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Separation of meiotic and mitotic effects of *claret non-disjunctional* on chromosome segregation in *Drosophila*

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The *claret* (*ca*) locus in *Drosophila* encodes a kinesin-related motor molecule that is required for proper distribution of chromosomes in meiosis in females and in the early mitotic divisions of the embryo. Here we demonstrate that a mutant allele of *claret non-disjunctional* (ca^{nd}), *non-claret disjunctional Dominant* (ncd^{D}), causes abnormalities in meiotic chromosome segregation, but is near wild-type with respect to early mitotic chromosome segregation. DNA sequence analysis of this mutant allele reveals two missense mutations compared with the predicted wild-type protein. One mutation lies in a proposed microtubule binding region of the motor domain and affects an amino acid residue that is conserved in all kinesin-related proteins reported to date. This region of the motor domain can be used to distinguish meiotic and mitotic motor function, defining an amino acid sequence criterion for classifying motors according to function. ncd^{D}'s mutant meiotic effect, but near wild-type mitotic effect, suggests that interactions of the *ca* motor protein with spindle microtubules differ in meiosis and mitosis.

Key words: claret non-disjunctional/kinesin-related motor molecule/meiotic, mitotic chromosome segregation

Introduction

The *claret* (*ca*) locus in *Drosophila* has been the object of unusual interest since Sturtevant (1929) reported that *ca Drosophila simulans* females produced very few offspring, many of which were abnormal. Genetic experiments showed that the abnormal offspring could be attributed to frequent non-disjunction and loss of chromosomes in meiosis. There was also a maternal effect of frequent loss of maternal chromosomes in early embryonic mitotic divisions, resulting in large numbers of gynandromorphs, which are mosaics of X/X female and X/O male tissue. A corresponding mutant in *Drosophila melanogaster* was recovered after X-irradiation (Lewis and Gencarella, 1952) and named *claret non-disjunctional* (ca^{nd}). Like the *ca* of *D.simulans*, ca^{nd} causes recessive phenotypes of claret eye color in both males and females, abnormal chromosome segregation in meiosis in females, and a maternal effect of frequent loss of maternal chromosomes in early embryonic cleavage divisions.

The effects of ca^{nd} on eye color and chromosome behavior can be separated genetically by mutant alleles that cause only ca eye color and by *non-claret disjunctional* (*ncd*) (O'Tousa and Szauter, 1980), an allele that causes abnormal chromosome segregation but does not affect eye color. Separation of the mutant effects of ca^{nd} on eye color and chromosome segregation suggested that two genes, *ca* and *ncd*, were present at the *ca* locus. This was confirmed by molecular analysis (Yamamoto *et al.*, 1989), which identified two divergently transcribed RNAs at *ca* and showed that mutations such as ca^{nd} are small deficiencies which affect both RNAs. ca^{nd} is therefore a null mutant for both ca^{+} and ncd^{+}.

Sequence analysis of cDNAs (Endow *et al.*, 1990) resulted in the finding that the predicted ncd^{+} protein is strikingly similar to the microtubule motor protein, kinesin (Vale *et al.*, 1985). The region of similarity corresponds to the 'motor' domain of kinesin and includes the ATP binding site and a region that can bind microtubules. A PCR clone that had been obtained using degenerate primers to the kinesin motor domain (McDonald and Goldstein, 1990) was subsequently identified as ncd^{+} by DNA sequence comparison. ncd^{+} is kinesin-related, but is distinct from kinesin in its predicted structure (Endow *et al.*, 1990; McDonald and Goldstein, 1990) and role within the cell. Its similarity to kinesin suggests that ncd^{+} is a motor molecule. The motility properties of ncd^{+} have now been examined *in vitro* with the surprising finding that ncd^{+} moves towards the minus ends of microtubules (McDonald *et al.*, 1990; Walker *et al.*, 1990), opposite to the direction of kinesin movement. The abnormalities in chromosome distribution caused by mutants, and its directionality of movement on microtubules implicate ncd^{+} in chromosome segregation in meiosis and mitosis.

non-claret disjunctional Dominant (ncd^{D}) is a mutant allele of ca^{nd} that shows wild-type eye color but abnormal chromosome segregation. ncd^{D} was originally isolated as an EMS-induced dominant mutant that caused high frequencies of non-disjunction in heterozygous females (Lindsley and Zimm, 1990). Here we show that ncd^{D} separates the meiotic and mitotic effects of ca^{nd}. Females carrying ncd^{D} show meiotic chromosome non-disjunction, but very low frequencies of mitotic chromosome loss. These females are mutant for the meiotic effect of ca^{nd} on chromosome segregation, but near wild-type for the maternal effect of ca^{nd} on early mitotic divisions. DNA sequence analysis of ncd^{D} indicates that two amino acid residues in the predicted ncd^{+} protein are changed in ncd^{D}. One of these residues is conserved in all eight of the kinesin-related proteins reported to date and is present in the motor domain, in a region that can bind microtubules. Loss of chromosomes in meiosis is consistent with a structural change in the microtubule binding site. Mitotic chromosome segregation, however, is not sensitive to the observed changes in predicted protein structure, since ncd^{D} is close to wild-type with respect to early embryonic mitotic chromosome loss.

Results

Although ncd^D was isolated as a dominant meiotic mutant, it now shows only weak semi-dominant and recessive effects on meiotic chromosome segregation. Recombination analysis places ncd^D between *Drop* (99.2) and *loboid* (102) on chromosome 3, close to or at *ca* (100.7). Its map position and its failure to complement the mutant chromosome behavior of ca^{nd} (see below) indicate that ncd^D is allelic to ca^{nd}.

ncd^D causes meiotic non-disjunction but not loss of the X chromosome

Segregation of only the X and 4th chromosomes was monitored in these studies; however, ca^{nd} affects meiotic segregation of all chromosomes in *Drosophila* females (Davis, 1969). The general conclusions from these studies are therefore assumed to apply to chromosomes 2 and 3, as well as to the X and 4th chromosomes.

Results of segregation tests for the X chromosome are shown in Table I. Abnormal offspring recovered from these crosses are non-disjunctional X/X/Y (B^S) females and X/O (y w) males, haplo-4 Minutes (M), gynandromorphs and haplo-4 mosaics. Because the recovery of haplo-4 offspring is highly variable, haplo-4 offspring are not shown in Table I and additional experiments were performed to demonstrate the effect of ncd^D on segregation of chromosome 4 (see below).

Control females (cross 1) produced only one abnormal male (X/O) among 2390 regular offspring. The X/O male is attributed to loss of the X chromosome in meiosis since no X/X/Y females were recovered, although it could have arisen as a product of X non-disjunction. Since only half of the gametes resulting from X chromosome non-disjunction or loss are recovered as viable offspring, the number of X/X/Y + X/O offspring is doubled in calculations of total gametes and frequencies of gametic non-disjunction and loss. The frequency of gametic X chromosome loss is 0.0008. In contrast to wild-type females, ca^{nd} females produced high frequencies of non-disjunctional X/X/Y females, X/O males and X chromosome somatic mosaics (gynandromorphs) (cross 5). The excess of X/O males relative to X/X/Y females is attributed to meiotic loss of the X chromosome (Sturtevant, 1929). The frequencies of gametic X chromosome non-disjunction and loss exhibited by ca^{nd} females are 0.107 and 0.080, respectively. The frequency of gynandromorphs is 0.129.

ncd^D/+ females (cross 2) produced eight X/X/Y females and six X/O males among 2347 offspring. The approximate equivalence of X/X/Y females and X/O males suggests that they arose as reciprocal products of X chromosome non-disjunction in female meiosis (Sturtevant, 1929). The frequency of gametic X chromosome non-disjunction observed is 0.012. Abnormal segregation of the X chromosome in ncd^D/+ females is significantly increased over the wild-type control ($\chi^2 = 13.2$, $P < 0.005$, 1 d.f.). ncd^D thus has a small dominant mutant effect on X chromosome segregation. The absence of gynandromorphs indicates that loss of the X chromosome is infrequent in early mitotic divisions of embryos produced by ncd^D/+ females.

Homozygous ncd^D females (cross 3) produced approximately equivalent numbers of X/X/Y females and X/O males, attributable to X chromosome non-disjunction. X/O males did not exceed the number of X/X/Y females, indicating that meiotic X chromosome loss is infrequent relative to non-disjunction. The frequency of gametic X chromosome non-disjunction is 0.072. The absence of gynandromorphs among 1788 offspring indicates that the X chromosome is lost mitotically at very low frequencies in embryos produced by ncd^D females. In repeat crosses, gynandromorphs were sometimes recovered at low frequencies among offspring of ncd^D females. Although some variability with genetic background was observed, the overall frequency for a total of 19 492 offspring was 0.0033. This is significantly lower than the frequency of 0.129 gynandromorphs produced by ca^{nd} females. Thus, ncd^D is close to wild-type for the mitotic X chromosome loss that is characteristic of ca^{nd}.

ncd^D/ca^{nd} females (cross 4) produced 49 X/X/Y females, 46 X/O males and 23 gynandromorphs among 1787 offspring. X/O males do not exceed the number of X/X/Y females as in offspring of ca^{nd} females, indicating that ncd^D complements ca^{nd} for X chromosome meiotic loss. The frequency of gametic X chromosome non-disjunction is 0.101, which is similar to the frequencies of 0.072 and 0.107 observed for ncd^D and ca^{nd} females. ncd^D partially complements ca^{nd} for mitotic loss of the X chromosome, as indicated by the frequency of 0.026 gynandromorphs produced by ncd^D/ca^{nd} females, compared with 0.129 gynandromorphs produced by ca^{nd} females.

Table I. Effect of ncd^D on X chromosome segregation and loss

Female parent	Offspring							Total gametes	Gametic X nd	Gametic X loss
	+ ♀	B^S ♀	B^S ♂	y w ♂	gyn	Other	Total			
+/+	1218		1171	1			2390	2391		0.0008
ncd^D/+	1259	8	1074	6			2347	2361	0.012	
ncd^D/ncd^D	916	36	793	30			1775	1841	0.072	
ncd^D/ca^{nd}	855	49	814	46	23		1787	1882	0.101	
ca^{nd}/ca^{nd}	553	35	427	87	82	1	1185	1307	0.107	0.080

Females of the indicated genotypes were mated to y^2 w^{hf}/B^SY males. Offspring of these matings were scored for non-disjunction [B^S (X/X/Y) females and y w (X/O) males] and mitotic loss [gynandromorphs (gyn)]. y w males can also arise after meiotic loss of a maternal X chromosome. Regular offspring are + females and B^S males. Haplo-4 (Minute) offspring and Minute mosaics were also scored but are not shown. 'Other' denotes an exceptional X/O male that carried a maternal X chromosome and arose following loss of a paternal X or Y chromosome. To adjust for the loss of half of the non-disjunctional and nullo-X gametes as X/X/X and O/Y embryos, the number of X/X/Y and X/O offspring was multiplied by two in calculations of the total gametes, and in estimates of the frequencies of X chromosome gametic non-disjunction (nd) and loss. The excess of X/O males relative to X/X/Y females is attributed to gametic loss of the X chromosome.

ncd^D causes meiotic non-disjunction and loss of chromosome 4

Flies that have lost a chromosome 4 show a Minute phenotype caused by deficiency for *Minute(4)*. Because of the low and variable viability of haplo-4 offspring, segregation in females of a marked chromosome 4 (*spa*^{Pol}) was monitored in crosses to compound-4 (*ci ey*^{R}) males (Table II). While wild-type females (cross 1) produced chromosome 4 non-disjunctional offspring at a frequency of 0.005, females heterozygous for *ncd*^{D} and a wild-type chromosome produced a frequency of 0.073 abnormal offspring (cross 2), consisting both of non-disjunctional products and products of meiotic loss. *ncd*^{D} thus has a dominant mutant effect of meiotic non-disjunction and loss of chromosome 4. *ncd*^{D} females produced chromosome 4 abnormal offspring at a frequency of 0.384 (cross 3). The large excess of ci ey, compared with spa, offspring is attributed to meiotic loss of chromosome 4. Thus, both meiotic non-disjunction and loss of chromosome 4 occur at high frequency in *ncd*^{D} females.

Results of tests of X and chromosome 4 segregation are summarized in Table III.

ncd RNA is present in adult *ncd*^{D} females

ncd^D is an EMS-induced mutation. *ncd* RNA is present in *ncd*^{D} females in approximately wild-type amounts (Figure 1). *ncd* is therefore expressed in *ncd*^{D} females but is mutationally altered. Analysis of *ncd*$^+$ RNA during development shows that it is present in wild-type adult females and in gradually decreasing amounts during embryogenesis (Figure 1), as expected for an RNA that is expressed maternally and persists in the embryo. The low amount of *ncd*$^+$ RNA present in first and second instar larvae is attributed to perdurance of a small fraction of the total. Expression apparently begins in third instar larvae and pupae, accounting for the gradual increase in amount. *ncd*$^+$ RNA is also present in low amounts in adult males, as noted previously (Yamamoto *et al.*, 1989).

ncd^D has changes in two amino acid residues, one of which is in a putative microtubule binding region

In order to determine the basis of its mutant phenotype, we sequenced *ncd*^{D}. We found two amino acid residue changes in *ncd*^{D} compared with *ncd*$^+$. The first change is at residue 556 of the predicted protein (Figure 2), where a G → T transversion converts GTT to TTT, resulting in the replacement of valine with phenylalanine. This change lies in the motor domain and corresponds to a region of kinesin that can bind microtubules (Yang *et al.*, 1989). Valine is found at this position in *ncd*$^+$ (Endow *et al.*, 1990; McDonald and Goldstein, 1990), *Drosophila* kinesin heavy chain (Yang *et al.*, 1989), squid kinesin heavy chain (Kosik *et al.*, 1990), *Caenorhabditis elegans unc104*$^+$ (Otsuka *et al.*, 1991) and *bimC*$^+$ of *Aspergillus* (Enos and Morris, 1990) (Figure 2). *KAR3* (Meluh and Rose, 1990), *cut7*$^+$ (Hagan and Yanagida, 1990) and *nod*$^+$ (Zhang *et al.*, 1990) contain an isoleucine instead of valine in this position. Valine and isoleucine are structurally very similar, differing only by an additional carbon present in the branched side chain of isoleucine. Phenylalanine, in contrast, contains an aromatic ring in place of the 3- or 4-carbon branched side chain of

Table II. Effect of *ncd*^{D} on chromosome 4 segregation

Female parent	Offspring			Total gametes	Gametic nd	Gametic loss
	+	spa	ci ey			
+/+	1018	3	2	1023	0.005	
ncd^D/+	1156	40	51	1247	0.064	0.009
ncd^D/*ncd*^{D}	303	34	155	492	0.138	0.246

Females homozygous for *spa*^{pol} and of the indicated genotypes with respect to *ncd*^{D} were mated to *C(4)RM, ci ey*^{R}*/O* males. Regular offspring of these matings are + (triplo-4) and spa M (haplo-4). spa M flies are not shown, since their recovery was highly variable. spa and ci ey offspring are diplo-4 non-disjunctional exceptions. ci ey progeny can also arise after meiotic loss of a maternal chromosome 4. The frequencies of chromosome 4 gametic non-disjunction (nd) and loss were calculated from the number of +, spa and ci ey offspring recovered. These represent half of both the regular and exceptional offspring, since spa M regular offspring are omitted and half of the non-disjunctional gametes, or gametes that have lost a maternal chromosome 4, are lost as 4,4/4-4 or nullo-4 lethal embryos.

Table III. Dominant and recessive effects of *ncd*^{D} on X and 4th chromosome segregation

	Dominant effects			Recessive effects		
	Nd	Meiotic loss	Mitotic loss	Nd	Meiotic loss	Mitotic loss
X chromosome	+	−	−	+	−	−
4th chromosome	+	+	−	+	+	−

Summary of effects of *ncd*^{D} on meiotic and mitotic chromosome segregation and loss. Mitotic loss of chromosome 4 was observed at very low frequencies in the crosses in Table I: *ncd*^{D}/+ females (cross 2) produced no Minute mosaics among 2347 offspring and *ncd*^{D} females (cross 3) produced 1 Minute mosaic among 1776 offspring. Nd = meiotic non-disjunction, + = observed, − = not observed.

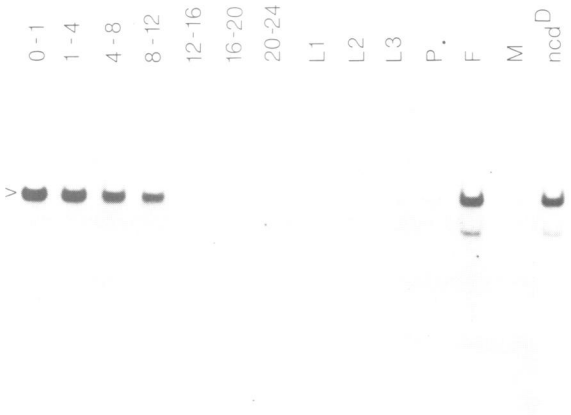

Fig. 1. *ncd*$^+$ RNA present in different developmental stages of *Drosophila*. Approximately 1 μg of poly(A)$^+$ RNA from each stage was separated on a 1.2% agarose−formaldehyde gel and transferred to a nitrocellulose filter. Hybridization was with an RNA probe synthesized from 8e2 cDNA (Yamamoto *et al.*, 1989). The lanes contain RNA from staged embryos of 0−1 h, 1−4 h, 4−8 h, 8−12 h, 12−16 h, 16−20 h, 20−24 h; first instar larvae (L1), second and third instar female larvae (L2, L3); 24−36 h female pupae (P); 3 day adult females (F) and males (M); and 3 day *ncd*^{D} females. The major band of hybridization (arrow) is ~2.2 kb in length. The low mol. wt band in two of the lanes is probably a degradation product since it was not present in previous Northern blots of the same RNA samples. The filter was hybridized with an actin DNA probe to ensure that the amounts of RNA in each lane were approximately equal.

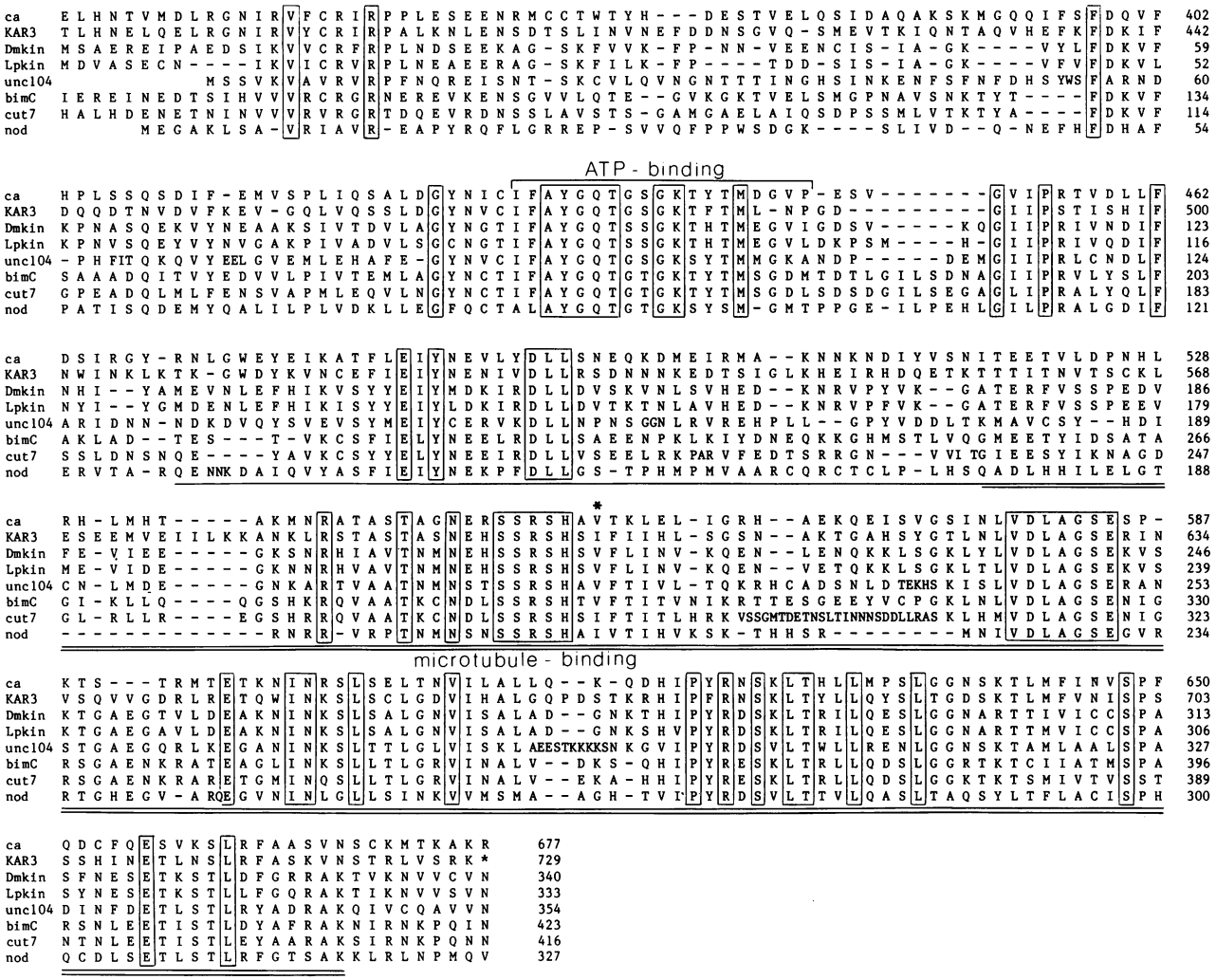

Fig. 2. The motor domain of eight kinesin-related proteins. Comparison of predicted amino acid sequences of *Drosophila ncd*$^{+}$ (ca), *Saccharomyces cerevisiae KAR3* (KAR), *Drosophila* kinesin heavy chain (Dmkin), squid kinesin heavy chain (Lpkin), *C.elegans unc-104*$^{+}$, *Aspergillus bimC*$^{+}$, *Schizosaccharomyces pombe cut7*$^{+}$ and *Drosophila nod*$^{+}$. Identical residues are boxed. The putative ATP binding site is indicated. Residues corresponding to the region of kinesin that can bind microtubules are underlined. Uncertainty in the N-terminal boundary of this region is indicated by a single line. The residue in the motor domain that is changed in *ncd*^{D} is marked with an asterisk.

valine or isoleucine. This large, rigid, completely hydrophobic structure is a striking structural change from valine or isoleucine. Its occurrence in a region of *ncd*$^{+}$ that can bind microtubules suggests that this change is the basis of the *ncd*^{D} mutant phenotype. This is substantiated by the finding of phenylalanine adjacent to the residue altered in *ncd*^{D} in all proteins except *ncd*$^{+}$ and *nod*$^{+}$, the only two of the eight proteins that are known to act in meiosis. The change of valine to phenylalanine in this region of the motor domain is apparently permitted for function in mitotic cells, but not in meiosis. The phenotype of *ncd*^{D} of meiotic dysfunction, but near wild-type mitotic function, is consistent with this interpretation.

The second amino acid change in *ncd*^{D} is outside of the motor domain at the carboxy-terminal end of the predicted protein at residue 696, four residues from the TAA stop codon. Here an A → G transition changes AAT to AGT and results in the replacement of asparagine with serine. Asparagine and serine are similar in that they both contain uncharged polar side chains. The side chain of asparagine is somewhat bulkier than that of serine. The replacement of asparagine with serine may therefore have an effect on the overall conformation of the protein.

Discussion

ncd^{D} is a mutant allele of *ca*nd that causes meiotic nondisjunction of the X and 4th chromosomes and meiotic loss of chromosome 4, but little or no mitotic loss of either the X or 4th chromosome. *ncd*^{D} therefore separates the meiotic and mitotic effects of *ca*nd on chromosome segregation, causing abnormal chromosome segregation in meiosis but not in mitosis. *ncd*^{D} contains two missense mutations. One mutation is in a proposed microtubule binding region of the motor domain. The altered residue is conserved in all eight of the kinesin-related proteins reported to date. Alteration of this residue from valine to phenylalanine is the apparent basis of the *ncd*^{D} mutant phenotype, since six of the eight kinesin-related proteins contain phenylalanine in the next adjacent position. The only two proteins that do not contain phenylalanine in this region of the motor domain are *ncd*$^{+}$ and *nod*$^{+}$, which are the only two kinesin-related proteins known to act in meiosis. Phenylalanine in this region of the motor domain is apparently permitted for mitotic function, but not for meiotic function. This defines a region of the motor domain which can be used to distinguish kinesin-related proteins that act in meiosis from those that function

in mitosis. Amino acid sequence criteria for classifying molecular motors according to function will be of importance in assigning roles to the large family of kinesin-related proteins that is now being discovered.

The second missense mutation is in the carboxy terminus of the protein. The first mutation is likely to be the basis of the mutant phenotype of ncd^D, although the second mutation may also affect the function of the protein.

These results indicate that changes in two amino acid residues, including one in a putative microtubule binding site, can cause a mutant phenotype in meiosis, but not in mitosis. This suggests that interactions of ncd^+ with spindle microtubules differ in meiosis and mitosis. Such differences may be due to meiosis- or mitosis-specific spindle components.

ncd^+ performs several functions during chromosome division. One function is involved in segregation of homologs in meiosis I, while two further functions are to prevent meiotic and mitotic chromosome loss, perhaps by ensuring chromosome attachment to the spindle. ncd^D is defective for the first function, wild-type with respect to the X but mutant with respect to chromosome 4 for the second, and near wild-type for the third function. Other alleles of ca^{nd}, including *ncd* (D.J.Komma and S.A.Endow, unpublished) are defective for all three functions.

The mutant effect of ca^{nd} on mitotic chromosome stability is transmitted as a maternal effect that acts on maternal, and only rarely on paternal chromosomes, representing a form of imprinting. The maternal effect implies that ncd^+ acts not only during meiosis in females, but also functions in early mitotic divisions of the embryo. This is consistent with results of Northern blot analysis showing that ncd^+ RNA is abundant in early embryos (Figure 1). ca^{nd} flies, however, do not show somatic loss of chromosomes throughout development. The lack of a mutant effect in later mitotic divisions is paralleled by very low amounts of ncd^+ mRNA in late embryo, larval and pupal stages, suggesting that ncd^+ does not function in these stages. During these stages, ncd^+ may be replaced by a corresponding zygotic product.

The effect on maternal, but not paternal, chromosomes may be in part a consequence of the fact that the first mitotic division after fertilization in *Drosophila* is gonomeric (Sonnenblick, 1950) with maternal and paternal chromosome sets remaining in separate groups even after attachment to the spindle. Mixing of the chromosome complements occurs only after completion of the first division. A maternal product, either chromosome- or spindle-associated, would be unlikely to affect paternal chromosomes before completion of the first mitotic division, during which much of the early chromosome loss observed for *ca* of *D.simulans* (Sturtevant, 1929) and ca^{nd} (Portin, 1978) occurs. Loss of maternal chromosomes probably also occurs, however, in the second and third mitotic divisions (Sturtevant, 1929; Portin, 1978), when maternal and paternal chromosome complements are expected to have mixed. This is most easily explained by mutation of a product normally associated with maternal chromosomes, and suggests that the *ca* segregation protein may be a chromosome-associated molecule that remains associated with maternal chromosomes throughout the early mitotic divisions of the embryo. That ca^{nd} affects a chromosome-associated product that is asymmetrically distributed during DNA replication was suggested earlier by Baker and Hall (1976).

Cytological observation of abnormal spindles in oocytes and embryos of *ca D.simulans* (Wald, 1936) and ca^{nd} females (Kimble and Church, 1983) has led to the hypothesis that the mutant defect lies in spindle organization. Although regulation of spindle organization is not well understood, tubulin incorporation studies show that kinetochore microtubules undergo flux *in vivo* at a slower rate than astral microtubules (Mitchison *et al.*, 1986), giving rise to the idea that spindle microtubules are stabilized by association with chromosomes. Abnormal spindles might therefore arise either from defects in spindle organization centers, or by failure of chromosomes to attach to spindle microtubules. Because the hypothesis that *ncd* represents a spindle organization defect does not account for the specific loss of maternal, but not paternal, chromosomes in early embryonic mitotic divisions, we favor the idea that the defect lies in a chromosome-associated molecule.

The segregation protein encoded at the *ca* locus thus performs several functions: it ensures proper segregation of homologs in meiosis I and prevents chromosome loss during meiosis and in early mitotic divisions of the embryo. Our results demonstrate that these functions can be separated by mutation and identify a region of the motor domain that can be used to discriminate between meiotic and mitotic motor function.

Materials and methods

Drosophila stock

ncd^D is described in Lindsley and Zimm (1990). It was isolated by E. Steiner as an EMS-induced mutant causing elevated non-disjunction in heterozygous females. Although isolated as a dominant mutant, it now shows only weak semi-dominant (this report) and recessive effects. Allelism to *ncd* was based on its map position and high non-disjunction frequencies in ncd/ncd^D females (Lindsley and Zimm, 1990). The stock was obtained from J.Kennison.

Recombination analysis

The meiotic and mitotic effects of ncd^D on chromosome segregation were mapped to the *ca* locus by recombination with *Drop ca brevis* and *ca loboid* chromosomes. Recombinant chromosomes were tested in homozygous females. The meiotic effect of ncd^D on chromosome segregation maps between *Drop* (*Dr*) (99.2) and *loboid* (*ld*) (102) on chromosome 3. This interval includes *ca* (100.7). Both *Dr* ncd^D and ncd^D *ld* recombinants caused very low frequencies of mitotic chromosome loss in embryos of homozygous females, indicating that this mitotic effect also maps to *ca*.

Chromosome segregation tests

Tests of X chromosome segregation were carried out by mating wild-type or mutant females to males carrying a recessively marked X chromosome (y^2 w^{bf}) and a dominantly marked Y chromosome (B^SY). Mitotic loss of the X and 4th chromosomes was also scored in these crosses. Offspring were scored for non-disjunctional exceptions (X/X/Y females and X/O males), gynandromorphs and haplo-4 (Minute) mosaics. Non-disjunction in female meiosis gives diplo- and nullo-X gametes. After fertilization by X- or Y-bearing sperm, approximately half of these non-disjunctional gametes are recovered as X/X/Y females or X/O males. The remainder of the gametes form non-viable triplo-X and O/Y embryos. X/X/Y females are recognized by the presence of the dominant B^S marker on the Y chromosome, resulting in narrow eyes, while X/O males are recognized by the absence of this marker. Gynandromorphs are mosaics of female and male tissue that arise after loss of an X chromosome from an X/X embryo during an early embryonic division, producing flies mosaic for X/X and X/O tissue. Exceptional gynandromorphs, mosaics of X/X/Y and X/Y tissue, arise after loss of an X chromosome from an X/X/Y embryo. Haplo-4, diplo-4 flies are mosaic for the Minute phenotype of small bristles, small body size and trident pattern on the thorax. They arise after a loss of a chromosome 4 during an early mitotic division.

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 compound chromosome 4 marked with *ci* and

ey^{R}. Regular segregation of chromosome 4 in females results in + triplo-4 and *M spa*^{pol} haplo-4 zygotes after fertilization by compound- or nullo-4 sperm, respectively. The reciprocal products of chromosome 4 non-disjunction in female meiosis are *spa*^{pol} diplo-4 and nullo-4 gametes, that are recovered as *spa*^{pol} or *ci ey*^{R} diplo-4 zygotes after fertilization by nullo- or compound-4 sperm, respectively. Since tetra- and nullo-4 zygotes either die as embryos or larvae, or are non-viable, only half of the non-disjunctional products are recovered.

Recovery of approximately equal numbers of diplo- and nullo-X, or diplo- and nullo-4 gametes is attributed to meiotic non-disjunction, while an excess of nullo-X or nullo-4 offspring is attributed to meiotic chromosome loss (Sturtevant, 1929).

Statistical tests

Standard chi-square tests were carried out on samples in which all expected values were ≥ 5. The null hypothesis was that the samples being tested were from the same population. The expected frequency of individuals in each category was calculated as the average number observed over all samples tested. In samples in which any expected value was <5, tests were carried out assuming a Poisson probability distribution. The probability of observing i events, p(i), is $(e^{-m} \times m^{i})/i!$ for a standard Poisson distribution, where m = number expected = (sample size) × (expected frequency).

Northern blots

Poly(A)$^{+}$ RNA was prepared from staged embryos (0–1 h, 1–4 h, 4–8 h, 8–12 h, 12–16 h, 16–20 h, 20–24 h), larvae (1st, 2nd, 3rd instar), pupae (24–26 h) and adults (3 day), and separated by electrophoresis on 1.2% agarose gels containing formaldehyde, as described previously (Yamamoto *et al.*, 1989). Transfer to nitrocellulose filters and hybridization with a single-stranded RNA probe were as previously described (Yamamoto *et al.*, 1989). Subsequent hybridization with an actin-encoding DNA fragment was used to monitor RNA amounts in each lane.

DNA sequence analysis

The dideoxy method (Sanger *et al.*, 1977) was used to sequence double-stranded DNA of cloned PCR-amplified *ncd*^{D} fragments. DNA templates were prepared from transformants in DH5α using the method described by Wang *et al.* (1988). The 2100 bp region sequenced was from the first ATG in the long open reading frame to the 3′ TAA termination signal. Oligonucleotides complementary to sequences at spaced intervals were used to sequence one strand completely and ~36% of the second strand. In regions where changes from the wild-type sequence were observed, both strands were sequenced. In the two cases in which nucleotide changes resulted in amino acid residue changes, the change was confirmed by sequencing the region from three combined, primary PCR mixes (for the V → F change) or by sequencing two DNA clones from an independent PCR mix (for the N → S change).

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