# *Drosophila dd4* mutants reveal that γTuRC is required to maintain juxtaposed half spindles in spermatocytes

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## Summary

**The weak spindle integrity checkpoint in *Drosophila* spermatocytes has revealed a novel function of the γ-tubulin ring complex (γTuRC) in maintaining spindle bipolarity throughout meiosis. Bipolar and bi-astral spindles could form in *Drosophila* mutants for *dd4*, the gene encoding the 91 kDa subunit of γTuRC. However, these spindles collapsed around metaphase and began to elongate as if attempting anaphase B. The microtubules of the collapsing spindle folded back on themselves, their putative plus ends forming the focused apexes of biconical figures. Cells with such spindles were unable to undergo cytokinesis. A second type of spindle, monopolar hemi-spindles, also formed as a result of either spindle collapse at an earlier stage or failure of centrosome separation. Multiple centrosome-like bodies at the foci of hemi-spindles nucleated robust asters of microtubules in the absence of detectable γ-tubulin. Time-lapse imaging revealed these to be intermediates that developed into cones, structures that also had putative plus ends of microtubules focused at their tips. Unlike biconical figures, however, cones seemed to contain a central spindle-like structure at their apexes and undergo cytokinesis. We conclude that spermatocytes do not need astral microtubules nucleated by opposite poles to intersect in order to form a central spindle and a cleavage furrow.**

Movies available online

Key words: Cell division, Mitotic spindle apparatus, Centrosome, Microtubules, Tubulin

## Introduction

γ-Tubulin is found in animal centrosomes and the spindle pole bodies of yeasts (Horio et al., 1991; Joshi et al., 1992; Oakley and Oakley, 1989; Sunkel et al., 1995). In budding yeast it is part of a complex with Spc97p and Spc98p at the inner and outer plaques of the spindle pole body (SPB) (Geissler et al., 1996; Knop and Schiebel, 1997). Counterparts of these two proteins also exist in animal cells and in *Drosophila* they are known as Dgrip84 and Dgrip91, respectively (Oegema et al., 1999). Together with some additional accessory conserved proteins, these constitute the γTuRC, the name reflecting the shape of the complex when visualized by electron microscopy (Moritz et al., 1995a; Moritz et al., 1995b; Zheng et al., 1995). Several *in vitro* studies point towards a role for the γTuRC in centrosome assembly and microtubule nucleation (Felix et al., 1994; Oegema et al., 1999; Stearns et al., 1994; Zheng et al., 1995). Centrosomes in vitro lose their ability to nucleate microtubules when stripped with high concentrations of salt. This activity can be restored by high-speed cytoplasmic extracts, provided that they contain the γTuRC (Moritz et al., 1998; Schnackenberg et al., 1998). Such assays also revealed that the integrity of the microtubule organising centre also requires the Abnormal spindle protein (Asp) from *Drosophila* cells (Avides and Glover, 1999).

*Drosophila* has two genes for γ-tubulin – one is located at salivary gland chromosome region 37C and is restricted in its expression to ovaries and precellularized embryos; the other is located at 23C and is expressed in somatic tissues and testes (Sunkel et al., 1995; Tavosanis et al., 1997; Wilson and Borisy, 1998). The brains of *γ-tub23C* Drosophila have highly condensed chromosomes, and spindles with defective poles (Sunkel et al., 1995). The gene encoding Dgrip91 is known as *discs-degenerate 4* (*dd4*) and has a similar phenotype, with highly condensed chromosomes, but shows a higher mitotic index as if arrested at the spindle integrity checkpoint (Barbosa et al., 2000). The poles of mitotically arrested *dd4* spindles contain some centrosomal antigens, whereas others may be dispersed rather than present in a single centrosomal body. Moreover, centrioles were found to be missing from one of the two spindle poles in a high proportion of mutant *dd4* cells. In many respects, the *dd4* spindles resemble those from *polo* neuroblasts, reflecting the requirement for *polo* to recruit γ-tubulin to the centrosomes and to phosphorylate and activate Asp (Avides et al., 2001; Donaldson et al., 2001). The disorganized *dd4* centrosomes are associated with fewer spindle microtubules, but nevertheless, stable bipolar spindles are formed and maintained in the mutant cells (Barbosa et al., 2000).

It has been particularly informative to study the phenotypes of *Drosophila* cell division cycle mutants to examine not only somatic cells, but also spermatocytes undergoing male meiosis. This is because the spindle assembly checkpoint is not effective at blocking spermatocyte division (Lin and Church, 1982; Miyazaki and Orr-Weaver, 1994; Rebollo and Gonzalez,

2000; Savoian et al., 2000) but only briefly delays meiotic progression and allows spermatocytes to complete division. Thus, examination of the mutant phenotypes of spermatocytes revealed, for example, that Polo kinase and Asp, known to function early in M phase to organize the spindle poles, also have additional functions in cytokinesis (Carmena et al., 1998; Riparbelli et al., 2002; Wakefield et al., 2001). A recent study of the meiotic phenotype exhibited in *γ-tub23C*$^{PI}$ males also suggested novel aspects of function of the γTuRC, as centrosomes were still capable of nucleating microtubules but conical structures formed in place of bipolar spindles (Sampaio et al., 2001). Such cones had centrosomes at their base and Klp3A and Polo kinase at their pointed ends, which, in some cases, could be the site of highly asymmetric cytokinesis.

In this paper we cast further light on the functions of the γTuRC through observations of meiosis in males in which γ-tubulin has been lost from the centrosome as a result of hypomorphic mutations in the gene for Dgrip91. We show that the nucleation of astral microtubules still occurs in such *dd4* males, and conical spindle structures are formed that strongly resemble those described in *γ-tub23C* testes (Sampaio et al., 2001). These *dd4* alleles have allowed us to identify a novel role for the γTuRC in maintaining the separation of spindle poles around the time of metaphase. Bipolar spindles that form in these cells collapse at this time and their poles re-associate. In some cells, spindle poles either never separate or collapse very early, and these nucleate robust asters of microtubules. If the chromosomes associated with such hemi-spindles organize bipolar arrays of microtubules, then contractile cytokinetic rings are able to form. We discuss the role of the γTuRC in the nucleation of asters, maintaining the separation of centrosomes and in the formation of the central spindle.

## Materials and Methods

### *dd4* Alleles used in this study

The lethal phase and phenotype of larval neuroblasts from *dd4*$^{S}$ males have already been described elsewhere (Barbosa et al., 2000). The *dd4*$^{3}$ allele was generated by ENU (ethyl nitrosourea) mutagenesis of a *y*$^{2}$ *cv v wy car* chromosome (A. T. Carpenter, unpublished data). Females carrying *dd4*$^{3}$ over *dd4*$^{S}$ have a similar phenotype as homozygous *dd4*$^{S}$ for viability and number of eggs laid. This will be described elsewhere. The *dd4*$^{3}$ allele has been kept in fertile males by double recombination into an X attached to a Y ring chromosome producing R(1,Y) *y v wy dd4*$^{3}$. The *dd4* mutation in these males is balanced by the free duplication Dp(1,f) LJ9 *y*$^{+}$ *dd4*$^{+}$, whose loss gives rise to identifiable individuals with *dd4* phenotype. All the stocks were kept at 25ºC. The fertility of adults with the genotypes *dd4*$^{S}$/Y and R(1,Y) *y wy dd4*$^{3}$ was tested through ten single crosses in which the flies were changed three times to fresh food at three day intervals. The progeny emerging in each vial was scored on the 10th, 12th, 14th and 17th day before being discarded. *y*$^{+}$ males from the *dd4*$^{3}$ stock were used as positive controls.

### Preparation of live testes for phase-contrast microscopy

Testes were dissected from young dark pupae in Testis Buffer (183 mM KCl, 47 mM NaCl, 10 mM Tris-HCl, pH 6.8, 1 mM EDTA) containing 1 μM PDMF protease inhibitor (Sigma), following the method described by Gonzalez and Glover (Gonzalez and Glover, 1993). The testes were transferred to a drop of the same buffer placed on a clean slide and cut with tungsten needles near the apical tip to release the cysts through the testes wall. The testes were gently squashed by placing a siliconized coverslip over them on the slide, and then applying a small piece of blotting paper to one of the edges of the coverslip. The process of squashing was monitored using a 40× phase-contrast objective. When the appropriate degree of squashing was attained, the blotting paper was removed. The criteria used to assess the degree of squashing were the integrity of the cysts outside the testes and the contrast of the phase-dense material within each cell. Specimens were screened for intact cysts of primary spermatocytes in phase-contrast with a Nikon Microphot-FX microscope at low magnification (25×). The morphology and number of cells in those cysts were analysed in photographs taken by a Nikon Coolpix 990 digital camera at higher magnification (60×). Onion-stage cysts were also photographed with the 60× objective but only isolated spermatids were counted on photographs at the same magnification. The nuclear diameter of the early spermatid cysts with one nucleus and one Nebenkern was measured (Gonzalez et al., 1988) on the digital photographs using OpenLab software, the ruler of which was calibrated with a scale that had minimum divisions of 1 μm. Ten *dd4*$^{3}$, *dd4*$^{S}$, and Oregon R (OrR) male pupae were dissected for this experiment.

### Spermatocyte culture and time-lapse microscopy

Spermatocytes were cultured as described by Church and Lin (Church and Lin, 1985) and the phase-contrast observations were made as in Rebollo and Gonzalez (Rebollo and Gonzalez, 2000), with a Leica DM IRB/E microscope equipped with a 63×/1.32 objective. Time-lapse images were captured with a Cohu camera at a rate of 20 frames/minute. Cultured *dd4*$^{S}$ spermatocytes from a *dd4*$^{S}$/FM6|Y were obtained from dark pupae stages and OrR spermatocytes from males of the same age were used as controls.

### Indirect immunolocalization of centrosomal and central spindle proteins in testes

Testes were dissected from young OrR and *dd4*$^{S}$ dark pupae and squashed as for phase-contrast observations, except that five pairs of testes were placed in one slide and up to three slides of each genotype were prepared per experiment. After squashing, the slide was immersed in liquid nitrogen and the coverslip flicked off with a scalpel blade. The preparation was fixed with methanol and acetone as described by Gonzalez and Glover (Gonzalez and Glover, 1993) and then 'blocked' by pre-incubation in 10% fetal calf serum in 1× phosphate buffered saline (PBS). Primary antibody incubations were done overnight at 4ºC in 1× PBS containing 10% fetal calf serum. Samples were washed in PBT (1× PBS, 0.1% Tween 20) before secondary antibodies diluted in PBT, 10% fetal calf serum were added. The secondary incubation was for at least 1 hour at room temperature. The preparation was then washed in PBT, rinsed in PBS and incubated for 15 minutes in PBS containing the DNA dye TOTO-3 (Molecular Probes) before being mounted. We used the monoclonal anti-body YL1/2 (Kilmartin et al., 1982) 10% diluted and anti-rat FITC-conjugated immunoglobulin G (Jackson Immunochemicals) to detect microtubules. γ-Tubulin was localized by using the monoclonal antibody from clone GTU88 (Sigma) diluted 1:50. Anti-Asp was a polyclonal rabbit serum Rb3133 (Saunders et al., 1997) and anti-Pav-KLP (Pavarotti) was Rb3301 (Adams et al., 1998) diluted 1:25. Centrosomin (CNN) was revealed with a rabbit CNN-specific R19 antibody (Heuer, 1995) at a dilution of 1:100, kindly provided by Thomas Kaufman (Indiana University, Bloomington, IN). Peanut was detected with MAb4C9 (Neufeld and Rubin, 1994) diluted 1:5. The secondary antibodies used to detect all antigens, with the exception of tubulin and F-actin, were conjugate with TexasRed, obtained from Jackson Immunochemicals, and were used according to the supplier's instructions. To visualize the distribution of F-Actin, fixed testes were incubated in a 1:200 dilution of rhodamine-conjugated phalloidin (Molecular Probes) in 1× PBS for 20 minutes. After washing with

**Table 1. Relative number of primary spermatocyte cysts and early spermatids in $dd4^S$ and $dd4^3$ testes**

| | Primary spermatocyte cysts* | | | | Early spermatids† | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Nucleus:Nebenkern‡ | | | | | | | |
| Genotype | 14-cell | 15-cell | 16-cell | Total | 0:1 | 1:1§ | 2:1 | 3:1 | 4:1 | 5:1 | 6:1 | Total |
| OrR | 0 | 0 | 7 | 7 | 1 | 242 (0) | 2 | 0 | 0 | 0 | 0 | 245 |
| $dd4^S$/Y | 1 | 3 | 7 | 11 | 4 | 88 (59) | 26 | 39 | 26 | 16 | 11 | 210 |
| R(1,Y)*y v wy* $dd4^3$ | 1 | 5 | 4 | 10 | 0 | 104 (104) | 15 | 6 | 0 | 0 | 0 | 125 |

*Number of intact cysts with morphologically normal primary spermatocytes found during general observation of preparations of testes with the indicated phenotype. Each column corresponds to cysts containing the indicated number of cells.

†Only loose early spermatids containing clearly identifiable nuclei and Nebenkerns were scored.

‡Number of cells containing the indicated ratio of nuclei per Nebenkern.

§In parentheses is the fraction of cells in which the diameter of both Nebenkern and nucleus was ≥5/2 that of the wild-type. Average size of both organelles was taken from a sample of $n$=15 (Gonzalez et al., 1989).

The protocol used for these observations does not allow the counting of spermatids in intact onion-stage cysts (see Materials and Methods and Fig. 1C,F).

PBS, testes were mounted as usual. Preparations were visualized using a Bio-Rad 1024 confocal scanning head coupled to a Nikon Optiphot microscope. The brightness and contrast of images collected for the figures were adjusted with Adobe Photoshop 5.5 software.

## Results

### Meiotic defects in *dd4* males are associated with two main categories of abnormal spindle

We have previously described an allelic series of mutations in *dd4*, the severity of which we assessed from the mitotic phenotype of hemizygous males and females trans-heterozygous with $dd4^S$ and the ability of such females to produce eggs (Barbosa et al., 2000). In this work we examine defects in male meiosis resulting from the $dd4^3$ and $dd4^S$ mutations. $dd4^S$ was considered to be an hypomorphic allele because females heterozygous for $dd4^S$ and a chromosome carrying a deficiency for the locus have reduced viability and a more severe mitotic phenotype in larval brains than seen in $dd4^S$ homozygous females (Barbosa et al., 2000). The phenotype of $dd4^3/dd4^S$ females is very similar to that of $dd4^S$ females for these same traits, suggesting that $dd4^3$ is also hypomorphic (our unpublished data). Males carrying $dd4^S$ or $dd4^3$ can develop to adults in favourable culture conditions although fewer $dd4^3$ males eclose than $dd4^S$ males when hemizygous (data not shown), suggesting that $dd4^3$ is more severe than $dd4^S$. Both $dd4^S$ and $dd4^3$ males were infertile and found to produce immotile sperm.

Analysis of squashed preparations of testes by phase-contrast microscopy revealed two main types of cytological abnormality. The first was in the appearance of cysts of primary spermatocytes. In wild type, a primary spermatogonial cell undergoes four mitotic divisions with incomplete cytokinesis to produce a cyst of 16 primary spermatocytes connected at ring canals through cytoplasmic bridges (Fuller, 1993). In both *dd4* alleles, several such cysts contained fewer than 16 cells (Table 1; Fig. 1D). The second type of abnormality was in the morphology of the meiotic spindles. In wild-type meiosis, a system of parafusorial membranes and mitochondria line up along the nuclear membranes and appear in the phase-contrast microscope as dark bands outlining the equatorial region of the spindle (Fig. 1B). In both $dd4^S$ and $dd4^3$, phase-dense membranous material similar to the wild type accumulated unevenly around the nuclear region (Fig. 1E, arrowhead). Very frequently, the dark material was organized in cone-like shapes with less dense material, probably corresponding to chromatin, sitting on the base of the cone (Fig. 1E, arrows). In some cases, biconical figures sharing the same base were found (Fig. 1E, arrowhead). Intact cysts of spermatocytes having the characteristics of meiosis II were not found in any sample taken from *dd4* males, suggesting that cells progress through the second cycle without dividing.

In wild type, the meiotic divisions normally result in a syncytium of 64 spermatids, each of which contains a single haploid (N) nucleus and a phase-dense spherical mitochondial aggregate, the Nebenkern (Fig. 1C). The morphology, size, and number of Nebenkerns per nucleus within a mutant spermatid indicate defects in organization of mitochondria on the spindle or failure in cytokinesis, or both. Similarly, the number and size of spermatid nuclei at this stage reflects the fidelity of chromosome segregation and karyokinesis (Cross and Shellenbarger, 1979; Gonzalez et al., 1988; Sunkel and Glover, 1988; Gonzalez et al., 1989). Cysts of early spermatids in both *dd4* mutants displayed fewer than 32 Nebenkerns with abnormal morphology and variable size (compare dark inclusions in Fig. 1C with those in Fig. 1F). Nuclei in the cysts were also variable in number and size (Table 1; Fig. 1F). More rarely, the abnormal meiotic divisions resulted in onion stages with only 16 cells (Table 1). 16-cell cysts at onion-stage with a 1:1 ratio of nuclei to Nebenkerns were found more frequently in $dd4^3$ than in $dd4^S$ males (Table 1). Such cells had nuclei of a size compatible with a tetraploid content of chromosomes (Gonzalez et al., 1989). In general, $dd4^3$ showed a stronger hypomorphic phenotype than $dd4^S$ mainly reflected in the relative viability of adult males, the number of early spermatid cysts with 4N nuclei, and the presence of sperm tails inside the testes (Fig. 1F, cyst on the right).

To clarify the nature of the spindle defects observed by phase-contrast microscopy, we carried out immunostaining to localize spindle microtubules. Morphologically, $dd4^S$ primary spermatocytes were indistinguishable from wild type except in the number of cells in some cysts (Table 1). Masses of microtubules were observed around presumably bivalent chromosomes at the onset of meiosis in *dd4* (Fig. 1J, arrows). Spindle-like structures then appeared to develop that fell into three main categories on the basis of their morphology. One category included conical structures with a radial and slightly concave microtubule array at the wider basal region and

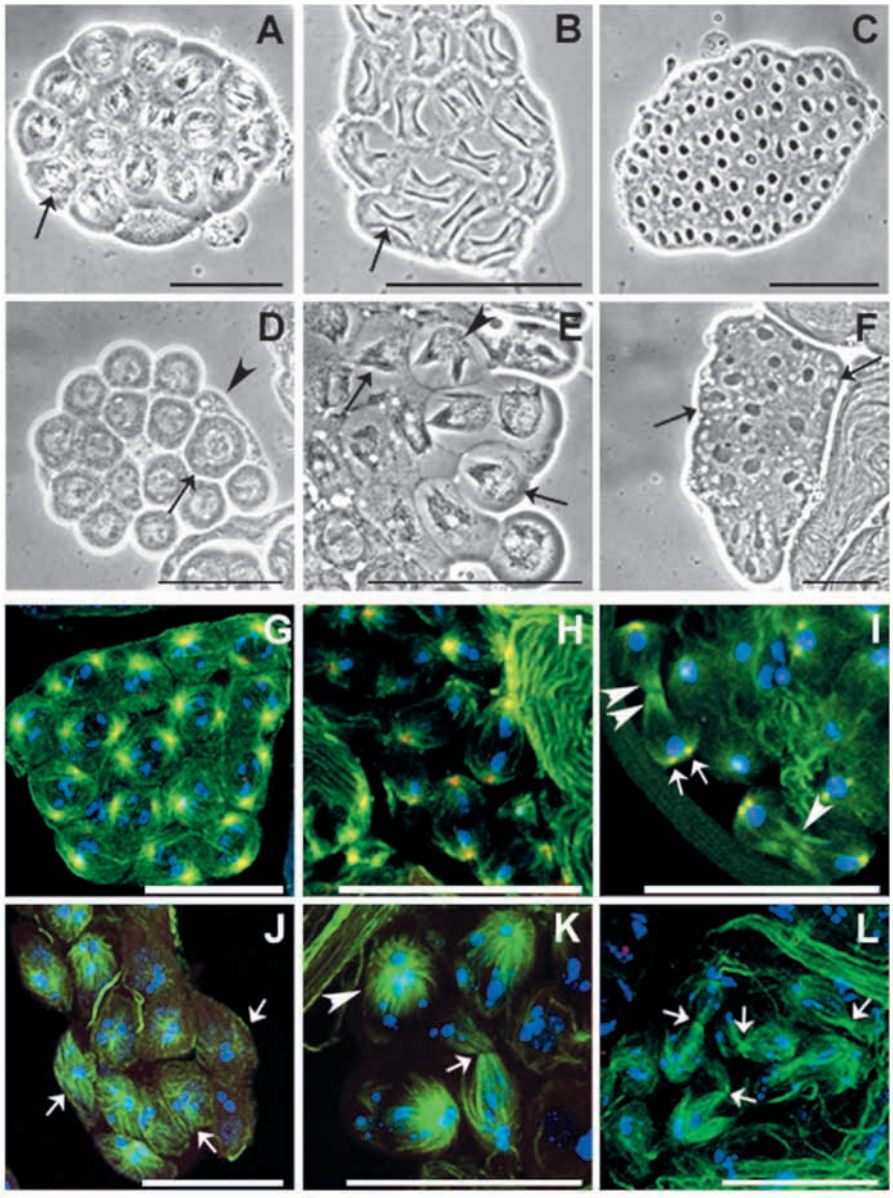


**Fig. 1.** Monopolar and conical spindles in *dd4* testes. (A-F) Living cells at different stages of meiosis in wild-type (A-C) and $dd4^S$ (D-F) testes viewed by phase-contrast light microscopy. (A) Cyst of primary spermatocytes at the onset of meiosis I with parafusorial membranes becoming visible (arrow). (B) This parafusorial material is seen along the meiotic spindle and concentrates in the equatorial region in late anaphase I (arrow). (C) A cyst at the onion stage showing spermatids, each containing a nucleus (light sphere) adjacent to a Nebenkern (phase dense sphere). (D) Cyst of primary spermatocytes from $dd4^S$ testis with an abnormal number of cells. One of the cells is larger than the others (arrow), whereas the morphology of both germline and somatic cells (cyst precursor cell, arrowhead) seems normal. (E) Example of the asymmetric distribution of the phase-dense material in $dd4^S$ meiocytes. Both conical (arrows) and biconical (arrowhead) figures are visible. (F) A cyst of early $dd4^S$ spermatids with very disorganized Nebenkerns (dark inclusions). The nuclei associated with each Nebenkern are variable in size and number (arrow). Note also what seem to be bundles of sperm tails on the right of this cyst. (G-L) Localization of γ-tubulin (red) with respect to spindle microtubules (green) in spermatocytes from fixed wild-type (G-I) and $dd4^S$ (J-L) testes. DNA is stained blue. (G) A wild-type16-cell cyst in prometaphase with duplicated and separated centrosomes. (H) Cells in anaphase before formation of the central spindle. (I) Late anaphase/telophase with fully formed central spindle marked by two bands of microtubules (paired arrowheads) separating the central spindle mid-zone (single arrowhead). The γ-tubulin-containing MTOCs have separated before meiosis II. (J) A $dd4^S$ 16-cell cyst in which condensing chromatin is surrounded by masses of microtubules (arrows) in early meiosis and γ-tubulin staining is undetectable. (K) Field of $dd4^S$ cells in meiosis showing one cone that has a central spindle-like structure (arrow) separating two masses of chromatin and three hemi-spindles. The DNA in the hemi-spindles is present both at the centre of the asters and around the periphery (e.g. arrowhead). (L) The left-most arrow points to a rare example of a bipolar spindle. The cones in this panel (remaining arrows) show a pronounced constriction at their apexes compared with the cone in panel K. Measurement of the frequency of the different types of defective meiotic figure in a sample of 162 cells indicated that 38% were hemi-spindles, 7% sharp cones and 33% biconical. 22% of this group of cells had a morphology that suggested either apoptosis or necrosis. Bars, (A-F), 20 μm; (G-L), 50 μm.

bundles of microtubules converging to an apex (Fig. 1K, arrow). Conical spindles usually displayed bundles of microtubules that extended distally from their apexes (Fig. 1K-L, arrows). In those instances a constriction around the apex together with a dark band interrupting the continuity of the microtubule ‘tracks’ suggested a rudimentary central spindle structure (Cenci et al., 1994; Gatti et al., 2000). The second category of abnormal spindle appeared as umbrella cup-like structures in which the only visible microtubules irradiated from the centre (Fig. 1K, arrowhead). These latter figures resembled astral arrays of microtubules or halves of normal spindles at early anaphase I in which the inter-polar microtubules were not yet visible (Fig. 1K, arrowhead). More rarely, ‘biconical’ figures such as those observed with the light microscope (Fig. 1E, arrowhead) were also found by indirect immunofluorescence of microtubules (Fig. 5D, arrows). We refer to these three types of spindles as ‘cones’, ‘hemi-spindles’, and ‘biconical figures’, respectively.

It was also evident that chromatin had a scattered distribution in most of the mutant meiotic figures (Fig. 1J-L). DNA was often found at two sides of the cones – namely, at the base and beyond the constriction, suggesting chromosome segregation on a bipolar structure. Generally, hemi-spindles contained chromatin in contact with the astral microtubules

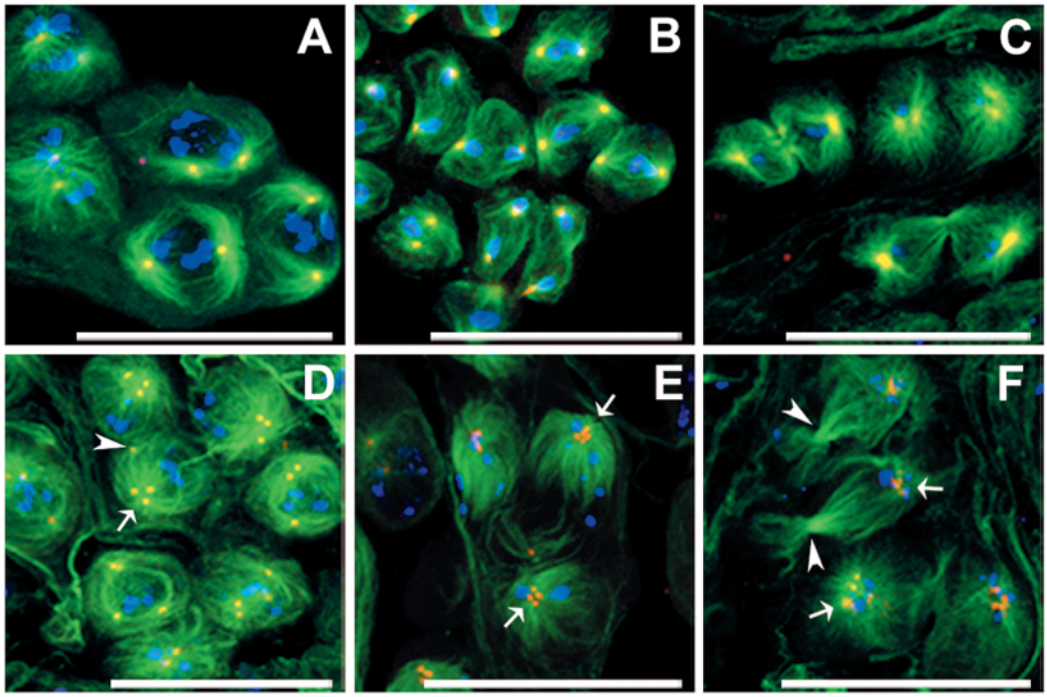


**Fig. 2.** CNN is associated with centrosomal MTOCs in *dd4*$^{S}$ spermatocytes. (A-C) Wild-type meiosis showing CNN (red) localized at the spindle poles during wild-type meiosis. Microtubules are stained green and DNA is stained blue. Cysts are shown at prometaphase (A), anaphase (B) and telophase/cytokinesis (C). (E-H) CNN localization in *dd4*$^{S}$ meiocytes. (D) Spermatocytes in early meiosis in which discrete bodies containing CNN do not form two discrete foci but are dispersed. In this field the three uppermost cells have at least three such bodies accumulated at the centre of the asters (arrow), although some CNN-containing bodies can be dispersed (arrowhead). (E) Hemi-spindles with variable numbers of CNN-containing bodies at the focus of the asters. (F) Two cones that show constrictions around the mid-zone (arrowheads). Punctate CNN staining is found at the astral poles of both cones and hemi-spindles (arrows). Bar, 50 μm.

varying, however, in its position relative to the main microtubule organizing centre (Fig. 1K).

We also stained such preparations to reveal γ-tubulin. In wild type, γ-tubulin concentrates in the duplicated centrosomes as the nucleus enters meiosis (Fig. 1G) and remains associated with centrosomes throughout meiosis (Fig. 1H,I). In late anaphase, the γ-tubulin-containing body at the spindle pole splits and the central spindle matures (Fig. 1I). In *dd4*$^{S}$ testes, however, we were unable to detect γ-tubulin in any particular structure of the meiotic apparatus of dividing spermatocytes (Fig. 1J-L).

## Defects in pole segregation of *dd4*$^{S}$ meiotic spindles are inferred from CNN localization

Two explanations may be offered to account for the abnormal meiotic spindles in *dd4*$^{S}$ spermatocytes. First, as with *dd4* neuroblasts, the microtubule organizing centres (MTOCs) are defective in duplication or segregation, or both. In male meiosis, where the spindle assembly checkpoint is less stringent, such a defect would be expected to have visible consequences for the later stages of the division cycle (Gonzalez et al., 1988; Sunkel and Glover, 1988). Second, the bipolar spindle in meiosis I first forms and then collapses around an abnormal central spindle, reuniting both poles and leaving an aneuploid complement of DNA per cyst cell similar to the effect proposed in *γTub23C* mutants (Sampaio et al., 2001).

In order to understand the behaviour of the spindle poles in the abnormal meiotic spindles of *dd4*$^{S}$ males, we studied the localization of centrosomin (CNN) in fixed preparations of *dd4*$^{S}$ testes. In wild-type meiosis, CNN appears in discrete bodies at the poles of the spindle that correlate with the expected number of centrosomes (Fig. 2A-C). As the chromosomes segregate and microtubules accumulate in the central spindle to organize cytokinesis, the CNN-containing bodies split in two, reflecting the distribution of this pericentriolar material (PCM) component around the centrioles (Li et al., 1998). In dividing *dd4*$^{S}$ spermatocytes, CNN-containing bodies accumulated together in a cluster (Fig. 2D-F, arrows) or showed an abnormal position relative to each other (Fig. 2D, arrow). These CNN-containing bodies were generally present in variable numbers (ranging from three to five) at the centre of the asters in both spindle types. However, independent CNN-containing bodies were, in some, cases found embedded in the microtubule network, distal from the rest of the CNN-staining fragments (Fig. 2D, arrowhead). Cones always contained CNN accumulated in the centre of their base (Fig. 2F). Hemi-spindles showed several CNN-stained bodies comparable to those in the bases of cones (Fig. 2E). The finding of some examples of both cones and hemi-spindles with greater than four CNN fragments per pole, in cysts undergoing meiosis (Fig. 2E,F), suggests that failure of centrosome segregation can occur from the gonial divisions onwards.

## Localization of Asp suggests the nature of hemi-spindles and reveals central spindle abnormalities in cones

As Asp participates in the organization of spindle poles (Avides and Glover, 1999) and in the subsequent stabilization of the central spindle (Wakefield et al., 2001; Riparbelli et al., 2002), we wished to examine its localization in the abnormal spindles in *dd4*$^{S}$ spermatocytes. Asp associates with the putative minus ends of microtubules and thus in wild-type meiosis, it is seen as a hemispherical cup-like structure overlying the spindle-facing side of the centrosomes of the meiotic spindle in late anaphase/telophase (Fig. 3B, arrowhead). It also localizes to the edges of the central spindle (Riparbelli et al., 2002) (Fig.

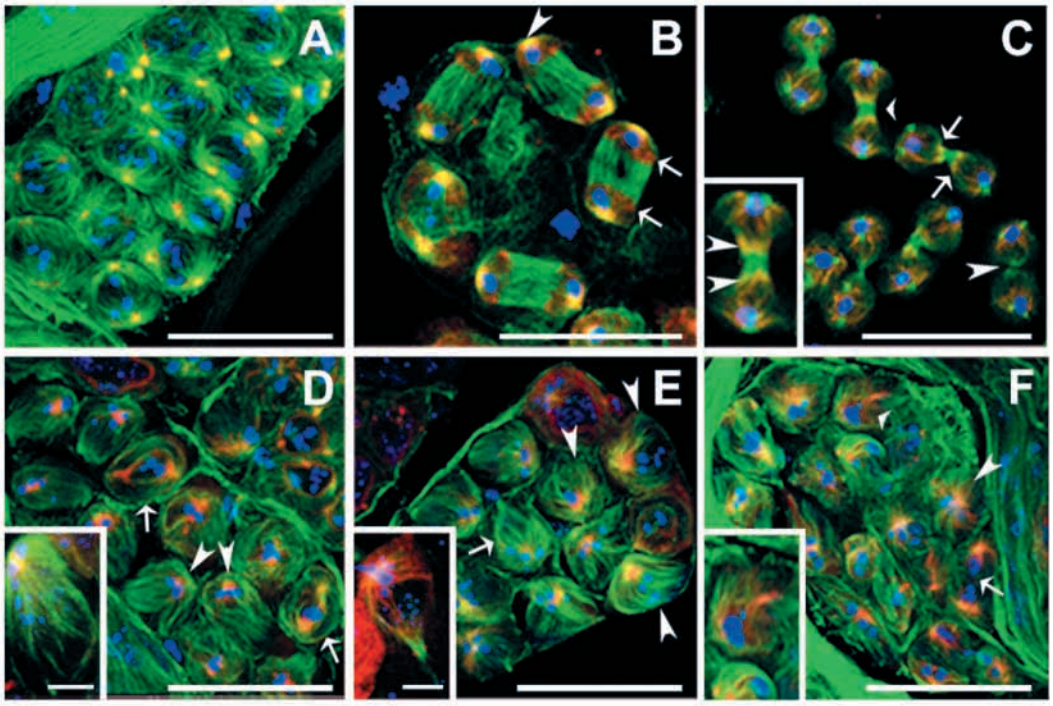


**Fig. 3.** Asp associates with the poles but not the central spindle of *dd4*$^{S}$ spermatocytes. Asp localization (red) in relation to microtubules (green) and DNA (blue) in meiotic wild-type (A-C) or *dd4*$^{S}$ (D-F) cells. Wild-type cysts are shown at prometaphase (A) and anaphase/telophase (B). Asp is localized at the spindle poles (arrowhead in B) and in association with the tips of microtubules in the central spindle during anaphase/telophase (arrows in B). (C) Later telophase stage in which cells show pronounced constriction around the central spindle mid-zone. Asp interacts with the putative minus ends of central spindle microtubules, more distant from the poles at this stage (arrows and arrowheads in the inset). The inset shows an enlargement of the spindle marked with small arrowhead. (D) Cyst of *dd4*$^{S}$ cells apparently at early meiosis with Asp staining at the centre of the hemi-spindle asters (arrowheads). The arrows indicate cells attempting to organize a bipolar spindle with defects in Asp distribution at the poles. The inset shows a higher magnification of the distribution of Asp in a hemi-spindle. (E) Cyst of *dd4*$^{S}$ cells in which the arrow points to a sharp cone with Asp staining only at the astral pole. The arrowheads highlight microtubule tips at the periphery of three hemi-spindles. Inset shows a more detailed view of a cone with fibrous Asp-containing material emanating from the astral pole and in this case also localizing near a central-spindle-like bundle of microtubules. (F) *dd4*$^{S}$ cyst displaying a anaphase cell lacking central spindle microtubules and with fragmented Asp staining at the poles (arrow). The large arrowhead points to a fibrous distribution of Asp emanating from the centre of a hemi-spindle aster. The inset shows a higher magnification of the spindle indicated by the small arrowhead showing a cone with some Asp localizing in the central-spindle-like region. Bars, 50 μm. Bar in insets of D and E, 10 μm.

3B, arrows). In *dd4*$^{S}$ spermatocytes, Asp was localized in an irregular manner at the core of the astral poles of the nascent spindles (Fig. 3D, arrowheads). Curiously, it was also possible to see Asp extending along fibres emanating from the asters in both cones and in hemi-spindles (Fig. 3E, inset; Fig. 3F, arrowheads). Although the fragmented distribution of Asp appeared restricted to the centre of the asters, in some cones the Asp-containing fibrous material extending from the asters seemed to reach the apex (Fig. 3F, inset). So, if Asp is marking the putative minus ends of microtubules it would appear that many have been released from the poles as seems to occur when the central spindle forms in wild type (Riparbelli et al., 2002). However, in this case discrete staining at the edges of the central spindle-like structure was not observed. This suggests that the central spindle-like structures associated with cones are not correctly organized and that the correct interaction of Asp with the putative minus ends of microtubules may require γTuRC function. In the hemi-spindle structures spreading of Asp from the organizing centre was restricted and it was never found around the periphery of these astral structures (Fig. 3E, arrowheads).

## Time-lapse studies indicate that bipolar spindles can form and subsequently collapse

To gain insight into the process that leads to formation of the abnormal spindle structures in *dd4*$^{S}$ spermatocytes, we followed meiosis in these cells by time-lapse microscopy. In wild-type meiosis (Fig. 4A-E), centrosome separation occurs around an intact nucleus (Fig. 4A). Subsequently, the spindle elongates and chromosomes align during prometaphase (Fig. 4B). Homologues separate in anaphase (Fig. 4C, arrows, and Fig. 4D) before the reformation of nuclei at telophase (Fig. 4E). In *dd4*$^{S}$ mutant spermatocytes we observed two main types of event: in one case (Fig. 4F-J) bipolar spindles assembled (Fig. 4F,G) and bioriented bivalents localized in the central region of the spindle (Fig. 4G). However, instead of further elongating, such spindles collapsed and their poles approached one another (Fig. 4H,I). After the collapse, microtubules continued to grow as if attempting to undertake anaphase B but with no evidence of segregation of homologues (Fig. 4H). These collapsed spindles thus produced biconical figures with a common basal region containing the asters (Fig. 4I). Finally, the spindle appeared to disassemble and nuclear-like vesicles formed (Fig. 4J). In the other type of event (Fig. 4K-O), asters remained very close to each other throughout. Phase dense membranous material (pdm) that would probably correspond to the parafusorial membranes accumulated along these apparently monopolar spindles. This material then became concentrated at the distal part (Fig. 4L,M), which developed an increasingly conical shape (Fig. 4N). In this process chromosomes were seen in rapid movements towards the astral

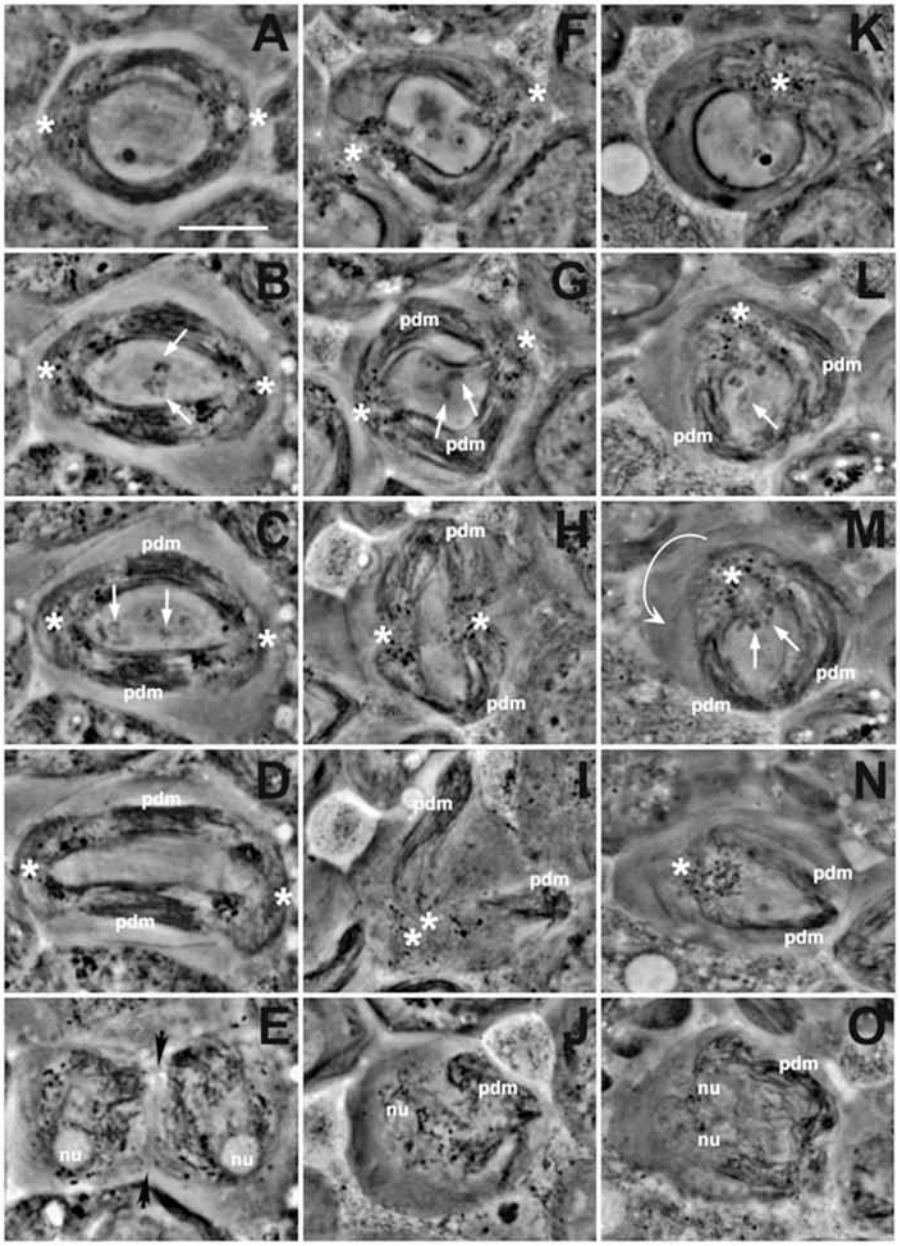


**Fig. 4.** Time-lapse series of meiosis I in wild-type and *dd4* spermatocytes. (A-E) Wild-type division. (A) Prophase with opposed asters (asterisks). (B) Prometaphase with elongated nuclear region within which bivalents (arrows) acquire biorientation. (C) Rapid movement of homologues (arrows) towards opposite poles as the phase-dense material (pdm) starts accumulating along the spindle. (D) Anaphase B where the parafusorial membranes are fully visible. (E) Telophase in which the cleavage furrow (arrowheads) will separate the daughter nuclei (nu). (F-J) Late spindle collapse in a $dd4^S$ spermatocyte. (F) Asters initially segregate. (G) pdm accumulates along the equatorial region of bipolar spindles as apparently bioriented homologues move between the poles (arrows). (H) The spindle poles approach each other before any visible segregation of homologues takes place. The pdm accumulates at the centre of the spindle, which becomes the apical regions of two nascent conical structures. (I) These cones elongate further and their apexes become darker. (J) The cell becomes disorganized as individualization of nuclear-like (nu) vesicles takes place. (K-L) Defective MTOC segregation or early spindle collapse in a $dd4^S$ spermatocyte. (K) In this cell, a bona fide biastral spindle never forms at the onset of meiosis and the hemi-spindle structure seen in this panel persists until later stages. (L) Individualized bivalents tend to undergo rapid movements towards the pole containing the aster (arrow) as the pdm accumulate distally. (M) In later stages all visible chromosomes localize in the vicinity of the astral pole without evident segregation of the homologues (the curved arrow indicates the direction of a rotation of the cell). (N) The 'hemi-spindle' then becomes conical as the pdm accumulates at the apex. (O) The pdm tends to fray and disorganize and several nuclear-like vesicles (nu) form in the region previously occupied by the asters. Bar, 10 μm.

pole (Fig. 4L, arrow) and sporadic slow movements away from it. In later stages most of the chromosomes seemed to localize in the vicinity of the pole (Fig. 4M, arrows). Hemi-spindles thus seem to be formed either as a result of a failure of centrosome separation or through a collapse of a bipolar spindle shortly after centrosome separation. Such hemi-spindles invariably developed into cones when observed by time lapse microscopy.

### Pav-KLP localizes to the putative plus ends of hemi-spindles and the apexes of cones

As our time-lapse observations indicated that the two major spindle defects arose at or shortly after metaphase I, we were curious about the extent to which events associated with cytokinesis occurred in $dd4^S$ males. To verify whether the formation of the contractile ring was compromised by the disorganization of the $dd4^S$ spindle, we first investigated the localization of Pav-KLP (Pav-KLP), a kinesin-like protein related to the mammalian MKLP-1 and essential for cytokinesis in mitosis (Fig. 5) (Adams et al., 1998). In *Drosophila* spermatogenesis, Pav-KLP localizes to the ring canals, remnants of the contractile rings from earlier divisions (Fig. 5A, arrow). It localizes to the central spindle in anaphase and concentrates in the mid-zone in telophase (Fig. 5B, arrow) (Carmena et al., 1998; Adams et al., 1998). In $dd4^S$ males, the localization of Pav-LKP in ring canals derived from pre-meiotic divisions (Fig. 5C, large arrow) did not seem to be affected. In the hemi-spindles of $dd4^S$ spermatocytes Pav-KLP was seen to localize at the putative plus ends of the microtubules (Fig. 5C, arrowheads). In cones with a morphology consistent with their derivation from hemi-

spindles, Pav-LKP was associated with microtubules at the point of constriction (Fig. 5C, small arrow; Fig. 5D, arrowheads). Biconical figures, which are probably derived from the collapse of bipolar spindles, showed Pav-KLP at the vertex of each cone (Fig. 5D, arrows). These observations support the hypothesis that hemi-spindles are initially monopolar structures. However, Pav-KLP never appeared in a ring shape in cones but rather as a 'knot' at their apexes. This could reflect abnormalities in the structure of the central spindle.

### Peanut fails to localize on hemi-spindles and forms rings around the rudimentary central spindles in cones

The *Drosophila* septin Peanut (Neufeld and Rubin, 1994) is a component of both mitotic and meiotic ring canals (Fig. 6A, inset) (Hime et al., 1996). Interestingly, Peanut was localized to the apex of a subset of 'cones' in $dd4^S$ spermatocytes, forming circular structures which usually ringed the very mid-zone of the putative central spindle structure whenever such a structure could be discerned (Fig. 6A, arrows). The rings of Peanut staining in these cones are morphologically similar to the Peanut-decorated contractile rings around the mid-zone of wild-type meiotic spindles during cytokinesis (Fig. 6A, inset). Peanut rings displayed a variable range of diameters correlated with the degree of constriction of microtubules (compare split channels in Fig. 6A). The pattern of distribution of Peanut in such cones suggests that it is part of a dynamic structure able to constrict around the abnormal central spindles leading to asymmetrical cytokinesis. A second set of cones that appeared to have no extension of microtubules showed only slight or no Peanut staining at their vertexes (Fig. 6B, arrow), whereas equivalent structures had Pav-KLP present in the same region (Fig. 5D, arrowheads). These observations suggest either that Peanut was lost or never associated with such structures. Hemi-spindles did not appear to contain any sort of Peanut staining in their microtubule arrays (Fig. 6B, arrowhead), suggesting that contractile rings cannot be properly assembled at the dispersed putative plus ends of their microtubules.

Actin filaments are part of the major contractile structure during cytokinesis (Hime et al., 1996). We stained both $dd4^S$ and wild-type testes with phalloidin to reveal actin and found a similar distribution to that of Peanut with respect to the contractile rings (data not shown). Thus, the central spindle would appear in some cells to be formed when microtubules were organized by a single pole and focused on a centrosomal aggregate on the one hand and by chromosomes on the other. In some cases sufficient of a central spindle was generated that enabled ring-like structures of Peanut and F-actin to form.

## Discussion

Two weak hypomorphic alleles of *dd4* have allowed us to study the stages of spermatogenesis that are most sensitive to the compromised function of the 91 kDa component of the γTuRC encoded by this gene. These mutant alleles appear usually able to provide sufficient functional protein for the four rounds of mitosis that precede meiosis but then show a variety of spindle defects during meiosis. This correlates with a loss of γ-tubulin staining from the spindle pole, suggesting substantial disruption of the γTuRC. The spindle abnormalities displayed in meiosis contrast in several respects to those we have previously described in mitotic divisions (Barbosa et al., 2000). *dd4* mutant larval neuroblasts arrest in mitosis at metaphase with bipolar spindles that have disorganized poles lacking γ-tubulin

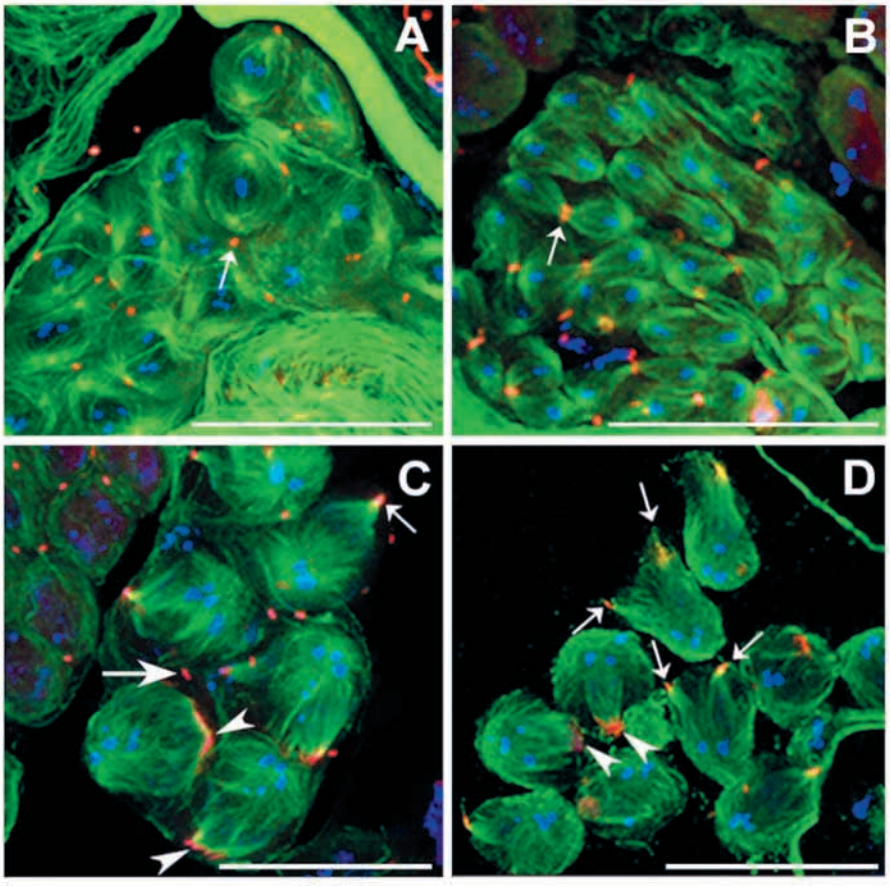


**Fig. 5.** Pav-KLP becomes focused at the apexes of cones. Pav-KLP (red), α-tubulin (green), and DNA (blue) in wild-type (A and B) and $dd4^S$ (C and D) meiocytes. (A) Wild-type cyst in prometaphase/metaphase revealing ring canals derived from pre-meiotic divisions containing Pav-KLP (arrow). (B) Meiocytes in telophase II showing contracting rings with Pav-KLP staining around the central-spindle mid-zone (arrow). (C) A $dd4^S$ cyst with Pav-KLP-containing ring canals (large arrow). This cysts also contains two hemi-spindles with Pav-KLP staining at the putative plus ends of microtubules at the periphery of the asters (arrowheads). A cone is also indicated by the small arrow in with Pav-KLP accumulated at its apex. (D) $dd4^S$ meiocytes with Pav-KLP at the constriction point of cones (arrowheads), and at the apex of two biconical structures (arrows). Bar, 50 μm.

and which do not have astral microtubules. $dd4^S$ meiocytes also lack γ-tubulin at their poles but, nevertheless, are capable of organizing arrays of astral microtubules. In contrast to *dd4* mitotic cells, stable bi-astral structures either fail to form or they collapse after their formation. The results of our combined application of real-time imaging of spermatocytes and immunolocalization of specific antigens in fixed preparations lead us to suggest a model for how the various abnormalities of the meiotic spindle arise (Fig. 7). In cells in which bi-astral spindles either never form or collapse very early, monopolar spindles first develop that we postulate correspond to the hemi-spindles seen in fixed preparations. After some time it seems that these can develop bipolarity as a result of chromatin accumulating on their periphery and a rudimentary spindle mid-zone can form in such structures. These are one type of cone-like spindle that, in some cases, may even complete cytokinesis to generate aneuploid daughter cells. Such structures correspond to similar conical spindles recently described in testes from mutants of the *γ-tubulin* gene at 23C ($\gamma Tub23C^{PI}$) (Sampaio et al., 2001). Our present work thus confirms that such structures arise from disruption of the γ-TuRC and extends it by showing hemi-spindles to be an intermediate in the formation of cones. It has also allowed us to show an alternative pathway by which conical structures can arise and thereby cast light on a novel role for the γ-TuRC in maintaining the stability of co-joined hemi-spindle structures in the normal bipolar meiotic spindle. This is indicated by our observations that bipolar spindles with well-defined poles could be formed but then collapsed around the time of the metaphase-anaphase transition, causing the two poles to move back together. As a consequence, the intervening spindle microtubules are displaced and the central region of the spindle folds back on itself at two points to form the apexes of biconical figures (Fig. 7). Our examination of the *dd4* mutant phenotype in testes has thus permitted three types of spindle defect to be identified: within asters themselves; in the spindle microtubules required for centrosome separation; and in the central region of the spindle, each of which we will discuss.

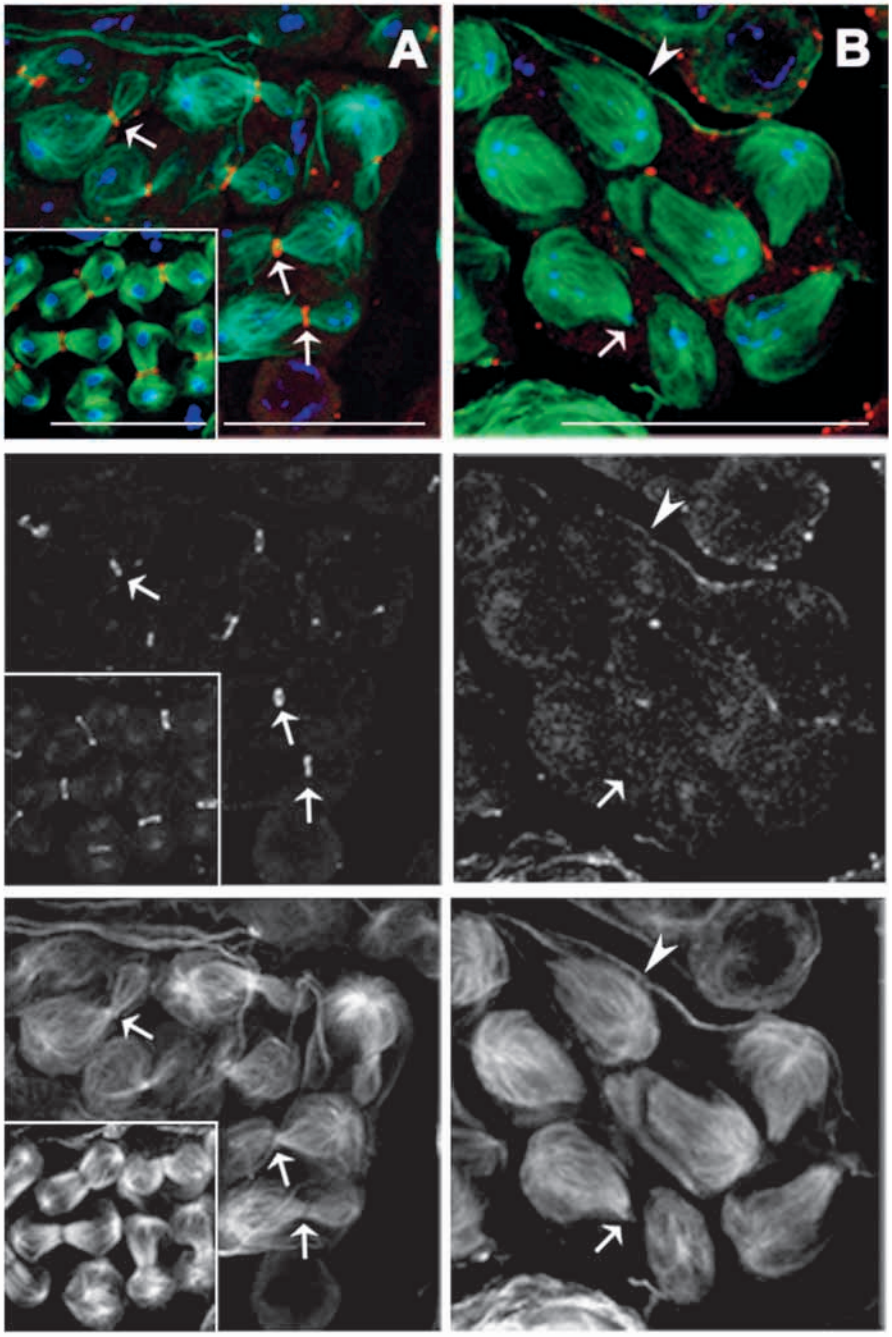


**Fig. 6.** Peanut localizes to the apexes of some cones. Merged images on the top row show the distribution of DNA (blue), Peanut (red), and α-tubulin (green) in $dd4^S$ (main panels) and wild-type (inset) cells. The individual channels for Peanut and α-tubulin are shown in the centre and bottom panels, respectively. (A) $dd4^S$ cells showing localization of Peanut in rings around the midzone-like region of cones (arrows). (B) Cone with sharp apexes and no detectable Peanut (arrow). The arrowhead points to a hemi-spindle devoid of Peanut staining. Bar, 50 μm.

## Aster formation

The spindle poles of *dd4* primary spermatocytes usually have the expected number of centrioles by the criteria of discrete bodies of CNN, a component of the PCM that has been described to closely surround the centrioles in such cells. However, the finding of some spermatocytes with more than four such bodies suggests that there can be failure in centriole separation in the pre-meiotic divisions as has been described in mutant *dd4* neuroblast divisions (Barbosa et al., 2000). The CNN-containing bodies in *dd4* spermatocytes appear either to

have never fully separated or have become reunited after spindle collapse and so the four such bodies are usually at the focus of the astral poles. In common with *dd4* mutant neuroblasts, these pole bodies lack the γTuRC but are associated with Asp. The ability of these poles to nucleate asters thus goes against the accepted dogma that the proper localization of γ-tubulin and centrosomal integrity is absolutely required for the function of a polar MTOC to direct the formation of asters (Bonaccorsi et al., 1998; Khodjakov and Rieder, 2001). At present we can only speculate why astral microtubule arrays are not seen in *dd4* neuroblasts (Barbosa et al., 2000) and yet appear robust in $dd4^S$ spermatocytes. On the one hand it could reflect a general deterioration of the spindle throughout a prolonged period of metaphase delay due to the more robust spindle integrity checkpoint in neuroblasts. On the other it could reflect underlying differences in spindle structure and function between these cell types (Casal et al., 1990). It is possible, for example, that Asp in the focus of asters in $dd4^S$ spindles may play more of a role in maintaining astral microtubules in spermatocytes than it does in neuroblasts. This would be consistent with the known function of Asp in the reorganization of radial arrays of microtubules around isolated *Drosophila* centrosomes (Avides and Glover, 1999). Moreover, meiotic spindles in *asp* spermatocytes are abnormal in shape, and the morphology of their asters is considerably affected (Gonzalez et al., 1990; Wakefield et al., 2001). However, it would seem that Asp may not be as efficient at stabilizing asters in the *dd4* larval CNS as in *dd4* spermatocytes.

Many of the astral structures revealed by the immunostaining of *dd4* testes appeared sufficiently asymmetric to have the appearance of hemi-spindles. These were truly monopolar by the criteria of having Asp at the focused putative minus ends of microtubules and with Pav-KLP located at their periphery, the putative plus ends. Such hemi-spindles are quite different structures from the asymmetric spindles sometimes observed in *dd4* mutant neuroblasts in which one Asp containing pole can be focused and the other comprised of scattered bundles of microtubules whose putative minus ends are associated with Asp. However, real-time imaging suggests the hemi-spindles seen in *dd4* meiocytes are an intermediary in the development of cones. In this process it seems that bipolarity is developed by the chromatin apparently acting to stabilize the diverging microtubules. Such spindles have one pole with multiple centrioles and the other with none.

## Spindle-pole separation

The difficulties in either establishing or maintaining the separation of spindle poles in male meiosis in *dd4* mutants point towards a novel role for the γTuRC in maintaining the function of spindle microtubules *per se*. It is possible that there could be two stages to this process that differ in their sensitivity to the compromised function of the γTuRC. This is suggested by the finding that in some cells, bipolar spindles either never form or collapse early (to form initially a hemi-spindle). Thus, the first crucial requirement of γ-tubulin function may be to nucleate a subset of spindle microtubules that maintain bipolarity. If a bipolar and bi-astral spindle does form then it seems to undergo a crisis around metaphase when it appears to collapse. The collapsing spindles do elongate however, suggesting that collapse may in part be driven by anaphase events. In some ways the spindle collapse is reminiscent of the consequences of inactivating γ-*Tub* function by RNAi in

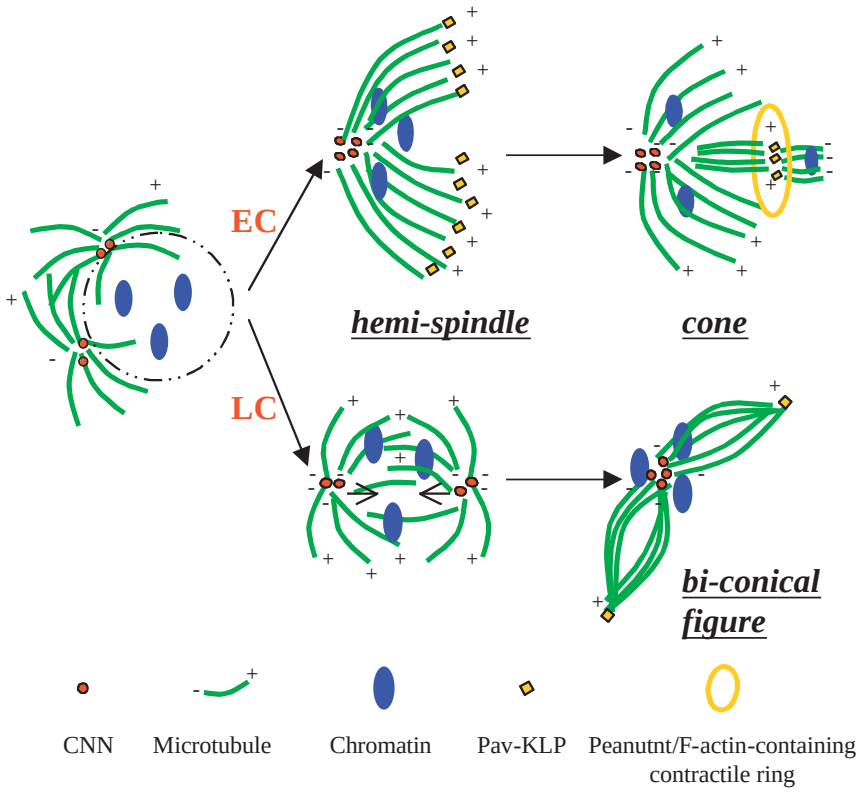


**Fig. 7.** Model to explain the abnormal microtubule structures during $dd4^S$ meiosis. Mutations in the γTuRC component Dgrip91 may result in either failure of separation of the MTOCs or the collapse of a bipolar spindle shortly after its formation in prophase I (EC, early collapse). Alternatively, a bipolar spindle may be assembled, only to undergo a late collapse (LC) before anaphase I. In the first case monopolar structures, that we term hemi-spindles, form and are able to attach bivalents (blue ellipses), which, because of the lack of biorientation do not congress at metaphase. Some of these chromatin masses, located distal to the astral pole, may stabilize microtubules (green lines) with an opposite polarity relative to those nucleated by the main spindle pole (+ and – represent the putative polarity of microtubules). A structure resembling a central spindle could then form. Only these cases (cones) seem to be able to assemble a contractile ring containing Peanut and F-actin (orange hoop) and undergo cytokinesis. LCs generally result in biconical figures that are probably unable to assemble a central spindle due to a drastic loss of orientation of bivalents relative to a single pole. Consistently, Pav-KLP (yellow squares), which localizes close to the putative plus ends of the microtubules (+) in hemi-spindles, is only seen at the apex of the cones and at the vertices of biconical figures.

*Caenorhabditis elegans* embryos that result in separated asters re-approaching each other at late prophase (Strome et al., 2001). Moreover, conical spindles in *γTub23C$^{PI}$* spermatocytes seem to appear from a collapse of bipolar spindles around prophase and elongate in a timeframe comparable to the assembly of the central spindle in wild type (Sampaio et al., 2001). It is possible that a second, stabilizing effect of the γTuRC at the minus ends of the microtubules (Wiese and Zheng, 2000) is specially required before metaphase in meiosis I. In vertebrate cells, low doses of taxol have shown to preferentially stabilize kinetochore microtubules plus ends leading to a slight collapse of the spindle around the time of metaphase (Waters, 1997; Compton, 2000). Perhaps the reduction of centrosomal γTuRCs in *γTub23C$^{PI}$* and *dd4* cells is reproducing this effect by destabilizing the minus ends of microtubules.

### Central spindle formation

The normal origin of the central spindle microtubules in wild-type cells is obscure. Treatment of cells with microtubule destabilizing agents after the onset of anaphase suggests that the central spindle may be assembled from newly nucleated microtubules and not from remains of the mitotic spindle material left in the cell equator (Canman et al., 2000; Gorbsky et al., 1998; Mastronarde et al., 1993; Shelden and Wadsworth, 1990). However, although the localization of γ-tubulin in the central spindle of mammalian dividing cells has been reported by several groups (Julian et al., 1993; Shu et al., 1995), the presence of γ-tubulin in *Drosophila* central spindle is still a matter of debate (Callaini, 1997; Carmena et al., 1998; Raff et al., 1993; Wakefield et al., 2000). The spindle collapse that occurs in *dd4* meiocytes could be related to the onset of reorganization of the spindle that occurs at the metaphase-anaphase transition when some microtubules appear to detach from the centrosomes as the central spindle structure begins to form. In wild-type meiocytes this is seen by the generation of a new set of central spindle microtubules with Asp at their putative minus ends (Fig. 3B,C) (Riparbelli et al., 2002). Central spindle microtubules never become fully organized in the *dd4* spermatocytes although this seems to progress further in cones. Consistently, Asp never undertakes its normal redistribution but rather adopts a fibrous pattern of organization extending from the spindle poles. If as it has been suggested, Asp works as an anchor to the putative minus ends of microtubules, it is possible that microtubules are released from the spindle poles and rather dispersed throughout the conical microtubule structure in *dd4* meiocytes. But the lack of Asp capped microtubules of central spindle-like structures in these cells suggests some degree of co-operation with the γ-TuRC is necessary to correctly co-ordinate this transition in spindle structure.

Despite the absence of clearly organized central spindle microtubules, the mutant cells do show several features typical of post-metaphase stages of meiosis that differ in two pathways of spindle development. The hemi-spindles that give rise to cones harbour homologues that are initially mono-oriented as they move towards and away from the asters without evidence of segregation. As cones develop from the hemi-spindles, bipolarity appears to arise from some ability of chromosomes to stabilize microtubules as discussed above. At this time, microtubules stabilized by distal chromatin in some hemi-spindles would appear to interdigitate with microtubules from the astral pole in an anti-parallel manner to form cones with the motor protein Pav-KLP then becoming associated with a 'knot-like' structure at the centre of the spindle but never forming a ring. Rings of septin and actin can then form around structures equivalent to those where the Pav-KLP 'knots' appear. Sometimes these enable cytokinesis to be achieved. In the pathway in which bipolar spindles collapse there is an elongation of spindle microtubules analogous to the lengthening that takes place in anaphase B. Such spindles have no arrangement of microtubules that resembles a central spindle. They lack the bipolarity usually associated with central spindle formation and unlike the hemi-spindles they appear to lack the ability for regenerating such a bipolar structure. The presence of Pav-KLP at the apexes of the biconical figures (Fig. 5D) suggests that although Pav-KLP is a known prerequisite for central spindle formation (Adams et al., 1998), this localization is in itself insufficient for this process. Thus, central spindle-like structures do not form in the biconical figures possibly reflecting the absence of interdigitating microtubules inherent in a bipolar structure and this in turn leads to a failure in formation of rings of septin and actin. Thus as previously observed in *γTub23C$^{PI}$* spermatocytes there seems to be some limited ability to organize some of the components required for cytokinesis when γTuRC function is compromised, the extent of which appears to reflect the ability to reorganize central spindle microtubules.

In summary our observations indicate that the γTuRC may provide several functions to the spindle. It is not absolutely essential for microtubule nucleation to form asters in all cell types. Rather, it may be required for the specific function of subsets of spindle microtubules that maintain pole separation. It appears to co-operate with other proteins associated with the minus ends of microtubules, notably Asp in *Drosophila* cells, and this appears to be important in the reorganization of the spindle that occurs following the metaphase-anaphase transition. Further work will be required to determine the extent to which defects in the reorganization of the central spindle at this stage reflect a direct requirement for the γTuRC or are a consequence of earlier defects in spindle organization.

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