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Genetics

A multimember kinesin gene family in *Drosophila*

(microtubule motor protein/superfamily)

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ABSTRACT **Degenerate primers to the kinesin motor domain were used in the polymerase chain reaction to amplify DNA sequences from *Drosophila* genomic DNA and cDNA libraries. The amplified DNA sequences were hybridized to polytene chromosomes and the map positions of the hybridizing sites were determined. More than 30 sites of hybridization were detected, indicating that the kinesin gene family may be much larger than previously thought. One new family member has already been identified as a result of this screen. The map positions should aid in the identification of further kinesin family members. Some of these kinesin-related genes are anticipated to function in previously undiscovered roles in the cell.**

Kinesin, a microtubule-based motor molecule, was first detected in giant axons of the squid (1), where it is thought to mediate fast axonal transport along microtubules. Methods developed for purification of the protein (2) led to the recovery of a *Drosophila* kinesin heavy-chain gene through the use of recombinant DNA methods and antibodies directed against the protein (3). Kinesin was once thought to be unique among the force-producing motility proteins in being the only member of its class (4). Recently, however, several genes have been discovered whose predicted products are related to kinesin. These proteins are implicated in nuclear fusion during karyogamy (5), spindle pole body separation (6, 7), and meiotic chromosome segregation (8–10). Identification of the first three of the kinesin-related genes (*KAR3, bimC, ncd*) was through database searches for amino acid sequence similarity. A fourth gene, *nod*, was identified as a kinesin-related gene based on the similarity of its mutant phenotype to ca^{nd}, after the kinesin-like motor domain in ncd^{+} had been discovered. In addition to *Drosophila* kinesin heavy chain, *KAR3, bimC, ncd*, and *nod*, the kinesin gene family includes squid kinesin heavy chain (11), *Schizosaccharomyces pombe cut7* (7), and *Caenorhabditis elegans unc104* (12). The region of similarity among these proteins corresponds to the kinesin motor domain and includes a putative ATP-binding site and a region that can bind microtubules. Some 35–45% of the ≈320 amino acids that comprise this domain are identical between each protein and *Drosophila* kinesin heavy chain. Outside of this region, however, the predicted amino acid sequences are dissimilar. The motor domain binds to microtubules and supports motility *in vitro* (13). The nonmotor regions of the kinesin proteins are thought to bind to and transport different cellular components.

The number of kinesin, or kinesin-related, molecules in eukaryotic cells is not known. The finding of three such molecules in *Drosophila* suggests that the kinesin family of proteins may have further, as yet undiscovered, members. Using degenerate primers to the kinesin motor domain, amplification of DNA sequences by the polymerase chain reaction (PCR), and hybridization of amplified sequences to polytene chromosomes, we have detected multiple sites of kinesin-related sequences in the *Drosophila* genome. Our finding suggests that the kinesin family of genes in *Drosophila* may be very large, comprising 30 or more members. The sites of hybridization have been mapped. They include loci that encode the three previously discovered *Drosophila* kinesin-related genes: kinesin heavy chain (3), ca^{nd} or *ncd* (8, 9), and *nod* (10). Further sites of hybridization may correspond to genes that, like *ncd* and *nod*, have a role in chromosome distribution.

MATERIALS AND METHODS

cDNA Libraries. A cDNA library prepared from 0- to 4-hr *Drosophila* embryos in the plasmid vector pNB40 was obtained from N. Brown (Harvard University; ref. 21). A *Drosophila melanogaster* ovary cDNA library and a *Drosophila hydei* larval testis cDNA library, both in λgt11, were from L. Kalfayan (27) and H. Bünemann (Universität Düsseldorf), respectively.

PCR. Primers used correspond to the Ile-Phe-Ala-Tyr-Gly-Gln-Thr motif in the proposed ATP-binding site of the kinesin motor domain (PCR1) and to sequences complementary to the conserved Val-Asp-Leu-Ala-Gly-Ser downstream of the ATP-binding site (PCR4) (Fig. 1). Primer PCR1 is 5′-AT(C/A)-TT(T/C)-GC(C/A)-TA(T/C)-GG(A/T)-CA(A/G)-AC-3′ and is 64-fold degenerate. Primer PCR4 is 5′-(T/A)-(G/A/T)CC-(G/A/C)GC-CA(G/A)-ATC-(C/A/G)AC-3′ and is 108-fold degenerate. Reaction mixtures (100 μl) contained 100 ng of genomic DNA or 0.5–2 ng of cDNA library, 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1 mM $MgCl_2$, 0.01% gelatin, 125 μM dNTPs, 1 μM PCR1, 1 μM PCR4, and 2.5 units of *Taq* DNA polymerase. PCR consisted of 40 cycles of 1 min at 92°C, 2 min at 52°C, 1 min ramping to 72°C, and 3 min at 72°C with a 5-sec increase each cycle. PCR-amplified DNA was purified by fractionation in agarose gels, followed by elution from excised bands.

DNA Sequence Analysis. Gel-purified DNA was cloned into *Eco*RV-digested pBluescript (Stratagene) and sequenced by the dideoxy chain-termination method (16).

***In Situ* Hybridization.** Gel-purified DNA was labeled with biotin-dUTP by oligonucleotide priming (17). Hybridization to polytene chromosome squashes (18) and detection of hybridized sequences (19) were as described previously.

Chromosome Mapping. Mapping of sites of hybridization was performed by photographing chromosomes with Kodak 4164 Tri-X professional film and comparing enlargements with photographs of the *Drosophila* chromosome maps (20).

RESULTS AND DISCUSSION

In order to recover kinesin-related gene sequences, degenerate oligonucleotide primers corresponding to two regions of

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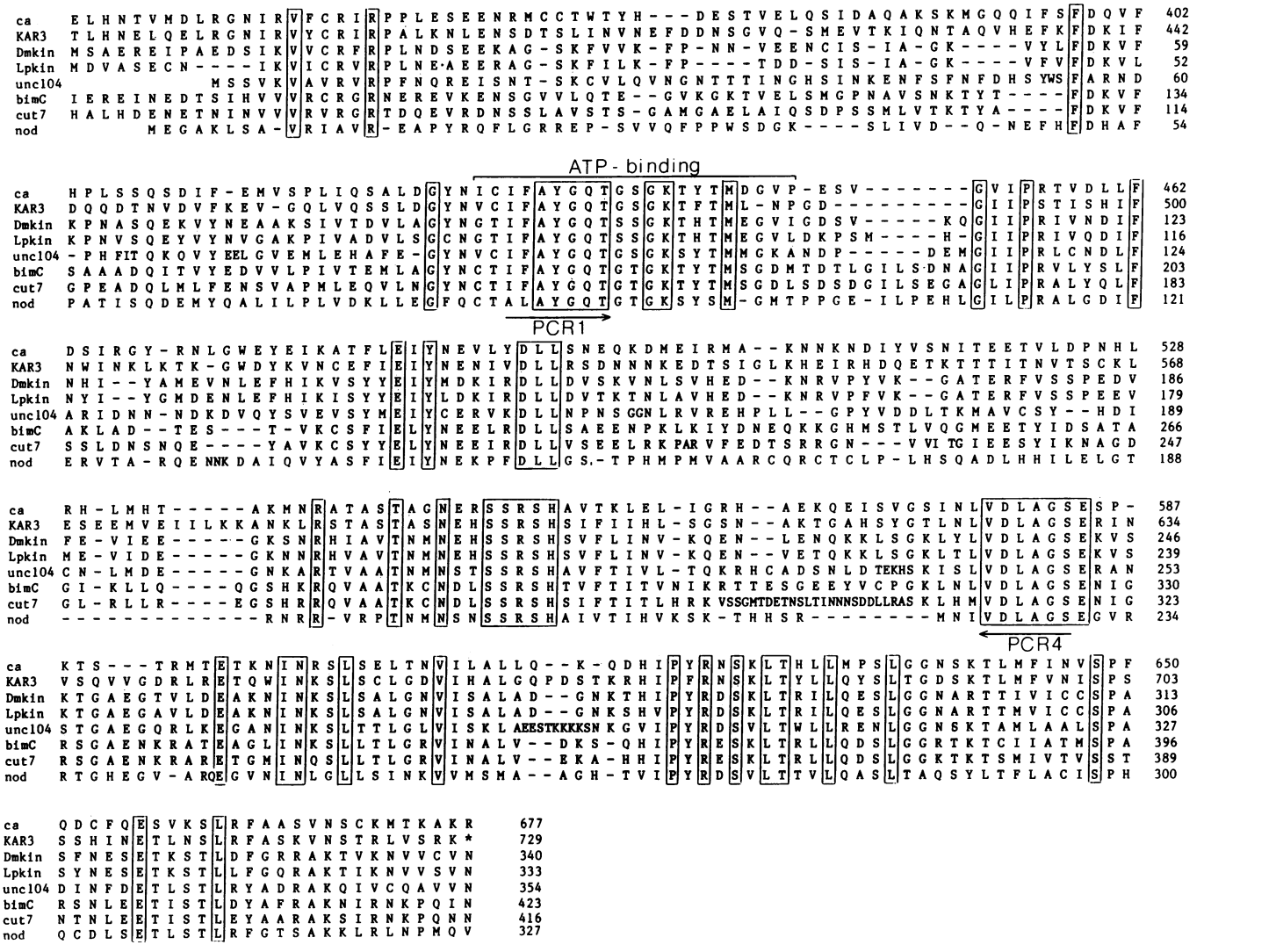

FIG. 1. Motor domain of kinesin family proteins. Comparison of predicted amino acid sequences of *ncd*$^+$ *(ca), KAR3, D. melanogaster* kinesin heavy chain (Dmkin; ref. 14), *Loligo pealii* kinesin heavy chain (Lpkin), *unc104*$^+$, *bimC*$^+$, *cut7*$^+$, and *nod*$^+$ (modified from figure 2 of ref. 15). Identical amino acid residues are boxed. The proposed ATP-binding site is indicated. Regions of conserved amino acid residues that correspond to the degenerate oligonucleotide primers used in these experiments, PCR1 and PCR4, are indicated.

conserved amino acid sequences within the motor domain were synthesized (Fig. 1). These sequences are conserved among the eight predicted kinesin or kinesin-related proteins from distantly related organisms, described above. The primers were used in the PCR to amplify sequences from total genomic *Drosophila* DNA. When amplified DNA 450–600 base pairs in length was hybridized *in situ* to *Drosophila* polytene chromosomes, 60–70 bands of hybridization were observed, including hybridization to the chromocenter. Although the sites of hybridization included the three previously identified kinesin-related *Drosophila* genes, the large number of sites and the chromocenter localization suggested that nonfunctional sequences were also being detected. To enrich for functional kinesin-related sequences, DNA was amplified from a *Drosophila* cDNA library prepared from 0- to 4-hr embryos (21) and amplified DNA of length ≈450 base pairs was hybridized to *Drosophila* polytene chromosomes. The sites of hybridization are listed in Table 1. They include the sites of the three *Drosophila* kinesin-related genes reported to date: kinesin heavy chain, *ncd*, and *nod* (Fig. 2). Localization to the chromocenter was not observed for sequences amplified from cDNA libraries. DNAs that hybridized to 99B/C and 53A were cloned from a PCR mixture and were confirmed by sequence analysis to be *ncd* and the kinesin heavy-chain gene, respectively.

At least three of the sites identified in this study correspond to known genes; however, not all of the loci detected may encode functional kinesin-related genes. Sequences homologous to these loci are present in a cDNA library, however, suggesting that they are transcribed. Their ability to be amplified using primers corresponding to conserved sequences within the kinesin motor domain suggests that they are kinesin-related. Furthermore, a fourth site identified in this screen has recently been mapped to a position in a "chromosome walk" consistent with its identity as lethal(1)zeste-white 4 [*l(1)zw4*] and found by sequence analysis to encode a predicted kinesin-related protein (B. Williams, E. Varak, and M. Goldberg, personal communication). Two sites (54D and 61F; L. S. B. Goldstein, personal communication) correspond to map locations for DNAs recovered by PCR that have been sequenced and shown to encode kinesin homologues (22). Finally, sequences that hybridize to 52A have been analyzed and found to encode a predicted kinesin-related protein that differs from kinesin heavy chain, which is encoded at 53A. Although not all of the sites of hybridization may encode functional kinesin-related genes, it seems likely, based on the conservation of the primer sequences in members of the kinesin gene family reported to date, that many of the kinesin-related genes are among the sites detected. The products of these genes are likely to

Table 1. Map positions of PCR-amplified DNA sequences homologous to the kinesin motor domain

Chromosome	Location	Embryo (0–4 hr)	Ovary	Larval testis
X	3A5–8 [*l(1)zw4*]	+	+	
	8A	+	+	
	8F	+		
	9E	+		
	10A	+		
	10C5–8 *nod*	+	+	
	11B	+		
	12B	+		
2L	26D1,2	+	+	
	32F	+	+	
	36C,D	+		
	38B	+		
2R	42D1,2		+	
	43E/F	+		
	48A	+	+	
	52A5–10	+	+	+
	53A3–5 kinesin HC	+	+	
	54D1	+	+	
	59F	+		
	60C	+		
	60E	+		
3L	61F3,4	+		
	64B	+		
	65F5–11	+	+	
	67C	+		
	68D	+	+	
	70B	+		
	72D	+	+	
3R	83B	+	+	
	85E1,2	+		
	86F3–7	+		
	87E3–12	+		
	96C1	+		
	98A	+	+	
	99B/C ca^{nd}	+	+	+

Sites of hybridization detected for DNA sequences amplified from a *Drosophila* embryo (0–4 hr), a *D. melanogaster* ovary, and a *D. hydei* larval testis cDNA library are listed. HC, heavy chain.

include minus-end-directed microtubule motors, like the ncd^+ gene product (23, 24), as well as plus-end-directed motors, like kinesin.

The use of tissue- and stage-specific cDNA libraries in PCRs provides information regarding gene expression. Sites of hybridization for sequences amplified from ovary and larval testis cDNA libraries are shown in Table 1. Amplified sequences from all three cDNA libraries hybridize to the site at 99B/C that encodes *ncd*. This is consistent with genetic and Northern blot data indicating that ncd^+ is expressed abundantly in ovaries and early embryos and in low amounts in males (15, 19). The role of ncd^+ in chromosome segregation in males is not certain.

Some of the loci detected in this study may encode further genes involved in meiotic chromosome segregation in females. These genes may be among the sequences amplified from ovary cDNAs. Among these loci may also be genes that perform the same function in chromosome distribution as ncd^+, but in mitosis. A candidate for such a gene is at 52A, a site detected by amplified sequences from all three cDNA libraries. Mutant phenotypes of mitotic chromosome nondisjunction and loss will help to identify these genes. A site at 28D was found for sequences amplified from genomic DNA. This site may encode paternal loss (*pal*), a gene whose product is predicted to function in male meiosis in a manner analogous to that of ncd^+ (25). Although this site was not

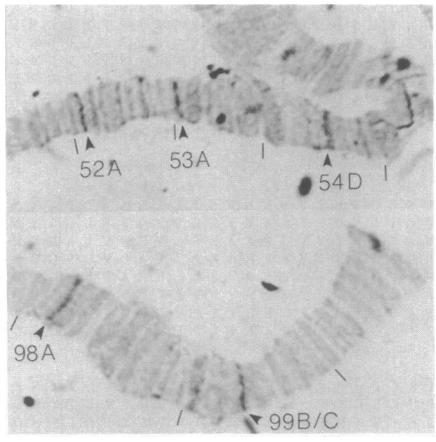

FIG. 2. Sites of hybridization corresponding to known genes. Loci shown are 53A (kinesin heavy chain) and 99B/C [claret nondisjunctional (ca^{nd})]. Sites of hybridization at 52A, 54D, and 98A are also indicated. PCR-amplified DNA was gel-purified, labeled with biotin-dUTP by oligonucleotide priming, and hybridized to polytene chromosome squashes.

detected using a probe amplified from larval testis cDNAs, sequences homologous to this site may be present in an adult testis cDNA library.

Other possible functions of the genes identified here may be in cytoplasmic transport. Although one *Drosophila* kinesin heavy-chain gene with a presumed role in axonal transport has been identified (3), the number of such molecules is not known. Another possible candidate for such a gene is *l(1)zw4*, which shows a neurogenic phenotype (26). Kinesin-related molecules may also play a role in cytoplasmic transport in non-neuronal cells. Correlation of sites of hybridization with mutant phenotypes may lead to the identification of genes with this function. The high degree of conservation of the motor domain even among distantly related organisms (Fig. 1) suggests that the PCR may be used to identify and isolate kinesin-related genes not only from *Drosophila* but also from other organisms.

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