp38B* is responsible for the defects seen in the mutants.

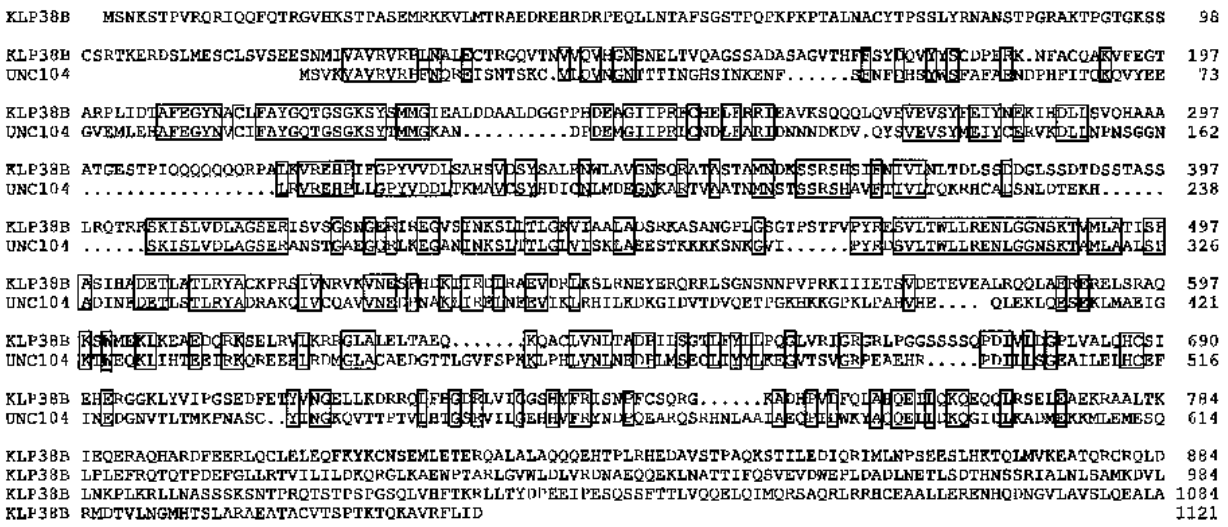

Fig. 4. Comparison of KLP38B with UNC-104, a kinesin-like protein from *C. elegans*. The entire amino acid sequences of KLP38B are shown in comparison with the N-terminal region of UNC-104. The remaining segment of UNC-104 shows little or no similarity. Only identical amino acid residues are marked.

type *fok* gene, these strains showed reduced adult viability, adult morphological defects, and cytokinesis defects identical to those seen in the original homozygous mutants. Two independent transformants gave identical results suggesting that the particular insertion site of the transforming DNA is not critical for expression. This was confirmed by northern blotting which showed that introduction of the 10 kb genomic fragment is sufficient to restore the expression of *fok* gene (Fig. 3). Thus we conclude it is disruption of the expression of *Klp38B* that is responsible for the morphological and cytokinesis defects in the mutants.

The primary sequence of the KLP38B

The sequence of the transcription unit predicts it to encode a protein of 1,121 amino acids in length, of 125 kDa molecular mass, and with an isoelectric point of 7.5. Searches of current databases revealed it has similarity to members of the kinesin superfamily. The most closely related protein is UNC-104 from *Caenorhabditis elegans* (Hall and Hedgecock, 1991, Fig. 4). However, the similarity is restricted to the motor domain which is situated in the amino-terminal region of the protein. In the motor domain, the identity between the amino acid sequences of KLP38B and UNC-104 is 49%. This is higher than the identity (36%) between KLP38B and the kinesin heavy chain of *Drosophila* but significantly lower than the identity (66%) between UNC-104 and its murine counterpart KIF1A in which the homology extends into the C-terminal region (Okada et al., 1995). UNC-104 and closely related proteins have roles in the transport of vesicles or mitochondria in axons and form one of several subfamilies of KLPs (Goldstein, 1993; Moore and Endow, 1995). Consideration of the structural and functional relationships of *Drosophila* KLP38B with other KLPs suggests that it may define a new sub-grouping of KLPs related to UNC104.

Klp38B mutants show adult defects typical of mutations affecting cell cycle progression

Adults homozygous for $Klp38B^{63/34}$ show a variety of morphological defects typical of mutations affecting cell cycle progression (Fig. 5). The eyes are rough as a result of missing omatidia. Bristles can also be missing but the bristle structure itself is normal. The mutation most commonly affects humeral bristles, but the effects are not restricted to them. Abdominal structures are also disrupted especially in females. In general, the movements of adults are not well coordinated. They tend to die earlier than heterozygous siblings. Although females are

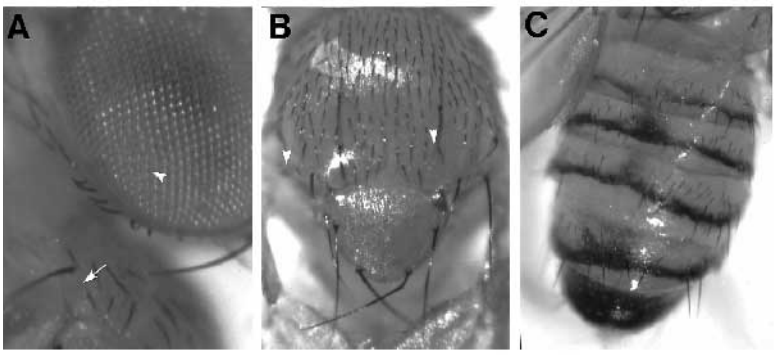

Fig. 5. Adult morphological defects in *Klp38B* mutants. Adult survivors homozygous for $Klp38B^{63/34}$ show morphological defects typical of many mitotic mutants. (A) Eye with abnormal alignment of omatidia (arrowhead). In addition, one of the two humeral bristles is missing (arrow). (B) Thorax with missing bristles (arrowheads). (C) Irregular pattern of abdominal cuticle.

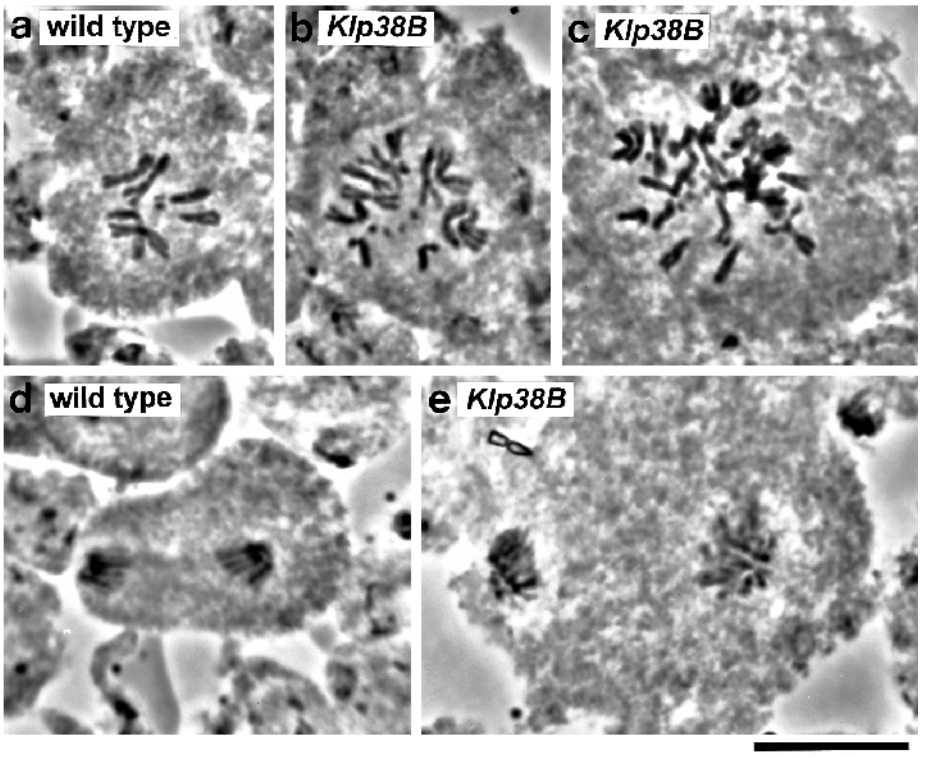

Fig. 6. Cytological phenotype in the central nervous system of *Klp38B* mutant larvae. Mitotic figures observed by orcein staining of squashed brain preparations from the third instar larvae of wild type (a,d) and *Klp38B*$^{63/34}$*/Df(2L)pr-A16* (b,c,e). (a) Wild-type diploid metaphase. (b) Mutant tetraploid metaphase. (c) Mutant octaploid metaphase. (d) Wild-type diploid anaphase. (e) Mutant tetraploid anaphase. Bar, 10 μm.

fertile, fertility is greatly reduced. Males appear to be sterile. However, cytological analysis of the testes indicates there are few, if any, meiotic defects and they can produce motile sperm. Male sterility observed in the mutants might therefore be due to behavioural and coordination problems. None of these defects are significantly changed in heterozygotes between the mutation and deficiencies uncovering the mutation, suggesting that the mutation is amorphic or a severe hypomorph.

The progeny of homozygous mutant females are subject to maternal effect lethality. All of the adult progeny of homozygous females and heterozygous males are heterozygotes indicating a maternal effect that can only be rescued by wild-type zygotic expression. We determined the lethal phase of the homozygous mutants lacking any wild-type maternal products by using a strain carrying the *T(2,3)TSTL, Tb* balancer to identify the contribution of paternal wild-type chromosomes to heterozygous offspring. As the only larvae we were able to observe were Tb and so carried this balancer chromosome, we conclude that homozygotes die at either embryonic or early larval stages in the absence of wild-type zygotic expression.

Mitotic progression in the central nervous system of *Klp38B* mutants

To determine whether mitotic defects could be detected during the development of homozygous *Klp38B*$^{63/34}$ mutants, we examined aceto-orcein stained preparations of larval brains. Whereas wild-type metaphase cells contain exclusively a diploid complement of chromosomes (two large pairs of autosomes, one pair of small fourth chromosomes, and the sex chromosomes; Fig. 6a), 7% of mitotic cells in homozygous mutant brains contain a tetraploid complement of chromosomes. In hemizygous mutants, the total proportion of polyploid cells increases to 17% of which an increased number become octaploid (Fig. 6; Table 1). Thus examination of the phenotype at the cellular level indicates the hypomorphic nature of this mutation.

Polyploid cells can be produced either by the failure of chromosome segregation or cytokinesis (Fig. 7). Defects in chromosome segregation lead to an increase in the frequency of mitotic cells with a lower proportion in anaphase, often coupled with a high degree of chromosome condensation as

Table 1. Mitotic parameters in *Klp38B* mutants

Genotype	Mitotic index*	Ploidy (%)				% Anaphase†
		2n	4n	8n	>8n	
Wild type	2.1	100	0	0	0	23
Klp38B$^{63/34}$	2.3	93	7	0.1	0	22
Klp38B$^{63/34}$*/Df*‡	1.9	82	13	3.8	0.7	19

*The number of mitotic cells per field using a ×60 objective, which roughly corresponds to 0.02 mm^2 and contains approximately 200 cells. A total of at least 340 fields from 4 larval brains were examined for each genotype.

†Frequency of anaphase cells in all mitotic cells.

‡*Df(2L)pr-A16* was used for a deficiency.

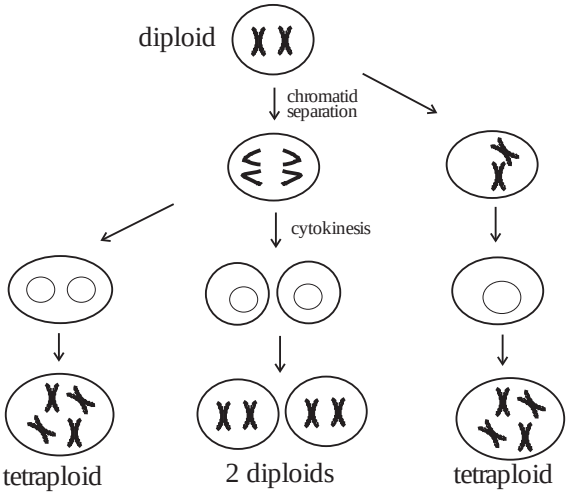

Fig. 7. Two possible mechanisms for the production of polyploid cells. The left and right columns indicate how tetraploid cells can arise from failure of cytokinesis and chromatid separation, respectively. Although failure of either chromosome segregation or cytokinesis could result in tetraploidy, binuclear cells arise only by a failure of cytokinesis. Thick solid bars represent chromosomes (n=1 for simplification). Large and small circles represent outlines of cell and nuclei, respectively.

cells become blocked or delayed at metaphase or prometaphase (Gatti and Baker, 1989). However, the frequency of mitotic cells and anaphase cells is comparable in the *Klp38B* mutant to wild type (Table 1). Moreover, we saw no chromosome over-condensation in diploid cells, and only in a low proportion of tetraploid cells (12% of tetraploid cells in homozygotes, and 19% in hemizygotes). Chromosome segregation also appears to occur normally in anaphase, even in polyploid cells (Fig. 6e). These observations suggest that polyploidy does not arise as a consequence of aberrant chromosome segregation in the mutant neuroblasts.

To further analyse chromosome segregation, we studied the three-dimensional structure of the mitotic spindle by immunostaining whole mount preparations of larval brains using an anti-αtubulin monoclonal antibody and the DNA binding dye propidium iodide (Fig. 8A,B). We were unable to find any obvious defects in the structure of spindle microtubules at this level of resolution, and observed polyploid complements of chromosomes at metaphase and anaphase in association with bipolar spindles. Interestingly, the polyploid metaphase figures had a number of poorly separated centrosomal bodies at two

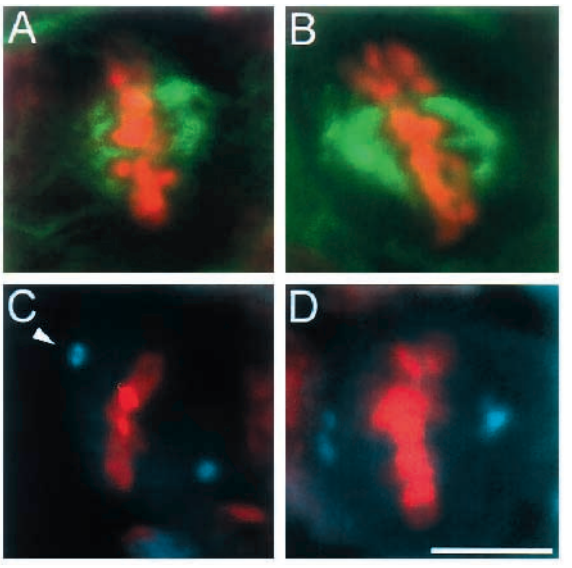

Fig. 8. Organisation of spindles and centrosomes in polyploid cells of *Klp38B* mutants. Whole mount preparations of mutant larval brains immunostained to reveal spindle microtubules and DNA (A,B), or centrosomes and DNA (C,D). Microtubules (green) are stained using the antibody YL1/2 (Materials and Methods), centrosomes (blue) using the antibody Rb188 (Whitfield et al., 1988), and DNA (red) using propidium iodide. The arrowhead points to centrosomes that appear to have duplicated. Bar, 10 μm.

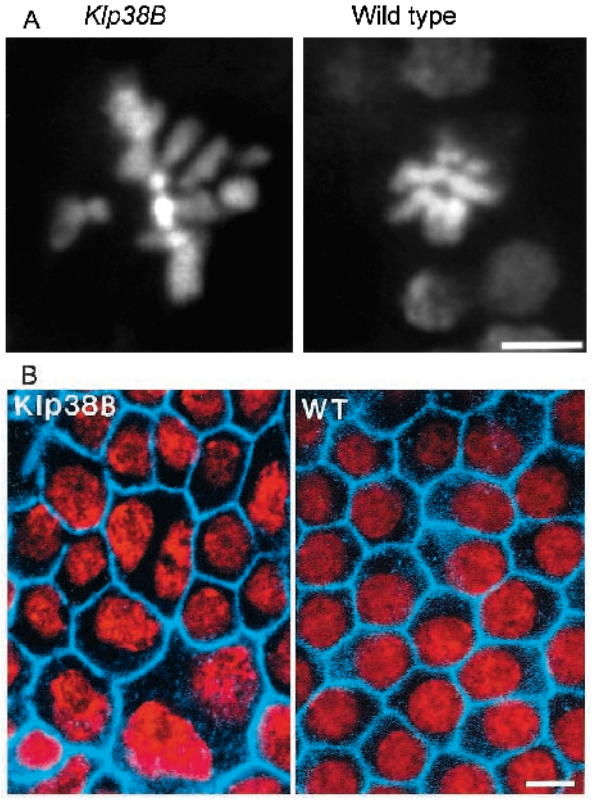

Fig. 9. Cytological phenotype in follicle cells of *Klp38B* mutants. (A) Polyploid metaphase follicle cells from *Klp38B*$^{63/34}$ (left) and euploid wild type (right). Ovaries were fixed and stained with a DNA probe, propidium iodide. (B) Interphase follicle cells from *Klp38B*$^{63/34}$ (left) and wild type (right). Cell outlines (blue) and nuclei (red) were visualised in fixed preparations of ovaries using anti-alpha spectrin antibody and propidium iodide, respectively. In *Klp38B* mutants, some cells contain two nuclei of roughly equal sizes. In this field, one binucleate cell has nuclei that appear from their size to be diploid (centre), whereas a second binucleate cell has larger (possibly tetraploid nuclei). The latter cell could have arisen as a result of two rounds of failed cytokinesis: the first round would have produced a binucleate cell also containing two centrosomes; the centrosomes would have nucleated a bipolar spindle in a second round of mitosis, and condensed chromosomes from the two nuclei congressed towards a common metaphase plate; the ensuing anaphase would generate the binucleate cell with polyploid nuclei. Bar, 10 μm.

poles as revealed by staining for the centrosome associated antigen CP190 (Whitfield et al., 1988; Fig. 8C,D). The number of centrosomes appears to be in proportion to the degree of polyploidy suggesting that the centrosome cycle is continuing in concert with the nuclear division cycle in these cells in the absence of cytokinesis.

Multinucleate ovarian follicle cells arise from cytokinesis defects

We also studied cell division in the ovaries of adult females. The majority of the homozygous *Klp38B* adult females lay either no eggs or very few. In general, their ovaries are small and produce a reduced number of mature oocytes. In the wild-type egg chamber, the nurse cells and oocyte are surrounded by a monolayer of somatically derived follicle cells. As the egg chamber enlarges, the number of follicle cells increase to 1,000 to maintain a monolayer. After four or five mitoses, these cells cease division but increase further in size by polytenisation (King, 1970). In contrast to wild-type follicle cells, polyploid sets of chromosomes are clearly visible in some mitotic cells in $Klp38B^{63/34}$ females (Fig. 9A). Moreover, whereas the nuclei of wild-type follicle cells in a given egg chamber are of uniform size and are aligned in an organised manner in cells of equally regular size, follicle cells in the mutant ovary vary in size, and contain some larger nuclei (Fig. 9B). Most strikingly, whereas in the wild-type, all follicle cells contain one nucleus of uniform size, in the mutants the larger follicle cells often contain two nuclei (Fig. 9B). The two nuclei in any one cell might sometimes be larger than wild-type nuclei, but are always of a similar size to each other. This indicates that the cell has undertaken proper nuclear division, but has failed to carry out cytokinesis in the previous mitosis. Thus our observations in cells of both the central nervous system and in follicles demonstrate that polyploidy arises in the mutants as a consequence of the failure of cytokinesis rather than of chromosome segregation.

DISCUSSION

Mitotic defects leading to semi-lethality

Previous screens for *Drosophila* mutants with aberrant mitoses have exploited the maternal contribution to the embryo. This is either because females homozygous for certain mutant alleles produce embryos that show mitotic abnormalities (e.g. Sunkel and Glover, 1988), or because expression of a single copy of a wild-type gene in a heterozygous mother can provide for survival of homozygous mutant progeny until late larval or pupal stages (e.g. Gatti and Baker, 1988). If the maternally contributed product is unstable, for example as with the *cdc25* homologue, *string*, cell cycle defects are seen after cellularisation of the embryo (Edgar and O'Farrell, 1989). We searched for mitotic mutants that show morphological defects in surviving adults. These arise since cell divisions can be tightly coupled with differentiation at very late stages of morphogenesis. For example, missing bristles can be seen if a sensory mother cell fails to undergo one of its two cell divisions to produce four cells, one of which becomes a bristle. Similarly, failure of cell division in the morphogenetic furrow of the developing eye disc can lead to rough eyes, and abnormal division of histoblasts can lead to malformations of the adult abdomen. Thus these adult morphological defects can be very sensitive markers for abnormal cell divisions, even at low frequencies, as with the mutant alleles of *Klp38B* under study in this paper.

Our results show that survival of some $Klp38B^{63/34}$ and $Klp38B^{57/2}$ mutants is at least partly due to the perdurance of wild-type maternal gene products. When there is no wild-type maternal contribution, homozygous embryos die at embryonic or early larval stages. However, our attempts to examine the cellular phenotypes in these embryos failed because very few eggs were laid by homozygous females. Survival may also result from residual gene activity because the two mutations under study are both hypomorphic.

Is *Klp38B* required for chromosome segregation?

Two lines of evidence indicate that the polyploidy resulting from failure to fully express this gene is due to defects in cytokinesis rather than chromosome segregation. First of all, the *Klp38B* mutations do not fit the general rule that mutations which prevent chromosome segregation result in an increased frequency of mitotic cells, a decreased frequency of anaphases, and overcondensation of chromosomes (Gatti and Baker, 1989). Mutations in *Klp61F* (kinesin-like protein at 61F), for example, which is required for bipolar spindle formation, result in about 15% of mitotic cells showing polyploidy. Frequencies of mitotic cells are twice that of wild-type; anaphases are rarely seen; most polyploid cells have overcondensed chromosomes; and even in diploid cells, chromosome overcondensation is very common (Heck et al., 1993, Y. Inoue, personal communication). However, severe loss of *Klp38B* expression leads to polyploidy in larval neuroblasts without appearing to affect mitotic progression drastically. The mitotic index and frequency of anaphases are both comparable to wild type, and only a low level of chromosome over-condensation is seen even in the polyploid cells. Direct evidence for failure in cytokinesis comes from our findings of binucleate follicle cells in which the size of the nuclei is roughly the same, one to the other. It is likely that failure of cytokinesis is not limited to these tissues, since the adult morphological defects suggest a low frequency of cell division defects in imaginal discs and histoblasts.

It is difficult to exclude the possibility that the *Klp38B* gene is also involved in chromosome segregation because our results suggest that the *Klp38B* mutants are not nulls, and the mitotic defects are enhanced when the mutations are heterozygous with a deficiency for the chromosomal region. Thus, there may be other roles for this kinesin-like protein in either spindle formation or function. This highlights a need to study additional alleles at the locus.

Spindle structure has not been examined in the larval neuroblasts of other mutants affecting cytokinesis, although orcein stained preparations appear to indicate the possibility of either multipolar polyploid anaphases in *l(3)7m62* (Gatti and Baker, 1989), or bipolar polyploid anaphases in *diaphanous* (Castrillon and Wasserman, 1994). The polyploid mitoses in the *Klp38B* mutants occur on bipolar spindles with multiple centrosomes at the poles. It would appear that such cells have failed to undergo cytokinesis in the previous cycle. They have inherited two sets of chromosomes and centrosomes that have each undergone replication, but whereas the two sets of repli-

cated chromatids undergo anaphase separation, the duplicated centrosomes remain clustered at the spindle poles. It is possible that the failure of centrosome separation with the preservation of the ability to form a bipolar spindle is an inherent consequence of the failure of cytokinesis in the preceding cycle. However, another possibility is that the kinesin-like protein might have a role in centrosome separation. In the light of the potential relationship between the structure of the spindle and the process of cytokinesis (see also below), it will be of considerable future interest to examine centrosome behaviour in other mutants affecting cytokinesis.

Requirements for kinesin-like proteins in cytokinesis

Kinesin-like proteins have been proposed to be involved in several mitotic or meiotic processes including formation of bipolar spindles, chromosome movement and spindle elongation, as well as in vesicle movements (for review, see Barton and Goldstein, 1996). Speculation about the functions of many of these molecules is based on their sub-cellular localisation, biochemical properties, or simply homology to other molecules. Genetic analysis has thus an important role for studying the function of this class of proteins. Of the 30 or so genes encoding kinesin-like proteins that exist in the *Drosophila* genome (Endow and Hatsumi, 1991), mutant phenotypes have been described in detail for only 5 genes (see Introduction). Of these, the *Klp3A* gene appears to be required for the initiation of cytokinesis. Mutations in this gene disrupt the interdigitation of microtubules in the central meiotic spindles of spermatocytes, a region to which the KLP3A protein normally localises during late anaphase and telophase (Williams et al., 1995). Interestingly KLP3A does not appear to be required for divisions of most somatic cells, but it is expressed in these tissues. This suggests there may be other genes which have an overlapping function with *Klp3A*, and it is not inconceivable that *Klp38B* could be one such gene. We have looked for cytokinesis defects in the testes of *Klp38B* mutants, and although we can find some spermatids with a large Nebenkern (mitochondrial aggregate) and two equal sized nuclei, indicating failure of cytokinesis in meiosis II, the frequency of such cells is extremely low.

It is a formal possibility that the KLP38B protein is directly involved in cytokinesis. However, to date there is no evidence that microtubules are directly involved in cytokinesis. Loss of the kinesin-like protein could affect some aspect of structure and function of the mitotic spindle which is not essential for nuclear division, resulting in the absence of a positive signal or production of a negative signal, to prevent cytokinesis. There is compelling evidence that in Echinoderm embryos asters can dictate the position of the cleavage furrow (reviewed by Rappaport, 1986), whereas in mammalian cells there is growing support for the notion that such a signal originates from the central spindle (Rappaport and Rappaport, 1974; Kawamura, 1977). Wheatley and Wang (1996) have found that when cultured mammalian cells are induced to undergo tripolar mitoses, in contrast to the results obtained with Echinoderm embryos, the position of cytokinesis is determined by the position of mid-zone microtubules. Furthermore, Martineau and colleagues (1995) have determined conditions in which dihydrocytochalasin B (DCB) will block cytokinesis and yet allow cells to exit mitosis. The resulting binucleate interphase-like cells retain a stable structure composed of a mid-zone array of microtubules associated with a telophase disc, a structure which normally forms only transiently at the site of the metaphase plate (Andreassen et al., 1991). This combined structure can persist for up to an hour during which time cytokinesis can be induced following removal of the DCB. Either or both the microtubules or telophase disc could transmit the signal for cytokinesis which can be physically blocked by creating a barrier by placing a blunt needle between this region of the spindle and the cell cortex (Cao and Wang, 1996). Kinesin-like proteins do play a role in organising the central spindle during late anaphase. They could thus facilitate the formation of the telophase disc, and could also transport other molecules that could serve to 'bridge' microtubules of the central spindle to the complex contractile structure that forms in the position of the cleavage furrow. This is clearly a possibility with *Klp3A* mutants, which lack the central spindle in male meiosis. In the case of *Klp38B* mutants, however, we can neither confirm nor exclude this possibility because of the difficulties of identifying the central spindle in neuroblasts and follicle cells. It is therefore of considerable future interest to be able to localise the KLP38B molecule in relation to the dynamic structure of the mitotic apparatus.

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