# *subito* Encodes a Kinesin-like Protein Required for Meiotic Spindle Pole Formation in *Drosophila melanogaster*

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ABSTRACT

The female meiotic spindle lacks a centrosome or microtubule-organizing center in many organisms. During cell division, these spindles are organized by the chromosomes and microtubule-associated proteins. Previous studies in *Drosophila melanogaster* implicated at least one kinesin motor protein, NCD, in tapering the microtubules into a bipolar spindle. We have identified a second Drosophila kinesin-like protein, SUB, that is required for meiotic spindle function. At meiosis I in males and females, *sub* mutations affect only the segregation of homologous chromosomes. In female meiosis, *sub* mutations have a similar phenotype to *ncd*; even though chromosomes are joined by chiasmata they fail to segregate at meiosis I. Cytological analyses have revealed that *sub* is required for bipolar spindle formation. In *sub* mutations, we observed spindles that were unipolar, multipolar, or frayed with no defined poles. On the basis of these phenotypes and the observation that *sub* mutations genetically interact with *ncd,* we propose that SUB is one member of a group of microtubule-associated proteins required for bipolar spindle assembly in the absence of the centrosomes. *sub* is also required for the early embryonic divisions but is otherwise dispensable for most mitotic divisions.

MEIOSIS utilizes two rounds of chromosome segregation to produce haploid gametes. During the first meiotic division, homologous chromosomes pair and segregate into two cells. The second meiotic division proceeds like mitosis, where the sister chromatids segregate. Prior to the first division, the homologs align and undergo recombination to produce crossovers, which then become chiasmata. Chiasmata hold the homologs together during spindle formation of meiosis I and are required for orientation of the homologs at metaphase until the kinetochores are pulled to their proper poles at anaphase. Mutations that reduce the rate of crossing over increase nondisjunction because homologous chromosome pairs lack chiasmata. In addition to chiasmata, chromosome segregation at meiosis I requires a bipolar attachment of bivalents on the spindle apparatus (Nicklas 1997). In many meiotic cells, microtubules organized by and growing from the centrosomes are captured by kinetochores. The chromosomes are stable and under tension when paired kinetochores are attached to microtubules emanating from opposite directions (or centrosomes). With the release of chiasmata the homologs are free to move to the poles, thus ensuring one copy of each chromosome in a cell.

In contrast to the canonical pathway of spindle formation, in diverse organisms such as mammals, nematodes, and insects, female meiotic spindles are organized in the absence of centrosomes and centrioles (McKim and Hawley 1995; Waters and Salmon 1995; Heald *et al.* 1996). It has also been shown in Drosophila oocytes that centrosome components such as γ-tubulin are not concentrated at the spindle poles (Matthies *et al.* 1996), despite its requirement for normal meiotic spindle function (Tavosanis *et al.* 1997). There is a substantial body of evidence that the chromosomes have a significant role in organizing the microtubules of a female meiotic spindle (McKim and Hawley 1995). In Drosophila oocytes, meiotic spindle formation begins with a mass of microtubules emanating from the chromosomes, suggesting that the chromosomes nucleate or capture microtubules that are later shaped into a bipolar spindle. In fact, individual chromosomes have been shown to form bipolar spindles in Drosophila oocytes (Theurkauf and Hawley 1992) and other organisms (Waters and Salmon 1995).

Mutants with defects in chromosome segregation during Drosophila female meiosis potentially identify genes that have a role in spindle organization or function. For example, *ncd* encodes a kinesin-like motor protein and was identified because mutations caused defects in chromosome segregation at meiosis I (Hatsumi and Endow 1992; Matthies *et al.* 1996). We present evidence here that *subito* (*sub*), which was originally identified by recessive female sterile alleles (Schupbach and Wieschaus 1989), is also required for chromosome segregation at meiosis I. Like *ncd* mutants, in the fertile $sub^{1794}$ mutation

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both chiasmate and achiasmate chromosomes nondisjoin. In both this mutant and the sterile *sub* mutants, there are severe abnormalities in meiotic spindle organization, indicating that *sub* is required for spindle pole formation during female meiosis. We have also shown that *Dub*, which was previously isolated because of its dominant nondisjunction phenotype (Moore *et al.* 1994), is an allele of *sub*. *sub* encodes a kinesin-like protein, and like NCD, SUB may be a microtubule motor functioning in the absence of centrosomes to organize the poles of the female meiotic spindle. Unlike *ncd*, however, *sub* has additional roles in male meiosis and the mitotic divisions of the early embryo.

## MATERIALS AND METHODS

**Source of *sub* alleles:** $sub^{1794}$ was isolated by screening a collection of ethyl methanesulfonate-treated second chromosomes, generated in the laboratory by Charles Zuker (E. Koundakjian and C. Zuker, personal communication), for elevated frequency of X chromosome nondisjunction. The original *Dub* mutation, which we refer to as $sub^{Dub}$, was isolated in B. Wakimoto's laboratory (cited in Moore *et al.* 1994).

**Genetic analysis:** Using an *al dp b pr cn c px sp* chromosome, $sub^{1794}$ was recombination mapped between *pr* and *c*. The map position was refined using complementation tests to deficiencies. $sub^{1794}$ failed to complement *Df(2R)PC4* (54D3-E10; 55D3-E11) and $Df(2R)Pcl^{XM82}$ (54D3-6; 55B7-12) and complemented *Df(2R)PCl11B* (55A1; 55C1-3), *Df(2R)PCl7B* (54E8-F1; 55B9-C1), *Df(2R)RM2-1* (54F2; 56A1), and *Df(2R)PC29* (55C1-2; 56B1-2). $sub^{Dub}$ was previously mapped to *Df(2R)PC4* (Moore *et al.* 1994).

X chromosome nondisjunction was assayed by crossing *y/y; sub/sub* females to *C(1;Y), v f B; C(4)RM, ci ey/0* males and the frequency was calculated as 2(exceptional progeny)/2(exceptional progeny) + (regular progeny). In these experiments nullo-4, but not diplo-4, ova were also recovered and the fourth chromosome nondisjunction frequency was calculated by doubling this number. Because chromosome loss may exceed the nondisjunction, this calculation may be an overestimate. X chromosome crossing over was measured by crossing *y cv f/ +++* females to *C(1;Y), v f B; C(4)RM, ci ey/0* males. In these crosses, crossing over followed by nondisjunction could also be detected. If a crossover bivalent nondisjoins, then in 50% of the second meiotic divisions a recombinant chromatid will segregate into the same product as a nonrecombinant chromatid carrying all of the recessive markers. The female this egg produces will carry two maternal X chromosomes (diplo-X) and be homozygous for all of the markers distal to the crossover site. X-Y nondisjunction in the male germline was measured by crossing $y/y^{+}Y$; *sub/sub* males to *y w; C(4)RM, ci ey/0* females. The nondisjunction frequency was calculated as (exceptional progeny)/(exceptional progeny) + (regular progeny).

Nondisjunction and crossing over on the second chromosome were tested by crossing *al dp b* $sub^{1794}$*/+ + +* $sub^{1794}$ females to males carrying compound chromosomes [*+/Y; C(2L), b; C(2R), px*]. In this cross, only the nondisjunction progeny survived, and as in the X chromosome experiment, if there was a crossover there was a 50% chance that the event would be detected. To calculate the crossover frequency among nondisjunctional chromosomes in either the X or second chromosome experiments, the number of diplo progeny with a crossover was divided by the total number of diplo progeny. Because only one-half of the events were detected, this number was multiplied by two to calculate the genetic map distance.

Egg viability was assayed by mating either $sub^{1794}$/+ or $sub^{1794}$/$sub^{1794}$ females on grape juice agar plates. After the parents were removed, the eggs were counted. Within the next 3 days the number of larvae was counted to determine how many of the embryos were viable.

**Genetic screen for new alleles of *sub*:** A *P*-element insertion [*EP(2)616*; Rorth *et al.* 1998] was found to be located 105 bp upstream of the *sub* coding region. Flies carrying *P{EP}EP(2)616* were crossed to a source of transposase (Δ2-3) to mobilize the *P* element. Specifically, *w; P{EP}EP(2)616/ CyO;* Δ*2-3, Sb/+* males were crossed to *y w/Y; Gla/SM6* females and excisions of the *P* element ($P\{w^{-}\}$/ *SM6* or *Gla*) were detected in the progeny by the loss of the $white^{+}$ marker gene. Individual white-eyed males were crossed to $sub^{1794}$*/SM6* females and the $P\{w^{-}\}$/ $sub^{1794}$ progeny were crossed to detect X chromosome nondisjunction. Those lines with elevated frequency of nondisjunction (>1%) were retested and stocks were made for further analysis. The extent of the deletions was determined by PCR. Genomic DNA was made from mutant homozygotes and primer pairs within and flanking the gene were used in PCR reactions. Failure to amplify in conjunction with a positive amplification using control primers indicated that there was a deletion of at least one of the primer sites.

Some mutations (*e.g.*, $sub^{72}$, $sub^{104}$, $sub^{158}$) clearly affected the intragenic region and therefore might reduce the expression of the *sub* neighbor CG10931. Furthermore, due to the close juxtaposition of *sub* and CG14487, we do not know if $sub^{22}$ affects the expression of the latter gene. However, three deletion mutations, $sub^{131}$, $sub^{202}$, and $sub^{218}$, affected *sub* only. Because these mutations should not influence the expression of CG10931 and CG14487, they represent the null phenotype of *sub*. Despite the caveats of whether the deletion affected an adjacent gene, we found that all nine mutants had similar effects on female and male meiosis and early embryogenesis (see RESULTS).

**Confocal microscopy:** Stage 14 oocytes were collected from 3- to 7-day-old yeast-fed females and fixed as described previously (Theurkauf and Hawley 1992; McKim *et al.* 1993). Oocytes were stained for DNA with 4′,6-diamidino-2-phenylindole (DAPI) or propidium iodide and for spindles with antitubulin conjugated to FITC [Sigma (St. Louis) monoclonal antibody DM1A]. For fluorescent *in situ* hybridization (FISH) to stage 14 oocytes, we used a Cy3-labeled oligonucleotide probe to a repeat sequence (AACAC) in the second chromosome centric heterochromatin. Labeling, fixation, and hybridization were as described by Dernburg *et al.* (1996). Images were collected on a Leica TCS SP confocal microscope.

**Sequencing and analysis:** The sequence of the *sub* gene was predicted by the genome sequencing project as CG12298 (Myers *et al.* 2000). *sub* cDNA clones were generated by Rubin *et al.* (2000) and obtained from Research Genetics (Huntsville, AL). The CG12298 genetic region was amplified by PCR from $sub^{1794}$ homozygotes and $sub^{Dub}$*/Df(2R)PC4* flies. The PCR product was blunt cloned into the pT7Blue vector using the Perfectly Blunt cloning system (Novagen). DNA clones for sequencing were prepared by alkaline lysis minipreps followed by polyethylene glycol (PEG) precipitation. Sequencing was performed by the University of Medicine and Dentistry of New Jersey sequencing facility. Sequence analysis utilized the Wisconsin Package Version 9.1 [Genetics Computer Group (GCG), Madison, WI]. Sequences from mutant DNA and another strain of the same genetic background were compared to identify the nucleotide changes.

## RESULTS

**$sub^{1794}$ causes nondisjunction during the first meiotic division:** The EMS-induced mutation, $sub^{1794}$, was iso-

**TABLE 1**

**Nondisjunction in *sub*$^{1794}$ mutants**

| Female genotype | Regular (X/0) | Regular (XX^Y) | Diplo-X (XX) | Nullo-X (X^Y) | % X-ND | Nullo-4 (4^4) | % 4-ND | Total progeny |
|---|---|---|---|---|---|---|---|---|
| *sub*$^{1794}$/*sub*$^{1794}$ | 184 | 219 | 33 | 51 | 29.4 | 114 | 34.6 | 571 |
| *sub*$^{1794}$/*Df(2R)PC4* | 286 | 125 | 51 | 32 | 28.8 | 69 | 23.9 | 577 |
| *sub*$^{1794}$/*sub*$^{131}$ | 421 | 46 | 82 | 21[a] | 30.6 | 43 | 13.4 | 673 |
| *sub*$^{1794}$/+ | 716 | 793 | 0 | 1 | 0.1 | 3 | 0.2 | 1510 |
| *y/FM7; sub*$^{1794}$/*sub*$^{1794}$ | 238 | 269 | 103 | 161 | 51.0 | 205 | 39.6 | 1035 |
| *y/FM7; sub*$^{1794}$/+ | 334 | 234 | 3 | 3 | 2.1 | 4 | 1.4 | 580 |
| *sub*$^{1794}$/*sub*$^{Dub}$ | 231 | 135 | 103 | 36[a] | 43.2 | 94 | 29.1 | 644 |
| *sub*$^{Dub}$/+ | 404 | 411 | 49 | 103 | 27.2 | 141 | 25.2 | 1119 |
| *sub*$^{1794}$/*EP616* | 292 | 319 | 0 | 0 | 0 | 0 | 0 | 611 |

ND, nondisjunction. Females were crossed to *C(1;Y), v f B; C(4)RM, ci ey/0* males. Regular progeny received one X and fourth maternal chromosome, whereas diplo-X progeny received two maternal X chromosomes, nullo-X progeny received no maternal X chromosomes, and nullo-4 progeny received no maternal fourth chromosome. The genotypes of these progeny are indicated, with *C(1;Y)* abbreviated as X^Y, and *C(4)RM* abbreviated as 4^4.

[a] The relatively low number of nullo-X progeny was due to low transmission of the paternal X chromosome (as shown by the recovery of XX^Y progeny).

lated in a screen for homozygous recessive mutations on the second chromosome that exhibited elevated levels of X chromosome nondisjunction (Table 1). Using three-factor crosses and deficiencies, *sub*$^{1794}$ was mapped to the cytological interval 54D3-6; 54F2. This region is deleted in two deficiencies, *Df(2R)PC4* and *Df(2R)Pcl*$^{XM82}$. The *sub*$^{1794}$/*Df(2R)PC4* females were fertile albeit with reduced fertility relative to the *sub*$^{1794}$ homozygotes. Additional alleles of *sub* were isolated in two previous studies. The first alleles were isolated as female sterile mutants by Schupbach and Wieschaus (1989). The sterile phenotype is due to a defect in embryogenesis that will be discussed after the description of the meiotic mutant phenotype. Another allele is the dominant mutation *Dub* (*Double or nothing*), which was shown by Moore *et al.* (1994) to affect meiotic chromosome segregation in males and females. This mutation was genetically mapped to the same region as *sub* (Moore *et al.* 1994). Our conclusion that *Dub* is an allele of *sub* (*sub*$^{Dub}$) was based on genetic as well as molecular evidence. *sub*$^{Dub}$/*sub*$^{1794}$ females had a higher frequency of X chromosome nondisjunction and lower fertility than either *sub*$^{Dub}$/+ or *sub*$^{1794}$/*sub*$^{1794}$ mutants (Table 1). The molecular evidence of allelism described below is that *sub*$^{Dub}$ and *sub*$^{1794}$ each have one amino acid change in the same gene.

Meiotic nondisjunction of the X, fourth (Table 1), and second (Table 2) chromosomes occurred at a high frequency in *sub*$^{1794}$ homozygotes, suggesting all chromosomes were affected. In several experiments there was a slight excess of eggs that received no X chromosome (nullo-X) relative to eggs that received two (diplo-X) from the *sub*$^{1794}$ mothers, indicating that chromosome loss might be occurring at a low frequency. A more pronounced effect was observed when we tested autosomal nondisjunction. The twofold excess of nullo-2 over diplo-2 progeny demonstrated that autosomal chromosome loss was frequent in *sub*$^{1794}$ mutants. To determine at which meiotic division chromosomes fail to segregate, *FM7, y B/y; sub*$^{1794}$/*sub*$^{1794}$ females were tested for nondisjunction (Table 1). *FM7* is a balancer marked with the semidominant *Bar* mutation and prevents crossing over between the X chromosomes. Nondisjunction at the first division results in eggs with one *y* and one *FM7* chromosome. Nondisjunction at the second division results in eggs with two copies of either the *y* or *FM7* chromosomes due to the failure to separate the sister chromatids. All 103 females carrying two maternal X chromosomes were *FM7/y*, demonstrating that most or all of the nondisjunction involved homologs at meiosis I.

***sub* is required for the segregation of chiasmate and achiasmate homologs:** The frequency of X chromosome crossing over in *sub*$^{1794}$ homozygotes was not significantly different than the controls (Table 2) and could not account for the high levels of nondisjunction. The alternative possibility, that in *sub*$^{1794}$ females there was a defect in segregation, could be demonstrated by assaying nondisjunction in *y cv f/+; sub*$^{1794}$/*sub*$^{1794}$ females. In the progeny of these females, diplo-X females homozygous for X-linked markers (one or more of the *y*, *cv*, or *f*) were frequently recovered. These progeny occurred when a crossover occurred but the homologs failed to segregate (Table 2 and materials and methods). These results demonstrated that *sub*$^{1794}$ was defective in chromosome segregation during meiosis I. A similar result was observed on the second chromosome; nondisjunction events were recovered where the two chromosomes involved had crossed over on chromosome 2L (Table 2). In these two experiments, the frequency and distribution of crossovers were not drastically different from controls. For example, the *cv–f* crossovers were the most

**TABLE 2**

**Crossing over and nondisjunction in *sub*$^{1794}$ females**

| Female genotype | Type of progeny | Diplo-X | Nullo-X | Genetic interval *y–cv* | Genetic interval *cv–f* | Genetic interval *f–cen* | Total |
|---|---|---|---|---|---|---|---|
| *y cv f/+; sub*$^{1794}$*/sub*$^{1794}$ | Regular[a] | | | 10.7 | 32.1 | —[b] | 2221 |
| | Diplo-X[a] | 161 | 178 | 9.9 (4) | 34.8 (14) | 7.4 (9) | |
| *y cv f/+; sub*$^{1794}$*/+* | Regular | | | 9.0 | 23.9 | —[b] | 500 |
| | Diplo-X | 0 | 0 | — | — | — | |
| Female genotype | Type of progeny | Diplo-2 | Nullo-2 | *al–dp* | *dp–b* | *b–cen* | Parents |
| *al dp b sub*$^{1794}$*/sub*$^{1794}$ | Regular[c] | | | 13.1 | 25.5 | 5.1 | |
| | Diplo-2 | 152 | 315 | 9.2 (7) | 13.2 (10) | 2.6 (2) | 137 |

*cen*, centromere. Two map distances are given: "Diplo-X" is the frequency of crossing over among chromosomes that failed to segregate (the number of crossover progeny is in parentheses) and "regular" is the frequency of crossing over among chromosomes that segregated properly.

[a] In the *y cv f/+* experiments the regular and diplo-X map distance data were collected in the same experiment.

[b] This interval could not be measured among the regular progeny in this cross. On the standard map it is 9.3 MU (Lindsley and Zimm 1992) and in a separate experiment with *sub*$^{1794}$*; y pn cv f* · *y*$^{+}$*/+* females, where we could determine the *forked* to centromere distance, it was 6.1 MU (total progeny, 511).

[c] For the *al dp b/+* experiment, the map data among the regular progeny were collected in a separate experiment.

common, which is consistent with this being the largest interval on the genetic map. Therefore, the position of the chiasma did not have a significant impact on whether the homologs would fail to segregate in *sub*$^{1794}$ females.

In addition to the chiasmate system of segregation, there is another system that segregates achiasmate chromosomes in Drosophila (Hawley and Theurkauf 1993). In the *FM7, y B/y; sub*$^{1794}$*/sub*$^{1794}$ females, the *FM7* balancer prevented crossing over between the X chromosomes. Therefore, the pair of X chromosomes in these females always segregated using the achiasmate system (Zhang and Hawley 1990). The high frequency of X chromosome nondisjunction in *FM7, y B/y; sub*$^{1794}$*/sub*$^{1794}$ females (51.0%; Table 1, line 5) was similar to that expected for random segregation. In contrast, the frequency of X chromosome nondisjunction in *y/y; sub*$^{1794}$*/sub*$^{1794}$ females (29.4%; Table 1, line 1) demonstrated that the presence of a chiasma reduced the chance that homologous chromosomes would fail to segregate in a *sub* mutant. Therefore, achiasmate chromosomes require *sub* for segregation, whereas in some instances chiasmate bivalents can segregate correctly in the absence of *sub*.

***sub*$^{1794}$ shows allele-specific genetic interactions with mutations in the kinesin *ncd*:** We tested for genetic interactions between *sub* and *ncd* mutations for two reasons. First, *ncd* encodes a kinesin-like protein and mutants have a similar mutant phenotype to *sub*. Second, genes previously shown to genetically interact with *ncd*$^{D}$, a missense allele, function in the meiotic spindle (Knowles and Hawley 1991; Komma and Endow 1997). Double heterozygote females with the genotype *sub*$^{1794}$*/+; ncd*$^{D}$*/+* showed a high frequency of X nondisjunction (Table 3). In contrast, *ncd*$^{1}$, a deletion allele that does not make a product, did not demonstrate a strong interaction with *sub*$^{1794}$. Similarly, the double heterozygote *Df(2R)PC4/+; ncd*$^{D}$*/+* showed less nondisjunction than the *sub*$^{1794}$ *trans*-heterozygote. These results show that *sub*$^{1794}$ has allele-specific genetic interactions with *ncd* and suggest that *sub* has a direct role in meiotic spindle function or assembly.

*nod* encodes a kinesin that is required in females for the segregation of achiasmate chromosomes and has been shown to genetically interact with *ncd* (Knowles and Hawley 1991). Females with the genotype *FM7, nod*$^{A}$*/y; sub*$^{1794}$*/+* had higher levels of X nondisjunction than the controls (Table 3). The interactions were less severe than those with *ncd*$^{D}$, possibly because *nod* is required only for achiasmate chromosome segregation. Alternatively, and similar to what was observed with *ncd*, the strongest interactions could occur between mutations that make an altered gene product. The *nod*$^{A}$ allele is a frameshift and does not make a protein (Rasooly *et al.* 1994).

These genetic interactions are so far limited to the meiotic kinesins *ncd* and *nod*. Genetic interactions were not observed with several other mutants known to affect the female meiotic spindle such as γ-*tub37C*$^{1}$ (Tavosanis *et al.* 1997), α-*tubu67C*$^{1}$ (Matthies *et al.* 1999), *msps*$^{P}$, *msps*$^{MJ208}$ (Cullen and Ohkura 2001), *d-tacc*$^{1}$ (Cullen and Ohkura 2001), and *wisp*$^{12\text{-}3147}$ (Brent *et al.* 2000); (data not shown).

***sub*$^{1794}$ is an allele of a kinesin motor protein:** Using the deficiencies described in materials and methods, we mapped *sub* to a region of chromosome 2 containing ~25 genes (Meyers *et al.* 2000). One of these genes,

**TABLE 3**

**Genetic interactions of *sub* with *ncd* and *nod***

| Female genotype | Regular (X/0) | Regular (XX^Y) | Diplo-X (XX) | Nullo-X (X^Y) | % X-ND | Nullo-4 (4^4) | % 4 ND | Total progeny |
|---|---|---|---|---|---|---|---|---|
| $sub^{1794}$/+; $ncd^{D}$/+ | 550 | 553 | 48 | 37 | 13.3 | 88 | 13.8 | 1273 |
| *Df(2R)PC4*/+; $ncd^{D}$/+ | 312 | 200 | 8 | 7 | 5.5 | 27 | 10.0 | 542 |
| *SM6*/+; $ncd^{D}$/+ | 465 | 331 | 5 | 1 | 1.5 | 19 | 4.7 | 808 |
| $sub^{1794}$/+; $ncd^{1}$/+ | 482 | 575 | 5 | 4 | 1.7 | 9 | 1.7 | 1075 |
| *FM7, y* $nod^{4}$*/y;* $sub^{1794}$/+ | 1072 | 919 | 25 | 27 | 5.0 | 90 | 8.6 | 2095 |
| *FM7, y/y;* $sub^{1794}$/+ | 334 | 234 | 3 | 3 | 2.1 | 3 | 1.4 | 580 |
| *FM7, y* $nod^{4}$*/y;* +/+ | 1483 | 1354 | 2 | 1 | 0.2 | 5 | 0.2 | 2843 |

See Table 1 legend for details.

CG12298, was predicted to encode a kinesin-like protein. CG12298 was a strong candidate for *sub* because of the cytological evidence for defective spindles in $sub^{1794}$ mutants (see below) and the genetic interactions with the kinesins *ncd*. The CG12298 coding region was sequenced in $sub^{1794}$ and found to contain a point mutation causing a cysteine to tyrosine substitution in the poorly conserved amino acid 152 (Figure 1). We also sequenced CG12298 in $sub^{Dub}$ and found a glutamic acid to lysine substitution in the highly conserved amino acid 385 (Figure 1), confirming that $sub^{1794}$ and $sub^{Dub}$ are alleles.

The predicted *sub* peptide is 628 amino acids and contains a conserved kinesin motor domain from amino acids 80–480 and a predicted coiled-coil region from amino acids 500–600 (Figure 1). From a comparison to other kinesin-like protein motor domains (Kim and Endow 2000), *sub* is most similar to the MKLP1 subfamily of kinesin proteins that includes proteins that function during mitosis (see also Miki *et al.* 2001). Kinesin-like proteins often have a "neck linker" between the motor and the coiled-coil domains that may be essential for the walking action of kinesin (Cross 2001) and has been proposed to function as a mechanical amplifier (Case *et al.* 2000). In SUB there is a possible neck-linker sequence between the motor domain and coiled-coil, but as is typical for members of the MKLP1 group, SUB has poor sequence similarity to other kinesin-like proteins in this region (Vale and Fletterick 1997).

Another notable feature of the SUB sequence is that it ends abruptly after the coiled-coil domain. While PAV and MKLP1 have almost 200 amino acids following their coiled-coil domains, SUB has <30. We confirmed the splicing pattern and early termination of the protein by fully sequencing the cDNA clone LD18884. In addition, a second cDNA clone, LD35138, was sequenced by Rubin *et al.* (2000; accession no. AY069597) with the same results. The region after the coiled-coil domain is usually the cargo-binding domain of the kinesins. While uncommon, the absence of a C-terminal domain has been observed before, as in Xklp2 of Xenopus (Boleti *et al.* 1996) and its homolog in sea urchin, $KRP_{180}$ (Rogers *et al.* 2000).

**Isolation and characterization of *sub* null alleles:** We

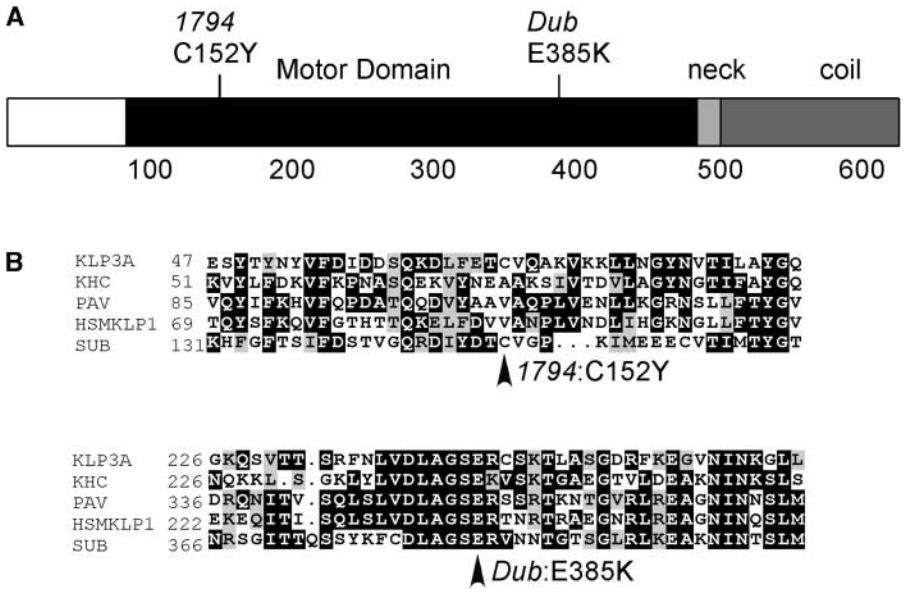


Figure 1.—Structure of the *sub* protein and amino acid changes in mutants. (A) Schematic of SUB showing the conserved domains. The motor domain of SUB extends from approximately amino acid 80 to 477. The neck-linker region defined by Vale and Fletterick (1997) continues to approximately amino acid 541. (B) The amino acid changes in $sub^{Dub}$ and $sub^{1794}$ are shown relative to an alignment of four other kinesins: Drosophila KLP3A (Williams *et al.* 1995), KHC (Yang *et al.* 1989), PAV (Adams *et al.* 1998), and human MKLP1 (Nislow *et al.* 1992).

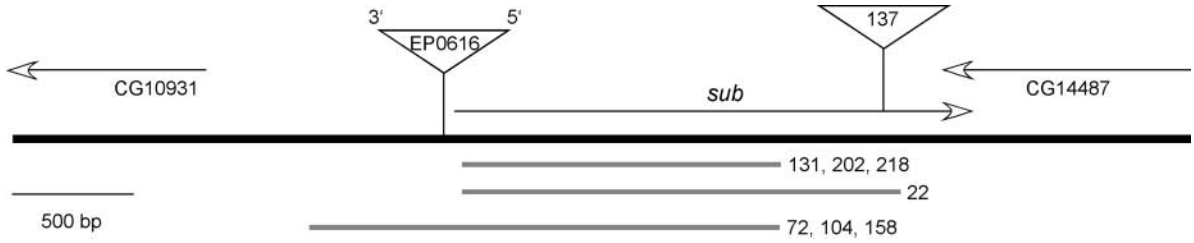


FIGURE 2.—Molecular map of the *sub* region. Above the line are *sub* and the flanking transcripts as well as the *P*-element insertion used to make the deletions and the one insertion. Below the line the deletions are indicated. The $sub^{137D}$ chromosome contains both the original EP element and a new one inserted into the coding region.

sought to isolate deletion alleles of *sub* by imprecise excision of a *P*-element insertion, *P{EP}EP(2)616*, located 105 bp upstream of the predicted ATG (Figure 2). This insertion complemented $sub^{1794}$ and therefore did not grossly affect gene expression (Table 1). We exposed this element to transposase and isolated events where the *P* element had moved and the resulting chromosome failed to complement $sub^{1794}$ (MATERIALS AND METHODS). Seven of the new mutations were shown by PCR to be deletions and are referred to as $sub^{null}$ alleles (Figure 2). In addition, one local transposition where the *P* element had disrupted the coding region ($sub^{137D}$, Figure 2) was isolated.

When $sub^{1794}$ was heterozygous to a deletion allele or *Df(2R)PC4*, the frequency of nondisjunction was similar to $sub^{1794}/sub^{1794}$ homozygotes (Table 1). These results suggested that the meiotic defect in $sub^{1794}$ was similar to a null allele. However, in other cell types we observed differences in their respective phenotypes, revealing that the deletion alleles had a more severe effect on SUB activity than $sub^{1794}$. Whereas $sub^{1794}$ females were fertile, all seven *sub* deletions and the insertion mutation were female sterile as homozygotes. Furthermore, their interactions with the dominant allele were different. $sub^{1794}/sub^{Dub}$ females were fertile but $sub^{null}/sub^{Dub}$ females were sterile. The sterile females also had rough eyes and clipped wings, two phenotypes associated with cell division defects. Therefore, we could construct an allelic series, $sub^{null}/sub^{Dub} > sub^{1794}/sub^{Dub} > +/sub^{Dub}$, suggesting that the $sub^{1794}$ product had low levels of wild-type activity.

We performed complementation tests against mutations isolated on the basis of a female sterile phenotype that mapped to the same region as $sub^{1794}$. Our alleles failed to complement previously isolated female sterile alleles of *sub* (SCHUPBACH and WIESCHAUS 1989). $sub^{131}/sub^{1}$ females were sterile and $sub^{1794}/sub^{1}$ females had elevated X chromosome nondisjunction in females.

Surprisingly, the $sub^{null}$ mutants also had an effect in males: There were elevated levels of X-Y chromosome nondisjunction in male meiosis. For example, in $sub^{202}$ homozygous males we observed 10.1% nondisjunction of the X and Y chromosomes ($n = 1257$). X-Y chromosome nondisjunction was not elevated in males homozygous for the parental *EP(2)616* chromosome (0/329) or in $sub^{1794}/sub^{1794}$ (2/1526 = 0.1%) and $sub^{1794}/Df(2R)PC4$ (4/849 = 0.5%) males. While some of the *sub* alleles are deletions, these phenotypes are not due to effects on neighboring genes. At least three of the mutants, $sub^{131}$, $sub^{202}$, and $sub^{218}$, affect only the *sub* coding region and have the female sterile and male nondisjunction phenotypes.

***sub* stage 14 oocytes have a defect in spindle pole formation:** To investigate the basis for the elevated nondisjunction in *sub* mutants, stage 14 oocytes of *sub* homozygotes were examined to determine if there were defects in meiotic spindle morphology. In wild type, meiosis arrests at metaphase I in stage 14 oocytes with the chromosomes located in a single mass (the karyosome) in the middle of a tapered bipolar spindle (Figure 3A). In $sub^{1794}$ mutant oocytes, a variety of defects were observed (Figure 3, C and D; Table 4). The most prominent defects were tripolar and monopolar spindles. In addition, some mutant oocytes had a split karyosome, spindles that were broken, and spindles that were frayed or did not taper at the poles. These results suggest that the reason for the high frequency of nondisjunction in *sub* mutants is the failure to organize a spindle with two poles.

Flies with the genotype $sub^{1794}/deficiency$ [$sub^{1794}/Df(2R)PC4$ or $sub^{1794}/Df(2R)XM82$] had similar defects to $sub^{1794}/sub^{1794}$ but were observed in a larger fraction of the oocytes (Figure 4C, Table 4). In addition, the *sub* null alleles had a higher frequency of spindle defects than $sub^{1794}$ (Figure 3, E and F; Table 4). The more severe phenotype when heterozygous to a deficiency or in the null alleles is consistent with the conclusion that $sub^{1794}$ is a hypomorph. However, this conclusion applies only to the frequency of abnormal spindles; qualitatively in these different genotypes, when there were abnormal spindles, the severity of the defects was similar (Figure 4).

Meiosis I spindles from $sub^{1794}/sub^{Dub}$ and $sub^{1794}/+; ncd^{D}/+$ females were also severely deformed: The microtubules were splintered, untapered, and again there was a failure to form a spindle with two well-defined poles (Figure 4). Although these genotypes do not represent simple loss of *sub* function, like $sub^{1794}$ females the ele-

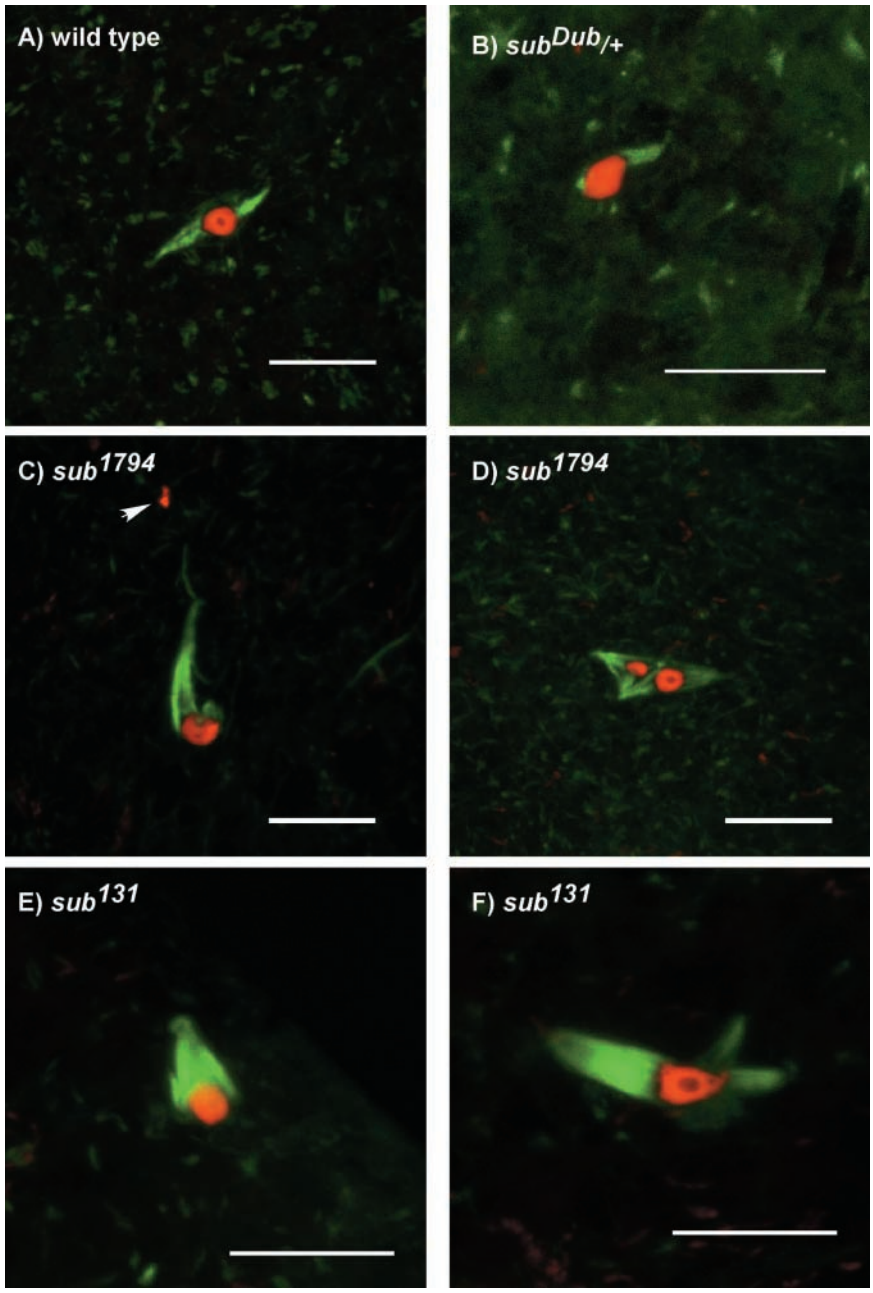


Figure 3.—Stage 14 oocytes from wild-type and *sub* mutants. DNA is in red and microtubules are in green. Bars, 10 μm. (A) A wild-type metaphase spindle with a bipolar spindle and round karyosome. (B) A typical monopolar spindle in $sub^{Dub}$/+ females. These females had relatively subtle defects in spindle organization. In addition, the spindles often appeared smaller than wild type. In $sub^{1794}/sub^{1794}$ females both unipolar (C) and tripolar (D) spindles are common. In C, a small chromosome, likely the fourth, has moved off the spindle (arrow). In D, the karyosome has split, although this is probably not anaphase (see Figure 5). In $sub^{131}/sub^{131}$ females (E and F) similar defects in spindle pole formation were observed.

vated frequency of nondisjunction appears to occur because of defects in spindle organization. We were thus surprised to find that $sub^{Dub}$/+ females had relatively minor defects in spindle organization (Figure 3B) even though nondisjunction was frequent. Like the recessive alleles, frayed spindles and monopolar spindles characterized the spindle defects. However, the defects were distinctly less severe than the recessive mutants. The mutant protein's dominant effects may occur by a defect in chromosome movement on the spindle rather than through a disruption in spindle organization.

A characteristic of $sub^{1794}$ mutant meiosis is that the karyosome was often abnormally shaped or even split. To investigate the organization of the chromosomes in *sub* mutation karyosomes, we performed FISH on $sub^{1794}$/*Df(2R)PC4* oocytes. A probe to the second chromosome centric heterochromatin was used to track the behavior of one pair of centromeres. In wild-type stage 14 oocytes, the centromeres are usually oriented toward the poles (Figure 5A and Dernburg *et al.* 1996). Frequently (8/17) in $sub^{1794}$/*Df(2R)PC4* females the centromeres were abnormally arranged: either on the same side of the karyosome or if the karyosome was split, in the same mass of chromosomes (Figure 5, B and C). The same type of abnormality was seen rarely (1/21) in wild-type controls. In all cases where the karyosome was split the two centromeres were in the same mass, suggesting that the oocyte had not entered anaphase. These results raise the possibility that in $sub^{1794}$ mutants there was a defect in chromosome organization within the karyosome.

**TABLE 4**

**Stage 14 oocyte cytology**

| Genotype | Metaphase[a] | Split karyosome[b] | Abnormal spindle morphology[b] |
|---|---|---|---|
| Wild type | 14 | 0 | 3 |
| $sub^{1794}/+$ | 22 | 0 | 0 |
| $sub^{1794}/sub^{1794}$ | 52 | 4 | 22 |
| $sub^{1794}/Df(2R)PC4$ | 22 | 3 | 17 |
| $sub^{1794}/Df(2R)XM82$ | 10 | 0 | 9 |
| $sub^{131}/sub^{131}$ | 14 | 0 | 14 |
| $sub^{1794}/+;\ ncd^{D}/+$ | 10 | 4 | 7 |
| $sub^{1794}/sub^{Dub}$ | 15 | 1 | 13 |
| $sub^{Dub}/+$ | 13 | 0 | 8 |
| $sub^{1794}/sub^{1794};$ $ncd^{1}/ncd^{1}$ | 20 | 10 | 20 |
| $ncd^{1}/ncd^{1}$ | 11 | 2 | 11 |

[a] The total number of meiotic figures observed.
[b] The number among the total with a defect in karyosome or spindle morphology.

***sub* and *ncd* have a similar function in oocyte spindle pole formation:** Our analysis of *sub* mutants has revealed an array of genetic and cytological phenotypes similar to *ncd*: Predominantly the poles were often frayed or there was an abnormal number (Matthies *et al.* 1996). Significantly, in oocytes of either mutant the activity of forming spindle poles is only partially disrupted. To determine if these genes have redundant roles in spindle pole formation, $sub^{1794};\ ncd^{1}$ double mutants were constructed. If the spindle poles that form in one mutant were dependent on the other gene, then we expected the double mutant to lack spindle poles. In fact, the majority of double mutant oocytes had spindles with the same defect as the single mutants. Spindle poles were able to form but there was an abnormal number and sometimes they were frayed (Figure 4, E and F). All of the double mutant spindles were abnormal, which was a higher frequency than that of the $sub^{1794}$ single mutant but the same as the $ncd^{1}$ single mutant (Table 4). Therefore, the increase in abnormal spindles in the double mutant relative to $sub^{1794}$ was a function of the $ncd^{1}$ mutation and not a synergistic effect. Because the spindle had a similar capacity to form poles in the absence of *ncd*, *sub*, or both gene products, these results are consistent with the conclusion that both genes function in the same process of spindle pole formation. While it would have been preferable to perform these experiments using $sub^{null};\ ncd^{1}$ females, these flies were not viable. Thus, while *ncd* and *sub* probably function in the same pathway during meiosis, they may have redundant roles in mitotic cells.

***sub* is required for the early mitotic divisions of the embryo:** The $sub^{null}$ mutants are viable, showing that *sub* is not essential for the postembryonic mitotic divisions (for many gene products the maternal contribution is sufficient for the embryonic divisions). The sterile phenotype of the $sub^{null}$ mutants demonstrated that *sub* is required for the embryonic mitotic divisions because early embryos survive entirely on the contributions from the mother.

To examine the mutant embryonic phenotype, we collected embryos from *sub* mutant mothers and wild-type controls at 2- or 3-hr intervals and examined the chromosomes and microtubules. In wild-type embryos we observed a range of developmental stages, usually with multiple nuclei (Figure 6, A and E). In contrast, the majority of embryos produced by $sub^{null}$ homozygous mothers were arrested at a very early stage, prior to the onset of the first embryonic mitoses (Figure 6, B and C). The most common mutant phenotype was an embryo with a polar body near one end and the development of one or two spindles in the middle. This phenotype was observed even when the embryos were 6–12 hr old, demonstrating that the embryonic arrest was rarely bypassed. The early embryonic arrest phenotype is also consistent with the description of the original *sub* alleles that were described as showing no visible signs of development (Schupbach and Wieschaus 1989).

The structure and number of spindles in the mutant embryos was variable, ranging from one normal-looking spindle to a low number of deformed and small spindles. A minority of the mutant embryos (~10%) had progressed beyond this early stage with a larger number of nuclei and spindles (Figure 6F). These late stage embryos had severely abnormal and variably sized mitotic figures. The variable size of the spindles in these mutants suggested that the spindles were forming around single chromosomes or severely aneuploid nuclei. Unlike the $sub^{null}$ mutants, in $sub^{137D}$ mutants the late stage abnormal embryos were the most common defect. Thus, the *P*-element insertion in $sub^{137D}$ may not completely eliminate gene function.

The sterility appears to be due to an embryonic defect because the abnormal $sub^{null}$ mutation meiotic spindles did not appear to be severe enough to produce inviable eggs and the female sterile phenotype. For comparison, *ncd* mutants have similar defects in meiotic spindle organization but are not sterile, while mutants in *Klp3A* do not have cytological defects during meiosis, but may have the same embryonic phenotype as *sub* (see discussion and Williams *et al.* 1997). The temperature-sensitive recessive lethal phenotype of the dominant allele $sub^{Dub}$ (Moore *et al.* 1994) is also consistent with a role for *sub* in mitotic cells.

Although $sub^{1794}$ females were fertile, it was still possible that the embryos had mitotic defects. Indeed, in early stage embryos from $sub^{1794}$ mothers, defects in spindle pole formation were common in addition to general disorganization of the spindle and of the chromosomes (Figure 6D). These effects were specific to early stage embryos, however, and later stage embryos appeared to develop normally (data not shown). The survival rate of embryos from $sub^{1794}$ mothers was determined by quantifying the fraction of eggs that produced viable

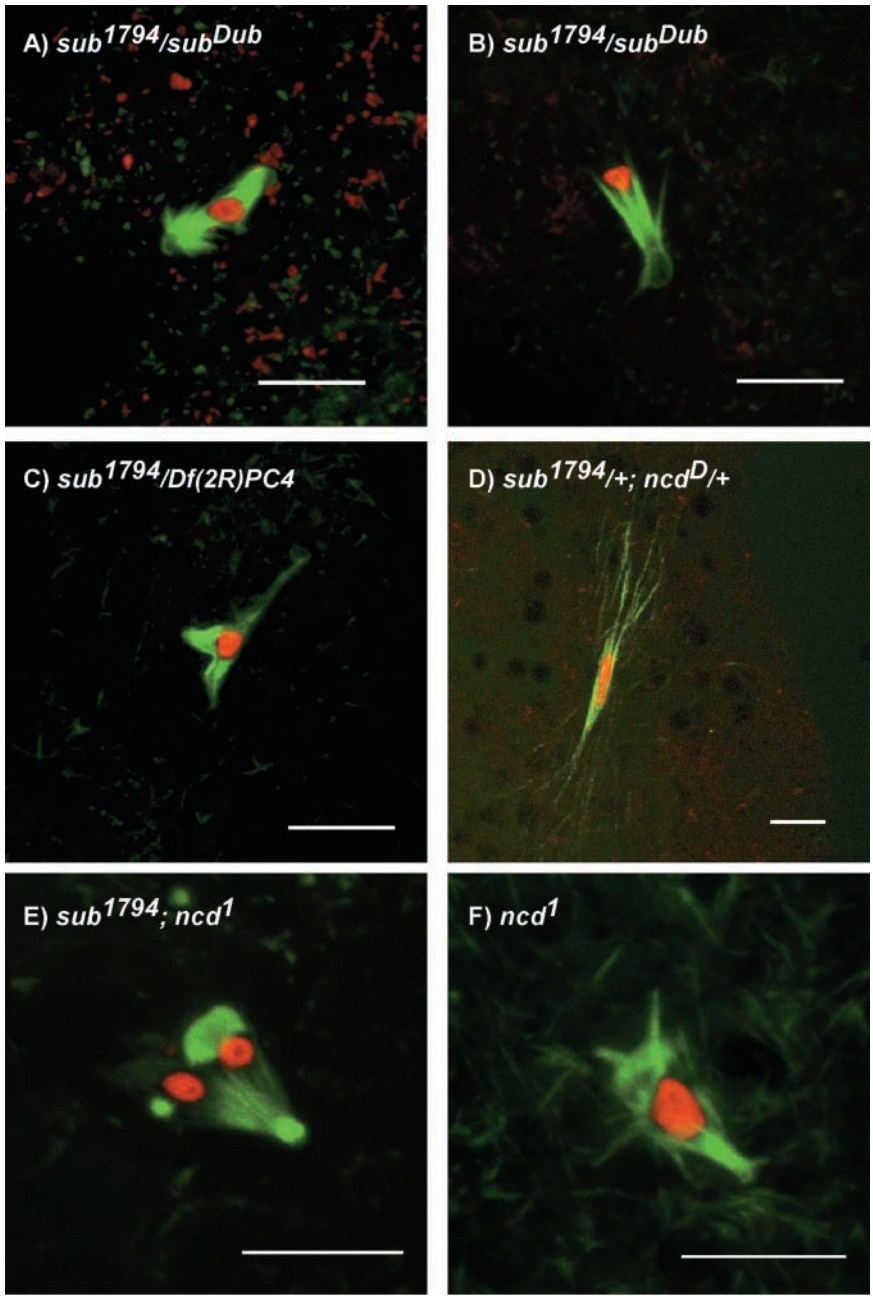


FIGURE 4.—Stage 14 oocytes from *sub*$^{1794}$ heterozygotes and double mutants. Bars, 10 μm. (A–F) The DNA is in red and the microtubules are in green. (A and B) *sub*$^{1794}$/*sub*$^{Dub}$ ; (C) *sub*$^{1794}$/*Df(2R)PC4*; (D) *sub*$^{1794}$/+; ncd$^{D}$/+; (E) *sub*$^{1794}$/*sub*$^{1794}$; *ncd*$^{1}$/*ncd*$^{1}$; (F) *ncd*$^{1}$/*ncd*$^{1}$.

larvae (MATERIALS AND METHODS). In controls, the embryo hatch rate was 92.6% ($n$ = 660) whereas from *sub*$^{1794}$ mothers the hatch rate was 37.3% ($n$ = 1091). This reduction, however, can be accounted for entirely by the aneuploidy caused by nondisjunction at meiosis I (BAKER and HALL 1976; MCKIM *et al.* 1993). Therefore, we suspect the embryos fully recover from the early mitotic abnormalities.

## DISCUSSION

**Genes required for bipolar spindle pole formation and homolog segregation in female meiosis:** The *sub* genetic and cytological mutant phenotypes are similar to those previously described for mutants in *ncd* (HATSUMI and ENDOW 1992; MATTHIES *et al.* 1996), which also encodes a kinesin-like protein. Most importantly, both mutants cause nondisjunction of homologous chromosomes at the first meiotic division but have no effect on the second meiotic division. On the basis of a live analysis, MATTHIES *et al.* (1996) proposed that NCD was required in the acentrosomal spindle to taper the microtubules into a pole with its minus-end-directed motor moving outward from the chromosomes, bundling together microtubules in the process. They also proposed that at least one additional motor was involved in the process because poles could still form in the absence of NCD. Thus, one possible function of SUB is to bundle microtubules to form that portion of the poles that is not handled by NCD. This model predicts

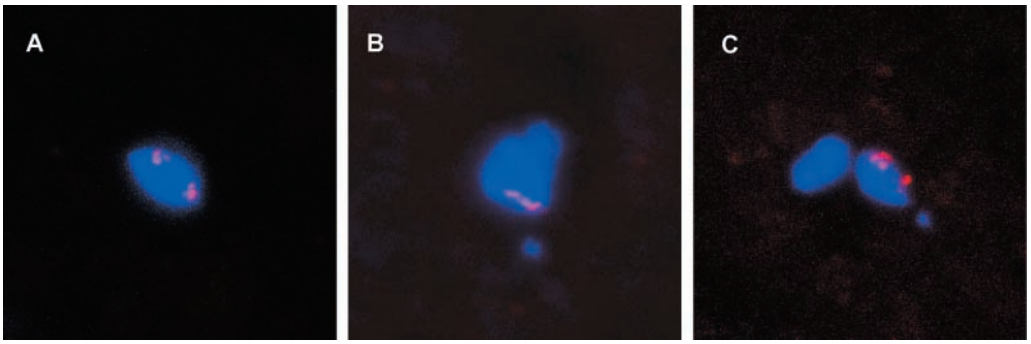


FIGURE 5.—FISH analysis of $sub^{1794}/Df(2R)PC4$ stage 14 oocytes. Each volume projection shows DNA in blue and a probe to the second chromosome centromeric heterochromatin in red. (A) In wild type the centromeres are oriented toward the poles. In $sub^{1794}/Df(2R)PC4$ the centromeres do not have a bipolar orientation (B) and when the karyosome is split (C) the centromeres remain associated, indicating that the chiasmata have not dissolved and that the cell has not entered anaphase.

that a *sub; ncd* double mutant would have a more severe defect in spindle pole formation than would either single mutant. Our double mutant analysis showed this not to be the case; the double mutant was able to make spindle poles with a similar array of defects as the single mutants. Therefore, we conclude that both *ncd* and *sub* are involved in the same process of spindle formation.

Mutants in genes encoding kinesin-like proteins are not the only ones to have defects in female meiotic spindle pole formation. Mutations in *mini spindles* (*msps*) and *transforming acidic coiled-coil protein* (*d-tacc*) also have meiotic phenotypes similar to *sub* and *ncd,* including a multipolar spindle phenotype (CULLEN and OHKURA 2001). MSPS and d-TACC localize to female meiotic spindle poles and there appears to be an interaction between these proteins and the motors because in *ncd* mutants the MSPS pattern is disrupted. Indeed, CULLEN and OHKURA (2001) proposed that NCD transports MSPS to the minus ends of microtubules where it stabilizes the spindle poles. The common mutant phenotype of these genes reveals a group of potentially interacting proteins with a fairly specific function. This function is not to make spindle poles *per se,* but to either organize or stabilize the poles such that there are only two of them, thus facilitating formation of a bipolar spindle. The failure of this system causes chiasmate and achiasmate chromosomes to nondisjoin at a high frequency. The observation that achiasmate chromosomes are more sensitive to this defect may be due to their sole dependence on the spindle for both orientation and segregation. In contrast, chiasmate chromosomes may properly orient in *sub* mutants, but are not able to segregate properly.

**The nature of the dominant *sub* allele:** The $sub^{Dub}$ mutation changes a highly conserved amino acid in the motor domain. The original study of the dominant $sub^{Dub}$ mutation did not distinguish between an antimorph or neomorph (MOORE *et al.* 1994). From our analysis the $sub^{Dub}$ meiotic phenotypes are almost identical to the null alleles, arguing that it is an antimorph. The kinesin motor *nod* also has a dominant antimorphic allele ($nod^{DTW}$) that is associated with a single amino acid change in a highly conserved region of the ATP-binding domain (RASOOLY *et al.* 1991). Similar to $sub^{Dub}$, $nod^{DTW}$ dominantly affects chiasmate and achiasmate chromosomes. Both the $sub^{Dub}$ and $nod^{DTW}$ proteins could have altered microtubule-binding activities that lead to interference with other proteins on the meiotic spindle. Both dominant mutations also cause lethality due to mitotic defects, and this phenotype is lessened by the presence of wild-type gene activity, suggesting that both *sub* and *nod* gene products interact with the mitotic spindle. Our observation that $ncd^{1}$ and $sub^{null}$ alleles are synthetically lethal also argues that *sub* has a function in mitotic cells.

**The role of SUB during spindle formation of female meiosis:** The Drosophila female meiotic spindle must organize in the absence of centrosomes and segregate homologs at the reductional division. Genes likes *sub* that are not required for the typical mitotic division or meiosis II may be required for these unique properties of the meiotic spindle. The simplest hypothesis is that SUB is a kinesin that interacts with spindle microtubules. This is supported by the specific genetic interactions with the $ncd^{D}$ and *nod* mutations. Interestingly, the SUB homologs MKLP1 and PAV have been shown to localize at centrosomes in mitotic metaphase (NISLOW *et al.* 1992; ADAMS *et al.* 1998). In addition, MKLP1 is known to bundle microtubules and be a plus-end-directed motor (NISLOW *et al.* 1992). Indeed, most of the members in the MKLP1 group have this property, although one, RB6K, has been reported to associate with the Golgi (ECHARD *et al.* 1998). Although a more recent report demonstrates that RB6K has an important role in cytokinesis (FONTIJN *et al.* 2001), we cannot rule out an indirect role for SUB in spindle formation.

On the basis of the ideas of THEURKAUF and HAWLEY (1992) and WALCZAK *et al.* (1998), spindle assembly in the absence of centrosomes can be divided into four

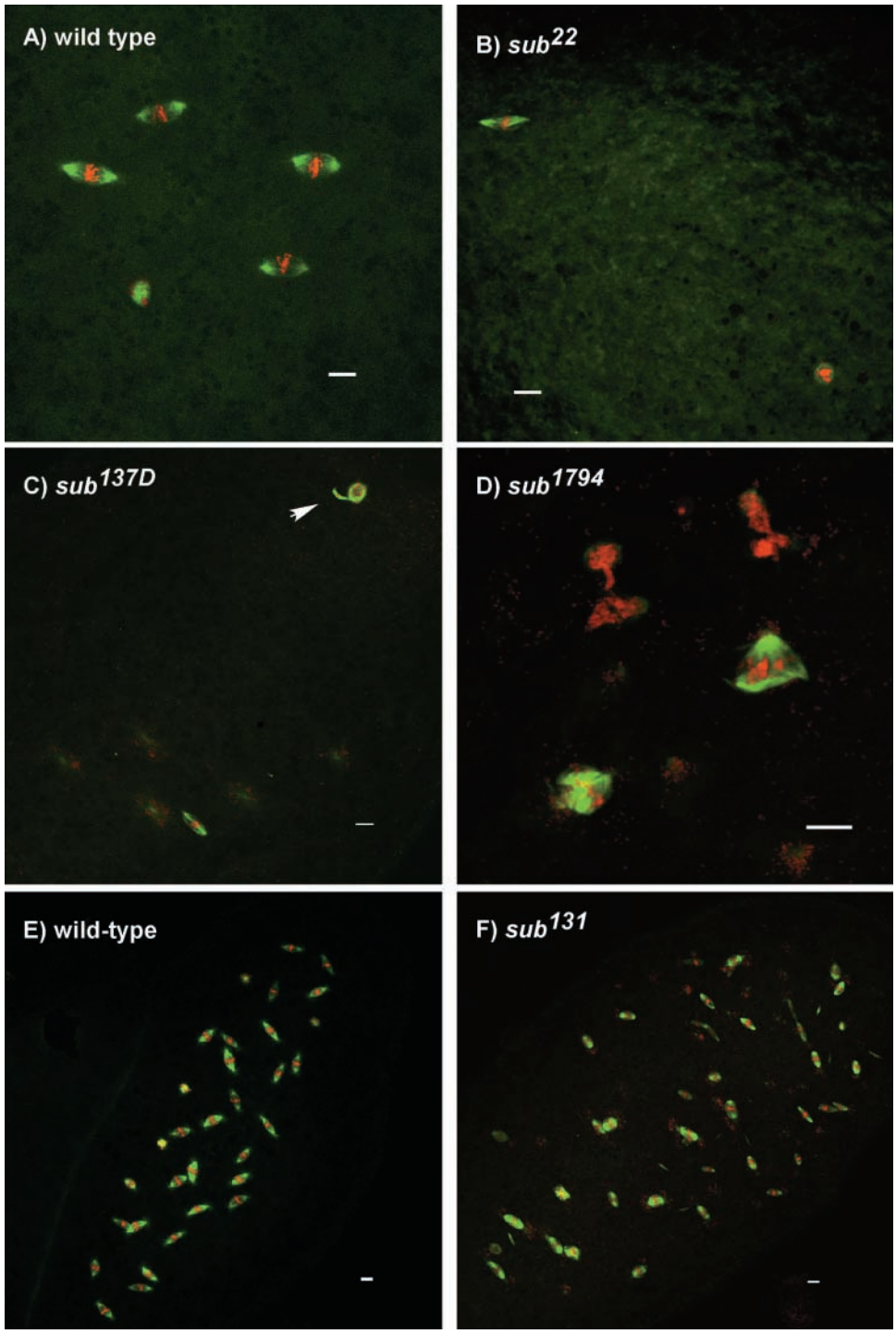


Figure 6.—Mitosis in embryos from *sub* homozygous and wild-type mothers. The DNA is in red and the microtubules are in green. Embryos were collected at 2- (wild-type) or 3-hr (mutant) intervals. Bars, 10 μm. (A) Early stage wild-type embryo with four spindles and a polar body. (B) An arrested embryo from a $sub^{22}$ mother with relatively normal looking spindle and polar body. This was a common phenotype in the mutants, but rare in wild type. (C) An embryo from a $sub^{137D}$ mother with an abnormally small spindle and a polar body with a spur of microtubules (arrow). In the same vicinity of the main spindle near the bottom of the frame, several short spindles are surrounded by fragments of chromosomal material. These were often observed in $sub^{null}$ mutants. These fragments stained with either propidium iodide or DAPI, showing they were probably chromosomal in origin. (D) An early embryo from a $sub^{1794}$ mother with abnormal spindles. (E) Late stage wild-type embryo. (F) Late stage embryo from $sub^{131}$ mother. Embryos at this late stage of development are rare (~10%) in most mutants but are the most common defect in $sub^{137D}$.

stages. The first stage is the nucleation or capture of microtubules by the chromosomes. Second, the microtubules are bundled together by proteins that can form bridges between parallel and/or antiparallel microtubules. The third step is extension of the spindle by antiparallel microtubule sliding or a "polar ejection force" exerted by motors associated with the chromosomes. Finally, the minus-ends of the microtubules are focused to produce defined spindle poles. Although these stages probably overlap and share genetic requirements, the function of SUB appears to be most important for the last stage of bipolar spindle formation. In *sub* mutants, microtubule arrays of wild-type length are able to form, but they fail to be focused into only two poles. It has been proposed that an inherent product of the microtubule bundling process is the formation of a single axis and therefore at least a crude bipolar spindle (Heald *et al.* 1996). A relationship between maintaining the integrity of the poles and constructing a spindle with only two poles would explain why in *sub*

mutants the spindle poles are often frayed and/or they are tripolar or monopolar.

One aspect of the *sub* mutation phenotype could be the inability to generate poleward forces. In *sub* mutants the position of the chromosomes within the karyosome is abnormal. Thus, SUB could facilitate interactions between the chromosomes and microtubules that are part of the process that organizes meiotic spindles. As was argued from an analysis of α-*Tubulin67C* mutants (Matthies *et al.* 1999) defects in *sub* could result in a disruption of poleward forces, leading to a failure in centromere positioning. This would provide a link between the chromosomes and spindle pole organization, which is plausible considering that the chromosomes have a role in organizing the spindle (McKim and Hawley 1995).

**SUB is required for male meiosis and mitosis:** In addition to female spindle formation, *sub* is required for at least two other cell divisions: male meiosis and the early embryonic cleavage divisions. There are significant differences between the Drosophila male and female meiotic divisions; for example, in male meiosis crossing over does not occur and centrosomes are present. In both male and female meiosis, however, there is a reductional division involving the segregation of homologs. Thus, the importance of SUB may not be for spindle pole formation, but to organize a spindle where bivalents must be oriented and segregated. It is noteworthy that the *sub* alleles are the only Drosophila mutants that are defective at the first meiotic division of both males and females without affecting the segregation of sister chromatids (Moore *et al.* 1994).

The null alleles of *sub* are female sterile due to a failure in the early embryonic cell divisions. The early defects in *sub* mutation embryos have some similarities to mutants in other genes with a variety of roles in spindle function such as *Klp3A* (Williams *et al.* 1997), α-*Tubulin67C* (Matthews *et al.* 1993), *polo* (Riparbelli *et al.* 2000), and *wispy* (Brent *et al.* 2000). In all these cases, it was suggested that the embryonic arrest was due to a defect in pronuclear migration, although the variety of defects observed in *sub* and the other mutants make it difficult to define a precise function for these proteins. In addition, pronuclear fusion may be a sensitive point for a wide variety of defects in microtubule-based processes, leading to the arrest prior to pronuclear fusion in many different mutants. It will be interesting to determine the nature of the *sub* function that is required for reductional meiotic divisions and an early event in embryogenesis.

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