Mutations in the $\alpha$-Tubulin 67C Gene Specifically Impair Achiasmate Segregation in Drosophila melanogaster

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Abstract. Drosophila melanogaster oocytes heterozygous for mutations in the $\alpha$-tubulin 67C gene ($\alpha$-tub67C) display defects in centromere positioning during prometaphase of meiosis I. The centromeres do not migrate to the poleward edges of the chromatin mass, and the chromatin fails to stretch during spindle lengthening. These results suggest that the poleward forces acting at the kinetochore are compromised in the $\alpha$-tub67C mutants. Genetic studies demonstrate that these mutations also strongly and specifically decrease the fidelity of achiasmate chromosome segregation. Proper centromere orientation, chromatin elongation, and faithful segregation can all be restored by a decrease in the amount of the Nod chromokinesin. These results suggest that the accurate segregation of achiasmate chromosomes requires the proper balancing of forces acting on the chromosomes during prometaphase.

Key words: chromosome • kinesin • meiosis • centromeres • meiotic spindle

The mechanisms by which chiasmate (or exchange) chromosomes achieve a stable bipolar orientation during meiotic prophase have been described by Nicklas (1974, 1997). The kinetochores of the two homologous chromosomes, tethered together by chiasmata, make attachments to opposite poles of the bipolar spindle. The stability of metaphase chromosome positioning is achieved by balancing the poleward forces between two oppositely oriented centromeres connected by chiasmata. The proper segregation of achiasmate chromosomes poses a rather different problem, since homologues are, by definition, not connected by chiasmata.

A chiasmate segregation is a well-characterized component of the meiotic process in Drosophila melanogaster oocytes (Hawley and Theurkauf, 1993; Dernburg et al., 1996; Karp et al., 1996; Matthies et al., 1996), and we recently completed a screen for Drosophila mutants defective in this process (Sekelsky et al., 1999). Mutants were isolated that exhibited high levels of X and/or 4th chromosome nondisjunction in FM 7/X females. FM 7 is a rearranged X chromosome, often referred to as a balancer, which recombines with a normal sequence X homologue with a frequency of $< 1\%$ of normal (Hawley et al., 1993). The use of this balancer chromosome allowed us to study the segregation of both the achiasmate X chromosomes and the obligately achiasmate 4th chromosomes (Hawley et al., 1993).

One of the mutants recovered from this screen, $\alpha$-tub67C$^{P40}$, resulted from the insertion of the P(lacW) transposable element into the coding region of the $\alpha$-tubulin 67C gene ($\alpha$-tub67C)$^1$. This insertion occurred at position 2173, 39 bp upstream of the 3' end, so that the last 13 amino acids of the normal $\alpha$-tub67C gene product are replaced by a novel sequence of five amino acids, followed by a stop codon. $\alpha$-tub67C, one of the most divergent of the $\alpha$-tubulins (Kalfayan and Wensink, 1982; Theurkauf et al., 1986), is transcribed primarily in the nurse cells, and is maternally loaded into the oocyte, eventually comprising $\sim 20\%$ of the $\alpha$-tubulin pool in normal oocytes (Matthews et al., 1993).

Materials and Methods

Drosophila Stocks

Two X chromosomes were used in this study: a normal sequence X chromosome marked with yellow (y) and white (w); and the FM 7 balancer chromosome. The FM 7 chromosome consists of three superimposed inversions: a nearly full-length paracentric inversion that moves most of the basal heterochromatin to the tip of the X and inverts all but the most distal bands of euchromatin; the di-49 inversion; and a third inversion, broken in the distally located heterochromatin (region 20) and in the euchromatin at position 15 D. FM 7 is as an excellent suppressor of exchange when heterozygous with a normal X chromosome.

$^1$ Abbreviation used in this paper: $\alpha$-tub67C, $\alpha$-tubulin 67C gene.
**Genetic Assays for Meiotic Nondisjunction**

We monitor X and 4th chromosome disjunction by crossing X/X; pol/pol females to attached X YO; C (4) RM, ci ey males. The frequencies of nondisjunction were calculated as described in Hawley et al. (1993).

**Confocal Cytology**

Oocytes were prepared and examined as previously described with minor modifications (Theurkauf and Hawley, 1992; Matthies et al., 1996). Egg chambers from 3-7-d-old females were extracted by quick pulses of a blender using a modified Robb's medium. The mixture was passed sequentially through a loose and fine mesh to separate late stage oocytes. The oocytes were fixed for 5 min on a rotator at room temperature in a hypertonic solution, therefore preventing hypotonic activation of the mature oocytes. After removal of the follicle cells, chorion, and vitelline membranes, the oocytes were permeabilized with 1% Triton X-100 in PBS. Oocytes were labeled with Y L1/2 (1:200) and Y OL1/34 (1:200) rat antitubulin mAbs (A. Kurcotic) and both Oligreen (Molecular Probes, Inc.; 1:10,000) and 1 MAB52 (1:500) anticore histone mouse monoclonal. In some experiments, oocytes were also labeled with ME1-5332 (1:500) guinea pig polyclonal antibody (generous gift of Drs. Tracy Tang and Terry Orr-Weaver, Massachusetts Institute of Technology, Cambridge, MA). Primary antibodies were then labeled with secondary antibodies (1:250) purchased from Jackson Immunoresearch Laboratories, conjugated in the following manner: Cy2 to anti-mouse; Cy3 to anti-rat; and Cy5 to anti-guinea pig.

Oocytes were examined using an MRC-1024 BioRad confocal microscope (K. Alman collection), and spindle and chromatin lengths and widths were determined using BioRad 3D software. Spindle and chromatin lengths were determined from maximum intensity projected spindles. Spindle lengths were measured from pole to pole and chromatin length from the sites of the chromatin found closest to either pole. Image stacks were converted to maximum intensity projections and subsequently converted to Photoshop images (Adobe Systems Inc.). Final images were produced on a dye sublimation printer (Tektronics Phaser 440).

**Results**

**The αtub67C (P40) Mutation Has a Dominant Effect on Chromatin Elongation**

Although the spindles of FM 7/X; αtub67C(P40)/+ oocytes are, on average, somewhat shorter than wild-type spindles (average spindle lengths are 12.2 versus 15.1 μm, respectively), confocal studies did not reveal obvious defects in spindle structure, as shown in Fig. 1 A. However, an examination of chromatin in FM 7/X; αtub67C(P40)/+ prometaphase oocytes revealed a failure of the chromatin mass to elongate along the axis of the spindle (see Fig. 1 B; compare top two rows).

Chromatin masses in wild-type spindles appear almost spherical as spindle assembly initiates, but elongate as spindle assembly progresses. However, in αtub67C(P40)/+ oocytes, the chromatin remains almost spherical, even on fully elongated spindles. A plot of the chromatin mass length from oocytes with two wild-type copies of the αtub67C gene reveals a wide distribution of chromatin lengths (Fig. 2 A). In contrast, a plot of the chromatin mass length from oocytes heterozygous for αtub67C(P40) exhibits a very narrow distribution (Fig. 2 B) that is centered at a much shorter length (Fig. 2 B). The chromatin mass ranged in length from 3.1-18 μm in control oocytes, whereas in FM 7/X; αtub67C(P40)/+ oocytes, chromatin length was observed to vary from 2.4-5.8 μm. Thus, in oocytes heterozygous for the αtub67C(P40) mutation, the chromatin mass fails to elongate properly.

To determine whether or not the observed defect in chromatin stretching was a consequence of the slightly shorter spindles found in FM 7/X; αtub67C(P40)/+ oocytes (Fig. 2, compare D and E), we calculated the axial ratio (length over width) of the chromatin and plotted this value against spindle length. This metric of axial ratios allowed us to evaluate the stretching of the chromosomes in a manner independent of spindle length. For FM 7/X; αtub67C(P40)/+ oocytes, the axial ratios range from one to two, whereas in oocytes obtained from FM 7/X; αtub67C(P40)/+ females, they range from one to two (Fig. 2, compare G and H).
Thus, even when differences in spindle length are taken into account, oocytes from FM7/X; αtub67C<sup>P40</sup>/+ females still display an obvious defect in chromatin stretching. Even in those FM7/X; αtub67C<sup>P40</sup>/+ oocytes with the longest spindles, little or no stretching of the chromatin mass is observed and the chromatin remains almost spherical. Taken together, these data reveal a defect in the stretching of chromosomes during prometaphase in FM7/X; αtub67C<sup>P40</sup>/+ oocytes, which is clearly distinct from an effect on spindle lengthening.

**The Defect in Chromatin Stretching in αtub67C<sup>P40</sup>/+ Oocytes Is Correlated with Abnormal Centromere Positioning**

Lengthening of the spindle and chromatin during meiotic prometaphase is paralleled by the coalescence of a *Drosophila* centromere-resident protein, MEI-S332, at the most poleward ends of the chromatin mass (Moore et al., 1998; Tang et al., 1998). We examined the distribution of MEI-S332 protein in prometaphase oocytes bearing the...
![Histone & MEI-S332 localization pattern from oocytes derived from FM 7/X; αtub67C<sup>p40</sup> females](Image)

| Genotype          | MEI-S332 Localization |
|-------------------|-----------------------|
| nod/αtub67C       |                        |
| +/+ ; -/+          |                        |
| +/- ; -/+          |                        |
| -/+ ; -/+          |                        |
| wt                |                        |

**Figure 3.** Failure of centromere positioning (MEI-S332 protein) in heterozygous αtub67C<sup>p40</sup> mutant oocytes. FM 7/X oocytes with the indicated genotypes were immunolabeled with anti-core histone, antitubulin, and anti-MEI-S332 antibodies. The first column displays the projections of MEI-S332 alone (red), whereas the second column displays the projections for both histone (green) and MEI-S332 (red). In the first column, the MEI-S332 immunoreactivity is found adjacent to the main chromosome mass, whereas the remainder of the protein is detected toward the center of the chromosomal mass. In the second panel, virtually all of the MEI-S332 immunoreactivity remains at one pole. The middle two rows depict oocytes heterozygous for αtub67C<sup>p40</sup>. In the oocyte displayed in the top row, a portion of the MEI-S332 immunoreactivity is found adjacent to the main chromosome mass, whereas the remainder of the protein is detected toward the center of the chromosomal mass. In the second panel, virtually all of the MEI-S332 immunoreactivity remains at one pole. The middle two rows are oocytes heterozygous for both αtub67C<sup>p40</sup> and nod (nod<sup>b17</sup>). In each of these oocytes, the MEI-S332 immunoreactivity is seen at opposite poles of the lengthening chromosomal mass, as is observed in wild-type oocytes (bottom row). Bar, 4 μm.

αtub67C<sup>p40</sup> mutation by immunolocalization. In wild-type spindles, the MEI-S332 protein is distributed over the surface of the chromatin in discrete foci during early prometaphase, and coalesces symmetrically at the extreme poleward tips during spindle assembly and elongation (M oore et al., 1998; T ang et al., 1998).

As expected, MEI-S332 protein was found on the poleward edges of the chromatin masses in FM 7/X; +/+ spindles (wild-type; see Fig. 3, bottom row). In contrast, the MEI-S332 localization pattern from oocytes derived from FM 7/X; αtub67C<sup>p40</sup>/ females indicated that the centromeres were positioned abnormally (Fig. 3, top two rows). In no case (0/15) was the MEI-S332 protein properly positioned at the distal tips of the elongating chromatin mass, as is observed in wild-type oocytes (M oore et al., 1998; Fig. 3, bottom row). Rather, MEI-S332 either failed to be completely localized at opposite poles of the main chromosome mass (Fig. 3, top row) or was found entirely on one side of the main chromatin mass (Fig. 3, second row). These cytological studies demonstrate that heterozygosity for the αtub67C<sup>p40</sup> mutation leads to a defect in both chromatin stretching and centromere positioning.

**Chromosome Missegregation Is Restricted to Achiasmate Bivalents**

The genetic studies of the αtub67C<sup>p40</sup> mutation were carried out in two types of females: females that were heterozygous for a normal sequence X chromosome and an FM 7 balancer chromosome, denoted FM 7/X, and females carrying two normal sequence X chromosomes, denoted X/X. In X/X females, the X chromosomes recombine at least once in >90% of these oocytes, while in FM 7/X females, the presence of the multiply inverted balancer chromosome reduces the frequency of X chromosome exchange to <1% of normal (H awley and T heurkauf, 1993). A comparison of the frequency of errors of meiotic segregation in FM 7/X and X/X females allowed us to differentially assess the effects of mutations such as αtub67C<sup>p40</sup> on the chiasmate and achiasmate segregation systems.

A s shown in Fig. 4, FM 7/X; αtub67C<sup>p40</sup>/ females display 20-fold higher levels of X chromosome nondisjunction than do FM 7/X; +/+ control females, suggesting that most, if not all, of these cases of X chromosome nondisjunction are due to a failure of achiasmate segregation. Indeed, even those few nondisjunction events that were observed in X/X; αtub67C<sup>p40</sup>/ females occurred in the 5–8% of the oocytes in which the two X chromosomes failed to undergo exchange (data not shown). An additional mutant allele, derived from αtub67C<sup>p40</sup> and denoted αtub67C<sup>p40</sup>, displayed an enhancement of meiotic chromosome missegregation relative to the original P element allele (Fig. 4). The αtub67C<sup>p40</sup> allele differs from the original P element insertion only in that a substantial internal portion of the P element has been removed. Heterozygosity for αtub67C<sup>p40</sup>, as assayed in FM 7/X females, leads to high levels of achiasmate nondisjunction; however, the αtub67C<sup>p40</sup> mutant has little or no effect on the segregation of chiasmate X chromosomes (in X/X females). For both of these alleles, the observed chromosome missegregation must primarily be due to nondisjunction rather than loss, as the frequency of diplo-X exceptions always equals or slightly exceeds that of nullo-X exceptions (data not shown).

FM 7/X; αtub67C<sup>p40</sup>/ and FM 7/X; αtub67C<sup>p40</sup>/ females also display elevated levels of 4<sup>th</sup> chromosome nondisjunction (again with an approximate equality of diplo-4 and nullo-4 exceptions). In both cases, the effect on 4<sup>th</sup> chromosome missegregation was less than half of the effect on X chromosome segregation. Two lines of evidence...
tion to levels comparable to those observed in females. X chromosome missegregation and not a direct effect of chromosome nondisjunction events are a secondary consequence of failures of Matthies et al. and reflects cases where the two 4th chromosomes segregate simultaneously nondisjunctional for the X chromosomes, chromosome nondisjunction occurs in oocytes that were nondisjunctional for the X chromosomes, and serves, in essence, as a stabilizing plateward force. As shown in Fig. 5 A, heterozygosity for a recessive loss-of-function mutation of nod (nod	extsuperscript{197}) strongly suppressed the meiotic effects of the αtub67C	extsuperscript{P40} and αtub67C	extsuperscript{P40}	extsuperscript{D} alleles. All four of the αtub67C mutations isolated by Matthews et al. (1993) are also suppressed by heterozygosity for a loss-of-function nod allele (data not shown). This effect is not allele-specific with respect to nod, because all three nod alleles tested, including a deficiency (Df(1)nod), exhibit a similar capability to suppress the phenotype of the αtub67C	extsuperscript{P40} allele. These data are consistent with the view that the relative abundance of functional αtub67C and Nod proteins is critical to the faithful segregation of achiasmate chromosomes.

The Effect of the αtub67C Mutations on Achiasmate Chromosome Segregation Is Suppressed by Decreasing the Dose of the Nod Chromokinesis Protein

The Nod kinesin-like protein is specifically required for achiasmate segregation in Drosophila (Carpenter, 1973; Zhang and Hawley, 1990; Zhang et al., 1990). Homozygosity for loss-of-function nod mutations specifically causes achiasmate chromosomes to nondisjoin or to be lost at high frequencies (Carpenter, 1973; Zhang and Hawley, 1990). Cytological studies of prometaphase oocytes from homozygous nod females reveal that achiasmate chromosomes are found precociously at the poles of the developing meiotic spindle and frequently dissociate from the spindle (Theurkauf and Hawley, 1992). During prometaphase, Nod protein is localized along the entire lengths of oocyte chromosomes (Afsheh et al., 1995a,b). Here, it acts to retard the poleward migration of achiasmate chromosomes and serves, in essence, as a stabilizing plateward force.

As shown in Fig. 5 A, heterozygosity for a recessive loss-of-function mutation of nod (nod	extsuperscript{197}) strongly suppressed the meiotic effects of the αtub67C	extsuperscript{P40} and αtub67C	extsuperscript{P40}	extsuperscript{D} alleles. All four of the αtub67C mutations isolated by Matthews et al. (1993) are also suppressed by heterozygosity for a loss-of-function nod allele (data not shown). This effect is not allele-specific with respect to nod, because all three nod alleles tested, including a deficiency (Df(1)nod), exhibit a similar capability to suppress the phenotype of the αtub67C	extsuperscript{P40} allele. These data are consistent with the view that the relative abundance of functional αtub67C and Nod proteins is critical to the faithful segregation of achiasmate chromosomes.

To test this hypothesis further, we measured chromosome segregation in oocytes with various levels of nod	extsuperscript{+} and αtub67C. We kept tubulin levels constant, and tested the effect of increasing the dose of nod	extsuperscript{+}. Sing a duplication of nod	extsuperscript{+}, we showed that three wild-type copies of nod	extsuperscript{+} in an otherwise wild-type background increased chromosome missegregation (see Fig. 5 B). The frequencies of X and 4th missegregation were lower than, but qualitatively similar to, the effect on achiasmate chromosome nondisjunction observed in oocytes from females heterozygous for αtub67C	extsuperscript{P40}. We also compared the effect of three copies of nod	extsuperscript{+} in the presence of different levels of wild-type αtub67C. X and 4th chromosome nondisjunction levels were substantially elevated in FM 7/X; αtub67C	extsuperscript{P40}/+ females carrying the nod	extsuperscript{+} duplication, compared with flies with the nod duplication and two wild-type copies of αtub67C (Fig. 5 B). Finally, we determined the effect of varying the number of copies of nod	extsuperscript{+} in the presence of constant levels of wild-type αtub67C, i.e., in αtub67C	extsuperscript{P40}/+ oocytes. A gain, in the presence of comparable αtub67C	extsuperscript{P40} levels, the levels of chromosome missegregation showed an almost linear relationship with the levels of nod (Fig. 5 C).
The Defect in Chromatin Stretching and Centromere Positioning can be Suppressed by Reducing the Number of Copies of the *nod* Gene

Reducing the copy number of *nod* suppresses the chromosome missegregation in oocytes from FM7/X; αtub67C<sup>P40</sup>/+ females. Therefore, we determined what effect a loss-of-function allele of *nod* (nod<sup>b17</sup>) has on the cytological defects observed in FM7/X; αtub67C<sup>P40</sup>/+ oocytes. We measured chromatin mass and spindle lengths in oocytes derived from FM7, nod<sup>b17</sup>/X; αtub67C<sup>P40</sup>/+ females (Fig. 1 B, bottom row and Fig. 2, C, F, and I), and found that chromatin mass length in FM7, nod<sup>b17</sup>/X; αtub67C<sup>P40</sup>/+ oocytes paralleled those of wild-type and ranged in length from 3–12.5 μm, with no chromatin mass shorter than 3 μm (Fig. 2, compare A and C). Plotting of the chromatin length or axial ratio versus the spindle length demonstrated a parallel elongation of chromatin and spindles in nod-suppressed oocytes (Fig. 2, compare D–F and G–I). Heterozygosity for nod with two copies of wild-type αtub67C has no effect on chromatin mass elongation (data not shown). Moreover, in FM7, nod<sup>b17</sup>/X; αtub67C<sup>P40</sup>/+ oocytes, MEI-S332 protein is once again normally localized (Fig. 3, third and fourth rows).

Reducing *Nod* levels restores the ability of chromatin to be elongated in mature spindles (Fig. 2, G–I) and restores proper centromere positioning. However, the effect of the αtub67C<sup>P40</sup> mutation on decreasing overall spindle length (average = 12.2 μm) in oocytes from αtub67C<sup>P40</sup>/+ females was not suppressed by reducing the dosage of nod<sup>+</sup> (average spindle length = 11.1 μm). Thus, reducing the dose of nod<sup>+</sup> can suppress the chromosome missegregation phenotype, the defect in chromatin elongation, and centromere mispositioning, but does not suppress the reduction in spindle length created by αtub67C<sup>P40</sup>. Altering Nod dosage in oocytes bearing the αtub67C mutations indicates that Nod plays a role in centromere positioning; this effect would have been impossible to detect using loss-of-function nod alleles alone.

Figure 5. The fidelity of achiasmate chromosome segregation is sensitive to the dose of both Nod and αtub67C proteins. Dark and light bars represent the frequencies of X and 4<sup>th</sup> chromosome missegregation, respectively, in the indicated genotypes. A. The effects of heterozygosity for αtub67C mutations on chromosome missegregation can be suppressed by heterozygosity for a loss-of-function allele of nod (nod<sup>b17</sup>). B. Increasing the dose of nod<sup>+</sup> to three copies (by use of a duplication) increases the frequency of X and 4<sup>th</sup> chromosomal missegregation, both in the females carrying two normal copies of the αtub67C gene, and, to an even greater extent, in females heterozygous for a mutation in the αtub67C gene. This enhancement of missegregation by the nod<sup>+</sup> duplication can be eliminated by using females that simultaneously bear a nod mutation on one of the two X chromosomes (i.e., flies that only carry two copies of nod<sup>+</sup>, despite carrying the nod<sup>+</sup> duplication; data not shown). This demonstrates that the enhancement observed in the presence of the duplication is due to the extra copy of nod<sup>+</sup>, since both X and 4<sup>th</sup> chromosome nondisjunction occur with the same frequencies in flies carrying two alleles of nod<sup>+</sup>, regardless of whether one comes from a duplication or from both the endogenous genes on the same chromosome. C. Comparison of the effects of one, two, and three doses of nod<sup>+</sup> in FM7/X; +/P40 females. N is the adjusted total of progeny scored (Hawley et al., 1993).
Discussion

A Model in Which the Balancing Of Forces Is Required for the Segregation of Achiasmate Chromosomes

Our data demonstrate that the fidelity of achiasmate chromosome segregation is sensitive to the relative levels of functional α\textsubscript{tub67C} and Nod proteins. The consequences of altering the level of one of these two proteins are ameliorated or exacerbated by changes in the level of the other. We propose that when the level of wild-type α\textsubscript{tub67C} protein is reduced, the poleward force(s) are compromised, and this leads to a failure of chromatid elongation and centromere positioning.

We can imagine four mechanisms to explain the disruption of poleward forces by α\textsubscript{tub67C} mutations. The first proposes that the presence of the aberrant α\textsubscript{tub67C}\textsuperscript{P40} subunits results in reduced production of poleward force from a minus end directed microtubule-based motor: a kinesin or a dynein (Kommala and Endow, 1997; Starr et al., 1998). Failure of these motors to generate poleward force could prevent the migration of kinetochores and/or the elongation of chromosome arms. The second hypothesis suggests reduced coupling between a plus end directed kinesin at the kinetochore and kinetochore microtubules (Duesbery et al., 1997; Schara et al., 1997; Wood et al., 1997). This could lead to poor coupling of kinetochore microtubules to the kinetochore, resulting in a failure of the mechanism that actually provides force for poleward kinetochore motility. A third possibility is that a plus end directed motor (Karpen et al., 1996) responsible for sliding antiparallel microtubules is compromised and this prevents sliding of microtubules bound to the chromatin. Finally, the observed impairment in poleward movement may reflect reduced binding of a microtubule-associated protein(s) or kinesin that regulates poleward flux (Wilson et al., 1994; Wilson and Forer, 1997; Desai et al., 1998) or microtubule dynamics (Karabay and Walker, 1999). Nod could also play a role in microtubule dynamics, and thereby regulate poleward and antipoleward forces.

As noted above, cytological and genetic studies are consistent with a model in which Nod functions in wild-type spindles to provide both a plateau force and a function which is important for centromere positioning. When poleward forces and chromatid stretching are reduced by heterozygosity for α\textsubscript{tub67C}\textsuperscript{P40}, decreasing the dose of nod\textsuperscript{+} ameliorates this defect. We speculate that this suppression occurs because the reduced plateau forces now more closely equal the impaired poleward forces. Similarly, increasing the amount of Nod in both wild-type and in α\textsubscript{tub67C}\textsuperscript{P40/+} oocytes should, and more importantly does, increase the frequency of meiotic errors in oocytes. These observations are consistent with a model in which Nod protein serves as a stabilizing plateau force for the segregation of achiasmate chromosomes, and that this force is balanced by poleward forces which are dependent on the level of functional α\textsubscript{tub67C} protein.

The model presented in the preceding paragraph does not require the physical interaction of the α\textsubscript{tub67C} and Nod proteins, only their separate roles in creating opposing forces. It is, however, at least possible that the two proteins do indeed physically interact. In this case, the observed defects in chromosome and centromere movements in oocytes carrying mutations in α\textsubscript{tub67C} gene might be the result of poisonous or rigor-like interactions between Nod protein bound to the chromatin and the mutant α\textsubscript{tub67C} protein.

The observation that the effects of these α\textsubscript{tub} mutations is restricted to achiasmate chromosomes is puzzling in terms of the more general defects observed cytologically. We can only surmise that the presence of chiasmata is prophylactic to the kinds of errors created by these tubulin mutations. Perhaps the types of kinetochore orientation mechanisms that successfully ensure the segregation of chiasmate bivalents are to some degree more fail-safe than those ensuring the segregation of achiasmate homologues.

We thank Drs. Kenneth Burtis, Don Cleveland, Larry Goldstein, Frank McNally, Jodi Nunrai, Kim McQuinn, Leslee Rose, Jon Scholey, Bill Theurkauf, Jeff Sekelsky, and all of the members of the Hawley and Scholey laboratories, especially Christina Boulton, for stimulating discussions. We especially would like to thank Drs. Tracy Tang and Terry Orr-Weaver, who generously supplied the MEI-S332 antibody, and Dr. Kathy Matthews for various alleles of α\textsubscript{tub67C}.

This work was supported by a grant from the National Institutes of Health to R.S. Hawley.

Submitted: 12 October 1999
Revised: 1 November 1999
Accepted: 3 November 1999

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