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Mutants of the *Drosophila* ncd microtubule motor protein cause centrosomal and spindle pole defects in mitosis

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SUMMARY

Nonclaret disjunctional (ncd) is a kinesin-related microtubule motor protein required for meiotic and early mitotic chromosome distribution in *Drosophila*. ncd translocates on microtubules with the opposite polarity to kinesin, toward microtubule minus ends, and is associated with spindles in chromosome/spindle preparations. Here we report a new mutant of ncd caused by partial deletion of the predicted coiled-coil central stalk. The mutant protein exhibits a velocity of translocation and ability to generate torque in motility assays comparable to near full-length ncd, but only partially rescues a null mutant for chromosome mis-segregation. Antibody staining experiments show that the partial loss-of-function and null mutants cause centrosomal and spindle pole defects, including centrosome splitting and loss of centrosomes from spindle poles, and localize ncd to centrosomes as well as spindles of wild-type embryos. Association of ncd with spindles and centrosomes is microtubule- and cell cycle-dependent: inhibition of microtubule assembly with colchicine abolishes ncd staining and centrosomal staining is observed in prometaphase, metaphase and anaphase, but diminishes in late anaphase/telophase. The cell cycle dependence of centrosomal staining and the defects of mutants provide clear evidence for activity of the ncd motor protein near or at the spindle poles in mitosis. The ncd motor may interact with centrosomal microtubules and spindle fibers to attach centrosomes to spindle poles, and mediate poleward translocation (flux) of kinetochore fibers, a process that may underlie poleward movement of chromosomes in mitosis. Together with previous work, our findings indicate that ncd is important in maintaining spindle poles in mitosis as well as in meiosis.

Key words: microtubule motor protein, centrosomal/spindle pole defects, spindle pole function

INTRODUCTION

Members of the expanding kinesin family of microtubule motor proteins are involved in diverse microtubule-related functions in the cell (Endow and Titus, 1992; Goldstein, 1991). Several kinesin proteins are implicated in meiotic and mitotic chromosome distribution (Sawin and Endow, 1993). The specific roles that each protein performs are presently being determined. The nonclaret disjunctional (ncd) microtubule motor protein is a kinesin family member (Endow et al., 1990; McDonald and Goldstein, 1990) required for proper chromosome segregation in *Drosophila* oocytes and early embryos (Endow et al., 1990). Mutants of ncd cause gametic and early zygotic chromosome nondisjunction and loss, resulting in high levels of embryonic lethality and aneuploidy (Sturtevant, 1929). The ncd motor protein translocates toward microtubule minus ends (Walker et al., 1990; McDonald et al., 1990), in contrast to the plus-end-directed kinesin, and is associated with meiotic and mitotic spindles in chromosome/spindle preparations (Hatsumi and Endow, 1992a).

Although spindle assembly and chromosome attachment to spindles in meiosis and mitosis are often considered to be similar processes, several features of the meiotic and mitotic spindle are known to differ strikingly from one another (Rieder et al., 1993; Sawin and Endow, 1993). Meiotic spindles in oocytes of several organisms, including *Drosophila*, *Xenopus* and the mouse, lack centrioles and astral microtubules, structures that are usually present at spindle poles in animal cells. Meiotic spindles of *Drosophila* and *Xenopus* oocytes consist of short microtubules that associate with the chromosomes prior to spindle formation (Hatsumi and Endow, 1992b; Theurkauf and Hawley, 1992; Gard, 1992), and the spindles that form differ in shape from mitotic spindles: the meiotic spindles are long and angular compared with the barrel-shaped mitotic spindles (Hatsumi and Endow, 1992b; Theurkauf and Hawley, 1992). Meiotic spindle assembly in *Drosophila* and *Xenopus* oocytes is thus unlikely to occur by the pathways that have been described for spindle assembly in *Xenopus* egg extracts (Sawin and Mitchison, 1991). The short microtubules that comprise the *Drosophila* and *Xenopus* oocyte meiotic spindles are assumed to be oriented with minus ends toward the poles, as has been found for microtubules in the mitotic spindles that have examined. The polarity of the short microtubules has not been determined, however, and requires experimental confirmation.

The observation of wide, diffuse or multipolar meiosis I

spindles in oocytes of *ncd* null mutants (Wald, 1936; Kimble and Church, 1983) has led to the idea that the primary defect is the oocyte microtubule organizing center (Kimble and Church, 1983). Localization of ncd to meiotic spindles from *Drosophila* oocytes (Hatsumi and Endow, 1992a) and the minus-end polarity of ncd movement on microtubules resulted in the proposal that the ncd motor protein functions to focus microtubule ends into spindle poles in meiosis I (Hatsumi and Endow, 1992a). This proposal was based on the observation that microtubules in *Xenopus* egg extracts form asterlike arrays in the presence of the minus-end-directed microtubule motor protein, cytoplasmic dynein (Verde et al., 1991), and is consistent with the defects observed in oocytes of *ncd* null mutants (Wald, 1936; Kimble and Church, 1983; Hatsumi and Endow, 1992b). The ncd motor protein is proposed to carry out a role in spindle pole organization in meiosis that is attributed to centrosomes in mitotic cells.

The ncd motor functions in mitosis as well as meiosis, based on the observation of frequent mitotic chromosome loss in embryos of the null mutant (Sturtevant, 1929; Lewis and Gencarella, 1952). The effects of ncd on chromosome segregation in meiosis and mitosis are separated by the mutant, ncd^D. Oocytes of ncd^D/ncd^D females exhibit defective meiotic chromosome segregation, but embryos of ncd^D/ncd^D females are near wild-type for mitotic segregation (Komma et al., 1991). Two amino acids are changed in ncd^D compared with ncd (Komma et al., 1991). One, D→S at residue 696, has also been found in a cDNA from an ncd^+ strain (McDonald and Goldstein, 1990) and therefore probably represents a polymorphism. The second change, V→F at residue 556, is apparently the cause of the ncd^D mutant effect - it occurs in a region of ncd^D that corresponds to the proposed microtubule-binding region of kinesin heavy chain (Yang et al., 1989). The mutant effect of ncd^D on meiotic, but not mitotic, chromosome segregation indicates that the ncd^D protein can overcome chromosome mis-segregation that occurs in the previous meiotic divisions. The partial loss-of-function ncd^D motor may promote capture of chromosomes by the spindle, performing a function in mitosis proposed for wild-type ncd of facilitating chromosome attachment and poleward movement of attached chromosomes (Hatsumi and Endow, 1992b). Different tubulin or microtubule-associated proteins must comprise or be associated with the meiotic and mitotic spindles to account for the mutant meiotic, but near wild-type mitotic, phenotype of ncd^D and its molecular change.

We now report a partial loss-of-function mutant that is caused by deletion of part of the ncd stalk region. The mutant shows incomplete rescue of the null mutant for chromosome mis-segregation. The partial loss-of-function and null mutants both show centrosomal and spindle pole defects in early embryonic mitotic divisions, including splitting of centrosomes and dissociation of centrosomes from poles. Localization of ncd in whole-mount *Drosophila* embryos using indirect immunofluorescence reveals staining not only of mitotic spindles, but also of centrosomes. Association of ncd with spindles and centrosomes of early mitotic spindles is microtubule- and cell cycle-dependent: embryos treated with colchicine to depolymerize microtubules show no spindle or centrosomal staining by anti-ncd antibody and centrosomal staining is observed in prometaphase, metaphase and anaphase, but diminishes in late anaphase/telophase. The cell cycle-dependent centrosomal staining and disruption of normal spindle pole structure in mutants indicate that ncd is active near or at the poles of early mitotic spindles, and that the motor functions to maintain spindle poles. Maintenance of spindle poles in mitosis is consistent with the previously recognized role of the protein in establishing and maintaining spindle bipolarity in meiosis (Hatsumi and Endow, 1992a).

MATERIALS AND METHODS

Enzymes and reagents

Restriction endonucleases and the Klenow fragment of DNA polymerase I were purchased from New England Biolabs, Inc. (Beverly, MA). AmpliTaq polymerase was from Cetus Corp. (Berkeley, CA). Sequenase polymerase, premixed dideoxynucleotides for DNA sequence analysis, and dioxane-free IPTG (isopropyl-β-D-thiogalactopyranoside) were from United States Biochemical (Cleveland, OH). G418 was obtained from Gibco (Chagrin Falls, OH) and DAPI (4′,6-diamidino-2-phenylindole) was from Boehringer Mannheim Corp. (Indianapolis, IN). Oligonucleotides were synthesized by A. Shore (Department of Botany, Duke University).

Antibodies and sera

Affinity-purified antibody directed against the N terminus of ncd (no. 6267) was prepared as described previously (Hatsumi and Endow, 1992a). Anti-Dmap190 antiserum (Raff et al., 1993), directed against the Dmap190 centrosomal protein (Kellogg and Alberts, 1992; Raff et al., 1993), and rhodamine-conjugated monoclonal anti-α-tubulin antibody were gifts from J. Raff and W. Theurkauf, respectively. Dmap190 has been reported previously as Bx63 (Frasch et al., 1986; Whitfield et al., 1988). Monoclonal anti-histone antibody was obtained from Chemicon International (Temecula, CA). FITC-conjugated anti-rabbit and anti-mouse IgG were purchased from Vector Lab. (Burlington, CA). Fetal calf serum (FCS) and normal mouse serum were obtained from Sigma Chem. Co. (St Louis, MO).

Construction of MC2

A plasmid encoding the partial loss-of-function mutant protein, MC2 (Modified Claret 2), was constructed by digesting the previously reported pET/N2 plasmid (Chandra et al., 1993) with *Bam*HI, repairing the ends with the Klenow fragment of DNA polymerase I, and digesting the repaired DNA with *Sac*II. A PCR product made using the primers 5′ TC CCC GCG GCC CAG AGC GAC AAC 3′ and 5′ TGC ATT CTT TAT TTA TCG 3′ was digested with *Sac*II and ligated to the *Sac*II-digested pET/N2 DNA. The gene was expressed in bacteria for motility assays (Chandra and Endow, 1993) and transferred into the $G418^R$ P element vector, pUChsneo (Steller and Pirrotta, 1985), for germline transformation of *Drosophila* embryos. The P element construct, pUC/MC2, is missing two introns present in the wild-type *ncd* gene (Endow et al., 1990) and is under the regulation of the wild-type *ncd* promoter. The MC2 coding region and restriction enzyme junctions used for pET/MC2 and pUC/MC2 plasmid construction were sequenced to determine if any nucleotides had been changed during the PCR or transfer of the PCR fragment into plasmids. Sequence analysis revealed four new amino acids (VPAA) present in-frame at the *Sac*II junction. No other sequence changes were found in the PCR-amplified region, restriction enzyme junctions, or remaining *ncd* coding region in pUC/MC2.

Genetic tests for chromosome disjunction

Tests of X chromosome segregation were carried out by mating wild-type or *ncd* mutant females to y^2w^{bf}/B^SY males. Nondisjunction in oocyte meiosis results in X,X or nullo-X eggs, instead of normal eggs with haplo-X chromosome complements. After fertilization by Y- or X-bearing sperm, nondisjunctional eggs develop into X/X/Y (B^S) female or X/0 (y w) male offspring, instead of regular X/X (+/y w)

female and X/Y (+/B^S) male offspring. Nullo-X eggs can also arise by X chromosome loss; the excess of X/0 males over X/X/Y females is therefore attributed to meiotic loss of the X (Sturtevant, 1929). Gynandromorphs, somatic mosaics of the X chromosome that contain patches of X/0 (y w) male tissue in a X/X (+/y w) female background, arise upon loss of the maternal X chromosome in early mitosis. Loss of the paternal X or Y chromosome occurs with higher frequency in oocytes and embryos of *ncd* mutants, compared with wild-type (Yamamoto et al., 1989; Nelson and Szauter, 1992), and results in X/0 (+) males that carry a maternal X chromosome and gynandromorphs with patches of X/0 (+) tissue in an X/X (+/y w) or X/Y (+/B^S) background. Offspring that showed loss or nondisjunction of chromosome 4 and exhibited a Minute phenotype, either coincident with, or independently of, X chromosome nondisjunction or loss, were scored but were excluded from calculations due to variability in recovery. To adjust for loss of half of the nondisjunctional and nullo-X gametes as nonviable X/X/X and 0/Y embryos, the number of X/X/Y and X/0 individuals recovered was multiplied by 2 in calculations of the total gametes, and gametic X chromosome nondisjunction and loss.

Tests of chromosome 4 segregation were carried out by mating wild-type or mutant females homozygous for a recessive chromosome 4 marker (*spa^{pol}*) to males carrying a dominantly marked (*ci ey^R*) compound chromosome 4. Nondisjunction of chromosome 4 in oocytes results in *spa^{pol}/spa^{pol}* and nullo-4 eggs, that develop into spa^{pol}/spa^{pol} and ci ey^R offspring after fertilization by nullo-4 or *ci ey^R* sperm. Half of the nondisjunctional offspring die as tetra-4 or nullo-4 embryos or larvae. Regular offspring are triplo-4 (*spa^{pol}/ci ey^R*) and haplo-4 (*spa^{pol}/0*). Haplo-4 offspring were omitted from calculations of chromosome 4 nondisjunction and loss due to their variable recovery, as above.

Viability tests were carried out by setting up ten single pair matings for each cross on day 1, using 3-day-old virgin females. Mating pairs were transferred to new vials on days 2-7 and discarded on day 8. Eggs were counted daily by inspection of the culture medium surface using a stereomicroscope, and egg counts for each vial were recorded. Offspring from each vial were scored for chromosome nondisjunction and loss, as described above. Viability was calculated as [total adults (including haplo-4 offspring)]/(total embryos).

Motility assays

MC2 protein for motility assays was prepared as described previously (Chandra et al., 1993; Chandra and Endow, 1993) from cells induced for 3 hours at 22°C. Briefly, frozen cells were thawed and lysed, and induced protein was concentrated from the 27,000 ***g*** supernatant by addition of ammonium sulfate to 40% of saturation, followed by incubation on ice with occasional mixing. Precipitated protein was recovered by centrifugation, solubilized in PB (10 mM $NaPO_4$, pH 7.4, 1 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT), dialyzed against PB, and stored at −70°C.

Motility assays were performed as described previously (Walker et al., 1990; Chandra et al., 1993) by mixing sequentially 5 μl of MC2 protein in PB, 1.6 μl of taxol-stabilized microtubules (tubulin concentration, 1 mg/ml; Mg^{2+}-GTP, 1 mM), 0.9 μl of 50 mM Mg^{2+}-ATP, and 1.5 μl of 6× salts solution (70 mM PIPES, pH 6.9, 480 mM NaCl, 1.4 mM EGTA, 0.7 mM $MgSO_4$, 30 mM $MgCl_2$, 4.8 mM DTT) on a clean glass coverslip. The coverslip was sealed onto a glass slide and movement was observed using video-enhanced differential interference microscopy (VE-DIC) (Walker et al., 1988). Flow chambers were used in some experiments instead of coverslips sealed to slides. Chambers were precoated with tubulin (Howard et al., 1989) before flowing in 5 μl MC2 protein + 1 μl 24 mM cytochrome *c* (Howard et al., 1989), followed by a mix consisting of 0.9 μl 50 mM Mg^{2+}-ATP, 1.6 μl of microtubules, 1.5 μl of 6× salts, and 5 μl of water. Polarity of MC2 movement and rotation of microtubules gliding on MC2 were determined using microtubules grown asymmetrically from the plus ends of sea urchin flagellar axonemes (Vale and Toyoshima, 1988; Walker et al., 1990; Hyman et al., 1991). Velocity of microtubule movement was determined using a mouse-driven video cursor overlaid on images played back from videotapes, as described previously (Walker et al., 1988).

Antibody staining experiments

MC2/MC2; ca^{nd}/ca^{nd} embryos were collected, dechorionated and devitelligenized as described previously (Hatsumi and Endow, 1992b), fixed with formaldehyde (Karr and Alberts, 1986) or methanol/EGTA (Hatsumi and Endow, 1992b), and stained with antibody (Hatsumi and Endow, 1992b). Embryos of *ca^{nd}/ca^{nd}* and wild-type (Oregon R) females were also prepared and examined. In some experiments, Oregon R embryos were treated with 0.5 mg/ml colchicine in Robb's *Drosophila* saline for 20 minutes at 22°C prior to fixation to depolymerize microtubules, as described (Raff et al., 1993). The anti-ncd antibody used in these studies was the previously reported anti-N terminus antibody (Hatsumi and Endow, 1992a), directed against the nonconserved 'tail' region of ncd. Following affinity purification, the antibody recognizes a single prominent band on a western blot of protein from a *Drosophila* cultured cell line, Schneider's line 2 (Hatsumi and Endow, 1992a). Schneider's line 2 is an embryo-derived line that expresses ncd protein. Affinity-purified antibody was diluted 1:3 or 1:5 with TBST (50 mM Tris-HCl, pH 7.3, 50 mM NaCl, 0.1% Triton X-100) containing 10% FCS (v/v) before use. Anti-centrosomal antiserum (anti-Dmap190) was used at a dilution of 1:400. Detection of the primary anti-ncd or anti-Dmap190 antibody was with FITC- or Texas Red-conjugated anti-rabbit IgG used at dilutions of 1:150 or 1:500, respectively. DAPI was added to one of the final washes at a concentration of 5 μg/ml to stain chromosomal DNA. In some experiments, embryos were incubated with monoclonal anti-histone antibody (1:500 dilution) to stain chromosomes, followed by incubation with FITC-conjugated anti-mouse IgG (1:250 dilution). Spindles were stained using a rhodamine-conjugated anti-α-tubulin monoclonal antibody at 1:200 dilution. Embryos stained with anti-histone antibody and FITC-conjugated anti-mouse IgG were incubated for >2 hours in TBST containing 10% normal mouse serum (v/v) prior to reaction with rhodamine-conjugated anti-α-tubulin monoclonal antibody. Embryos stained with anti-Dmap190 antiserum and FITC-conjugated anti-rabbit IgG were incubated for >2 hours in TBST containing 10% preimmune rabbit serum (v/v) prior to reaction with anti-ncd antibody, followed by Texas Red-conjugated anti-rabbit IgG.

Coverslips containing pelleted spindles and associated chromosomes were prepared from lysates of early embryos and stained with anti-ncd and anti-tubulin antibodies, as described previously (Hatsumi and Endow, 1992a). Embryos of *MC2/MC2;ca^{nd}/ca^{nd}* females, *ca^{nd}/ca^{nd}* females and wild-type (Oregon R) females were used to prepare chromosome/spindle coverslips.

Images were collected using a Bio-Rad MRC 600 scanning confocal imaging system attached to a Zeiss Axiophot microscope. A 63X/1.4 NA Planapochromat objective was used to collect images and image processing was performed using the Bio-Rad Som and Comos software. Reproductions were made by photographing images from the monitor screen onto 35 mm Tri-X film.

RESULTS

Partial deletion of the stalk results in a partial loss-of-function *ncd* mutant

The mutant, *MC2 (Modified Claret 2)*, was constructed by deleting amino acids 183-247 corresponding to ~one-third of the predicted α-helical coiled-coil stalk region of ncd (Fig. 1). The *MC2* gene, cloned in a P element vector under the regulation of the *ncd* promoter, was injected into *Drosophila* embryos in order to recover germline transformants. A single

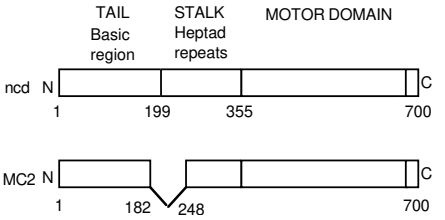


Fig. 1. The MC2 protein. The numbers refer to amino acid residues in the full-length ncd protein. MC2 lacks amino acids 183-247. The tail, stalk and motor domains of the ncd protein are described by Chandra et al. (1993).

transformant was obtained and put into stock. Offspring were mated to flies carrying the *Δ 2,3* P element transposase gene (Laski et al., 1986) on a dominantly marked chromosome in order to mobilize the *MC2* transgene and obtain further single insertion lines.

The insertion in the original *MC2* line was transferred into a ca^{nd} (*ncd* null mutant) background. The *MC2* transgene from the original stock was tested for rescue of the mis-segregation phenotype of the null mutant. The transgene showed dosage-dependent, partial rescue of the chromosome segregation defect of the ca^{nd} null mutant in tests of X chromosome segregation (Table 1). One copy of the *MC2* transgene resulted in an overall frequency of 0.237 X chromosome nondisjunction and loss, compared with 0.381 for the null mutant (Table 1). Two copies of the transgene resulted in a frequency of 0.100 X chromosome nondisjunction and loss (Table 1). Embryo viability was improved by one copy of the transgene, compared with the null mutant, and further improved by two copies (Table 1). Segregation test results, together with viability tests, thus indicate that two copies of the transgene rescues the null mutant to a greater degree than one copy, but that rescue of the null mutant is incomplete for females carrying either one or two copies of the transgene.

Chromosome 4 segregation tests also showed partial rescue of the ca^{nd} null mutant by one or two copies of the *MC2* transgene (not shown). In these tests, a dosage effect was not observed: mis-segregation of chromosome 4 was reduced to 0.152 by one copy of *MC2* and to 0.155 by two copies, compared with 0.604 in the null mutant. The absence of a dosage effect for chromosome 4 segregation may reflect the small size of the chromosome, rendering it sensitive to a small amount of active motor protein, but insensitive to an increased amount.

Tests of *MC2* in a wild-type background showed no effect of one or two copies of the transgene on distribution either of the X or chromosome 4. These results indicate that the MC2 protein shows no detectable interference with the wild-type ncd protein.

To determine if the partial rescue of the null mutant by *MC2* could be attributed to a position effect, a second insertion was examined. The original *MC2* transgene was mapped by in situ hybridization to 35F. A mobilization that inserted at 26A also showed partial rescue of ca^{nd} in *MC2* or *MC2/MC2* females. The original and mobilized *MC2* transgenes were analyzed by Southern blot hybridization to ensure that the transgenes and promoter regions were unaltered. DNA from both lines showed the restriction enzyme fragment patterns predicted for intact promoter and coding regions, based on the *pUC/MC2* fragment patterns. Because the *MC2* transgene lacks two introns that are present in the wild-type *ncd* gene, transformants carrying a full-length *ncd* cDNA regulated by the *ncd* promoter were recovered and tested to control for any effects due to the missing introns. The *ncd cDNA* transgene fully rescued the null mutant, yielding 2 y^+ males among 2,262 offspring of *ncd cDNA;*ca^{nd}/ca^{nd} females and 1 y^+ male among 1,998 offspring of *ncd cDNA/ncd cDNA;*ca^{nd}/ca^{nd} females. The frequency of mis-segregation for one or two copies of *ncd* cDNA (<0.001) is comparable to that observed for +/+ flies (Table 1).

The *MC2* mutant therefore shows a partial loss-of-function phenotype of chromosome nondisjunction and loss that cannot be attributed to insertional position of the transgene or inappropriate regulation due to deleted intervening sequences.

MC2 protein is expressed in transformants

Evidence based on genetic analysis, including partial rescue of the null mutant and the dosage effect for the X chromosome, indicates that the MC2 protein is expressed in transformants. We also carried out immunofluorescence experiments to demonstrate MC2 expression. The MC2 protein was not detected after incubating whole-mount *MC2/MC2;*ca^{nd}/ca^{nd} embryos with anti-ncd antibody; however, spindles in chromosome/spindle preparations of *MC2/MC2;*ca^{nd}/ca^{nd} embryos were stained by the antibody (Fig. 2), confirming that the MC2 protein is expressed in transformants. Control spindles prepared from embryos of the null mutant showed no staining by the anti-ncd antibody, while spindles of wild-type embryos were brightly stained.

MC2 shows microtubule motility in vitro comparable to wild-type ncd

MC2 was expressed in bacterial cells and tested for motility in vitro. Induced protein was concentrated by ammonium sulfate precipitation from a high-speed supernatant, followed by dialysis against low-salt buffer for use in motility assays. The MC2 protein translocated microtubules at a velocity of 6.3±0.4 μm min^{-1} ($x \pm$ s.d., n=31) in assays containing 5 mM Mg^{2+}-ATP, bundled microtubules, and supported rotation of micro-

Table 1. Partial rescue of the ca^{nd} null mutant by the *MC2* transgene

Female parent	Offspring						Total gametes	Gametic X ND	Gametic X loss	Mitotic X loss	Total* adults	Total embryos	Viability
	+ ♀	B^S ♀	B^S ♂	y w ♂	gyn	+ ♂							
ca^{nd}/ca^{nd}	52	3	57	27	9	3	181	0.066	0.265	0.050	246	1,463	0.168
MC2;ca^{nd}/ca^{nd}	240	8	202	54	14	2	582	0.055	0.158	0.024	665	2,784	0.239
MC2/MC2;ca^{nd}/ca^{nd}	527	8	468	34	27	2	1,108	0.029	0.047	0.024	1,159	1,898	0.611
+/+	603		467			1	1,071	<0.001	<0.001	<0.001	1,073	1,316	0.815

gyn, gynandromorph; ND, nondisjunction.
*Including haplo-4 (Minute) offspring.

tubule-axoneme complexes. Assays of MC2 protein in 1 mM or 2.5 mM Mg^{2+}-ATP resulted in somewhat lower velocities of microtubule translocation, presumably due to the presence of other ATPases in the unpurified protein. Microtubules were only partially attached to the surface in assays containing 7.5 mM Mg^{2+}-ATP and did not bind to the glass surface in 10 mM Mg^{2+}-ATP. The velocity of microtubule translocation by MC2 in 5 mM Mg^{2+}-ATP is comparable to the velocities observed for partially purified, near full-length ncd of 6 μm min^{-1} (Cole et al., 1992) and 8-10 μm min^{-1} (Chandra et al., 1993). MC2 thus shows microtubule motor activity in vitro comparable to near full-length ncd, including microtubule bundling and ability to generate torque, but shows a partial loss-of-function mutant phenotype in vivo.

MC2 and an *ncd* null mutant show centrosomal and spindle pole defects in mitosis

Embryos of *MC2/MC2;cand/cand* females were examined to determine the effect of *MC2* on spindle structure. *MC2/MC2;cand/cand* embryos, stained with anti-α-tubulin antibody to visualize spindles, showed curved or 'bent' spindles and metaphase/early anaphase spindles with split centrosomes at one or both spindle poles (Fig. 3). Centrosomal staining was confirmed by double immunofluorescence experiments with anti-tubulin antibody and anti-Dmap190 antiserum (Raff et al., 1993), directed against a centrosomal antigen (Frasch et al., 1986; Whitfield et al., 1988; Kellogg and Alberts, 1992; Raff et al., 1993). The split centrosomes observed in metaphase and early anaphase *MC2* spindles contrasts with wild-type spindles in which doubled centrosomes appear in late anaphase. Centrosomes in early *MC2/MC2;cand/cand* embryos were frequently displaced from spindle poles, missing from one or both spindle poles, or free in the cytoplasm. Split centrosomes and dissociation of centrosomes from spindle poles were observed in *MC2/MC2;cand/cand* embryos as early as metaphase of cycle 1 (Fig. 3).

Embryos of the *cand* null mutant were also examined using immunofluorescence experiments. Early mitotic spindles in embryos of *cand/cand* mutant females were frequently curved or bent, like those of *MC2/MC2;cand/cand* embryos, with metaphase and early anaphase spindles showing split centrosomes at one or both spindle poles (Fig. 3). Centrosomes in early embryos of *cand/cand* were often observed separated from spindle poles and free in the cytoplasm, as in *MC2/MC2;cand/cand* embryos. The centrosomal defects were observed in early mitotic divisions, including cycle 1, of embryos of the null mutant.

ncd localizes to centrosomes and spindles of wild-type embryos

ncd was localized in whole-mount wild-type embryos using an antibody specific for the N-terminal tail of the protein (Hatsumi and Endow, 1992a). Double immunofluorescence experiments with anti-α-tubulin antibody were carried out. Embryos showed staining of spindles by both antibodies throughout early cleavage divisions, as late as cycle 12-13, with the anti-ncd antibody staining closely paralleling that of the anti-tubulin antibody. Stage 14 embryos were stained by anti-tubulin antibody, but anti-ncd antibody staining was greatly diminished. The maternally expressed ncd protein therefore persists in the early embryo until cellular blastoderm.

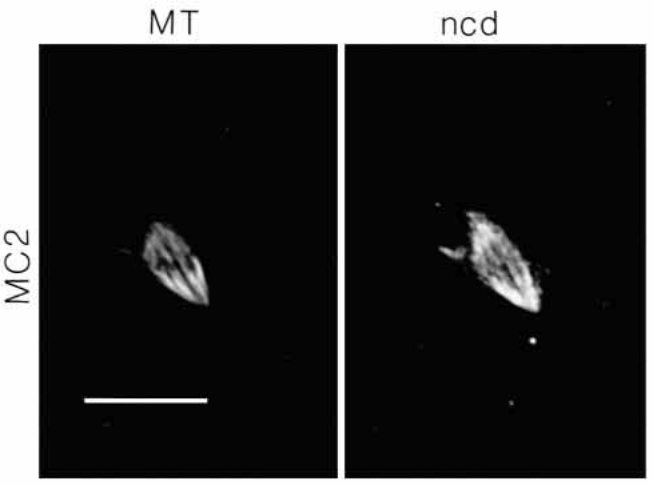


Fig. 2. MC2 expression in early embryos. Spindles of *MC2/MC2;cand/cand* embryos, prepared as described previously (Hatsumi and Endow, 1992a), were stained with anti-α-tubulin (MT) and anti-ncd (ncd) antibody. The spindle shows staining by both antibodies, indicating that MC2 protein is expressed in transgenic flies. Centrosomes are not present at the spindle poles, due to loss from the spindle during the homogenization or centrifugation step of the preparation procedure. Spindles prepared from embryos of *cand/cand* females were stained by anti-tubulin antibody but showed no staining by the anti-ncd antibody. Bar, 10 μm.

The anti-ncd and anti-tubulin antibodies stained prometaphase, metaphase and anaphase mitotic spindles, including centrosomes, of early wild-type embryos (Fig. 4). Both the anti-ncd and the anti-tubulin antibody staining were concentrated at the spindle poles and centrosomes in prometaphase spindles, and distributed throughout the spindle and centrosomes in metaphase and anaphase. Embryos in late anaphase/telophase showed bright staining of the spindle midbody by anti-ncd antibody, but faintly stained or unstained centrosomes, although both the spindle midbody and centrosomes were visibly stained by anti-tubulin antibody. Centrosomal staining was confirmed by double immunofluorescence experiments with anti-tubulin antibody and anti-centrosomal (anti-Dmap190) antiserum (not shown). Control embryos of *cand/cand* null mutant females showed only nonspecific staining by the anti-ncd antibody.

The ncd motor protein is therefore present on spindle fibers and associated with centrosomes in early mitotic spindles, but localization of ncd to centrosomes is dependent on the stage of the cell cycle: ncd is associated with centrosomes in prometaphase of early mitotic divisions, remains centrosome-associated in metaphase and anaphase, and dissociates in late anaphase/telophase, around the time of centrosome doubling in wild-type spindles.

Embryos treated with 0.5 mg/ml colchicine for 20 minutes at room temperature prior to fixation were stained with anti-ncd and anti-tubulin antibody, or anti-ncd antibody and anti-centrosomal (anti-Dmap190) antiserum. Colchicine-treated embryos showed no spindle or centrosomal staining by either anti-ncd or anti-tubulin antibody, although centrosomes of treated embryos were stained by anti-Dmap190 antiserum (Fig. 5). DNA staining showed condensed chromosomes near the centrosomes (not shown), indicating that the colchicine treatment blocked embryo divisions in metaphase. Staining of

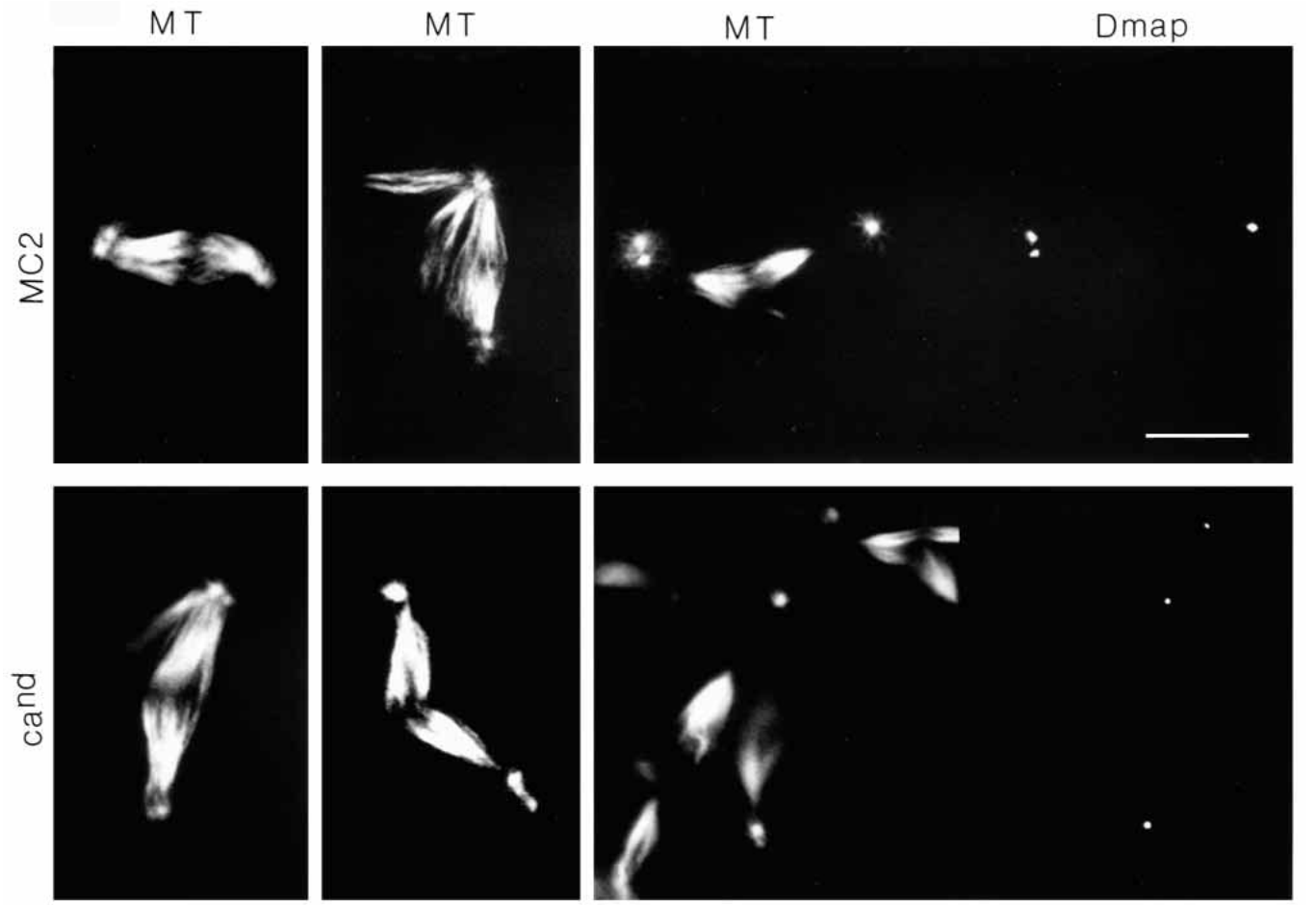
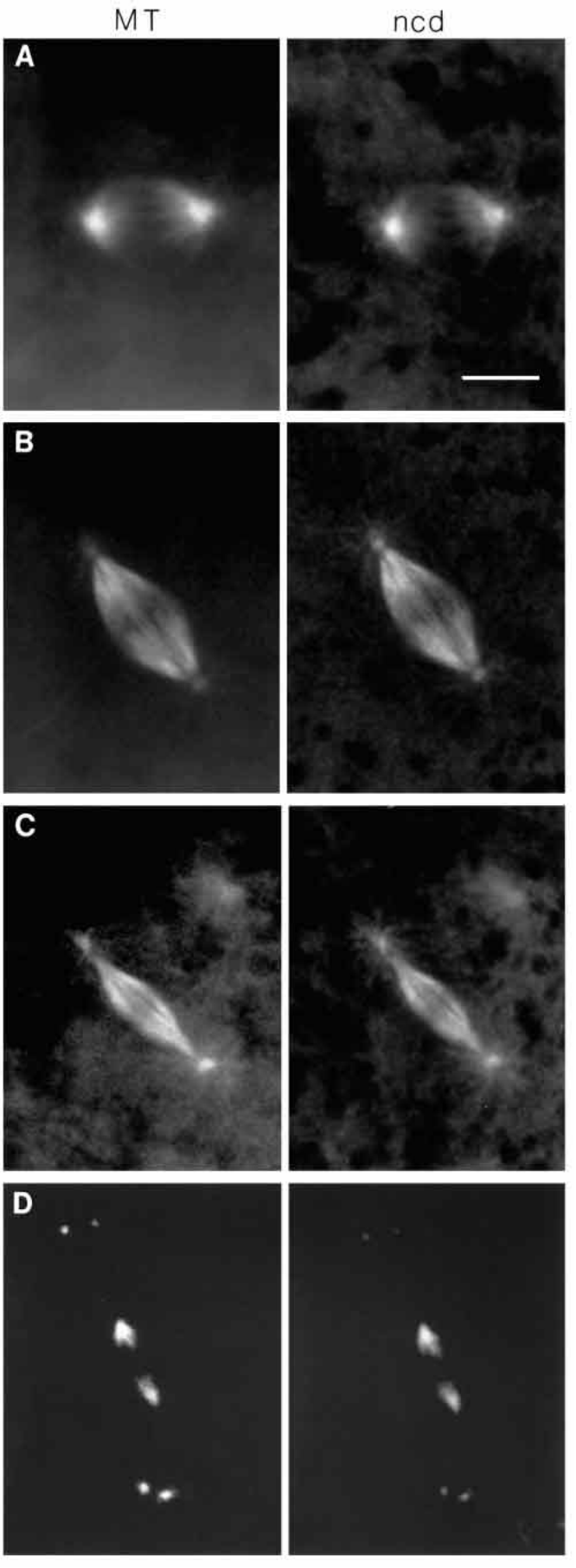


Fig. 3. Centrosomal and spindle pole defects caused by mutants of *ncd*. Early embryos of *MC2/MC2;ca*nd*/ca*nd and *ca*nd*/ca*nd, a null mutant, were stained with anti-α-tubulin antibody (MT) and anti-Dmap190 antiserum (Dmap) (Raff et al., 1993), directed against a centrosomal antigen (Frasch et al., 1986; Whitfield et al., 1988; Kellogg and Alberts, 1992; Raff et al., 1993). Spindles shown are as follows: *MC2*, curved early anaphase cycle 6 spindle with two centrosomes at one pole and one at the other; metaphase cycle 1 spindle with two centrosomes at one pole and three at the other; late anaphase cycle 4 spindle with two centrosomes at one pole and one at the other; *ca*nd, early anaphase cycle 3 spindle with two centrosomes at each pole; bent early anaphase cycle 4 spindle showing dissociation of one of the two centrosomes from one pole; early anaphase cycle 8-9 spindles and half-spindle showing centrosome loss and free centrosomes. Images were overexposed to emphasize centrosome structure, resulting in diminished astral microtubules. Bar: (MC2 images) 25 μm, 14 μm, 25 μm; (cand images) 25 μm, 10 μm, 10 μm.

colchicine-treated embryos by anti-Dmap190 antiserum was observed in early (pre-cycle 11) embryos, corresponding to stages of nontreated control embryos that showed bright spindle and centrosomal staining by anti-ncd and anti-tubulin antibody. The lack of staining by anti-ncd antibody is therefore not due to the destruction of centrosomes by the colchicine treatment or the stage of the embryos, but is correlated with the lack of anti-tubulin antibody staining, indicating the absence of intact microtubules. These results demonstrate that ncd association with centrosomes can be abolished under conditions in which microtubule assembly is inhibited by colchicine. ncd is therefore unlikely to be an integral component of the centrosome, but requires the presence of intact microtubules to associate with centrosomes.

DISCUSSION

We report here a new partial loss-of-function *ncd* mutant, *MC2*, that lacks ~one-third of the predicted α-helical coiled-coil central stalk of the ncd motor protein. *MC2* partially rescues the *ca*nd null mutant, exhibiting a dosage-dependent effect on rescue of X chromosome mis-segregation. In our video motility assays the MC2 motor translocates on microtubules with a velocity comparable to near full-length ncd, supports microtubule rotation during translocation (Walker et al., 1990), and bundles microtubules (McDonald et al., 1990; Chandra et al., 1993), as has been reported for near full-length ncd protein. Failure to completely rescue the null mutant may be due to lower expression of the MC2 protein in transformants. Alternatively, partial deletion of the predicted α-helical coiled-coil stalk may cause the MC2 protein to misfunction in vivo or disrupt interactions with other proteins in the cell. Our results do not distinguish between partial loss of function due to lower expression of the MC2 protein or to a conformational change in the protein. However, a similar effect has been reported for a cytoplasmic myosin II protein partially deleted for the coiled-coil tail (Kubalek et al., 1992). The partially



deleted cytoplasmic myosin protein translocates on actin filaments in vitro with the same velocity as the wild-type myosin II and is expressed at a level comparable to the wild-type protein, but exhibits frequent failure of cytokinesis in vivo. Our results suggest that the coiled-coil stalk of the ncd motor protein, like the coiled-coil tail of cytoplasmic myosin II, is required for wild-type protein function in vivo.

The *MC2* partial loss-of-function mutant and the ca^{nd} null mutant both cause centrosomal and spindle pole defects in early mitosis. The defects include centrosome splitting and dissociation of centrosomes from spindle poles. The bent or curved spindles that are observed in mutant embryos could be produced by an imbalance in spindle forces resulting from partial or complete loss of ncd activity. Mutations in loci that are not known to encode centrosomal proteins also cause centrosomal defects in *Drosophila*. An example is the maternal-effect lethal mutation, *gnu*, that uncouples DNA synthesis from nuclear division and results in replication of centrosomes independently of nuclei in fertilized mutant embryos (Freeman et al., 1986). Inhibition of DNA synthesis in syncytial embryos by microinjection of aphidicolin similarly uncouples centrosomal and nuclear division, resulting in multiple rounds of centrosome division in the absence of DNA replication and dissociation of extra centrosomes from the nuclei (Raff and Glover, 1988). These observations indicate the need for caution in interpreting centrosomal function based on mutant phenotype alone.

However, consistent with ncd mutant effects of centrosome splitting and detachment of centrosomes from spindle poles, we find that the ncd motor protein is associated with both centrosomes and spindle fibers in mitotic spindles of early wild-type embryos, and that localization of ncd to centrosomes is dependent on microtubule assembly and the stage of the cell cycle. ncd is associated with centrosomes in prometaphase, remains centrosome-associated in metaphase and anaphase, and dissociates in late anaphase/telophase, around the time of centrosome doubling. The *MC2* partial loss-of-function and ca^{nd} null mutants both show disruption of centrosome attachment to the ends of the spindle fibers, and centrosome splitting in metaphase and early anaphase, consistent with the idea that wild-type ncd acts to attach centrosomes to spindle poles and prevent centrosome splitting until late anaphase. Cell cycle-dependent localization of the ncd protein to centrosomes,

Fig. 4. Centrosomal and spindle localization of ncd in wild-type embryos. ncd is associated with centrosomes and spindles in early cycle divisions of whole-mount wild-type embryos. Embryos were stained with anti-α-tubulin (MT) and anti-ncd (ncd) antibodies. (A) Cycle 5 prometaphase/early metaphase spindle showing bright staining of centrosomes and spindle poles by both anti-ncd and anti-tubulin antibodies. (B) Cycle 1 metaphase spindle with anti-ncd and anti-tubulin antibody staining distributed throughout the spindle and spindle poles. (C) Cycle 4 anaphase spindle showing bright staining of centrosomes, the spindle and the developing midbody by both antibodies. (D) Cycle 3 telophase spindle showing staining of the spindle midbody by both antibodies, but diminished centrosomal staining by anti-ncd compared with anti-tubulin antibody. The telophase spindles were overexposed to emphasize the difference in centrosomal staining, resulting in diminished astral microtubules. Control null mutant (ca^{nd}/ca^{nd}) embryos were stained by the anti-α-tubulin antibody, but showed no specific staining by the anti-ncd antibody. Bar: (A) 7.5 μm, (B-D) 10 μm.

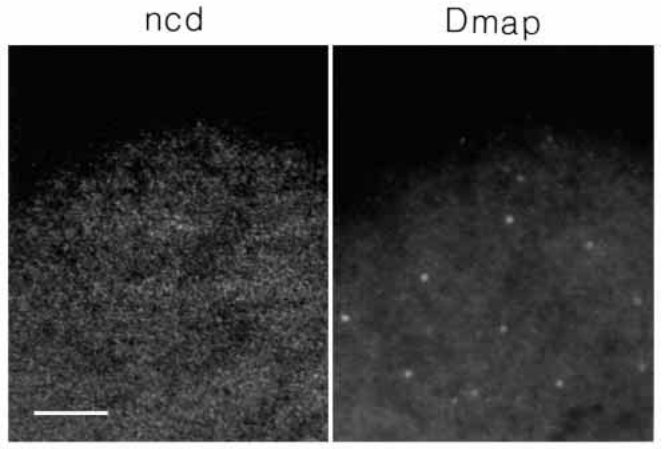


Fig. 5. Centrosomal staining of colchicine-treated embryos. Embryos were treated with colchicine prior to fixation, to depolymerize microtubules, and stained with anti-ncd antibody (ncd) and anti-centrosomal (anti-Dmap190) antiserum (Dmap). DAPI was used to stain the chromosomes. A stage 10 embryo is shown with centrosomes brightly stained by anti-Dmap190 antiserum. Metaphase-arrested chromosomes were positioned between pairs of centrosomes (not shown). No specific staining by anti-ncd antibody was observed. Bar, 10 μm.

together with the effects of the *MC2* and ca^{nd} mutants on centrosomes, provide clear evidence for activity of the ncd microtubule motor protein near or at the spindle poles. The ncd motor protein is therefore important not only in establishing and maintaining spindle bipolarity in meiosis, but also in maintenance of spindle poles in mitotic cells.

How does ncd function to maintain the junction between centrosomes and poles? The microtubule depletion experiment indicates that ncd is not an integral component of the centrosome, since embryos treated with colchicine to depolymerize microtubules exhibit no centrosomal staining by anti-ncd (or anti-tubulin) antibody. Centrosomes remain intact following colchicine treatment, however, and can be visualized by staining colchicine-treated embryos with antiserum directed against a centrosomal antigen. These observations suggest that ncd maintains centrosome attachment to spindle fibers at the poles by binding to microtubules and crosslinking to microtubules associated with the centrosome, rather than binding to centrosomes directly.

In contrast to the cell cycle-dependent association of ncd with centrosomes, the ncd protein is present in the spindle from prometaphase through late telophase. The presence of ncd on spindle fibers throughout anaphase and telophase, together with its minus-end polarity of translocation, suggest that the motor may mediate poleward translocation of microtubules. Poleward translocation of microtubules (flux) is a process that is thought to underlie spindle structure and dynamics, and contribute to poleward movement of chromosomes during mitosis (Mitchison, 1989). While the basis of poleward microtubule flux has not yet been determined, it is likely to involve several components, including microtubule dynamics and microtubule motors (Mitchison and Salmon, 1993). A possible model of how ncd could function both in centrosome attachment to spindle poles and poleward microtubule flux is by binding preferentially and tightly by its basic tail to kinetochore microtubules and, at the same time, by its ATP-sensitive microtubule binding site to dynamic polar microtubules attached to the centrosomes. Minus-end translocation by the motor would pull the more-stable kinetochore microtubules poleward while pulling the centrosomes into the ends of the kinetochore microtubules, thus serving both to translocate chromosomes poleward and to maintain attachment of centrosomes to spindle poles.

Activity of ncd at the poles of mitotic spindles reinforces the idea that ncd functions in the oocyte meiotic divisions to establish and maintain spindle bipolarity (Hatsumi and Endow, 1992a). Our studies provide direct evidence that the ncd motor is active in mitosis as well as in meiosis, and demonstrate that the motor has spindle pole-related functions in both mitosis and meiosis. Kinesin motors like ncd are likely to perform similar microtubule-dependent roles in mitosis and meiosis in other organisms.

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REFERENCES

Chandra, R. and Endow, S. A. (1993). Expression of microtubule motor proteins in bacteria for characterization in *in vitro* motility assays. *Meth. Cell Biol.* **39**, 115-127.

Chandra, R., Salmon, E. D., Erickson, H. P., Lockhart, A. and Endow, S. A. (1993). Structural and functional domains of the *Drosophila* ncd microtubule motor protein. *J. Biol. Chem.* **268**, 9005-9013.

Cole, D. G., Cande, W. Z., Baskin, R. J., Skoufias, D. A., Hogan, C. J. and Scholey, J. M. (1992). Isolation of a sea urchin egg kinesin-related protein using peptide antibodies. *J. Cell Sci.* **101**, 291-301.

Endow, S. A., Henikoff, S. and Soler-Niedziela, L. (1990). Mediation of meiotic and early mitotic chromosome segregation in *Drosophila* by a protein related to kinesin. *Nature* **345**, 81-83.

Endow, S. A. and Titus, M. A. (1992). Genetic approaches to molecular motors. *Annu. Rev. Cell Biol.* **8**, 29-66.

Frasch, M., Glover, D. M. and Saumweber, H. (1986). Nuclear antigens follow different pathways into daughter nuclei during mitosis in early *Drosophila* embryos. *J. Cell Sci.* **82**, 155-172.

Freeman, M., Nusslein-Volhard, C. and Glover, D. M. (1986). The dissociation of nuclear and centrosomal division in *gnu*, a mutation causing giant nuclei in *Drosophila*. *Cell* **46**, 457-468.

Gard, D. L. (1992). Microtubule organization during maturation of *Xenopus* oocytes: assembly and rotation of the meiotic spindles. *Dev. Biol.* **151**, 516-530.

Goldstein, L. S. B. (1991). The kinesin superfamily: tails of functional redundancy. *Trends Cell Biol.* **1**, 93-98.

Hatsumi, M. and Endow, S. A. (1992a). The *Drosophila claret* microtubule motor protein is spindle-associated in meiotic and mitotic cells. *J. Cell Sci.* **103**, 1013-1020.

Hatsumi, M. and Endow, S. A. (1992b). Mutants of the microtubule motor protein, nonclaret disjunctional, affect spindle structure and chromosome movement in meiosis and mitosis. *J. Cell Sci.* **101**, 547-559.

Howard, J., Hudspeth, A. J. and Vale, R. D. (1989). Movement of microtubules by single kinesin molecules. *Nature* **342**, 154-158.

Hyman, A., Drechsel, D., Kellogg, D., Salser, S., Sawin, K., Steffen, P., Wordeman, L. and Mitchison, T. (1991). Preparation of modified tubulins. *Meth. Enzymol.* **196**, 478-485.

Karr, T. L. and Alberts, B. M. (1986). Organization of the cytoskeleton in early *Drosophila* embryos. *J. Cell Biol.* **102**, 1494-1509.

Kellogg, D. R. and Alberts, B. M. (1992). Purification of a multiprotein complex containing centrosomal proteins from the *Drosophila* embryo by chromatography with low-affinity polyclonal antibodies. *Mol. Biol. Cell* **3**, 1-11.

Kimble, M. and Church, K. (1983). Meiosis and early cleavage in *Drosophila melanogaster* eggs: effects of the claret-non-disjunctional mutation. *J. Cell Sci.* **62**, 301-318.

Komma, D. J., Horne, A. S. and Endow, S. A. (1991). Separation of meiotic and mitotic effects of *claret nondisjunctional* on chromosome segregation in *Drosophila*. *EMBO J.* **10**, 419-424.

Kubalek, E. W., Uyeda, T. Q. P. and Spudich, J. A. (1992). A *Dictyostelium* myosin II lacking a proximal 58-kDa portion of the tail is functional in vitro and in vivo. *Mol. Biol. Cell* **3**, 1455-1462.

Laski, F. A., Rio, D. C. and Rubin, G. M. (1986). Tissue specificity of Drosophila P element transposition is regulated at the level of mRNA splicing. *Cell* **44**, 7-19.

Lewis, E. B. and Gencarella, W. (1952). Claret and non-disjunction in *Drosophila melanogaster*. *Genetics* **37**, 600-601 (abstract).

McDonald, H. B. and Goldstein, L. S. B. (1990). Identification and characterization of a gene encoding a kinesin-like protein in Drosophila. *Cell* **61**, 991-1000.

McDonald, H. B., Stewart, R. J. and Goldstein, L. S. B. (1990). The kinesin-like *ncd* protein of Drosophila is a minus end-directed microtubule motor. *Cell* **63**, 1159-1165.

Mitchison, T. J. (1989). Mitosis: basic concepts. *Curr. Opin. Cell Biol.* **1**, 67-74.

Mitchison, T. J. and Salmon, E. D. (1993). Poleward kinetochore fiber movement occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J. Cell Biol.* **119**, 569-582.

Nelson, C. R. and Szauter, P. (1992). Timing of mitotic chromosome loss caused by the *ncd* mutation of *Drosophila melanogaste*r. *Cell Motil. Cytoskel.* **23**, 34-44.

Raff, J. W. and Glover, D. M. (1988). Nuclear and cytoplasmic mitotic cycles continue in Drosophila embryos in which DNA synthesis is inhibited with aphidicolin. *J. Cell Biol.* **107**, 2009-2019.

Raff, J. W., Kellogg, D. R. and Alberts, B. M. (1993). *Drosophila* γ-tubulin is part of a complex containing two previously identified centrosomal MAPs. *J. Cell Biol.* **121**, 823-835.

Rieder, C. L., Ault, J. G., Eichenlaub-Ritter, U. and Sluder, G. (1993). Morphogenesis of the mitotic and meiotic spindle: conclusions obtained from one system are not necessarily applicable to the other. In *Chromosome Segregation and Aneuploidy* (ed. B. K. Vig), vol. 1472, pp. 183-197. Berlin: Springer-Verlag.

Sawin, K. E. and Endow, S. A. (1993). Meiosis, mitosis and microtubule motors. *BioEssays* **15**, 399-407.

Sawin, K. E. and Mitchison, T. J. (1991). Mitotic spindle assembly by two different pathways in vitro. *J. Cell Biol.* **112**, 925-940.

Steller, H. and Pirrotta, V. (1985). A transposable P vector that confers selectable G418 resistance to *Drosophila* larvae. *EMBO J.* **4**, 167-171.

Sturtevant, A. H. (1929). The claret mutant type of *Drosophila simulans:* a study of chromosome elimination and of cell-lineage. *Z. Wiss. Zool.* **135**, 323-356.

Theurkauf, W. E. and Hawley, R. S. (1992). Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* **116**, 1167-1180.

Vale, R. D. and Toyoshima, Y. Y. (1988). Rotation and translocation of microtubules in vitro induced by dyneins from Tetrahymena cells. *Cell* **52**, 459-469.

Verde, F., Berrez, J.-M., Antony, C. and Karsenti, E. (1991). Taxol-induced microtubule asters in mitotic extracts of *Xenopus* eggs: requirement for phosphorylated factors and cytoplasmic dynein. *J. Cell Biol.* **112**, 1177-1187.

Wald, H. (1936). Cytologic studies on the abnormal development of the eggs of the claret mutant type of Drosophila simulans. *Genetics* **21**, 264-281.

Walker, R. A., O'Brien, E. T., Pryer, N. K., Soboeiro, M. F., Voter, W. A., Erickson, H. P. and Salmon, E. D. (1988). Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J. Cell Biol.* **107**, 1437-1448.

Walker, R. A., Salmon, E. D. and Endow, S. A. (1990). The *Drosophila claret* segregation protein is a minus-end directed motor molecule. *Nature* **347**, 780-782.

Whitfield, W. G. F., Millar, S. E., Saumweber, H., Frasch, M. and Glover, D. M. (1988). Cloning of a gene encoding an antigen associated with the centrosome in *Drosophila*. *J. Cell Sci.* **89**, 467-480.

Yamamoto, A. H., Komma, D. J., Shaffer, C. D., Pirrotta, V. and Endow, S. A. (1989). The *claret* locus in *Drosophila* encodes products required for eyecolor and for meiotic chromosome segregation. *EMBO J.* **8**, 3543-3552.

Yang, J. T., Laymon, R. A. and Goldstein, L. S. B. (1989). A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. *Cell* **56**, 879-889.

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