Nuclear structure and chromosome segregation in Drosophila male meiosis depends on the Ubiquitin ligase dTopors


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ABSTRACT

In many organisms, homolog pairing and synapsis at meiotic prophase depends on interactions between chromosomes and the nuclear membrane. Male Drosophila lack synapsis, but nonetheless, their chromosomes closely associate with the nuclear periphery at prophase I. To explore the functional significance of this association, we characterize mutations in *nuclear blebber (nbl)*, a gene required for both spermatocyte nuclear shape and meiotic chromosome transmission. We demonstrate that *nbl* corresponds to *dtopors*, the Drosophila homolog of the mammalian dual ubiquitin/SUMO ligase Topors. We show that mutations in *dtopors* cause abnormalities in Lamin localizations, centriole separation and prophase I chromatin condensation, and also cause anaphase I bridges that likely result from unresolved homolog connections. Bridge formation does not require *mod(mdg4) in meiosis*, suggesting that bridges do not result from misregulation of the male homolog conjunction complex. At the ultrastructural level, we observe disruption of nuclear shape, an uneven perinuclear space and excess membranous structures. We show that dTopors localizes to the nuclear lamina at prophase, and also transiently to intranuclear foci. As a role of *dtopors* at gypsy insulator has been reported, we also asked if these new alleles affected expression of the gypsy-induced mutation *ct6*, and found that it was unaltered in *dtopors* homozygotes. Our results indicate that dTopors is required for germline nuclear structure and meiotic chromosome segregation, but in contrast, is not necessary for gypsy insulator function. We suggest that *dtopors* plays a structural role in spermatocyte lamina that is critical for multiple aspects of meiotic chromosome transmission.
INTRODUCTION

Associations between chromosomes and the nuclear envelope during meiotic prophase are a widely conserved phenomenon important for proper chromosome transmission. In many species, such interactions are required for bouquet formation, an arrangement in which telomeres cluster in association with the nuclear envelope to facilitate homolog pairing and synapsis (reviewed in (SCHERTHAN 2007)).

In *C. elegans*, analogous interactions between nuclear envelope proteins and chromosomes are mediated by zinc finger proteins that connect chromosome-specific pairing sites to integral nuclear membrane proteins (PHILLIPS *et al.* 2009). These connections establish bridges across the nuclear envelope and allow for interactions between meiotic chromosomes and cytoskeletal actin. Chromosome movements dependent on these connections are important for homolog pairing (SATO *et al.* 2009).

The association of meiotic chromosomes with the nuclear periphery is particularly striking in Drosophila males, in which paired homologs occupy discreet domains closely apposed to the nuclear membrane. The relevance of this organization to meiotic chromosome segregation in this organism, however, has not been explored. Drosophila males have an unconventional meiosis in which homologs pair but do not assemble synaptonemal complex (MEYER 1960), and do not undergo crossing over. The mechanism of pairing is unknown. Pairing sites have been defined for the sex chromosomes (MCKEE AND KARPEN 1990), however they have not been demonstrated to associate with the nuclear envelope.

Towards understanding the relationship of nuclear structure to meiotic chromosome segregation in this organism, we have examined mutations in *nbl*. Mutant *nbl* males exhibit defects in spermatocyte nuclear shape and also show a high frequency of fourth chromosome loss (WAKIMOTO *et al.* 2004). This suggested that further characterization of *nbl* might reveal aspects of nuclear organization important for meiotic chromosome transmission. Here we show that the *nbl* gene corresponds to *dtopors*,...
the fly homolog of Topors, a mammalian tumor suppressor that has both ubiquitin and SUMO ligase activities (Saleem et al. 2004; Rajendra et al. 2004; Pungaliya et al. 2007). Our results reveal a requirement for dtopors in both male germline nuclear envelope structure and in events associated with meiosis I chromosome segregation. We suggest that both requirements may be related to a role at the nuclear lamina. As dtopors has been implicated in gypsy insulator function, we also examine the effects of these new alleles on gypsy insulator function, and find that in contrast to meiosis, dtopors is not required at gypsy insulators.

MATERIALS AND METHODS

Drosophila stocks: Fly stocks were cultured on standard cornmeal molasses medium at 25°C. The Z mutations were provided by C. Zuker lab at the University of California at San Diego, and are described in Wakimoto et al. (Wakimoto et al. 2004). All other stocks were obtained from the Bloomington stock center and are described in FlyBase (Tweedie et al. 2009).

Mapping nbl mutations: The nbl mutations were mapped to the cytogenetic interval 56A1-B2 by failure of both Df(2R)Exel6068 (56A1-B5) and Df(2R)PC66 (55D2-56B2) to complement the fourth chromosome loss and the spermatocyte nuclear phenotypes. This localization was consistent with recombination mapping of nbl to the region between pr and px. Additional complementation tests revealed that Df(dtoporsAA), a small deletion that removes dtopors and the adjacent gene prod (Secombe and Parkhurst 2004), failed to complement the nuclear and chromosome segregation defects of nbl. Furthermore, an insertional mutation in dtopors, P(Bac[WH]Topors^f05115, failed to complement nbl, whereas an insertional mutation in prod, P[lacW]prod^k08810, fully complemented.

Genetic assays of meiotic chromosome segregation: To test if dtopors mutations caused sex chromosome and fourth chromosome nondisjunction, y/y+Y; dtopors ; spad^pol males were crossed to y w
sn; C(4)RM ci ey/0 females. In these tests, normal progeny were y+w sn sons and y daughters, whereas sex diplo-exceptional progeny that received both the X and Y from their fathers were distinguished as y+ females, and sex nullo-exceptional progeny that failed to receive a paternal sex chromosome were distinguished as y w sn males. Diplo-XY exceptions are indicative of nondisjunction at meiosis I, whereas nullo-XY exceptional progeny could be a consequence of loss or nondisjunction at either MI or MII. Fourth chromosome nondisjunction was monitored simultaneously in these crosses. Diplo-4 exceptional sperm gave rise to spa progeny, and nullo-4 exceptional sperm gave rise to ci ey progeny. These crosses did not allow distinction between MI and MII fourth chromosome segregation errors. The relative frequencies of nullo- versus diplo- exceptional progeny, however, are an indication of whether errors were largely due to nondisjunction (in which case a 1:1 nullo:diplo ratio is expected), or chromosome loss (in which case the frequency of nullo-exceptions would exceed that of diplo-exceptions).

To directly assess MII nondisjunction, yw/y+Y; dtopors; spa/+ males were crossed to C(1)RM y v/Y; C(4)RM ci ey/0 females. From these crosses, progeny produced from diplo-XY sperm resulting from MI nondisjunction (y+ w sons), diplo-XX sperm resulting from meiosis II nondisjunction (y w daughters) and nullo-XY sperm resulting from either MI or MII nondisjunction (y v daughters) could be distinguished from normal y w sons and y+ v daughters. Simultaneously, we could detect MII fourth chromosome diplo-exceptions as spa progeny, and MI or MII nullo-exceptions as ci ey progeny. For both MI and MII nondisjunction tests, a minimum of 100 males of each genotype were tested.

To assay for autosomal nondisjunction, crosses were performed between dtopors males and females bearing either Compound 2 or Compound 3 chromosomes. In these females, autosomal (A) homologs are attached to a single centromere, resulting in the production of diplo-A and nullo-A eggs. Because aneuploidy for either of the two major autosomes (chromosome 2 or 3) is lethal, progeny of
C(A) females mated to chromosomally normal males survive only if produced from aneuploid sperm. We used a metric of “progeny per male” to determine if dtopors males produced a greater number of viable progeny than their heterozygous siblings, which would indicate an increased incidence of aneuploidy for the major autosomes.

For assays of male fertility of dtopors; mnm allelic combinations, we also determined progeny produced per male. Crosses were performed using single y/y+Y; spa males and five y w sn; C(4)RM ci ey/0 females. Parents were allowed to reproduce for 8 days before removal, and progeny produced were scored until 18 days after the cross was initiated. Sex and fourth chromosome nondisjunction were monitored as above.

**PCR and DNA sequencing:** Genomic DNA was PCR amplified from Drosophila genomic DNA using the following primers:

5′ CTGTTAACATGGCGGAGGAGAATCCC 3’,
5′ CCAGATCTATACGGCAGTAGTCCCTGAT 3’,
5′ CCAGATCTACGCCTCACAATGTGGTAACG 3’,
5′ CCAGATCTGTAAACGTTTATGTCACTACGG 3’,
5′ CCAGATCTTGCGGCCTCCAGCGAATAGGC 3’,
5′ CTGTTAACATGGATCATGTGGTGCAGTATTCG 3’,
5′ CTGTTAACATGGATCATGTGGTCAGTATTGC 3’,
5′ CTGTTAACATGGATCTAGTTGGCGAATCA 3’

PCR products were purified using QIAquick columns (Qiagen), and sent for commercial sequencing to Eurofins, MWG Operon.
Indirect immunofluorescence and confocal microscopy: Testes were dissected in Schneider’s insect tissue culture medium (Invitrogen), and gently squashed under silanized coverslips. Coverslips were removed after freezing in liquid nitrogen, and tissues fixed 10 min. in cold methanol. Primary antibodies were used at the following dilutions in Phosphate buffered saline (PBS) + 1.0% bovine serum albumin, fraction V (Fisher Scientific): rabbit-anti-GFP 1:1000 (Invitrogen), mouse anti-dTopors, 1:1000 (SECOMBE and PARKHURST 2004), mouse anti-β tubulin 1:500 (E7, Iowa Hybridoma Bank), mouse anti-γ tubulin 1:2000 (GTU-88, Sigma Scientific), mouse anti-Lamin Dm0 1:10 (LC28) and mouse-anti-Lamin C (ADL67) 1:100 (Iowa Hybridoma Bank), rabbit anti-pH3 (Sigma 06570) 1:500, anti-centrosomin 1:500 (Li et al. 1998), anti-dTACC 1:2000 (GERGELY et al. 2000), anti-Aurora A 1:1000 (BERDNIK and KNOBLICH 2002) and anti-HP1 (CA19) 1:500 (Iowa Hybridoma Bank). Secondary antibodies used were Alexafluor 546 goat-anti-mouse, 1:1000 (Molecular Probes) and Alexafluor 488 goat-anti-rabbit (Molecular Probes). Primary incubations were 1 hr rt followed by 3 x 5’ washes in PBS. Secondary incubations were 45’ rt followed by 3 x 5’ washes in PBS. Slides were stained by 1’ incubation in 1 ug/ml 4’,6-diamidino-2-phenylindole (DAPI, Sigma) and mounted in 50% glycerol. For each antigen examined, a minimum of 200 MI cells at prophase and 30 MI cells between prometaphase and telophase I from at least five individuals were observed.

Condensation assay: Testis squashes were prepared as above and stained with DAPI and anti-γ tubulin antibodies. To minimize variations in staining, all slides were processed simultaneously in identical solutions. Twenty S6 cells from at least 5 different males per genotype were selected based on staining with DAPI and antibodies to gamma tubulin, as above. Images were captured using standardized settings using an Olympus FV500 confocal laser scanning microscope and Fluoview software. The nuclear area of each DAPI image was selected and pixel intensity histograms generated.
using Adobe photoshop software. Pixels with an intensity value of less than 10 (on a scale of 0-250) were considered negative, and were used to determine the area of each nucleus devoid of chromatin.

**Western blotting:** Testes were dissected from 1 day-old adults, sonicated in Sample buffer (1% SDS, 10% glycerol, 0.01% bromophenol blue, 5% beta-mercaptoethanol, 250mM Tris pH 6.8) plus 2μM MG132, 0.2 mM IAA, 80mM NEM and proteinase inhibitor cocktail P2714 (Sigma), then separated by SDS-PAGE. After transfer to Immobilon membrane (Millipore), blots were probed with anti-Drosophila SUMO (N- terminal), (AP1287a, Abgent), diluted 1:500 in Tris buffered saline, pH7.4, mouse-anti-Lamin C (1:100), mouse anti-β tubulin (1:500) or mouse anti-Lamin Dm0 (1:10), and detected using goat-anti-mouse HRP-conjugated secondary antibodies (1:10,000) (Jackson Immunoresearch) with Supersignal West Pico Chemiluminescence reagents (Thermo Scientific). Signals were captured and analyzed using a Bio-Rad Chemidoc XRS image acquisition system. Comparisons of antigen abundance were based on the average signal intensities relative to control β tubulin signals from triplicate blots.

**Electron microscopy:** Spermatocytes were dissected in Schneider’s Tissue culture media (Sigma), centrifuged onto Thermanox plastic coverslips (Nunc), and immediately fixed in 2% glutaraldehyde , 0.1M cacodylate, 3% sucrose and 3 mM CaCl. Fixed tissues were stained in 1 μm DAPI and examined using fluorescence microscopy to identify cells at stages of interest. Selected cells were then fixed in reduced osmium (MCDONALD 1984) and stained en bloc with 2% aqueous uranyl acetate, dehydrated in graded ethanol and embedded in Eponate-12 (Ted Pella). Thin sections were examined using a Hitachi 7600 TEM at an accelerating voltage of 80Kv. All images were captured with an Advanced Microscope Techniques, digital camera (AMT 16000-S).

**RESULTS**

9
**nuclear blebber corresponds to dtopors:** Two mutations in *nuclear blebber* (*nbl*) had been isolated in a screen for viable recessive *male chromosome loss* (*mcl*) mutations that caused increased incidence of fourth chromosome loss among progeny of mutant males (WAKIMOTO *et al.* 2004). The original designations were *nbl* \textsuperscript{Z1837} and *nbl* \textsuperscript{Z4522}. In addition to the chromosome loss phenotype, *nbl* males display a striking nuclear dysmorphology in primary spermatocytes (see Figure 2, (WAKIMOTO *et al.* 2004)). We mapped the *nbl* to the *dtopors* locus by recombination and deficiency mapping (see MATERIALS AND METHODS). Henceforth, we will refer to these mutations as *dtopors*\textsuperscript{Z1837} and *dtopors*\textsuperscript{Z4522}.

The *dtopors* genes were PCR-amplified from flies homozygous for either *dtopors*\textsuperscript{Z4522}, *dtopors*\textsuperscript{Z1837} or the wildtype progenitor *cn bw* second chromosome, and DNA sequences determined. Comparison of wildtype and mutant sequences revealed a single basepair difference from the progenitor chromosome for each *dtopors* allele. Each EMS allele is predicted to encode a dTopors protein bearing a single amino acid substitution. In *dtopors*\textsuperscript{Z4522}, this substitution lies in an 84 amino acid region of high conservation (41% identity, 56% similarity) between mammalian Topors homologs, but for which no particular function has been ascribed (Figure 1). The substitution in the *dtopors*\textsuperscript{Z1837} allele is 124 amino acids closer to the N-terminus, in the same general region. Neither mutation affects a residue conserved in mammals, but the Z4522 mutation alters a residue conserved among Drosophilids. Overall, human Topors and dTopors share only limited homology outside of the RING domain and this region. For the full-length proteins, only 122/1038 of the fly amino acids are identical in the human homolog.

**Mutations in dtopors disrupt segregation of all chromosomes at MI in males:** As the original *mcl* screen monitored only fourth chromosome loss from mutant males, we performed genetic tests to determine 1) if *dtopors* mutations affect the meiotic transmission of other chromosomes, 2) at which meiotic division defects in chromosome segregation occur, and 3) if the effects are male-specific.
In addition to the EMS-induced alleles, we made use of an existing deletion, \( Df(dtopors^{AA}) \) (Secombe and Parkhurst 2004), and a piggyBac insertion allele, \( dtopors^{f05115} \). Conceptual translation of the piggyBac insertion allele sequence predicts that it encode a protein truncated after amino acid 47, and therefore is likely a null allele. Homozygous \( dtopors \) males bearing each possible combination of the four alleles showed near random segregation of both sex and fourth chromosomes (Table 1). The frequencies of progeny that resulted from simultaneous sex and fourth nondisjunction were similar to predictions based on independence, suggesting that the two sets of chromosomes were not influencing each other’s behavior. Consistent with a severe chromosome segregation defect, the fecundity of \( dtopors \) males was greatly reduced, producing on average fewer than 5 progeny per male. We were unable to assay nondisjunction in \( dtopors^{Z1837} \) homozygotes, as these males were essentially sterile, producing only 2 progeny from over 500 matings. This allele also had a weak dominant effect, as we observed low frequencies of exceptional progeny from heterozygous \( dtopors^{Z1837}/+ \) fathers. All other alleles were recessive. As predicted, \( dtopors^{f05115} \) was similar to that of the deletion, producing random segregation of both sex and fourth chromosome homologs, suggesting that it is a null allele. The \( dtopors^{Z4522} \) homozygotes produced significantly fewer exceptional progeny than \( Df(dtopors^{AA}) \) homozygotes, and frequency of exceptional progeny from transheterozygous \( dtopors^{Z4522}/Df(dtopors^{AA}) \) was intermediate. These data indicate that the \( dtopors^{Z4522} \) allele is hypomorphic. As in null mutants, sex and fourth chromosome segregation were equally perturbed in \( dtopors^{Z4522} \) males, suggesting that the two pairs of chromosomes are equally sensitive to the \( dtopors \) defect.

The frequencies of chromosome 4 \( nullo \)- and \( diplo \)-exceptions amongst progeny of \( dtopors \) males were roughly equal, consistent with nondisjunction rather than loss as the primary consequence of the \( dtopors \) defect. Among sex chromosome exceptions, \( nullo \)-exceptions significantly outnumbered
diplo-exceptions. This likely reflects meiotic drive, which favors the recovery of sperm with lower chromatin content (Peacock et al. 1975), rather than chromosome loss.

We also tested for meiosis II nondisjunction, using both null Df(dtopors$^{A4}$) and hypomorphic dtopors$^{24522}$ males (see MATERIALS and METHODS). While we observed frequencies of MI exceptions comparable to previous tests (see Table 1), we observed no evidence for MII nondisjunction (Table 2). This suggests that the effects of dtopors mutations are specific to meiosis I.

To ask if transmission of the major autosomes was also affected in dtopors males, we crossed mutant males and their heterozygous siblings to females bearing compound autosomes (C(A)), and counted the viable progeny per male produced as an indication of autosomal nondisjunction (see MATERIALS and METHODS). From crosses involving either C(2) or C(3) females, we observed significantly more progeny per male from dtopors vs. dtopors/+ males (Table 3, p<0.05). Paternal nullo-A and diplo-A exceptional progeny were observed for both chromosome 2 and 3, indicating that dtopors causes nondisjunction of both major autosomes. As for the sex chromosomes, an excess of autosomal nullo-exceptions was observed, likely as a consequence of meiotic drive which also occurs as a result of autosomal missegregation (Dernburg et al. 1996).

In contrast to males, we detected no increase in chromosome transmission errors in dtopors females. We crossed females of each dtopors allelic combination to In(1)EN, X.Ys, y f/0 ; C(4) ci ey/0 males, and scored at least 1000 progeny from each cross (data not shown). In each case, we observed fewer than 0.5% sex chromosome exceptions and fewer than 1% fourth chromosome exceptions, and the frequencies of each did not significantly differ from those observed among progeny of control dtopors/+ mothers. Nor did we observe any significant reductions in female fertility. We conclude that the effects of dtopors on meiotic chromosome transmission are limited to meiosis I in males.
**dtopors causes precocious centriole separation and Anaphase I bridges:** To determine the cause of the *dtopors* meiosis I chromosome segregation defects, we compared the progression of meiosis I in wild type and *dtopors* mutant spermatocytes. To assess spermatocyte stage, chromosome morphology and microtubule organization were monitored in fixed spermatocytes using the DNA-specific dye DAPI and antibodies to γ and β tubulin, as described by Cenci et al. (Cenci et al. 1994). Males homozygous for each of the four *dtopors* alleles were examined, and similar phenotypes were observed for each allele. The *dtopors*<sup>f05115</sup> allele was characterized most extensively, as this allele was expected to be a null based the predicted protein product (Figure 1).

In wildtype meiosis, the centriolar pair is duplicated in prophase of MI, and segregates as a perpendicular pair to opposite poles to establish the MI spindle. When stained with anti-γ tubulin antibodies, it appears as a “V” shape. At telophase of MI, each centriolar pair separates without duplication, and the single centrioles migrate to opposite poles to establish two parallel MII spindles (Li et al. 1998).

Staining with anti-γ tubulin antibodies revealed an abnormal separation of centrioles at meiosis I in *dtopors*<sup>f05115</sup> males (Figure 2A). This defect occurred rarely at late prophase S6 (2/501), but was seen much more frequently at prometaphase (9/32). Of 43 cells examined at meiosis I metaphase through telophase, all were abnormal with respect to γ tubulin staining. In 22 of these, centrioles were slightly separated at each pole, giving the appearance of telophase I. Examination of spindles and chromosomes, however, suggested these cells were at metaphase to early anaphase. In the remaining cells, the chromosomes also appeared to be at metaphase or early anaphase I, yet the centrioles had an MII configuration, resulting in tetraropolar spindles. In 30/31 cells at mid to late anaphase I, chromatin bridges were observed connecting the separating masses of chromatin (Figures 2A and B). Because our genetic data indicated MI homolog nondisjunction, and we failed to observe abnormal connections between
non-homologs at prometaphase (e.g. Figure 2A), we conclude that these bridges likely result from the failure to separate homologs at anaphase I.

To verify that the extra \( \gamma \) tubulin foci resulted from centriole separation rather than an additional round of duplication, we examined EM serial sections of ten MI \textit{dtopors}^{05115} spermatocytes. None of ten cells contained more than four centriole halves, and centriole morphology was normal. Antibody staining of centrosomal antigens centrosomin, dTACC and Aurora A showed wildtype patterns of associations of these antigens with each \( \gamma \) tubulin focus (data not shown).

We also noted a subtle defect in chromatin condensation in \textit{dtopors} spermatocytes. DAPI staining appeared normal from early-mid prophase stages S1-S4, but at late prophase stages S5 and S6 the chromosomes appeared slightly less condensed than normal. This mild condensation defect was apparent until stage M1a (Figure 2A). Pairing appeared grossly normal, as paired homologs occupied territories adjacent to the nuclear periphery at this stage. No differences between \textit{dtopors} and \textit{wildtype} were detected in antibody staining patterns for HP1 (\( n=133 \) and 136, respectively) or S10-phosphorylated H3 (\( n=179 \) and 145, respectively).

To ask if progression through meiosis was altered in \textit{dtopors}, we assessed the frequency of MI stages in \textit{dtopors}^{05115} versus \textit{dtopors}^{05115/+} siblings (\( n=435 \) and \( n=467 \), respectively, Figure 2C), based on DAPI staining and phase contrast imaging of spindle morphology. A greater proportion of cells were observed at prometaphase (M1a, M1b and M2) in \textit{dtopors}^{05115} males, suggesting a delay is associated with the defect in condensation. In addition, a greater proportion of cells were observed in anaphase in the mutant, consistent with a delay in anaphase progression owing to bridge formation. These observations raise the possibility that the centriole separation defect may be a consequence of continued progression of the centriole cycle concomitant with a block or delay in the chromosome cycle. We do not know, however, if centriole defects are limited to cells that are delayed in meiosis.
Determination of whether dTopors directly or indirectly affects the centriole cycle will require real-time observations of chromosome and centriole behavior at meiosis.

In summary, our observations suggest that there are two main defects leading to chromosome missegregation: an asynchrony between the centrosome and chromosome cycles, leading to tetrapolar MI spindles, and inappropriate or unresolved connections between homologs at anaphase I.

**Epistasis tests between dtopors and mod(mdg4):** In somatic cells, dTopors has been demonstrated to physically interact with components of *gypsy* chromatin insulators, including the chromatin protein Modifier of mdg4 (Mod(mdg4)), (CAPELSON and CORCES 2005). Sumoylation of one isoform, Mod(mdg4)2.2, has been shown to be negatively regulated by dTopors, which correlates with enhanced insulator function and formation of insulator bodies (CAPELSON and CORCES 2006). This suggested a model in which interactions between insulators could be modulated by the activity of dTopors, leading to the formation of insulator bodies.

The *mod(mdg4)* locus is complex, producing 31 protein isoforms, some of which are produced via trans-splicing (DORN et al. 1993). One of these isoforms, named Mod(mdg4) in Meiosis (MNM), is required for male meiotic chromosome segregation. Mutations in *mnm* precociously disrupt conjunction between paired homologs at MI in males, leading to homolog nondisjunction. MNM has been proposed to form a pairing complex with two other proteins, Stromalin in Meiosis (THOMAS et al. 2005) and Teflon (ARYA et al. 2006), to mediate bivalent stability. All Mod(mdg4) isoforms share a BTB protein interaction domain at their carboxyl termini, and may form homodimers through self-interactions of this domain (DORN et al. 1993). By analogy to its role at insulators, we wondered if dTopors might also regulate germline Mod(mdg4). Specifically, the lack of dTopors might alter the MNM-dependent pairing complex formation and/or stability. The anaphase I bridges and nondisjunction in *dtopors* mutants might be a consequence of prolonged associations between homologs resulting from
misregulation of MNM-dependent conjunction. To test this, we examined meiosis in males singly or
doubly mutant for null alleles of dtopors (dtopors\textsuperscript{f05115}) and mnm (mnm\textsuperscript{Z5578}).

At anaphase/telophase I, chromosome segregation appeared normal in dtopors\textsuperscript{f05115/+} ;
mnm\textsuperscript{Z5578} males (n=30). In dtopors\textsuperscript{f05115/+} ; mnm\textsuperscript{Z5578}, individual lagging chromosomes were observed, as
was reported for mnm mutants (THOMAS et al. 2005), but no anaphase I bridges were seen (n = 34). In
both dtopors\textsuperscript{f05115} ; mnm\textsuperscript{Z5578} and dtopors\textsuperscript{f05115} ; mnm\textsuperscript{Z5578} males, nearly all anaphase/telophase
figures had bridges (52/56 and 19/21, respectively). This demonstrates that the connections between
chromosomes that lead to anaphase bridge formation in dtopors mutants are not dependent on MNM.

Homozygous dtopors males are nearly sterile, and we used this near-sterility as a second assay
for interactions between mnm and dtopors. Although flies homozygous for mnm exhibit nearly random
segregation of autosomes, they are significantly more fertile than dtopors flies. We predicted that if the
dtopors-induced anaphase bridges were dependent on MNM that we might see an increased fertility of
dtopors; mnm doubly mutant flies relative to dtopors; + flies. No increase in fertility was observed,
nor did frequencies of nondisjunction of sex or fourth chromosomes indicate an interaction (Table 4).
Consistent with our cytological observations, these results suggest that the dtopors anaphase bridges are
not dependent on MNM.

We also compared chromosome morphology at late prophase in singly and doubly mutant males
(Figure 3). Both chromosome condensation and homolog associations appeared normal at S6 in
dtopors\textsuperscript{f05115/+} ; mnm\textsuperscript{Z5578} males. In dtopors\textsuperscript{f05115/+} ; mnm\textsuperscript{Z5578} males, the previously described mnm
phenotype of precocious separation of homologs was observed (THOMAS et al. 2005) and chromosomes
were noticeably more diffuse. A mild condensation defect was also apparent in dtopors\textsuperscript{f05115} ;
mnm\textsuperscript{Z5578} cells. In doubly mutant males, the condensation defect was more severe than either single
mutant, suggesting that the effects of the two mutants were additive, and therefore that the genes act in
distinct pathways. To quantify these differences, we compared the distribution of chromatin within nuclei of 20 S6 stage spermatocytes of each genotype, matched as closely as possible based on centriole morphology and position as revealed by anti-γ tubulin staining. Confocal images of DAPI-stained nuclei were analyzed to determine the percentage of pixels below an arbitrary low intensity level (i.e. black pixels), as an estimate of nuclear space between chromosomes, which was expected to reflect the degree of condensation. Values obtained were consistent with our qualitative impressions (mean percentage +/- standard deviations: dtopors/+; mnm/+ 16.7 +/- 1.5, dtopors; mnm/+ 13.6 +/- 3.0, dtopors/+; mnm 7.7 +/- 3.9 and dtopors; mnm 1.7 +/- 0.8, p < 0.05 for each pairwise comparison).

**Germline nuclear lamina assembly or stability is disrupted in dtopors mutants:** As dTopors physically interacts with lamin Dm0 (CAPELSON and CORCES 2005), we wondered if the nuclear blebbing phenotype might reflect a defect in the nuclear lamina. To test this, we used EM to examine serially sectioned whole-mounted testes from dtopors and wildtype males (Figure 4). We selected S5-S6 stage cells based on nucleolar morphology, which breaks down at S6, position of the centrioles, which complete their migration from a position adjacent to the cell membrane to a perinuclear location by S5, and the presence of condensed chromatin. In 9 wildtype cells examined (5 S5 and 4 S6), spermatocyte nuclei were not perfectly round as in flattened preparations, but had a generally round shape, with only minor involutions and protrusions of the nuclear membrane. The perinuclear space between the inner and outer nuclear membranes was very regular around the entire nucleus in all cells. In contrast, in 11 S5 and 13 S6 Df(dtopors^{AA}) homozygous spermatocytes examined, nuclear membranes were highly involuted in each cell. In cross-section, these involutions often appeared as finger-like projections (Figure 4). These protrusions may represent “weak” spots in the nuclear structure that result in the blebs observed in flattened live preparations. In addition, in each of these cells the perinuclear space was irregular. The distance between the inner and outer nuclear membrane varied as much as ten-fold at
different positions (Figure 4 inset). These results suggest that interaction between dTopors and Lamin Dm0, or lack thereof, has structural consequences on nuclear lamina assembly in the germ line.

In more than 100 dtopors testes examined, we only observed blebs in late stage spermatocytes (S4-6), and never in gonial cells. The frequency of blebs was highly variable and dependent of the degree of squashing and on genetic background. Among S4-6 cells from dtopors\textsuperscript{Z1837}, dtopors\textsuperscript{Z4522}, dtopor\textsuperscript{f05115} and Df(dtopors\textsuperscript{AA}) males, we observed 165/413, 243/760, 140/480 and 153/596 cells with blebs, respectively. This suggested that defects were limited to meiotic prophase. Alternatively, as prophase spermatocytes have a greater nuclear volume than gonial cells, and are much more abundant, our observations may have reflected either a greater susceptibility of spermatocytes to blebbing, or merely an ascertainment error. In a prior examination of dtopors\textsuperscript{Z1837} and dtopors\textsuperscript{Z4522} spermatocytes, Wakimoto et al. (2004) reported the presence of micronuclei (i.e. blebs) in gonial cells as well. To examine this more closely, we looked at testes of males doubly mutant for dtopors and benign gonial cell neoplasm (bgcn). In bgcn males, gonial cells overproliferate but do not differentiate into spermatocytes (GONCZY et al. 1997). Nuclear blebs were observed in testes of these males, indicating that the defect is not specific to the spermatocyte stage (data not shown). We also examined somatic tissues in dtopors males, including testis sheath cells, larval neuroblasts and the giant nuclei of the larval salivary gland cells, but failed to detect a similar nuclear defect. This suggests that dtopors may have a unique role in germline nuclear structure.

As dTopors localizes to the nuclear lamina in Drosophila both diploid cells and polytene salivary gland cells (CAPELSON and CORCES 2005), we asked if we could also detect it at the nuclear lamina in spermatocytes. Using confocal microscopy and indirect immunofluorescence with anti-dTopors antibodies (kindly provided by S. Parkhurst (SECOMBE and PARKHURST 2004)), we found that dTopors is indeed associated with the nuclear lamina in primary spermatocytes (Figure 5). Although the signal at
nuclear periphery was weak, this localization was detectable as early as S1, but most easily seen in late stage (S6) spermatocytes. In addition to this lamina staining, we observed a stronger dTopors signal localized to punctate nuclear spots. These first appear at S3, become largely chromosome-associated by S6, and disappear at prometaphase I. Using confocal microscopy, we counted the spots in 0.5 µm optical sections through entire nuclei for 50 S6 primary spermatocytes, and found that they ranged in number from 6 to 25, with a mean of 15.6 +/- 3.3 spots per cell. To verify that these staining patterns were dTopors-specific, we identically stained mutant dtopors testes (bottom panel, Figure 5). Neither the lamina localization nor the nuclear spots were detected.

To determine if dtopors mutations disrupted the nuclear lamina by altering assembly of lamina components, we compared localizations of the two Drosophila lamins, Lamin Dm0 and Lamin C, in wildtype and dtopors spermatocytes. Both localized uniformly to the nuclear periphery in wildtype cells. In dtopors cells, both Lamins localized to the nuclear lamina as well, even in regions included in the blebs. In addition, however, prominent Lamin Dm0 and Lamin C staining was observed in the internal regions of the nucleus. The Lamin C staining appeared in large intranuclear foci, whereas the Lamin Dm0 staining showed a finer-grained distribution. A similar distribution of Lamins was observed for each allele (Figure 6). These results suggest that dtopors is required for the normal assembly of both Lamin Dm0 and Lamin C in the male germline.

In somatic tissue, dTopors has E3 ubiquitin ligase activity (SECOMBE and PARKHURST 2004) and also influences the sumoylation of protein components of gypsy insulator complexes (CAPELSON and CORCES 2005). This raised the possibility that the accumulation of Lamins in the nuclear interior of spermatocytes might result from either an increased abundance resulting from decreased ubiquitin-mediated degradation, or improper targeting due to changes in sumoylation. To examine this, we performed Western blotting using anti-Lamin antibodies on wildtype and dtopors testis proteins that
were isolated in the presence of SUMO isopeptidase inhibitors and proteasome inhibitors. Blots were probed with antibodies to β-tubulin to control for loading. Additional control blots were probed with anti-SUMO and anti-ubiquitin antibodies to verify the activity of the inhibitors (data not shown). Blots were performed in triplicate and average signal intensities compared. A representative blot is shown in Figure 6B. No changes in the abundance or migration of Lamin Dm0 or Lamin C relative to β-tubulin were detected in dtopors versus wildtype extracts. This suggests that dTopors may play a structural role in Lamin assembly or stability at the spermatocyte nuclear lamina, rather than regulating the turnover or abundance of lamins.

**New dtopors alleles affect gypsy insulators function:** The two EMS-induced mutations in dtopors alter amino acids in the same general region of the protein, and we wondered if these changes might affect meiosis-specific functions, or if they might also affect gypsy chromatin insulators. In genetic assays using gypsy-induced mutations, a heterozygous deletion of dtopors acts dominantly to disrupt the ability of gypsy insulators to block interactions between regulatory elements and gene promoters. Reduction of dTopors by in vivo RNAi expression has a similar effect (Capeelson and Corces 2005). We generated males heterozygous or homozygous for each of the EMS dtopors alleles and the piggyBac insertion allele, and assayed the phenotype of a gypsy-induced mutation in the cut gene (ct<sup>6</sup>). This mutation is caused by the insertion of gypsy between a wing margin enhancer and the promoter of the cut gene (Gause et al. 2001). When a functional gypsy insulator is established, it prevents proper expression of ct, resulting in a wing with a notched edge.

We found a similar dominant effect in tests of each dtopors allele, resulting in a disruption of insulator function in dtopors/+ flies (Figure 7). Surprisingly, however, this effect was not observed in flies homozygous for any of the dtopors alleles. Rather, insulator function appeared normal in dtopors homozygotes, and wing phenotypes were indistinguishable from those of dtopors+ flies. As controls
for insulator-independent effects of \textit{dtopors} on \textit{ct} expression, we examined both strong and weak hypomorphic alleles of \textit{ct}, \textit{ct}^\textit{n} and \textit{ct}^\textit{K}, respectively. We observed no effect of \textit{dtopors}^{Z1837/+} or \textit{dtopors}^{Z4522/+} on \textit{ct}^\textit{n}, and only a very slight suppression of the posterior notching for the \textit{ct}^\textit{K} allele. We conclude that \textit{dtopors} regulates the activity of \textit{gypsy} insulators in a dose-dependent manner, but this regulation is not essential for insulator function. Additionally, the amino acid substitutions in the EMS alleles affect dTopors function both in meiosis and at insulators, suggesting that the mutated amino acids may be part of a domain required for both germline and somatic activities.

DISCUSSION

Our results indicate that dTopors is required in the Drosophila male germ line for proper nuclear lamina and envelope formation, centriole behavior and chromosome segregation at meiosis I. We found no essential function of dTopors in mitotic cells nor in female meiosis, as homozygous \textit{dtopors} null flies are viable and females are fertile and do not exhibit increased errors in meiotic chromosome segregation. This contrasts with findings in mouse in which the absence of Topors leads to perinatal lethality and increased aneuploidy in Mefs (MARSHALL \textit{et al.} 2010), and in zebrafish, in which translational knockdown of Topors using morpholinos indicates an essential role in development (CHAKAROVA \textit{et al.} 2011). It is unknown if these different requirements for Topors in flies and vertebrates reflects functional redundancy for \textit{dtopors} in flies, additional targets of Topors modification in vertebrates, or both.

\textbf{dTopors and the nuclear lamina}: The \textit{dtopors} phenotype may be a consequence of improper ubiquitination or sumoylation of multiple targets, and it is unclear if the pleiotropic meiotic defects result from alterations of multiple pathways. In light of the male specificity, however, we favor the hypothesis that the primary defect may be disruption of interactions between meiotic chromatin and the
nuclear lamina. Drosophila males have evolved an unconventional system of meiotic homolog conjunction, which may depend on chromatin-lamina interactions. As chromosomes condense during prophase I, bivalents are spatially separated from one another and are closely apposed to the nuclear lamina, and retain this association until prometaphase (Cenci et al. 1994). During prophase I, associations between homologous loci change, as monitored by LacI-GFP fusion protein-labeling of integrated lacO arrays. Homologous lacO loci are tightly paired and appear as a single spot early in prophase, but by mid-prophase they separate into distinct spots (Vazquez et al. 2002). These observations indicate that interactions between homologs are refined or redistributed while chromosomes occupy lamina-associated domains. In dtopors males, an altered lamina may disrupt prophase progression and result in the establishment or persistence of inappropriate interactions between homologs, interfering with their subsequent separation at anaphase. Thus, the observed condensation defect in prophase, anaphase I bridge formation and meiosis I-specific nondisjunction could all be secondary consequences of an abnormal interaction between chromatin and the nuclear lamina.

A number of observations support this notion. First, dTopors is localized to the nuclear lamina in spermatocytes. Although lamina is still formed in dtopors spermatocytes, our observations of abnormal nucleoplasmic foci of both Lamin Dm0 and Lamin C indicate that dTopors regulates or facilitates some aspect of lamin assembly. This is unlikely to occur via regulation of Lamin turnover, as we found no differences in abundance of lamins in testis. dTopors also physically interacts with Lamin Dm0 in somatic cells, and this interaction is functionally significant, as dtopors mutation or knockdown disrupts gypsy chromatin insulators (Capecion and Corces 2005).

Second, there is precedence for chromosome condensation and segregation defects resulting from disruption of chromatin-lamina interactions in other organisms. Immunodepletion of lamins in mammalian cell lines alters chromosome condensation in dividing cells (Burke and Gerace 1986;
Ulitzur and Gruenbaum 1989; Dabauvalle et al. 1991; Ulitzur et al. 1992) and RNAi knockdown of lamins in C. elegans results in improper mitotic chromosome movement (Liu et al. 2000). Expression of unprocessed or truncated lamin A disrupts both lamina structure and causes aneuploidy in MEFs (Liu et al. 2005). Expression of mutant forms of human Lamin B Receptor, a protein that physically connects chromatin to lamins via Heterochromatin Protein 1 (HP1), causes a phenotype similar to that seen in dtopors spermatocytes in that it results in expansion of the perinuclear space and an accumulation of excess endoplasmic reticulum (Zwerger et al. 2010). In Topors -/- MEFs, HP1α shows a diffuse nuclear distribution rather than its normal pericentric localization (Marshall et al. 2010), raising the possibility that Topors may affect chromatin-lamina links indirectly through alterations of HP1α distribution. We failed to observe any similar alterations in HP1 distribution in dtopors spermatocytes by IIF, however we cannot rule out the possibility that dtopors may cause subtle changes in heterochromatin that impact chromatin-lamina interactions.

Third, indirect evidence also suggests that most or all aspects of the dtopors meiotic phenotype may result from a single primary defect. The dtopors phenotype is strikingly similar to that described for a no longer extant mutation, ms(1)244 (Lifschytz and Meyer 1977), which caused a similar spermatocyte nuclear dysmorphology, multipolar MI spindles and chromosome segregation defects. These similarities suggest either that ms(1)244 and dtopors affect a similar spectrum of downstream targets, or more parsimoniously, that they affect the same single target or pathway. We failed to identify any X-linked genes with extensive homology to dtopors in homology searches, making it unlikely that ms(1)244 encodes a protein with similar biochemical properties.

An alternative, but not mutually exclusive possibility is that some aspects of the meiotic dtopors defects result from perturbation of the nuclear envelope and a resulting disruption of protein complexes that span the inner and outer nuclear membranes. These LINC complex proteins, including SUN and
KASH domain proteins, are classes of integral membrane proteins that interact in the perinuclear space, and have been proposed to form a communication bridge between the cytoskeleton and nucleoplasm, that is important for coordinating cell cycle events (Padmakumar et al. 2005; Crisp et al. 2006; McGee et al. 2006). In C. elegans, meiotic pairing is facilitated by interactions between chromosome-specific zinc finger proteins and the SUN and KASH domain proteins SUN-1 and ZYG-12, and disruption of these protein complexes has been demonstrated to affect meiotic chromosome behavior (Sato et al. 2009).

The precocious separation of centrioles we observed at meiosis I in dtopors spermatocytes may also be related to disruption of LINC complexes. In Drosophila, human fibroblasts and C. elegans, altered interactions between LINC complex proteins in the nuclear membrane have consequences on both centrosome attachment (Patterson et al. 2004; Salpingidou et al. 2007; Starr 2009) and chromosome movement (Starr 2009). Alternatively, dTopors may have a more specific function at meiotic centrosomes. It has recently been shown that Topors localizes to basal bodies in ciliated mammalian retinal cells, and to centrioles in retinal cell lines (Chakarova et al. 2011). Although we failed to detect dTopors at centrosomes, we cannot rule out the possibility that the amount of centrosome-associated protein is below our limits of detection, or that epitopes recognized by our antibodies are masked at centrosomes.

**Intranuclear dTopors foci:** In addition to a lamina localization, we also observed dTopors localization to intranuclear foci. The functional significance of this is unknown. Although in mitotic and salivary gland cells, dTopors localizes to Mod(mdg4)-containing insulator bodies, the meiotic dTopors foci are unlikely to be insulator bodies. When an antibody to the common BTB domain shared by all Mod(mdg4) isoforms is used to stain late prophase spermatocytes, the only staining observed is associated with the nucleolus, the site of the XY pairing sites (Soltani-Bejnood et al. 2007).
Antibodies to the insulator protein CP190 also fail to recognize intranuclear foci in late prophase spermatocytes (data not shown). As mammalian Topors is localized to Promyelocytic Leukemia (PML) bodies (Rasheed et al. 2002; Weger et al. 2003), and the dTopors foci we observed are similar in number to PML bodies in mammalian cells, they could perhaps represent a functional analog of these structures. The Drosophila SUMO homolog, Smt3, forms similar intranuclear foci when expressed in either fly or human cells, suggesting that structures analogous to PML bodies may form in Drosophila (Lehembre et al. 2000). Mammalian PML bodies are dynamic, chromatin-associated structures, and our observations of a redistribution of dTopors foci to condensed chromosomes at late prophase is consistent the process of condensation of chromatin-associated foci.

**Regulation of homolog separation:** Both our genetic and cytological observations suggest that dTopors function is important for homolog separation. We failed to observe any gross alterations in homolog pairing, as homolog domains were clearly established in late prophase nuclei. The chromatin bridges that we observed in nearly all dtopors anaphase and telophase I cells therefore likely represent unresolved attachments between homologs. We suggest that in male flies, interactions between the chromosomes and the nuclear lamina may be critical for prophase I chromatin condensation. Our data, however, do not eliminate the possibility that such interactions are also important for homolog pairing as in other organisms, as pairing in males may be precede the requirement for dtopors.

To further investigate the role of dTopors in homolog separation, we performed tests of epistasis with a null mutation in the mod(mdg4) isoform mnm. Homolog conjunction in male flies depends on three proteins, MNM, Stromalin-in-meiosis SNM (Thomas et al. 2005) and Tef (ArYa et al. 2006), that have been proposed to interact in a conjunction complex (Thomas et al. 2005). There were two suggestions that the dtopors-induced anaphase bridges might be dependent on the activity of this conjunction complex. First, dTopors physically interacts with another Mod(mdg4) isoform, 2.2, at
gypsy chromatin insulators (CAPELSON and CORCES 2005). MNM and Mod(mdg4)2.2 share a carboxyl terminal BTB protein interaction domain (DORN et al. 1993), and thus it seemed possible that a similar interaction might also occur between dTopors and MNM in meiosis. Second, mutations in the condensin II subunit Cap-H2 result in similar anaphase bridges and condensation defects in male meiosis. The frequency of these anaphase bridges is significantly reduced in tef Cap-H2 double mutants, suggesting that their formation is dependent on the conjunction complex (HARTL et al. 2008). Condensin complex subunits in yeast have been identified as sumoylation substrates in proteomic screens (WOHLSCHLEGEL et al. 2004; DENISON et al. 2005), and although it is not known if condensins are targets of dtopors-directed modification, the similarities between the dtopors and Cap-H2 phenotypes suggested a possible relationship.

Our results did not, however, support a direct regulation of the MNM-dependent homolog conjunction complex by dTopors, nor a requirement for the conjunction complex for bridge formation. Removal of MNM did not abolish anaphase I bridge formation nor restore fertility of dtopors males. This suggests that there are homolog connections, in addition to those mediated by the conjunction complex, that are either prevented or resolved by dTopors activity. In wildtype flies, such interactions either do not occur, or they are resolved by dTopors prior to the activity of the conjunction complex, as their presence would not allow for the precocious separation of homologs observed in mnm, snm and tef mutants.

With regard to these persistent homolog interactions, it may be relevant that in mouse, lack of Topors results in alteration of the pericentric heterochromatin (MARSHALL et al. 2010). In fly male meiosis, it has been proposed that homolog interactions may be relegated to the pericentric heterochromatin during late prophase, a stage when both homologous euchromatic loci and centromeres
are separated (Vazquez et al. 2002). Thus, persistent homolog attachments in dtopors may reflect an inability to resolve pericentric connections as a result of an abnormal chromatin structure.

**dTopors role at Gypsy insulators:** While we found that dTopors activities are essential for meiosis, we were surprised to find that its role at gypsy insulators, as assessed by expression of the ct^6 mutant phenotype, was dispensable. Gypsy insulator function is compromised in flies heterozygous for a deletion of the dtopors gene, and knockdown of dTopors by *in vivo* expression of RNAi results in a similar effect (Capeelson and Corces 2005). These data led to a model in which dTopors facilitates coalescence of insulator complexes into functional insulator bodies by anchoring chromatin loops to the nuclear lamina. While our data do not negate a role of dTopors at insulators, they indicate that its role is not required for insulator function.

Rather, our results suggest that when present, dTopors regulates insulator function in a dose-dependent manner, but when absent, this regulation is unnecessary. This paradoxical result suggests that dTopor’s role at insulators evolved as part of a regulatory mechanism after functional insulators already existed. It might be explained if two activities of dTopors, (e.g. the localization of gypsy insulator complexes to the nuclear lamina and the negative regulation of sumoylation of insulator components Mod(mdg4)2.2 and CP190 (Capeelson and Corces 2006)), must be in balance to properly regulate insulator function. For example, dTopors biochemical activities may be disruptive of insulator function unless the complexes are lamina-associated. The physical lamina association might be more sensitive to small changes in dTopors stochiometry, whereas the enzymatic activities may be less dose-sensitive. Thus, a 50% reduction in dTopors may result in a 50% reduction in lamina association of insulators, while having no significant effect on SUMO or Ubiquitin ligase activities. A complete deletion of dTopors, however, would equally ablate both activities. This model may be tested using separation-of-
function mutations in dTopors, or by expressing independent domains and assessing their effect on gypsy insulator function.

Interestingly, while both meiotic and insulator functions of dTopors appear to be related to its association with the nuclear lamina, a lamina localization has not been observed for mammalian Topors homologs (HALUSKA et al. 1999; ZHOU et al. 1999; RASHEED et al. 2002; WEGER et al. 2003). Given the domain conservation between the fly and human proteins, however, it seems likely that their activities are mechanistically similar. Further studies identifying the downstream targets of Topors and dTopors ubiquitination and sumoylation, and functional tests of the lamina association will be necessary to shed light on the significance of this difference.

ACKNOWLEDGEMENTS

We wish to thank Susan Parkhurst, Juergen Knoblich, Michelle Moritz, Bill Theurkauf and Jordan Raff for generously providing antibodies, and Susan Parkhurst and Charles Zuker for providing fly lines. Antibodies were also obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242. This work was supported by NIH GM079780-01A1 grant to J.T. and UNCG undergraduate research assistantships to M.M., K.S., and M.H.
LITERATURE CITED


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H. sapiens
Z1837
G721A
Z4522
G160A

M. musculus
SAEFRG
G221S
NPA
E345K

D. melanogaster
SAFRYNPAQ

Consensus
SA F+R NPA +HRL+PW R++ L VN V ++ + L F L P+L RT HFIHE +FARSP+++
Figure 2

A  

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C  

![Histogram of stage of meiosis](image17.png)

% of total cells in meiosis I

Stage of meiosis
Figure 3
Figure 4
Figure 5

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Figure 6

A

Lamin C  DNA  Merge

+  

Df(dtopors^{AA})

+  

Df(dtopors^{AA})

B

dtopors allele

+  Z4522  Z1837  f01151  Df(dtopors^{AA})

Lamin C

Lamin Dm0

βTubulin
Figure 7

### dtopors alleles

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Figure Legends:

FIGURE 1: Structure of dTopors. Positions of EMS-induced mutations and a piggyBac insertion in dtopors are shown on a diagram of the dTopors protein (modified from (SECOMBE and PARKHURST 2004)). Numbers below each mutation indicate positions of the substituted basepair in the gene and the substituted amino acid in the protein. Conceptual translation of the piggyBac insertion allele predicts a protein truncated after K47. PEST sequences involved in protein degradation are indicated by solid bars, a RING domain essential for ubiquitin and/or SUMO ligase function is indicated by gray shading, an Arginine/Serine-rich region (RS) is indicated by diagonal hatch marking, and a region of four consensus bipartite nuclear localization domains by underlining. A region of high conservation between mammalian and fly homologs is indicated by horizontal striping, corresponding to amino acids 281-364 in dTopors. The sequence of this region shown below with the position of the dtoporsZ4522 mutation indicated.

FIGURE 2: Abnormal meiosis I in dtopors. Wildtype and dtopors05115 meiocytes at the indicated meiotic stages stained with DAPI (red) and (A) anti-γ tubulin antibodies (green) or (B) anti-β tubulin antibodies. In (A) the stage of wild type cells is indicated above, and the stage of dtopors cells in shown below each panel. Arrows point to chromatin bridges. Bar = 10 µm. C) Percentage of meiosis I cells in dtopors05115 vs. dtopors05115/+ classified by stage (according to Cenci et al. (1994)).

FIGURE 3: Chromosome morphology in DAPI-stained dtopors and mnm single and double mutants. The dtopors genotypes are indicated to the left of each row; mnm genotypes are indicated above each column. The top four panels depict S6 spermatocytes, staged by anti-gamma tubulin staining showing
paired centrioles adjacent to the nucleus (arrows). The lower panels depict meiocytes at late anaphase I/telophase I. Bar = 10 µm.

FIGURE 4: Spermatocyte Ultrastructure. Electron micrographs of sections of S6 spermatocytes from wildtype (A) and dtopors (B) males. Note the accumulation of excess membrane surrounding the nucleus in the mutant. Enlargements of the nuclear membrane of wildtype (C) and dtopors (D and E), showing an irregular perinuclear space (arrows) in the mutant. Bars = 1 µm.

FIGURE 5: Localization of dTopors in primary spermatocytes. Wildtype primary spermatocytes stained with DAPI (red) and anti-dTopors antibodies (green). Stages of development according to Cenci et al. (1996) are indicated at left. Arrows point to staining at the nuclear periphery. The bottom row shows homozygous dtopors$^{AA}$ mutant S4-5 stage primary spermatocytes stained with DAPI (red) and anti-dTopors antibodies (green). Bar = 10 µm.

FIGURE 6: Lamin localizations in primary spermatocytes. (A) Wildtype and Df(dtopors$^{AA}$) S5 stage primary spermatocytes stained with DAPI (red) and anti-lamin C or anti-lamin Dm0 antibodies (green). Bar = 10 µm. (B) Representative Western blot of whole testis extracts from flies of the indicated dtopors genotypes, probed with anti-Lamin C, anti-Lamin Dm0, and anti-β tubulin antibodies.

FIGURE 7: Modification of ct$^6$ phenotypes by dtopors mutations. Wing phenotypes of flies either heterozygous or homozygous for the indicated dtopors alleles and either the gypsy-induced ct$^6$ mutation or control ct$^n$ or ct$^K$ hypomorphic point mutations.
TABLE I

Sex and fourth chromosome disjunctional data from crosses of y/y+; *dtopors; spa* males to y w sn; C(4)RM ci ey /0 females

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<th>dtopors&lt;sup&gt;Z4522&lt;/sup&gt;/Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</th>
<th>dtopors&lt;sup&gt;f05115&lt;/sup&gt;/Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</th>
<th>dtopors&lt;sup&gt;Z1837&lt;/sup&gt;/dtopors&lt;sup&gt;Z4522&lt;/sup&gt;</th>
<th>dtopors&lt;sup&gt;Z1837&lt;/sup&gt;/dtopors&lt;sup&gt;f05115&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>dtopors&lt;sup&gt;Z1837&lt;/sup&gt;/SM1,Cy</td>
<td>185</td>
<td>299</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dtopors&lt;sup&gt;Z4522&lt;/sup&gt;</td>
<td>216</td>
<td>273</td>
<td>229</td>
<td>52</td>
<td>52</td>
<td>53</td>
<td>63</td>
<td>63</td>
<td>9</td>
<td>5</td>
<td>27</td>
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<tr>
<td>dtopors&lt;sup&gt;Z4522&lt;/sup&gt;/SM1,Cy</td>
<td>503</td>
<td>979</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dtopors&lt;sup&gt;f05115&lt;/sup&gt;</td>
<td>33</td>
<td>50</td>
<td>48</td>
<td>31</td>
<td>10</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>24</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</td>
<td>16</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>10</td>
<td>15</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)/SM1,Cy</td>
<td>206</td>
<td>342</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dtopors&lt;sup&gt;Z1837&lt;/sup&gt;/Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</td>
<td>48</td>
<td>61</td>
<td>75</td>
<td>32</td>
<td>22</td>
<td>9</td>
<td>27</td>
<td>16</td>
<td>40</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>dtopors&lt;sup&gt;Z4522&lt;/sup&gt;/Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</td>
<td>48</td>
<td>67</td>
<td>32</td>
<td>25</td>
<td>8</td>
<td>5</td>
<td>13</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>dtopors&lt;sup&gt;f05115&lt;/sup&gt;/Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</td>
<td>28</td>
<td>35</td>
<td>53</td>
<td>20</td>
<td>11</td>
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<td>8</td>
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<td>27</td>
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<td>270</td>
<td>324</td>
<td>153</td>
<td>60</td>
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<td>46</td>
<td>46</td>
<td>65</td>
<td>46</td>
<td>21</td>
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<tr>
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<td>26</td>
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<td>15</td>
<td>16</td>
<td>15</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>$\text{dtopors}^{\text{Z4522}}$/$\text{dtopors}^{\text{f05115}}$</td>
<td>61</td>
<td>77</td>
<td>67</td>
<td>30</td>
<td>13</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>11</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
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<td>---</td>
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</tr>
<tr>
<td>% Nondisjunction</td>
<td>nullo XY</td>
<td>diplo XY</td>
<td>nullo 4</td>
<td>diplo 4</td>
<td>XY</td>
<td>4</td>
<td>XY + 4*</td>
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<td></td>
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<tr>
<td>$\text{dtopors}^{\text{Z1837}}$/SM1, Cy</td>
<td>1.9</td>
<td>1.2</td>
<td>2.3</td>
<td>1.4</td>
<td>3.1</td>
<td>3.6</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{dtopors}^{\text{Z4522}}$</td>
<td>22.9</td>
<td>9.4</td>
<td>13.3</td>
<td>14.3</td>
<td>32.3</td>
<td>27.6</td>
<td>5.8 (8.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{dtopors}^{\text{f05115}}$</td>
<td>31.4</td>
<td>21.2</td>
<td>17.1</td>
<td>16.7</td>
<td>52.6</td>
<td>33.8</td>
<td>20.4 (15.9)</td>
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</tr>
<tr>
<td>$\text{Df(}d\text{topors}^{\text{AA}})$</td>
<td>33.3</td>
<td>15.2</td>
<td>26.7</td>
<td>21.0</td>
<td>48.5</td>
<td>47.7</td>
<td>25.7 (23.1)</td>
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<td></td>
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</tr>
<tr>
<td>$\text{dtopors}^{\text{Z1837}}$/Df($\text{dtopors}^{\text{AA}})$</td>
<td>35.8</td>
<td>14.8</td>
<td>19.9</td>
<td>21.8</td>
<td>50.6</td>
<td>41.7</td>
<td>21.8 (21.1)</td>
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</tr>
<tr>
<td>$\text{dtopors}^{\text{Z4522}}$/Df($\text{dtopors}^{\text{AA}})$</td>
<td>20.2</td>
<td>16.9</td>
<td>11.1</td>
<td>18.1</td>
<td>37.1</td>
<td>39.2</td>
<td>13.6 (10.8)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$\text{dtopors}^{\text{f05115}}$/Df($\text{dtopors}^{\text{AA}})$</td>
<td>40.8</td>
<td>15.6</td>
<td>22.3</td>
<td>13.3</td>
<td>56.4</td>
<td>35.6</td>
<td>21.8 (20.0)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\text{dtopors}^{\text{Z1837}}$/$\text{dtopors}^{\text{Z4522}}$</td>
<td>33.0</td>
<td>16.0</td>
<td>12.9</td>
<td>13.3</td>
<td>49.0</td>
<td>26.2</td>
<td>12.8 (12.8)</td>
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<td>$\text{dtopors}^{\text{Z1837}}$/$\text{dtopors}^{\text{f05115}}$</td>
<td>30.8</td>
<td>22.3</td>
<td>19.4</td>
<td>25.1</td>
<td>53.1</td>
<td>44.5</td>
<td>21.1 (23.6)</td>
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</tr>
<tr>
<td>$\text{dtopors}^{\text{Z4522}}$/$\text{dtopors}^{\text{f05115}}$</td>
<td>28.3</td>
<td>14.0</td>
<td>11.1</td>
<td>12.4</td>
<td>32.3</td>
<td>23.5</td>
<td>10.7 (7.6)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Frequencies of simultaneous sex and fourth chromosome nondisjunction. Observed and (Expected based on independence).
**TABLE 2**

Sex and fourth chromosome disjunctional data from crosses of *yw/y<sup>+</sup>Y; dtopors; spa<sup>pol</sup>/+ males
to  *C(1)RM y v/ Y ; C(4)RM ci ey /0 females*

<table>
<thead>
<tr>
<th>Recovered male gametes:</th>
<th>Y;4</th>
<th>X;4</th>
<th>0;4</th>
<th>X/Y;4</th>
<th>Y;0</th>
<th>Y;4/4</th>
<th>X;0</th>
<th>X;4/4</th>
<th>0;0</th>
<th>X/X;4</th>
<th>XX;0</th>
<th>XX;4/4</th>
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<tbody>
<tr>
<td>Paternal Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*dtopors&lt;sup&gt;c&lt;sup&gt;4522&lt;/sup&gt;&lt;/sup&gt;</td>
<td>66</td>
<td>26</td>
<td>29</td>
<td>7</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*dtopors&lt;sup&gt;c&lt;sup&gt;4522&lt;/sup&gt;/SM1,Cy&lt;/sup&gt;</td>
<td>517</td>
<td>194</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</em></td>
<td>58</td>
<td>31</td>
<td>34</td>
<td>21</td>
<td>17</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td><em>dtopors&lt;sup&gt;AA&lt;/sup&gt;/SM1,Cy</em></td>
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<td>1</td>
<td>0</td>
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</table>

<table>
<thead>
<tr>
<th>% Nondisjunction</th>
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<tbody>
<tr>
<td>Sperm Genotype</td>
</tr>
<tr>
<td>Errant Division</td>
</tr>
<tr>
<td>*dtopors&lt;sup&gt;c&lt;sup&gt;4522&lt;/sup&gt;&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Df(dtopors&lt;sup&gt;AA&lt;/sup&gt;)</em></td>
</tr>
</tbody>
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TABLE 3

Results of crosses of 50 dtopors males to 100 C(2)EN, bw sp or C(3)EN, st cu e females

<table>
<thead>
<tr>
<th>Maternal Genotype</th>
<th>C(2)EN, bw sp</th>
<th>F1 /male</th>
<th>C(3)EN, st cu e</th>
<th>F1 /male</th>
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</thead>
<tbody>
<tr>
<td>Sperm genotype</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>dtopors\textsuperscript{Z4522}</td>
<td>49</td>
<td>230</td>
<td>5.58</td>
<td>54</td>
</tr>
<tr>
<td>dtopors\textsuperscript{Z4522}/SM1,Cy</td>
<td>0</td>
<td>4</td>
<td>0.08</td>
<td>4</td>
</tr>
<tr>
<td>Paternal Genotype</td>
<td>males tested</td>
<td>progeny per male</td>
<td>% XY nd</td>
<td>% 4 nd</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>-----------------</td>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>$dtopors^{f05115}/+ ; +$</td>
<td>59</td>
<td>15.0</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>$dtopors^{f05115}/+ ; mnm^{Z5578}/+$</td>
<td>39</td>
<td>16.7</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>$dtopors^{f05115}/+ ; mnm^{Z5578}$</td>
<td>42</td>
<td>4.8</td>
<td>39.9</td>
<td>49.3</td>
</tr>
<tr>
<td>$dtopors^{f05115} ; +$</td>
<td>135</td>
<td>1.6</td>
<td>46.8</td>
<td>37.0</td>
</tr>
<tr>
<td>$dtopors^{f05115} ; mnm^{Z5578} /+$</td>
<td>63</td>
<td>2.2</td>
<td>40.4</td>
<td>37.5</td>
</tr>
<tr>
<td>$dtopors^{f05115} ; mnm^{Z5578}$</td>
<td>59</td>
<td>0.9</td>
<td>38.9</td>
<td>48.1</td>
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</table>