Cytological characterisation of the mutant phenotypes produced during early embryogenesis by null and loss-of-function alleles of the $\gamma$Tub37C gene in Drosophila

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SUMMARY

We have studied the mutant phenotypes brought about during early embryogenesis by mutation in the $\gamma$Tub37C gene, one of the two isoforms of $\gamma$-tubulin that have been identified in Drosophila. We have focused our attention on fs(2)TW1 and fs(2)TW1RU34, a null and a hypomorph allele of this gene, whose sequences we report in this work. We have found that the abnormal meiotic figures observed in mutant stage 14 oocytes are not observed in laid oocytes or fertilised embryos, suggesting that these abnormal meiotic figures are not terminally arrested. We have also concluded that both null and hypomorph alleles lead to a total arrest of nuclear proliferation during early embryogenesis. This is in contrast to their effect on female meiosis-I where hypomorph alleles display a much weaker phenotype. Finally, we have observed that null and hypomorph alleles lead to some distinct phenotypes. Unfertilised laid oocytes and fertilised embryos deficient for $\gamma$Tub37C do not contain polar bodies and have a few bipolar microtubule arrays. In contrast, oocytes and embryos from weaker alleles do not have these microtubule arrays, but do contain polar bodies, or polar-body-like structures. These results indicate that $\gamma$Tub37C is essential for nuclear proliferation in the early Drosophila embryo.

Key words: Drosophila, Meiosis, Mitosis, MTOC, Mutant, $\gamma$-tubulin, $\gamma$Tub37C.

INTRODUCTION

The centrosome is the major microtubule organising center (MTOC) in most animal cells. Centrosomes organise microtubules by initiating their assembly and anchoring them at their minus ends, thus facilitating microtubule growth at the rapidly growing plus ends. Because of its central role in many essential aspects of cell physiology, both in interphase and during cell division, an intense research activity has been carried out to characterise this organelle since it was discovered over a hundred years ago (Fulton, 1971; Mazia, 1984; Kalnins, 1992; Kimble and Kuriyama, 1992; Kalt and Schliwa, 1993; Kellog et al., 1994).

The morphology, subcellular localisation and molecular composition of MTOCs vary across different species (Balczon, 1996; Mignot, 1996). There is also a considerable variability among the MTOCs of the different cell types within single species. In Drosophila, for instance there are clear differences between the centrosomes found at the poles of the mitotic spindles during early embryogenesis, the MTOCs of the meiotic spindles of both sexes and the MTOCs of terminally differentiated cells, to mention only a few examples of divergent MTOCs (reviewed by Gonzalez et al., 1998). Most of what we have learned about MTOCs comes from experimental model systems like tissue culture cells, algae, frog egg extracts, and genetically amenable yeast and fungi (Balczon, 1996). Therefore, our current view is restricted to the centrosomes of single proliferative cell types where the role of centrosomes is limited to the organisation of the interphase cytoskeleton and the mitotic spindle. To get a better understanding of the roles of MTOCs in metazoans we, and others, have chosen Drosophila as a model system. Like any other higher eukaryote, Drosophila displays many kinds of cell division which have their own cytological landmarks. Meiosis has many features which make it different from mitosis. Male and female meiosis are dramatically different and so are two meiotic divisions. There is also considerable variability among the mitotic divisions observed at different developmental stages.

The study of MTOC plasticity during development has only started. In Drosophila, a major goal at this stage is the identification of new centrosomal components and the characterisation of their function within the MTOCs of different developmental stages. One of the few integral centrosomal components identified so far in Drosophila is $\gamma$-tubulin, a member of the tubulin superfamily of proteins which was first identified as the product of the mipA gene in A. nidulans (Weil et al., 1986; Oakley and Oakley, 1989). All the eukaryotes examined contain at least one homologue of the mipA gene, including Plasmodium falciparum (Maessen et al., 1993), Schizosaccharomyces pombe (Horio et al., 1991), Ustilago violacea (Luo and Perlin, 1993), Anemia phyllitidis...
Oakley et al., 1990), the centrosomes of two alleles. In the absence of differences between the mutant phenotypes produced by these alleles, phenotypes brought about during early embryogenesis by (Tavosanis et al., 1997). The meiotic spindle is also severely disrupted in these mutants of the chromatin masses which characterise wild-type female polar body-like structures to be formed, suggesting that observed in females homozygous for lack-of-function alleles them have nuclear proliferation defects. The meiotic figures abnormal meiotic spindles and that the embryos derived from restricted to ovaries and early embryos (Tavosanis et al., 1997).

...the open-ring shape of the approximate diameter of a microtubule (Zheng et al., 1995; Moritz et al., 1995). Some of the components of these complex are highly conserved (Murphy et al., 1998; Tassin et al., 1998; Martin et al., 1998).

It has now been consistently shown by a combination of different approaches that γ-tubulin provides an essential MTOC function. This conclusion is substantiated by blocking experiments with anti γ-tubulin antibodies as well as by γ-tubulin overexpression (Joshi et al., 1992; Ahmad et al., 1994; Shu and Joshi, 1995; Julian et al., 1993); immunodepletion and biochemical manipulation of in vitro reconstituted centrosomes from Xenopus eggs extracts (Stearns and Kirschner, 1994; Moudjou et al., 1996) which has an open-ring shape of the approximate diameter of a microtubule (Zheng et al., 1995; Moritz et al., 1995). Some of the components of these complex are highly conserved (Murphy et al., 1998; Tassin et al., 1998; Martin et al., 1998).

There are two γ-tubulin genes in Drosophila. The γTub23C isofrom is essentially ubiquitous and is required for viability and microtubule organisation during mitosis and male meiosis (Sunkel et al., 1995; P. Sampaio, C. E. Sunkel and C. González, unpublished). In contrast, the expression of γTub37C is restricted to ovaries and early embryos (Tavosanis et al., 1997). We have previously shown that Drosophila females homozygous for mutations in the γTub37C gene display abnormal meiotic spindles and that the embryos derived from them have nuclear proliferation defects. The meiotic figures observed in females homozygous for lack-of-function alleles of γTub37C lack the bilateral symmetry and linear arrangement of the chromatin masses which characterise wild-type female meiotic figures during metaphase-I (Tavosanis et al., 1997). The meiotic spindle is also severely disrupted in these mutants (Tavosanis et al., 1997).

We have now carried out a detailed analysis of the mutant phenotypes brought about during early embryogenesis by different mutant alleles of this gene. We have focused on two alleles, fs(2)TW1 and fs(2)TW1RU34. We have sequenced these alleles and confirmed previous evidence suggesting that they correspond to a null and a partial loss of function of the γTub37C gene, respectively. We have found significant differences between the mutant phenotypes produced by these two alleles. In the absence of γTUB37C no polar bodies are formed, while partial depletion of the γTub37C gene allows for polar body-like structures to be formed, suggesting that meiosis is not arrested in these embryos. Interestingly, we have found that the abnormal fs(2)TW1 female meiotic figures that we have previously described (Tavosanis et al., 1997) are not terminally arrested and discuss the relevance of this observation which suggests the presence of a major transition point in terms of microtubule organisation. Despite these differences, both mutant conditions impair severely nuclear proliferation during early embryogenesis. These observations allow us to conclude that the lack of nuclear proliferation is not a downstream effect of the meiotic defects. We have also found that no centrosomes are made in embryos laid by fs(2)TW1 females, but there are some in those laid by fs(2)TW1RU34 females, thus suggesting that γ-tubulin function might be required for centrosome formation. We also show that these centrosomes are unlikely to be the MTOCs of the spindles with which they associate. In addition, we have localised CNN (Heuer et al., 1995), a major component of Drosophila centrosomes, to the aster that connects the two second meiotic spindles in wild-type embryos.

MATERIALS AND METHODS

Drosophila cultures and stocks

Cultures were maintained on standard Drosophila medium supplemented with active dry yeast and were raised at 25°C. fs(2)TW1RU34 was isolated by Schüpbach and Wieschaus (1989); fs(2)TW1 and Df(2L)VA23 are described by Lindsley and Zimm (1992). The original fs(2)TW1 and fs(2)TW1RU34 chromosomes were recombined with a multiple marked chromosome carrying al dp b pr c px sp.

Western blots

Western blots were performed as described by Tavosanis et al. (1997) using a 1:1000 dilution of affinity purified mouse polyclonal m152, raised against bacterially-expressed full-length γTUB37C protein. For detection we used a secondary antibody coupled to horseradish peroxidase, and the ECL system from Amersham.

Sequencing

Sequencing was carried out on genomic DNA amplified by PCR. To this aim genomic DNA was extracted from wild-type, fs(2)TW1 or fs(2)TW1RU34 flies and the region spanning from the initial ATG to the STOP codon of each of these alleles was amplified in three fragments: from nucleotides 1 to 448, using the oligos #3 (5’ GTT GTA CAA CCA GGA GAA TGT) and #444 (5’ GCC TCG CTC A TT CCC ACG CCC #444); from nucleotides 426 to 994 using the oligos #5 (5’ GTT GTA CAA CCA GGA GAA TGT) and #4 #444 (5’ TGG GAA TGA GCG AGG CGG); and from nucleotides 946 to 1607 using the oligos #444 (GCC TCG CTC ATT CCC ACC GAG ATT ATA) and #2 (5’ ACA CAT TCT CCT GGT TGT AC); from nucleotides 426 to 994 using the oligos #3 (5’ GTT GTA CAA CCA GGA GAA TGT) and #5 (5’ TGG GAA TGA GCG AGG CGG); and from nucleotides 946 to 1607 using the oligos #444 (GCC TCG CTC ATT CCC ACC GAG ATT ATA) and 37CSTOP (5’ gga gga aga tct TTA ACC GGC TTA CCC ACC GAG ATG). The amplifications were carried out using Taq polymerase (Perkin Elmer, Weiterstadt, Germany). The fragments were cloned into PCRScript (Stratagene, La Jolla, CA), and transformed into XL1Blue cells. Three independent colonies for each fragment were chosen for double-stranded sequencing which was carried out at the EMBL sequencing service.

Cytology

Unfertilised eggs and embryos were collected by forcing the females to lay under CO2. They were immediately fixed and processed for immunofluorescence. Some embryos were kept at 25°C for longer intervals before fixation to study the evolution of the mutant phenotype. Immunostaining of embryos was performed using methanol fixation as described by Gonzalez and Glover (1993). Taxol...
was never included. Microtubules were visualised with the DM1-a anti-α-tubulin antibody (Sigma). Centrosomes were visualised with antibodies against CNN (Heuer et al., 1995) and CP190 (Whitfield et al., 1988). DNA was stained with TOTO-3 (Molecular Probes). For double and triple labeling we used secondary antibodies conjugated with Texas Red and FITC (Jacksons).

RESULTS

Molecular characterisation of fs(2)TW1¹ and fs(2)TW1¹Ru34

We have previously shown that the yTub37C gene corresponds to complementation group fs(2)TW1¹ (Stathakis et al., 1995; Tavosanis et al., 1997). Based on the phenotypic analysis of some of the extant mutant alleles of this gene we concluded that fs(2)TW1¹ represents a very severe lack of function, probably a null allele of this gene while fs(2)TW1¹Ru34 is a leaky one. These two alleles were chosen for this study. Here we present their molecular characterisation.

Western blot analysis with affinity purified m152, a mouse polyclonal antibody raised against full-length, bacterially expressed yTUB37C protein (Fig. 1A), shows that no product is detected in fs(2)TW1¹ homozygous females. The same antibody recognises a single band of the expected molecular mass in fs(2)TW1¹Ru34 hemizygous females. We have determined by PCR and RT-PCR that there are no major DNA rearrangements in the coding region of these two genes and that mRNA is produced in both fs(2)TW1¹ and fs(2)TW1¹Ru34 mutant females (data not shown). We have sequenced these two mutants and found that fs(2)TW1¹ contains a point mutation (G to A) which changes Tryptophan 352 into a STOP codon (Fig. 1B). This results in a truncated protein from which the last 105 carboxy-terminal amino acids are missing. This domain is thought to be important for proper protein folding (Burns and Surridge, 1994). Thus, the failure of the m152 antibody to detect the truncated yTUB37C produced by fs(2)TW1¹ may be due to the rapid degradation of this truncated form. Alternatively, the truncated form may be present, but may not be recognised by the m152 antibody. We believe that this possibility is unlikely because other polyclonal antibodies raised against the full-length protein in mice and rabbits give similar results (data not shown). These observations are in agreement with previous results, suggesting that fs(2)TW1¹ is a null or a severe lack-of-function allele (Tavosanis et al., 1997).

The second mutant allele, fs(2)TW1¹Ru34, contains a point mutation (G to A) that modifies a Glu 343 to Lys (Fig. 1B). This activates meiosis which is otherwise arrested in metaphase of the first meiotic division. Female meiosis-II figures are characterised by the presence of two tandemly arranged spindles connected by an aster of microtubules (Fig. 2A and B). The distal poles of the meiotic spindles are not associated with asters. The full sequence corresponds to the wild type. The amino acids modified in the two mutant alleles, E343K and W352STOP in fs(2)TW1¹Ru34, are not in the central aste centrosomes, is also present in the central aste and is not detectable at the spindle poles (Fig. 2A and B). In fertilised embryos, CP190, and γ-tubulin, can also be observed in the aste organised around the head of the sperm (Riparbelli and Callaini, 1996, 1998). We have found that Centrosomin (CNN; Heuer et al., 1995), another known component of mitotic centrosomes, is also present in the central aste and is not detectable at the spindle poles (Fig. 2A and B). In fertilised embryos, CP190, and γ-tubulin, can also be observed in the aste organised around the head of the sperm (Riparbelli and Callaini, 1996, 1998; S. Llamazares and C. Gonzalez, unpublished). The presence of an astral array between the two meiosis-II spindles does not necessarily mean that pericentriolar material is getting assembled at this stage. In fact Riparbelli and Callaini (1996, 1998) have shown that at least the localisation of CP190 and γ-tubulin in the center of the aste is microtubule-dependent
since it disappears following colchicine treatment. The association of CP-190 and $\gamma$-tubulin to mitotic centrosomes is not microtubule dependent.

Completion of meiosis leads to the segregation of four haploid nuclei; one female pronucleus and three polar bodies that do not participate in singamy. Polar bodies are monopolar structures organised by a dense aster of microtubules (Fig. 2C). The chromosomes within polar bodies are condensed and are oriented like radial spokes with their centromeric regions pointing towards the center of the aster. The three polar bodies of an embryo often fuse to form a single larger polar body. In fertilised oocytes, the chromosomes in the polar bodies remain condensed as the chromatin of the proliferating nuclei undergo successive cycles of condensation and decondensation. The polar bodies are disassembled around the time of syncytial blastoderm formation (Rabinowitz, 1941). Pericentriolar markers like CNN (Li and Kaufman, 1996), $\gamma$-tubulin (Sunkel et al., 1995; Tavosanis et al., 1997) and CP190 (Whitfield et al., 1988), which are found in the centrosomes of the mitotic spindles of the embryo, are not detected in polar bodies.

The embryos derived from $fs(2)TW1^1$ homozygous mothers depart from the wild type in three major regards (Fig. 3). Firstly, they do not contain polar bodies. Secondly, mitotic spindles of the kind found in wild-type embryos are never observed (Fig. 3A,B,C). Finally, the vast majority of them contain one, some times two, spindle-like structures, (Fig. 3D,E,F) together with a few other microtubule arrays (Fig. 3G,H,I). These features are common to unfertilised laid oocytes and fertilised embryos derived from $fs(2)TW1^1$ mothers. The pole-to-pole distance of the spindle-like structures found in $fs(2)TW1^1$ derived embryos is quite variable. The smallest that we have observed measured 17 $\mu$m (Fig. 3F), marginally larger than the average size of a meiosis-I spindle (Theurkauf and Hawley, 1992), but others reached over 40 $\mu$m in length (Fig. 3D). They were always considerably thicker than a wild-type meiosis-I spindle. They also contain variable amounts of chromatin. Sometimes, the chromosomes are located in the equator, like a metaphase plate (Fig. 3F), but they can also be found scattered along the spindle (Fig. 3D and E). Chromosome condensation is also variable. It is always high, comparable to that observed in mitotically arrested cells or even higher so that the chromosomes, or perhaps chromosome fragments appear as tiny dots (Fig. 3D). There are never asters at the poles of these spindles and we have been unable to detect any significant amounts of CP190 and CNN associated with them. The other microtubule arrays found in these embryos are organised around small masses of chromatin (Fig. 3G,H,I). It is in fact a very distinct feature of the embryos derived from $\gamma$Tub37C mutant mothers that almost every chromatin spot, regardless of size, is associated with a microtubule array. Some are only a few microns in length (Fig. 3I), but others were almost as long as the major spindle, although narrower. Most of these can be categorised as bipolar.

**Weak loss-of-function mutations**

Unlike the embryos derived from $\gamma$-tubulin deficient females, the embryos derived from females homozygous for the hypomorph allele $fs(2)TW1^{RU34}$ contain polar bodies and do
not display large bipolar structures (Fig. 4A,B,C,D). Nevertheless, like in the γ-tubulin deficient embryos, nuclear proliferation is severely affected and normal mitotic spindles are not observed. The DNA content and the size of the polar bodies found in the mutant are variable. In some cases, they are similar to the ones found in wild-type embryos, although there are clear differences among the four meiotic products of a given egg. Moreover, most of these polar bodies do not display the round, homogeneous appearance of their wild-type counterparts (Fig. 4E,F,G,H). Chromosome content and chromatin condensation within these polar bodies are also variable and different from the wild type. In these embryos we also observed some spindle structures which were bipolar and had chromatin at the equator. These were never found in an anaphase or anaphase-like stage. Most of these spindles have centrosomal material associated with either one or the two
poles of the spindle (Fig. 4I,K,L). Cases in which more than one centrosome was found associated with a single spindle pole or in which the centrosome and the spindle pole were a few microns apart were also observed (Fig. 4I,K,L). We also detected centrosomal material in some monopolar microtubule arrays which looked like polar bodies (Fig. 4J). While some of these centrosomes showed a small microtubule aster around, others did not. Taken all together, these observations suggest that these centrosomes may not be fully functional and that despite their association to the spindles the may not be serving as their microtubule organising centers.

The polar body-like structures found in fertilised embryos from $fs(2)TW1RU^{34}$ mothers are significantly different from those found in the wild-type, both in terms of microtubule organisation and chromosome content. Most of the mutant polar bodies are considerably large, some of them more than 3 times larger in diameter than wild-type polar bodies. Moreover, although their main body is organised in a spherical fashion similar to the wild type, the polar bodies in the mutant have many mini spindle-like structures associated with them (Fig. 4E). In some embryos, more than one of these polar bodies could be observed connected by the small associated minispindles. These large polar body-like structures contain many, very often tens, of chromosomes. When chromatin condensation allows, it is possible to observe that the chromosomes within them are organised as a bouquet structure like the chromosomes in wild-type polar bodies. The associated minispindles also contain chromatin which is located in the middle of the spindle. There seems to be a good correlation between spindle size and chromatin content to the extent that some of the smallest minispindles appear to contain only one chromosome or a chromosome fragment, although, the extreme chromatin condensation makes it impossible to perform any karyotypic determination. We have not been able to detect CP-190 or CNN on these polar body-like structures or their associated mini-spindles.

**DISCUSSION**

There are three major conclusions that can be drawn from this work regarding the function of the $\gamma$Tub37C gene. The first conclusion is that abnormal meiotic figures of the kind found in mutant stage 14 oocytes (Tavosanis et al., 1997) are not observed in laid oocytes or fertilised embryos. This observation suggests that these abnormal meiotic figures are not terminally
arrested. The same observation has been reported by Wilson and Borisy (1998). We do not know how these figures evolve, but it is likely that the natural course of activation triggered by passage through the oviduct exerts some effect in the mutant oocytes which results in the progression from the abnormal meiotic figures of the ovarian oocytes into the mutant phenotypes observed in laid eggs. We have also concluded that both hypomorph and lack-of-function alleles lead to a total arrest of nuclear proliferation during early embryogenesis. This is in contrast to their effect on female meiosis-I where hypomorph alleles display a much weaker phenotype. Thus, there seems to be a clear difference in the requirements for $\gamma$-tubulin function between meiosis-I and mitosis so that while low levels of $\gamma$-tubulin function may be sufficient to carry out normal female meiosis, they are not sufficient for nuclear proliferation during early embryogenesis. If the effect of the different mutant alleles on meiosis-II is similar to their effects on meiosis-I, then this observation suggests that the absence of nuclear proliferation is not just a pleiotropic effect brought about by the abnormal meiosis displayed by mutant oocytes. Finally, we have observed that despite the lack of nuclear proliferation which is common to null and hypomorph alleles, each of these kind of alleles lead to some distinct phenotypes. Unfertilised laid oocytes and fertilised embryos deficient for $\gamma$TUB37C do not contain polar bodies and have one or two large bipolar microtubule arrays together with a few minor ones. In contrast, oocytes and embryos from weak alleles do not have these microtubule arrays, but do contain polar bodies, or polar-body-like structures.

The presence of bipolar microtubule arrays organised around chromatin in embryos derived from $\gamma$Tub37C deficient mothers is remarkable for two reasons. Firstly, because it occurs in the same mutant background in which the female meiotic spindle often fails to be formed. Secondly, because it takes place in the absence of the major isoform of $\gamma$-tubulin present at that stage. The presence of bipolar arrays of microtubules in laid oocytes and embryos derived from females which were unable to organise a proper meiosis-I spindle argues that these two structures, the wild-type meiotic spindle and the bipolar microtubule arrays are organised by different mechanisms. This is not surprising since we are comparing a wild-type functional structure which has evolved to ensure segregation of homologue chromosomes and an aberrant microtubule array. The only suggestion of similitude between these two structures comes from their bipolar shape and the fact that they are anastral. However, this argument is not very solid. Spindle-like microtubule arrays which are bipolar and anastral can also form around microinjected naked DNA in Xenopus eggs (Karsenti et al., 1984) and around beads coated with DNA in Xenopus egg extracts (Heald et al., 1996). Furthermore, functional spindles which are bipolar and anastral have been reported during the syncytial mitotic divisions which occur in parthenogenetic Sciara embryos (de Saint Phalle and Sullivan, 1998). Finally, even at the morphological level, there are some differences between the spindle structures found in $fs(2)TW1^{1}$ derived laid oocytes and embryos and the wild-type female meiosis-I spindle. The former are often larger (sometimes more than twice as long as the wild type), considerably thicker and often contain much more chromatin. Since we have not followed these spindle structures in real time, we cannot positively rule out that they may drive chromosome segregation, but we find it unlikely since we have never observed them in an anaphase or anaphase-like stage. In fact, the stability of these structures, which are present in relatively old embryos, indicate that they are fairly static and that chromosome segregation never occurs. Together with the one or two major spindles discussed above, $fs(2)TW1^{1}$ derived laid oocytes and embryos have always a variable number of smaller spindle-like arrays, which also contain chromatin. We interpret the presence of these groups of large and small spindles associated with large and small masses of chromatin as a consequence of the abnormal meiosis observed in mutant oocytes. Interestingly, similar figures are produced in a fraction of eggs from grauzone and cortex mothers, which arrest in meiosis-I (Page and Orr-Weaver, 1996).

Whichever their origin and nature, the microtubule arrays found in $\gamma$Tub37C deficient embryos seem to argue that microtubule polymerisation is possible in the absence of this isoform. Nevertheless, this observation must be interpreted with caution. Although extremely unlikely, the possibility that non-detectable amounts of the truncated product of the $fs(2)TW1^{1}$ allele may promote microtubule nucleation cannot be ruled out. In a $\gamma$Tub37C null embryo, microtubule polymerisation could be supported by the small amounts of $\gamma$TUB23C which are present at this stage, or a third as yet not identified $\gamma$-tubulin isoform. Alternatively, these microtubule arrays may not depend at all on $\gamma$-tubulin for their polymerisation and organisation. In any case, the presence of a few microtubule arrays in $\gamma$Tub37C deficient embryos should not be interpreted as evidence that microtubule polymerisation is normal since we have no information whatsoever on the dynamics of microtubule polymerisation in these mutant embryos. Contrary to the phenotype displayed by laid oocytes and embryos derived from females deficient for the $\gamma$TUB37C isoform, the laid oocytes and embryos from females carrying hypomorph alleles of this gene contain polar bodies. This observation is in agreement with the weak meiotic phenotype reported for this allele in which only 15% of the meiotic figures can be classified as abnormal (Tavosanis et al., 1997). The presence of polar bodies in this mutant condition indicates that meiotic chromosome segregation takes place, although their abnormal chromosome content and microtubule structure suggests that meiosis did not proceed normally. Both, the spindle-like and the polar-body-like structures found in the two mutant conditions studied in this work seem to be able to grow while normal nuclear proliferation does not take place. In both cases, chromatin content can reach very high levels, much greater than 4N, suggesting that DNA replication is taking place within these structures. The associated microtubule mass seems to increase accordingly.

From our phenotypic analysis of embryos derived from $\gamma$Tub37C mutant females we believe that the main defect caused by mutation in this gene during embryogenesis is that normal functional centrosomes cannot be assembled. This conclusion is based on the observation that organised centrosomes are always absent in the case of $fs(2)TW1^{1}$ and there are only a few in $fs(2)TW1^{R/34}$. These few centrosomes were never observed organising dense asters and showed an erratic association with neighboring microtubule arrays. Although these observations could be partially due to a downstream effect of the abnormal meiotic divisions displayed...
by these oocytes we believe that, most likely, they reflect a direct requirement for γTUB37C to organise functional mitotic centrosomes. This can be due to either structural or functional reasons. It is possible that the absence or reduction of γ-tubulin levels affects centrosome organisation so that the organelle cannot be assembled. Alternatively, the first centrosomes may be assembled correctly, but be unable to organise microtubules. Either case would result in the absence of functional centrosomes which could lead to a total arrest of the wild-type programme of nuclear proliferation within mutant embryos.

We still do not know whether the mutant phenotypes produced by mutation in the γTUB37C gene are due to a specific requirement for this isoform or simply to the fact that it is the most abundant one during these stages. Since the γTUB23C isoform is present at low levels during early embryogenesis (Tavosanis et al., 1997) and it does not seem to be associated with centrosomes (Wilson et al., 1997), it has been suggested that some functional incompatibility between the two isoforms may exist at this stage (Wilson et al., 1997). A similar argument could be made for the abnormal meiosis displayed by γTUB37C deficient females despite the presence of small amounts of the γTUB23C isoform (Tavosanis et al., 1997). To unequivocally answer this question we are studying the ability of the γTUB23C isoform to rescue these phenotypes when expressed under the control of the γTUB37C regulatory regions.

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REFERENCES


Drosophila γTub37C gene mutant phenotypes


