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Isolation and characterization of the gene encoding the heavy chain of *Drosophila* kinesin

(cell motility/kinesin/cDNA cloning)

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ABSTRACT **An antiserum that recognizes the heavy chain of *Drosophila* kinesin was used to isolate *Drosophila* cDNA clones. Immunoblot analysis of the proteolytic fragments of the protein produced by one of the cDNA clones has demonstrated that the cDNA clones encode the heavy chain of *Drosophila* kinesin. The *in vitro*-synthesized product of the largest cDNA comigrates with *Drosophila* kinesin heavy chain on $NaDodSO_4$/polyacrylamide gels and binds to taxol-stabilized microtubules in the presence of the nonhydrolyzable analogue of ATP, 5′-adenylyl imidodiphosphate, but not in the presence of ATP or 0.1 M KCl. Analysis of the cDNA clones suggests that there is a single gene encoding kinesin heavy chain in *Drosophila* located at polytene chromosome position 53A. However, Southern hybridization analyses suggest the presence of related sequences in the *Drosophila* genome.**

In eukaryotic cells, microtubule-based motility is thought to be involved in many cellular processes including mitosis, cell-shape changes, and organelle transport. To understand the molecular mechanism of microtubule-based motility, it is essential to identify and analyze the "motors" that generate the motile forces. Recently, a force-generating protein called kinesin was discovered in extracts of squid axoplasm (1), in unfertilized sea urchin eggs (2), as well as in chicken and bovine brain (1, 3).

Kinesin is thought to be a multiprotein complex with a heavy chain ranging in size in different species from 110 kDa to 134 kDa and light chains of 60–80 kDa. In the presence of ATP, kinesin induces purified microtubules to move on glass or relative to one another in solution. By using axonemes or astral arrays of microtubules polymerized from centrosomes, it was shown that kinesin-induced movement has a polarity corresponding to the anterograde direction in axons (4). Biochemical analyses have shown that kinesin binds to microtubules in the presence of nonhydrolyzable ATP analogues such as 5′-adenylyl imidodiphosphate p[NH]ppA; (sometimes referred to as AMP-PNP), and releases from microtubules when ATP is added (1). Moreover, there is evidence indicating that kinesin is a microtubule-activated ATPase (5, 6). It has been proposed that kinesin may generate movement along microtubules by cyclic cross-bridge interactions with microtubules, coupled to ATP hydrolysis.

Since kinesin was originally isolated from a soluble fraction of squid axoplasm that induces organelle movement on microtubules, it has been suggested that kinesin may power organelle translocation during axoplasmic transport in squid giant axon (1). In addition, a recent immunolocalization study of sea urchin kinesin has raised the possibility that this protein is present in the mitotic spindle, thus suggesting a role for kinesin in mitosis (2, 7). As yet, there are no *in vivo* data to support these hypotheses.

To understand the *in vivo* functions of proteins, one powerful approach is to utilize genetic analysis of *Drosophila melanogaster*. Recently, immunological, biochemical, and *in vitro* motility data have shown that kinesin exists in *Drosophila* embryos, larvae, adults, and tissue culture cells (8). *Drosophila* kinesin binds to microtubules in the presence of p[NH]ppA and induces ATP-dependent microtubule gliding over a glass substrate. The heavy chain of *Drosophila* kinesin has been isolated and found to migrate on $NaDodSO_4$/polyacrylamide gels with a relative molecular mass of 115 kDa (this polypeptide is referred to as the *Drosophila* 115 in the following text). It has been shown that the *Drosophila* 115 is immunologically related to other kinesins. The antisera against squid and sea urchin kinesin recognize *Drosophila* 115; similarly, a polyclonal antiserum raised against *Drosophila* 115 recognizes squid and sea urchin kinesin (8).

In this report, we present our isolation of cDNA clones encoding *Drosophila* 115. Studies of the protein products made from these cDNA clones have demonstrated that the clones identify the *Drosophila* kinesin heavy-chain gene and suggest that the isolated heavy chain possesses both the ATP and the microtubule-binding activities of the intact kinesin complex.

MATERIALS AND METHODS

Isolation of Microtubules Containing Kinesin. Microtubules were isolated from Schneider's line 2 (S2) *Drosophila* tissue culture cells as described (9) except that the high-speed supernatant, which was incubated with taxol and GTP, was incubated with 2.5 mM p[NH]ppA at room temperature for 10 min before it was layered over a sucrose cushion. The microtubule pellet from the sucrose cushion contains kinesin. We note that in our gel system, the *Drosophila* 115 is slightly larger (120 kDa) than reported by Saxton *et al.* (8). For consistency, however, we refer to this polypeptide as *Drosophila* 115.

Isolation of cDNA Clones and Induction of Lysogens. An adult *Drosophila* head cDNA library in λgt11 was provided by P. Salvaterra (10). Anti-115 antiserum was used as the probe. This antiserum is a total rabbit serum specific for the heavy chain of *Drosophila* kinesin (referred to as anti-115 antiserum in the following text). The antiserum was raised and fully characterized by Saxton *et al.* (8). The methods for phage screening and lysogen induction were those of Young and Davis (11) as modified by Goldstein *et al.* (9).

Affinity Purification of Antibody. Protein samples were run on a $NaDodSO_4$/polyacrylamide "curtain" gel and electro-

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Abbreviation: p[NH]ppA, 5′-adenylyl imidodiphosphate (sometimes referred to as AMP-PNP).

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phoretically transferred to a nitrocellulose filter. The curtain gel was prepared by casting the stacking gel with a wide (7–12 cm) central lane and one (2 mm) peripheral lane for the molecular size standards. Bands of interest were excised from the filter and incubated with anti-115 antiserum overnight at 4°C. Antibodies were eluted from the filter as described (9).

Isolation of Genomic Clones. Genomic clones were isolated from a library made from the *Drosophila* strain *dp cl cn bw* by R. Blackman (Howard University). In this strain, all second chromosomes are identical by descent and hence no polymorphism on chromosome 2 should exist. The library was screened by plaque hybridization (12) using cDNA clone 1 as the probe.

Hybridization Conditions. For hybridizations to genomic DNA, DNA from *de cl cn bw* embryos was digested with restriction enzymes, run on 0.7% agarose gels, transferred to nitrocellulose, and hybridized as described elsewhere.

For RNA blot analysis, RNA was isolated from *Drosophila* heads following a modified procedure of O'Hare *et al.* (13). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (12). Poly(A)$^+$ RNA was run on a 0.7% agarose gel, in 2.2 M formaldehyde/20 mM 2-(*N*-morpholino)propane sulfonic acid/5 mM sodium acetate/0.5 mM EDTA, and transferred to nitrocellulose. Hybridization and washing were done as described elsewhere (9).

In situ hybridization to polytene chromosomes was done by the procedure of Bonner and Pardue (14) with tritiated cDNA and genomic clones as probes. Polytene chromosomes were prepared from Canton S flies.

Probes for all hybridizations were labeled by nick-translation (12).

Subcloning into pEV Expression Vector. pEV expression vectors were constructed by Crowl *et al.* (15). These vectors contain synthetic DNA sequences comprising a computer-generated model ribosomal binding site, translational start, and three restriction sites, all located downstream from the regulated phage λ P_L promoter. To accommodate all three translational reading frames, each vector differs by the number of adenine residues located between the ATG and the first restriction site, the *Eco*RI site. cDNA was inserted into the *Eco*RI site in the vectors, and a subclone that expressed a protein recognized by anti-115 antibody was identified by the *in situ* immunological screening method described in their paper (15).

Cyanogen Bromide Digestion. Proteins from microtubule preparations or from transformed bacteria were run on a 10% $NaDodSO_4$/polyacrylamide gel, and the bands of interest were visualized by Coomassie blue staining and excised from the gel. The gel slices were washed in water for 20 min, then incubated at room temperature for 1 hr in 50 mg of cyanogen bromide per ml, which was diluted with 0.1 M HCl, 0.4% 2-mercaptoethanol from a stock (700 mg/ml) (in 88% formic acid). After incubation, the slices were washed twice with water and once in 1:4 diluted stacking gel buffer. They were then placed in the wells of a 12–16% polyacrylamide gradient gel and electrophoresed at 60 V for 15 hr. Proteins from this second gel were electrophoretically transferred to a nitrocellulose filter, which was then incubated with affinity-purified anti-115 antiserum at 4°C overnight. After incubation in primary antibody, the filter was rinsed and incubated with alkaline phosphatase-conjugated secondary antibody (1:500 dilution) at 37°C for 1 hr. It was then developed with a solution containing 0.6 mg of nitroblue tetrazolium per ml in 0.15 M Tris·HCl and 60 mg of 5-bromo-4-chloro-3-indolyl phosphate per ml in dimethyl sulfoxide.

***In Vitro* Transcription and Translation.** The insert of cDNA clone 1 was subcloned into the Bluescript vector (Stratagene, San Diego, CA) oriented such that a T7 promoter was upstream of the 5′ end of the insert. For *in vitro* transcription, the plasmid was linearized by cutting at the *Bam*HI site in the polylinker region downstream of the 3′ end of the insert. Transcription was carried out in a 20-μl reaction mixture containing 500 ng of linearized DNA, 10 mM dithiothreitol, 40 mM Tris·HCl (pH 7.4), 6 mM $MgCl_2$, 20 mM NaCl, 2 mM spermidine, 0.5 mM each ribonucleotide (ATP, UTP, CTP, GTP), 40 units of RNase inhibitor, and 10 units of T7 RNA polymerase. TE (10 mM Tris·HCl, pH 7.5/1 mM EDTA) was added instead of DNA as a no-template control. The reaction was carried out at 37°C for 30 min. For *in vitro* translation, a rabbit reticulocyte lysate system was used as described by Jackson and Hunt (16). [^{35}S]Methionine was used in the translation reaction to label the translation product.

Microtubule-Binding Experiment. The *in vitro* translation mixture was clarified by spinning at 50,000 × g_{max} for 30 min. Taxol-stabilized microtubules lacking microtubule-associated proteins were prepared as described by Goldstein *et al.* (9). The binding mixture (10 μl) contained 2 μl of the *in vitro* translation mixture (with or without added RNA), 1 mg of microtubules per ml, 2.5 mM p[NH]ppA (or 2.5 mM ATP or 0.1 M KCl), 20 μM taxol, 2 mM GTP, 0.1 M Pipes, 1 mM $MgCl_2$, 2 mM EGTA, and 2 mM dithiothreitol. Mixtures were incubated at room temperature for 15 min and spun at 50,000 × g_{max} for 30 min. The pellets were resuspended in 0.1 M Pipes/1 mM $MgCl_2$/2 mM EGTA/2 mM dithiothreitol, and run on a 7.5% $NaDodSO_4$/polyacrylamide gel. The gel was stained with Coomassie blue, dried, and exposed to an x-ray film.

RESULTS

Isolation and Immunological Characterization of cDNA Clones Whose Products Are Recognized by Anti-115 Antiserum. A *Drosophila* head cDNA library in λgt11 was screened with the anti-115 antiserum specific for *Drosophila* 115 (this antiserum was fully characterized as described in ref. 8). Six phage clones producing products recognized by the antiserum were isolated from $\approx 1 \times 10^6$ recombinant phages. Immunoblots of lysates from induced lysogens of the six clones were stained with anti-115 antiserum, which was affinity-purified on the *Drosophila* 115 protein. Each lysogen produced a polypeptide that was recognized by the antiserum. However, the intensity of staining by the antiserum was very weak, suggesting that only a very small amount of these polypeptides were produced in these lysogens. Moreover, none of these polypeptides had a size larger than that of β-galactosidase. One explanation is that the polypeptides produced were not fusion proteins but were encoded by messages not transcribed from the strong *lac* promoter. Further assays of plaques showed that the clones produced proteins that were recognized by anti-115 antiserum even without isopropyl β-D-thiogalactoside induction. This result supports the suggestion that the transcripts of interest were not transcribed from the *lac* promoter (data not shown; formally equivalent data on immunoreactivity are shown in Fig. 2).

To determine that the proteins produced by the *Drosophila* cDNA inserts of the clones were not recognized by contaminating non-anti-115 antibodies in the antiserum, antibodies were affinity-purified on these proteins. Antibodies eluted from each of the six proteins specifically recognized *Drosophila* 115 in both microtubule and *Drosophila* head homogenate preparations (data not shown). Antibodies eluted from a random protein band in the λgt11 lysogen lysate, which did not contain a cDNA insert, did not recognize *Drosophila* 115 in either of the preparations. These results indicate that the isolated clones and *Drosophila* 115 are recognized by the same antibodies.

Hybridization Analyses and Restriction Mapping of the cDNA Clones. Cross-hybridization analysis and restriction mapping were conducted to determine whether the isolated clones are related. The inserts of all six clones cross-hybridize and share the same restriction sites (Fig. 1). These results suggest that the six clones have cDNA inserts derived from the same gene.

The insert from clone 1 was used to probe an RNA blot of poly(A)$^+$ RNA isolated from *Drosophila* head. The probe hybridized to a 4000-nucleotide message, which is sufficient to encode a 115-kDa polypeptide (see Fig. 5).

Expression of Clone 18 in the Expression Vector pEV-vrf. In initial experiments to study the protein products of the isolated cDNAs, we used bacterial expression vectors. To express DNA in bacteria efficiently, it is necessary to have the start of the protein-coding region of the DNA an appropriate distance from a Shine–Dalgarno sequence (17). Therefore, it is not straightforward to express in bacterial cells complete eukaryotic cDNA clones, which may have leader sequences upstream of the coding region. For this reason, cDNA clone 18, which is less than full length and likely to be lacking the 5′ leader sequences, was chosen for initial experiments. The insert of clone 18 [3 kilobases (kb)] was subcloned into the *Eco*RI site in each of the three pEV vectors, which have different reading frames at the *Eco*RI site, relative to the start of translation. After *in situ* immunological screening with anti-115 antiserum, a subclone designated pVJ18 was isolated. The subclone expresses a 105-kDa polypeptide that is recognized by the polyclonal anti-115 antiserum and by affinity-purified anti-115 antibody, but not by the preimmune rabbit serum (Fig. 2). To ensure that the antibodies that recognized the 105-kDa polypeptide were indeed the antibodies recognizing *Drosophila* 115, an affinity-purification experiment was conducted. The antibodies eluted from the 105-kDa protein exclusively recognized *Drosophila* 115 from both microtubule and *Drosophila* head homogenate preparations. In addition, the 105-kDa polypeptide is also recognized by an anti-squid kinesin antibody provided by R. Vale (University of California, San Francisco), showing that the antibodies against kinesins from two distant species both recognized the 105-kDa polypeptide (data not shown). To determine the orientation of the cDNA inserts, restriction analysis of pVJ18 was carried out. Since this clone makes immunoreactive protein, we can infer that the 5′ end of the coding sequence is proximal to the promoter. Therefore, positions of restriction sites indicate direction of transcription (Fig. 1).

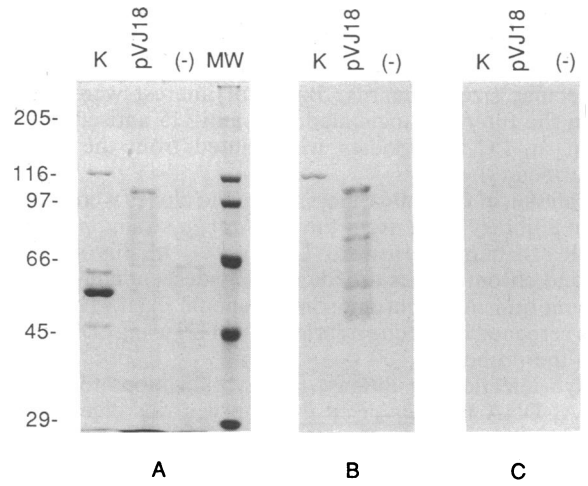

FIG. 2. Immunoreactivities of *Drosophila* 115 and the 105-kDa polypeptide produced by transformed bacteria. $NaDodSO_4$/-polyacrylamide gels loaded with kinesin isolated from *Drosophila* cells (lanes K), lysate of bacteria transformed with pVJ18 (lanes pVJ18), and lysate of bacteria transformed with pEV-vrf2 (lanes −) were stained with Coomassie blue (*A*) or electrophoretically transferred to nitrocellulose (*B* and *C*) and stained with affinity-purified anti-115 antiserum (*B*) or preimmune serum (*C*). Lane MW, molecular mass markers. Numbers on left are kDa.

Comparison Between the Proteolytic Fragments of *Drosophila* 115 and the 105-kDa Polypeptide Produced by pVJ18. To confirm that the cDNA clones encode *Drosophila* 115, the primary structures of *Drosophila* 115 and the 105-kDa polypeptide produced by pVJ18 were compared by analyzing proteolytic fragments of the two proteins. If the 105-kDa polypeptide is part of the *Drosophila* 115, we would expect to observe similar proteolytic fragments from the two proteins. To conduct this analysis, both proteins were isolated in gel slices, digested with cyanogen bromide, and run on a second gel. An immunoblot of the separated proteolytic fragments probed with affinity-purified anti-115 antiserum shows that cyanogen bromide digestion generated essentially the same set of epitope-containing fragments from *Drosophila* 115 as from the 105-kDa polypeptide, although there are minor differences (Fig. 3). We suggest that the few differing fragments arise as a result of the 105-kDa polypeptide being truncated, presumably at the amino terminus. In view of the substantial similarity between the two sets of

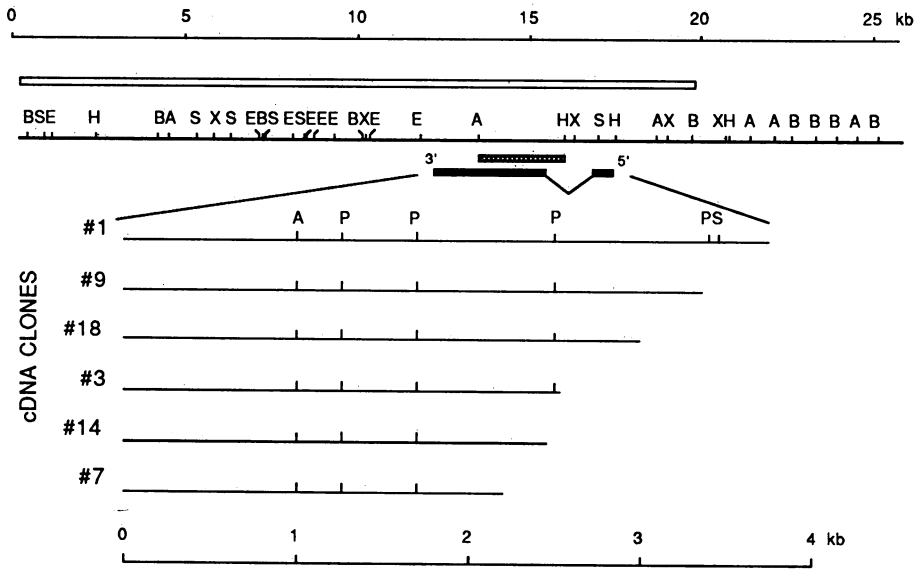

FIG. 1. Restriction maps of genomic and cDNA clones encoding *Drosophila* 115. A, *Sac* I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; S, *Sph* I; X, *Xba* I. Bars represent probes used in hybridization analyses. Open bar, genomic DNA probe used in *in situ* hybridization analysis; solid bar, cDNA clone 1, used as probe in Southern and RNA blot analyses; shaded bar, the 2.7-kb *Hin*dIII/*Sac* I fragment used as a probe in Southern blot analysis.

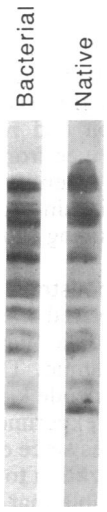

FIG. 3. Antibody staining of proteolytic fragments generated from the 105-kDa polypeptide made in transformed bacteria (bacterial) and native *Drosophila* 115 (native). Both proteins were digested with cyanogen bromide (50 mg/ml) at room temperature for 1 hr. The proteolytic fragments were separated on a $NaDodSO_4$/polyacrylamide gel, electrophoretically transferred to nitrocellulose, and reacted with affinity-purified anti-115 antiserum.

epitope-containing fragments, we conclude that pVJ18 encodes most of the kinesin heavy chain and, therefore, that the clones we have isolated are derived from the gene encoding *Drosophila* 115.

Microtubule Binding of the *in Vitro* Translation Product of cDNA Clone 1. To begin analyzing the *in vitro* properties of the product of these cDNA clones, the largest cDNA clone (clone 1) was transcribed and translated *in vitro*. The *in vitro* translation product of the clone comigrated on a $NaDodSO_4$/polyacrylamide gel with *Drosophila* 115 isolated from cultured cells (Fig. 4). To see if the *in vitro*-produced polypeptide has functional properties similar to those of native kinesin, an experiment was carried out to assess nucleotide-dependent microtubule binding. The results (Fig. 4) show that most of the *in vitro*-produced *Drosophila* 115 copellets with microtubules in the presence of 2.5 mM p[NH]ppA but not in the presence of 2.5 mM ATP or 0.1 M KCl. This result indicates that the *in vitro*-translated product of cDNA clone 1 binds to microtubules specifically in the presence of p[NH]ppA, which is a characteristic property of kinesin. The p[NH]ppA specific binding of the product of the cDNA clone provides further strong evidence that the cDNAs we have isolated encode the heavy chain of *Drosophila* kinesin.

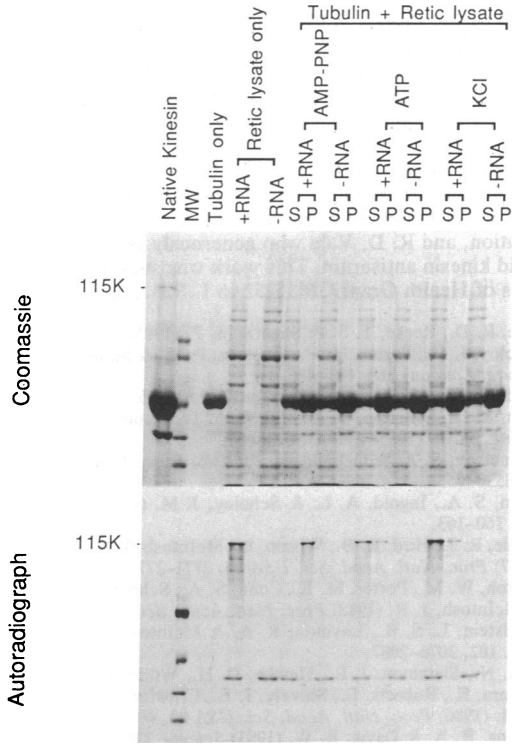

FIG. 4. p[NH]ppA-dependent microtubule-binding of *Drosophila* 115 produced *in vitro*. Reticulocyte lysate with or without the *Drosophila* 115 mRNA (+RNA or −RNA) was incubated with tubulin in the presence of p[NH]ppA (AMP-PNP), ATP, or 0.1 M KCl at room temperature for 15 min. The mixture was subjected to centrifugation to give a supernatant (S) and a pellet (P) fraction prior to analysis on a $NaDodSO_4$/polyacrylamide gel. (*Upper*) Reproduction of the Coomassie blue-stained gel. (*Lower*) Autoradiogram of the same gel.

Studies on the Organization and Genomic Location of the *Drosophila* 115 Gene. To study the organization of the *Drosophila* 115 gene, six genomic clones were isolated. Restriction analyses have shown that all of the genomic clones are overlapping since they share many restriction sites and positions. A combined restriction map of these genomic clones is diagrammed in Fig. 1. The cDNA clones share the *Sac* I site and the *Sph* I site with the genomic clones, which are proximal to the 3′ end and the 5′ end of the cDNAs, respectively. They do not share the two restriction sites (*Hin*dIII and *Xba* I) that are located between *Sac* I and *Sph* I on the genomic fragments. In addition, the *Sac* I/*Sph* I restriction fragment derived from the cDNA is ≈1.4 kb smaller than that from the genomic DNA. Therefore, there appears to be an intron ≈1.4 kb long in the region, as indicated on the restriction map (Fig. 1).

The map was compared to the data obtained by probing Southern blots of restriction enzyme-digested *Drosophila* genomic DNA with cDNA clone 1 (Fig. 5). As shown, some of the bands are less intense and do not fit the predictions of the restriction map. These bands may represent *Drosophila* 115 coding regions not included in the set of genomic clones, or they may be sequences from genes related to the *Drosophila* 115 gene. To distinguish between the two possibilities, a 2.7-kb *Hin*dIII/*Sac* I fragment from one of the genomic clones, which contains only an internal segment of the kinesin-coding region, was used to probe Southern blots of digested genomic DNA. This probe again hybridized to the bands that did not fit the predictions of the restriction map (Fig. 5). This result indicates that the unexpected restriction

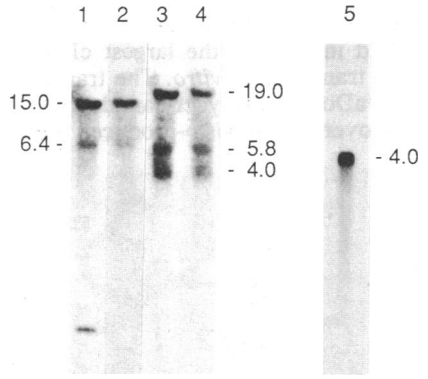

FIG. 5. Autoradiograms of hybridizations to Southern blots showing that there are sequences related to the *Drosophila* 115 gene in the *Drosophila* genome (lanes 1–4) and to an RNA blot showing the size of the transcript encoding *Drosophila* 115 (lane 5). In Southern blot analyses, *Drosophila* genomic DNA was digested with *Hin*dIII (lanes 1 and 2) or *Eco*RI (lanes 3 and 4) and transferred to nitrocellulose. cDNA clone 1 (lanes 1 and 3, indicated as solid bar in Fig. 1) or a *Hin*dIII/*Sac* I fragment of a genomic clone (lanes 2 and 4, indicated as shaded bar in Fig. 1) were used as the probes. The bands ranging in size from 6.4 to 4.0 kb do not fit the prediction of the restriction map (Fig. 1). In the RNA blot analysis, labeled cDNA clone 1 was hybridized to an RNA blot of poly(A)$^+$ RNA isolated from *Drosophila* head.

fragments do not result from exons that are included in the cDNA but not in our set of genomic clones. This conclusion is also supported by another set of hybridization analyses, in which Southern blots of restriction enzyme-digested *Drosophila* DNA were probed with the cDNA clones 3 and 7, as well as a 2.8-kb *Eco*RI/*Sac* I fragment of cDNA clone 1. cDNA clones 3 and 7 are missing a portion of DNA sequences at the 5′ end of cDNA clone 1 (none of these clones hybridized to the 1.5-kb 5′ *Hin*dIII fragment that hybridized to clone 1). On the other hand, the 2.8-kb *Eco*RI/*Sac* I fragment of cDNA clone 1 is missing a portion of the 3′ end of the clone. All of these probes hybridized to the same bands that are not accounted for by the restriction map of the region from which the cDNAs are all derived (data not shown). Thus, these data suggest that there are one or more DNA sequences in the *Drosophila* genome that share homology with the *Drosophila* 115 gene that we have isolated.

To map the genomic location of the *Drosophila* 115 gene, an *in situ* hybridization experiment was carried out using cDNA clone 1. The cDNA clone appeared to hybridize to a single site in the polytene chromosomes, at position 53A of chromosome 2R. However, in our initial experiments the signal was rather weak. To enhance the signal, we used a 19-kb genomic clone as probe, which hybridized to the same single site as the cDNA clone (Fig. 6).

DISCUSSION

Several lines of evidence show that we have obtained cDNA and genomic clones encoding the *Drosophila* kinesin heavy chain. The strongest evidence comes from two sets of experiments on products of the cDNA clones. The first set of experiments (described in Figs. 2 and 3) utilized bacteria to produce a large amount of protein from cDNA clone 18. A 105-kDa polypeptide produced by these bacteria was recognized by both anti-*Drosophila* and anti-squid kinesin heavy chain antisera, indicating that the cDNA expresses a polypeptide that shares antigenic determinants with kinesins from two evolutionarily distant species. Comparison between the proteolytic fragments of this protein product and those of the *Drosophila* 115 showed that the primary structures of the two proteins are almost identical, even though the polypeptide produced by the transformed bacteria is smaller than *Drosophila* 115. In the second set of experiments (described in Fig. 4), the largest cDNA clone was transcribed and translated *in vitro*. The translation product comigrates in $NaDodSO_4$/polyacrylamide gels with *Drosophila* 115. Moreover, this *in vitro*-produced protein binds to microtubules in the presence of p[NH]ppA but does not bind in the presence of ATP. This is one of the primary biochemical characteristics used to identify and isolate kinesin. Even though different cDNA clones were used in these two sets of experiments, Southern cross-hybridization and restriction mapping data have shown that they are derived from a single gene. We conclude that these cDNA clones identify the gene encoding the heavy chain of *Drosophila* kinesin and that the largest cDNA clone contains the entire coding sequence of the protein.

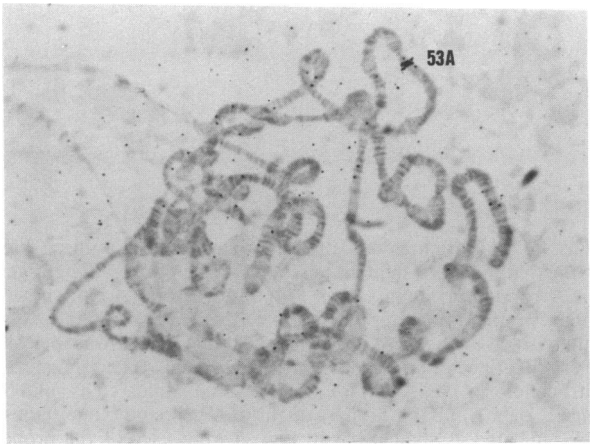

FIG. 6. *In situ* hybridization of a genomic clone (indicated as open bar in Fig. 1) to polytene chromosomes of Canton S.

While our hybridization analyses demonstrate that the cDNA and genomic clones we have isolated are derived from a single gene, our hybridization data indicate the presence of related sequences in the genome. Thus, it is possible that there are related genes that encode kinesin-like proteins. Our current *in situ* hybridization experiments have not revealed the location of these sequences since even after long exposures, additional sites of hybridization to polytene chromosomes are not observed. It is possible that the other related genes are clustered at the same chromosomal region as the kinesin gene we have identified, or that the conditions in our *in situ* hybridization studies are too stringent to detect other genes that might only be partially homologous to the *Drosophila* 115 gene. Further work is necessary to locate and characterize these genes.

Isolation of the *Drosophila* 115 gene now makes it possible to study the protein with molecular and genetic techniques. For example, the nucleotide-specific interaction of the *in vitro*-expressed gene product with microtubules shows that *Drosophila* 115 may possess the microtubule-binding and the ATP hydrolysis activities of kinesin in the absence of the light chains. It may now be possible to localize the microtubule- and ATP-binding sites by generating and analyzing deletions of the clone. It may also be possible to analyze the motility properties of the kinesin heavy chain in a similar way. Eventually, analyses of mutations defective in kinesin function, in concert with structure–function analyses, will allow us to elucidate the function of kinesin *in vivo*.

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