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Localization of a myosin heavy chain-like polypeptide to *Drosophila* nuclear pore complexes

(nuclear transport)

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ABSTRACT **Antibodies previously used for immunofluorescence localization of a myosin heavy chain-like polypeptide to the nuclear envelope in higher eukaryotic cells crossreact with both muscle and nonmuscle isoforms of *Drosophila* myosin heavy chain. Analyses of *Drosophila* tissue culture cells and premyogenic embryos suggest that it is the nonmuscle isoform that is associated with the nuclear envelope. Further immunofluorescence and immunoelectron microscopy indicate that this polypeptide is associated with nuclear pore complexes. These data support the hypothesis put forward previously that myosin or myosin-like molecules play a role in pore complex architecture.**

The histochemical localization of an ATPase activity to the nuclear pore complex (1–5) as well as recent evidence demonstrating energy requirements for specific nuclear protein import in cell-free systems (6–9) has led to considerable interest in nuclear envelope ATPases. Previously, we reported the identification of a high molecular mass ATPase polypeptide found in nuclear envelope-enriched fractions from *Drosophila* as well as a variety of vertebrate tissues (10). In *Drosophila*, this polypeptide was originally thought to be closely related to or identical with muscle myosin heavy chain and was localized to the nuclear envelope *in situ* (11). On the basis of these observations and on the known structure of myosin, we proposed a model in which myosin molecules were major constituents of the nuclear pore complex. A similar but less explicit model was suggested by LeStourgeon (12).

Shortly after publication of our previous article (11), Kiehart and Feghali (13) reported that *Drosophila* tissue culture cells possessed a distinct myosin heavy chain of identical size to muscle myosin heavy chain. They designated this isoform cytoplasmic myosin heavy chain and suggested that it was the product of a gene different from that coding for the muscle isoform. This suggestion was recently confirmed (14).

Drosophila tissue culture cells apparently lack the muscle myosin heavy chain isoform (13). If true, this would rule out the possibility previously suggested (11) that muscle myosin heavy chain was a pore complex component since tissue culture cells obviously contain nuclear pores. To gain further insight into the potential role of myosin-like molecules as nuclear pore complex components in *Drosophila*, we initiated a survey of myosin heavy chain isoforms in nonmuscle cells and during embryogenesis. Our observations were consistent with those of others (13, 14). Affinity-purified antibodies against muscle myosin heavy chain crossreacted with the cytoplasmic (nonmuscle) isoform and were used for further immunofluorescence and immunoelectron microscopy. Results of these experiments, which show punctate staining of male accessory gland nuclei at the light microscopic level and specific labeling of nuclear pore complexes by immunoelectron microscopy, lend support to our previous proposal that myosin or myosin-like molecules play a role in the structure and function of the nuclear pore complex. Together, these results also suggest that it is the nonmuscle isoform of *Drosophila* myosin that is involved.

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MATERIALS AND METHODS

The sources of the materials and most of the methods have been described (11, 15). Antibodies directed against *Drosophila* nuclear myosin heavy chain (anti-DNMHC) were affinity purified from rabbit serum as were anti-*Drosophila* lamin antibodies (16). Antiserum directed against *Drosophila* nonmuscle myosin (anti-DCM) was the generous gift of D. Kiehart (Harvard University). Monoclonal antibody directed against *Drosophila* tubulin was a generous gift of L. Goldstein (Harvard University). Monoclonal antibody 414 (mAb 414) ascites fluid directed against rat liver nuclear pore complex glycoprotein p62 (17) was a generous gift of L. Davis and G. Blobel (The Rockefeller University). *Drosophila melanogaster* (Oregon R, P2 strain) were grown in mass culture, and embryos were collected according to Allis *et al.* (18). $NaDodSO_4$/PAGE was on 7% polyacrylamide gels (19). One-dimensional $NaDodSO_4$/PAGE peptide mapping was according to Cleveland *et al.* (20) as previously described (11). Immunoblot analysis was according to Smith and Fisher (21). K_c cells generously provided by J. Watson (University of California at San Francisco) were grown in suspension culture on defined medium (22). Indirect immunofluorescence analyses of adult male *Drosophila* accessory glands were performed in an immunofluorescence chamber (23) as described (11). *Drosophila* K_c cells, grown in suspension culture, were harvested by centrifugation (2000 × *g* for 10 min), resuspended, and washed twice by recentrifugation in 140 mM NaCl and 10 mM KPO_4 at pH 7.5, and finally resuspended in 10 pellet volumes of standard extraction buffer (15, 24) supplemented with Triton X-100 to a final concentration of 1% (vol/vol). A nuclear pellet fraction was prepared and digested with nucleases at 37°C exactly as described for *Drosophila* embryo nuclei (24). Fractions were denatured with $NaDodSO_4$ and subjected to $NaDodSO_4$/PAGE and immunoblot analysis as described in the legend to Fig. 2. Immunoaffinity purification of myosin from *Drosophila* embryo extracts was by chromatography on columns of protein A-Sepharose to which affinity-purified anti-DNMHC antibodies had been covalently crosslinked with glutaraldehyde. Immunoaffinity columns were prepared and run according to Lin and Fisher (25). For indirect immunoelectron microscopy, accessory glands from the adult male genital tract (26) were dissected under a stereo microscope in MSM-Pipes (27) and were gently squashed between a clean microscope slide and a plastic coverslip (Bel-Art Products).

Abbreviation: mAb, monoclonal antibody.

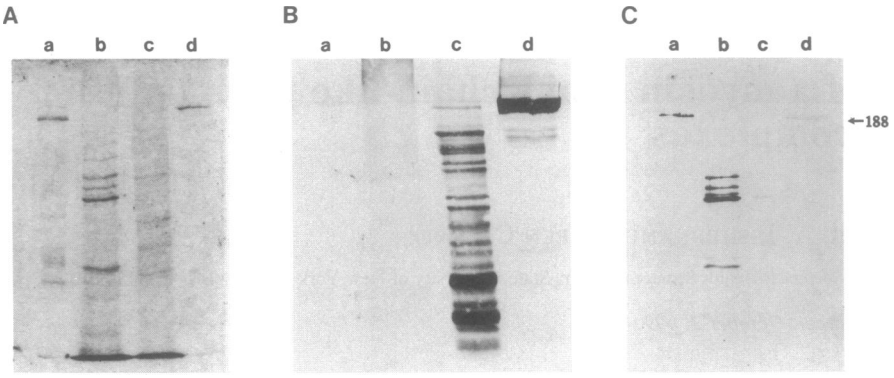

FIG. 1. Chymotryptic peptide map comparison of *Drosophila* myosin heavy chain isoforms purified from early embryos and adult muscle. Myosin was purified from 100,000 × *g* supernatants of *Drosophila* 0- to 4-hr embryo homogenates by immunoaffinity chromatography on columns of protein A-Sepharose to which affinity-purified anti-DNMHC antibodies had been crosslinked with glutaraldehyde. Immunoblot analysis of total extract and unadsorbed fractions demonstrated complete removal of immunoreactive species in a single pass through the column (data not shown). *Drosophila* myosin fractions immunoaffinity purified from 0- to 4-hr embryos (lanes a and b) and biochemically purified from adults (lanes c and d) were partially digested with chymotrypsin as described (11) and electrophoresed on $NaDodSO_4$/7% polyacrylamide minigels. (*A*) Coomassie blue-stained gel. (*B*) Immunoblot of a parallel gel probed with affinity-purified anti-DNMHC antibodies at a final concentration of 750 ng/ml. (*C*) Immunoblot of a second parallel gel probed with a 1:500 dilution of anti-DCM antiserum. Bands of immunoreactivity were visualized colorimetrically (21). Lanes: a, ≈500 ng of immunoaffinity-purified early embryo myosin before digestion with chymotrypsin; b, 10 times that much after digestion; d, ≈500 ng of adult muscle myosin before digestion; c, 10 times that much after digestion. In this figure and Figs. 2 and 3, the arrows indicate molecular masses in kilodaltons.

They were then fixed for 4 min with a freshly prepared solution of 2.6% (vol/vol) paraformaldehyde and 0.1% (vol/vol) glutaraldehyde in MSM-Pipes. Incubation with the primary antibody diluted in MSM-Pipes was for 1 hr at 37°C. Samples were washed with three changes of MSM-Pipes while mixing for a total time of ≈15 min and then incubated for 2 hr at 37°C with 10-nm gold-conjugated affinity-purified goat anti-rabbit IgG diluted in MSM-Pipes. This was followed by three washes of 5 min each in 560 mM NaCl, 10 mM KPO_4 (pH 7.5), 0.1% Triton X-100, 0.02% (wt/vol) $NaDodSO_4$. Samples were fixed according to Barros and Berrios (28) and examined with a Jeol JEM 1200EX transmission electron microscope.

RESULTS

The results of Kiehart and colleagues (13, 14) suggested that, in *Drosophila*, nonmuscle cells express a distinct myosin heavy chain isoform not encoded by the muscle myosin heavy chain gene. Affinity-purified anti-DNMHC (anti-*Drosophila* nuclear myosin heavy chain) antibodies prepared previously (11) react strongly with the muscle myosin heavy chain isoform. To characterize further the specificity of these antibodies, we probed immunoblots containing homogenates from *Drosophila* tissue culture cells and embryos collected at different developmental stages. Results from these experiments (data not shown) demonstrated that affinity-purified anti-DNMHC antibodies recognize a polypeptide with the expected one-dimensional $NaDodSO_4$/PAGE mobility of *Drosophila* muscle myosin heavy chain both in tissue culture cells and in premyogenic embryos.* However, when parallel blots were probed with anti-DCM antiserum prepared against the nonmuscle isoform of *Drosophila* myosin (13), far greater immunoreactivity of what apparently was the same polypeptide was seen (data not shown).

Affinity-Purified Anti-DNMHC Antibodies Crossreact with *Drosophila* Nonmuscle Myosin. To demonstrate directly that affinity-purified anti-DNMHC antibodies recognized both muscle and nonmuscle isoforms of myosin heavy chain, we used these antibodies for immunoaffinity chromatography. Anti-DNMHC antibodies were covalently conjugated to protein A-Sepharose, and high-speed supernatants (100,000 × *g*)† from developmentally staged *Drosophila* embryo extracts were passed over the column. Myosin was eluted from the column and subjected to $NaDodSO_4$/PAGE and immunoblot analysis.

The immunoaffinity column used for purification of myosin heavy chain from the embryo extracts was effective for this purpose with all of the samples tested (data not shown), thus proving the immunocrossreactivity of the anti-DNMHC antibodies with each of the myosin heavy chain forms. However, when the same anti-DNMHC antibodies were used to probe a blot containing the same immunoaffinity-purified myosin fractions, it was clear that heavy chain immunoreactivity varied among these samples. The myosin heavy chain isoform immunoaffinity purified from the early (premyogenic) embryo extract was only weakly crossreactive relative to that from older (postmyogenic) embryos (data not shown). In contrast, when similar experiments were performed with anti-DCM antiserum, the opposite pattern was observed. Myosin heavy chain immunoaffinity purified from premyogenic embryos was highly immunoreactive relative to that obtained from postmyogenic embryos or adult muscle (data not shown).

One-Dimensional $NaDodSO_4$/PAGE Peptide Map Comparison of *Drosophila* Myosin Heavy Chain Isoforms. The immunochemical distinction between myosin heavy chain isoforms immunoaffinity purified from embryos of different ages was corroborated biochemically by one-dimensional $NaDodSO_4$/PAGE peptide map analyses. Different myosin fractions were partially digested with either chymotrypsin or CNBr and subjected to one-dimensional $NaDodSO_4$/PAGE and immunoblot analysis using either affinity-purified anti-DNMHC antibodies or anti-DCM antiserum. The results of some of these experiments are presented in Fig. 1. In Fig. 1*A*,

*Myogenesis in *Drosophila* begins sometime after the temporal midpoint (11 hr at 25°C) of embryo development (see ref. 14).

†Later in embryogenesis, nuclear lamina and pore complex proteins are generally not found in the 100,000 × *g* supernatant fraction of *Drosophila* embryo extracts. Early in embryogenesis, however, large soluble pools of these proteins are present, presumably derived from germinal vesicle breakdown at the end of oogenesis. These pools apparently provide the karyoskeletal building blocks necessary for rapid formation of new nuclei early in development. The presence of any given polypeptide in the 100,000 × *g* supernatant of the *Drosophila* early embryo extract is therefore consistent with a role as a nuclear envelope component.

a Coomassie blue-stained NaDodSO$_4$/PAGE gel is shown; Fig. 1*B* shows an immunoblot of a parallel gel probed with affinity-purified anti-DNMHC antibodies while Fig. 1*C* shows an identical blot probed with anti-DCM antiserum. In Fig. 1, lanes a and b were loaded with a myosin fraction immunoaffinity purified from premyogenic *Drosophila* embryos; lanes c and d were loaded with myosin purified from adult muscle. In Fig. 1, lanes b and c were loaded with samples after digestion with chymotrypsin; lanes a and d were loaded with samples that had not been digested.

From the results shown in Fig. 1*A*, it is clear that the pattern seen for the myosin heavy chain isoform immunoaffinity purified from premyogenic embryos was distinct from that seen with the muscle isoform. This distinction was substantiated by immunoblot analyses (Fig. 1 *B* and *C*). Similar distinctions could be made after CNBr treatment (data not shown). When myosin fractions immunoaffinity purified from postmyogenic embryos were digested with CNBr or chymotrypsin, patterns seen were highly similar with those of adult muscle (data not shown).

Subcellular Distribution of *Drosophila* Nonmuscle Myosin Heavy Chain in K$_c$ Cells. Anti-DCM antiserum was used to study the distribution of nonmuscle myosin heavy chain after subfractionation of *Drosophila* K$_c$ tissue culture cells (Fig. 2*A*). Although the majority of this species remained in the postnuclear supernatant after low-speed centrifugation (compare Fig. 2*A*, lanes 1 and 2), a significant minority of this antigen was reproducibly recovered in the nuclear pellet fraction (Fig. 2*A*, lane 5). For comparison, identical blots prepared in parallel were probed with either affinity-purified anti-*Drosophila* lamin antibodies (Fig. 2*B*) or monoclonal anti-*Drosophila* tubulin antibody (Fig. 2*C*). Results of these experiments clearly demonstrated both the efficiency of nuclear isolation from *Drosophila* K$_c$ cells as indicated by results obtained with the anti-lamin antibodies (Fig. 2*B*) as well as the purity of these nuclei relative to cytoplasmic contamination as indicated by results obtained with anti-tubulin antibodies (Fig. 2*C*).

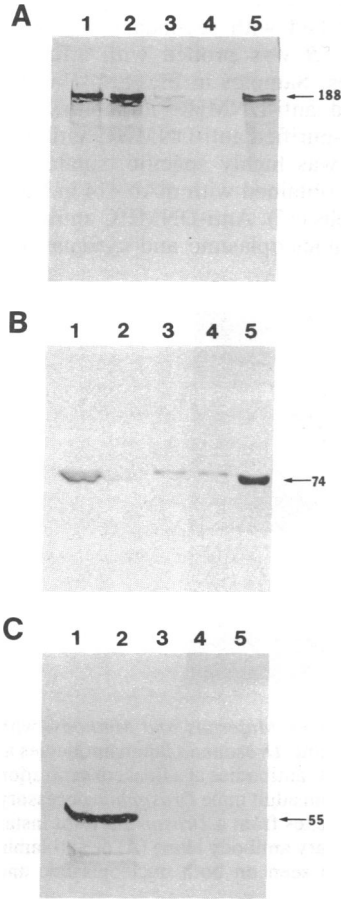

FIG. 2. Subcellular distribution of *Drosophila* nonmuscle myosin heavy chain from K$_c$ cells. *Drosophila* K$_c$ tissue culture cells were homogenized in standard extraction buffer supplemented with 1% Triton X-100; nuclei were prepared by low-speed sedimentation (2000 × *g* for 10 min) and washing as described (15). Equivalent amounts (derived from ≈1 mg of packed cells) of each subcellular fraction generated were electrophoresed on NaDodSO$_4$/7% polyacrylamide gels, proteins were transferred to nitrocellulose, and the resulting blots were probed with antibodies. The blot shown in *A* was probed with anti-DCM antiserum as described in the legend to Fig. 1*C*; the blot shown in *B* was probed with affinity–purified anti-*Drosophila* lamin antibodies at a final concentration of ≈500 ng/ml; the blot shown in *C* was probed with monoclonal anti-*Drosophila* tubulin antibody hybridoma supernatant diluted 1:500. Lanes: 1, total cell homogenate; 2, postnuclear supernatant; 3, first wash supernatant; 4, second wash supernatant; 5, purified nuclei.

One-Dimensional NaDodSO$_4$/PAGE Peptide Map Comparison Between Nonmuscle Myosin Heavy Chain Fractions from K$_c$ Cells. The immunochemical crossreactivity between myosin heavy chain species found in K$_c$ cell postnuclear supernatant and nuclear pellet fractions facilitated further comparison by one-dimensional NaDodSO$_4$/PAGE peptide map analyses. Crude subcellular fractions were partially digested with chymotrypsin and subjected to NaDodSO$_4$/PAGE and immunoblot analysis with anti-DCM antiserum (Fig. 3). It was clear that, while not absolutely identical, the digestion pattern seen for myosin heavy chain from the postnuclear supernatant was highly similar to that seen for myosin heavy chain present in the nuclear pellet. These patterns also appeared similar to that seen after partial chymotryptic digestion of the myosin heavy chain isoform isolated from premyogenic embryos (see Fig. 1*C*, lane b).

Anti-DNMHC Antibodies Stain *Drosophila* Nuclei with Similar Patterns to mAb 414, a Monoclonal Antibody Specific for the Rat Liver Nuclear Pore Complex. Our original suggestion that myosin or myosin-like molecules were part of the nuclear pore complex was based largely on results of indirect immunofluorescence analyses performed with affinity-purified anti-DNMHC antibodies (11). Since then several papers have appeared demonstrating that antibodies directed against nuclear pore complex components produce a characteristic punctate pattern of peripheral nuclear fluorescence (see, for example, refs. 17, 29, and 30). Within the context of our underlying hypothesis that myosin or myosin-like molecules were nuclear pore complex components, there were at least three possible explanations to be considered in evaluating further the smooth staining pattern we observed: (*i*) that myosin heavy chain-like molecules were in fact not pore complex components; (*ii*) that salivary gland nuclear envelopes contained pore complex components not fully assembled into pore complexes and that this interpore complex material was still recognized by antibodies, leading to diffuse fluorescence images; or (*iii*) that the large number of pore complexes found in these nuclei resulted in a closeness of packing that obscured any punctate pattern that might have been discernible had the pore complexes been further apart.

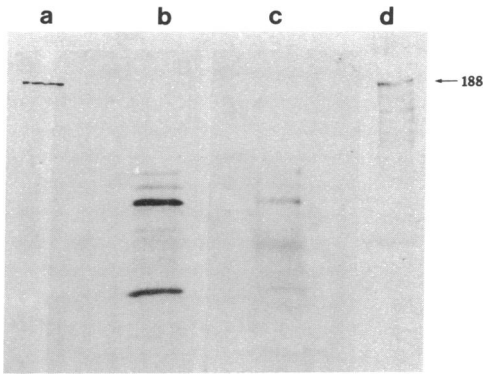

FIG. 3. Chymotryptic peptide map comparison of *Drosophila* K$_c$ cell cytoplasmic and nuclear nonmuscle myosin heavy chain fractions. Nuclear pellet and postnuclear supernatant fractions, generated as described in the legend to Fig. 2, were digested with chymotrypsin (11) and subjected to NaDodSO$_4$/7% PAGE and immunoblot analysis with anti-DCM antiserum as described in the legend to Fig. 1*C*. Lanes: a, undigested postnuclear supernatant derived from 0.2 mg of cells; b, 16-min digest of postnuclear supernatant derived from 2 mg of cells; c, 16-min digest of nuclear pellet derived from 10 mg of cells; d, undigested nuclear pellet derived from 2 mg of cells.

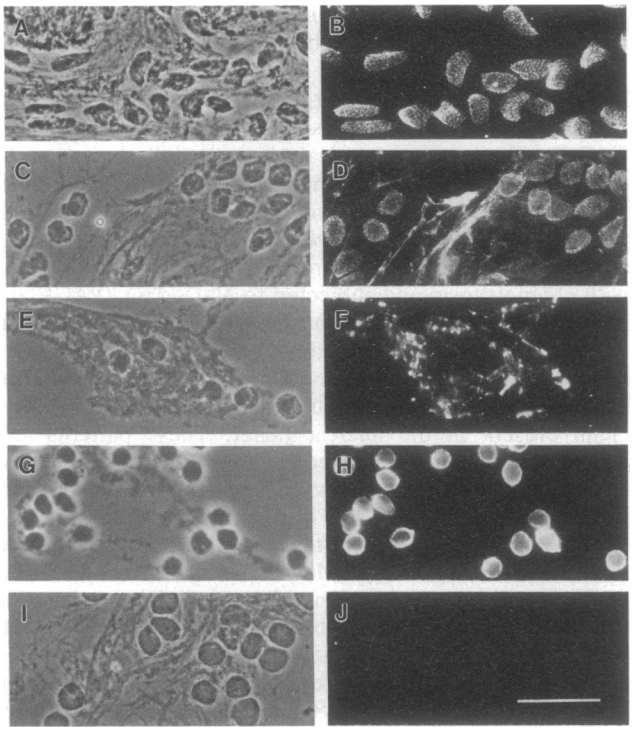

FIG. 4. Immunofluorescence staining of adult male *Drosophila* accessory gland cells. Phase contrast (*A*, *C*, *E*, *G*, and *I*) and epifluorescence (*B*, *D*, *F*, *H*, and *J*) micrographs of accessory gland cells probed with primary antibodies are shown. (*A* and *B*) mAb 414 ascites fluid diluted 1:10. (*C* and *D*) Affinity-purified anti-DNMHC antibodies at a final concentration of ≈7.5 μg/ml. (*E* and *F*) Anti-DCM antiserum diluted 1:500. (*G* and *H*) Affinity-purified anti-*Drosophila* lamin antibodies at a final concentration of ≈5 μg/ml. Samples shown in *A* and *B* were probed with rhodamine-conjugated goat anti-mouse IgG F(ab')$_2$ at 1:1000. Samples in *C–J* were probed with an affinity-purified rhodamine-conjugated goat anti-rabbit IgG at 1:1000. Photography was on Kodak 2415 film. (For *A–J*, bar = 25 μm.)

To explore these several possibilities, we first probed *Drosophila* salivary gland giant cell nuclei with mAb 414, a monoclonal antibody directed against rat liver pore complex component p62; mAb 414 gives a punctate pattern of peripheral nuclear fluorescence in Buffalo rat liver tissue culture cells and reportedly gives nuclear rim staining in *Drosophila* cell lines (17). Although mAb 414 showed strong reactivity with the nuclear envelopes of these giant cell nuclei, staining appeared smooth rather than punctate, even when the microscope was focused on the nuclear surface (data not shown). This pattern was similar to that seen with anti-DNMHC antibodies (11). These results in conjunction with those of electron microscopic analyses of salivary gland nuclei (27) were consistent with the notion that closeness of packing of salivary gland nuclear pore complexes obscured the punctate staining that might have been observed, at least with mAb 414.

Nuclei from adult male *Drosophila* accessory glands have fewer pore complexes than salivary gland nuclei. We therefore chose this tissue for further studies. Indirect immunofluorescence staining of accessory gland nuclei with mAb 414 (Fig. 4 *A* and *B*) gave a distinctive punctate pattern comparable to that seen with this antibody in Buffalo rat liver tissue culture cells (17). A similar staining pattern of accessory gland nuclei was seen with affinity-purified anti-DNMHC antibodies (Fig. 4 *C* and *D*). In addition, anti-DNMHC antibodies apparently decorated cytoskeletal filaments in these cells. In contrast, anti-DCM antiserum decorated cytoskeletal myosin in these cells but apparently did not stain nuclei (Fig. 4 *E* and *F*). Samples probed with affinity-purified anti-lamin antibodies showed the characteristic smooth perinuclear staining pattern (Fig. 4 *G* and *H*). A sample probed with secondary antibody alone is shown in Fig. 4 *I* and *J*. Similar results were obtained when samples were probed with nonimmune primary antibodies before addition of secondary antibody (data not shown).

Anti-DNMHC Antibodies Decorate *Drosophila* Nuclear Pore Complexes. Immunofluorescence studies performed with affinity-purified anti-DNMHC and anti-lamin antibodies were complemented by immunogold electron microscopy (Fig. 5). Fig. 5*A* shows a sample probed with secondary antibody alone. The sample in Fig. 5*B* was probed with affinity-purified anti-lamin antibodies. Samples in Fig. 5 *C–F* were probed with affinity-purified anti-DNMHC antibodies. In samples probed with affinity-purified anti-DNMHC antibodies, pore complex labeling was highly specific, similar to previously published results obtained with mAb 414 in Buffalo rat liver tissue culture cells (17). Anti-DNMHC antibody labeling was seen on both nucleoplasmic and cytoplasmic

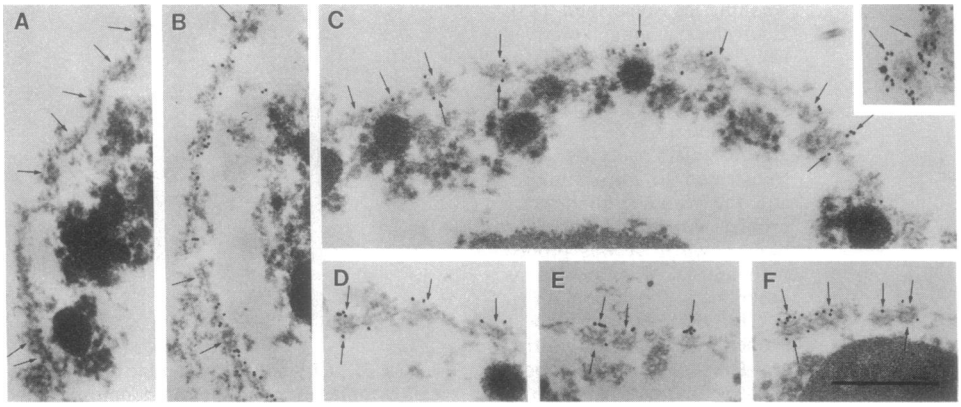

FIG. 5. Immunogold electron microscopy of *Drosophila* nuclei. Samples were prepared and probed (see *Materials and Methods*) with secondary antibody (10-nm gold-conjugated affinity-purified goat anti-rabbit IgG) only (*A*), affinity-purified anti-*Drosophila* lamin antibodies at a final concentration of ≈5 μg/ml followed by secondary antibody as in *A* (*B*), or affinity-purified anti-DNMHC antibodies at a final concentration of 10 μg/ml followed by secondary antibody as in *A* (*C–F*). All samples except that shown in *C inset* were from adult male *Drosophila* accessory glands. (*C Inset*) Immunogold labeling of cross sections through morphologically recognizable pore complexes from a *Drosophila* third instar larval salivary gland. Arrows in *A* and *B* designate recognizable pore complexes, unlabeled either by secondary antibody alone (*A*) or anti-lamin antibodies (*B*). Arrows in *C–F* indicate immunogold-labeled pore complexes only. Where labeling was seen on both nucleoplasmic and cytoplasmic faces, arrows so indicate. Photography was on Kodak 4489 film. (For *A–F*, bar = 500 nm.)

sides of pore complexes and within pores in appropriate cross sections (see, for example, Fig. 5C *Inset*).

DISCUSSION

Previously, we reported the *in situ* localization of a myosin heavy chain-like polypeptide to the nuclear envelope in a variety of higher eukaryotic cells (11). Although we suggested initially that in *Drosophila*, our primary organism of study, this polypeptide was similar to or identical with muscle myosin heavy chain, results of others (13, 14), which have been both confirmed and extended in our current study, require that we modify this hypothesis. It now seems probable that if myosin heavy chain is indeed a nuclear envelope component, it is the nonmuscle isoform described by Kiehart and Feghali (13) that is involved. Our previous immunocytochemical results may be explained by the fact that the affinity-purified anti-DNMHC antibodies used, which are primarily reactive with *Drosophila* muscle myosin heavy chain, crossreact with the nonmuscle isoform.

We have taken advantage of this crossreactivity to perform additional immunocytochemical studies with affinity-purified anti-DNMHC antibodies. Punctate immunofluorescent staining of male *Drosophila* accessory gland cells and immunogold electron microscopic labeling of accessory gland nuclear pore complexes lend support to a model, presented in detail previously (11), for the organization of myosin molecules in the nuclear pore complex. The model proposes that the annular subunits of the nuclear pore complex are formed by the heads of myosin molecules; the cylindrical wall of the pore lumen is formed by myosin tails.

In considering this model, certain caveats must be introduced. We were led to propose this model by immunocytochemical observations presented here and previously (11). These observations are supported by the results of cell fractionation studies demonstrating the presence of myosin heavy chain-like molecules in nuclear envelope-enriched fractions prepared from a number of higher eukaryotes (10). Yet at later stages of *Drosophila* embryogenesis, we have found that a muscle myosin heavy chain isoform apparently contaminates the nuclear pellet fraction (M.B. and P.A.F., unpublished results). Indeed, it was probably primarily this isoform against which anti-DNMHC antiserum was originally prepared (15) and which dominated one-dimensional NaDodSO$_4$/PAGE peptide maps presented previously (11). It is therefore still possible that the myosin heavy chain isoform associated with isolated nuclear fractions that reacts strongly with anti-DCM antiserum is a cytoplasmic contaminant and that results of *in situ* localization studies reflect coincidental crossreactivity between myosin heavy chain and an authentic pore complex component.

In this context, it is of some concern that anti-DCM antiserum, while staining cytoskeletal filaments intensely (ref. 13 and Fig. 4), apparently does not stain nuclei. It seems possible that the epitopes recognized by the anti-DCM antiserum are not accessible for immunocytochemical staining in intact pore complexes. Additional experimentation and/or preparation of new antisera will be necessary to explore further this possibility. It is also not clear what, other than apparent subcellular localization (as defined by cell fractionation), distinguishes nuclear from cytoplasmic forms of nonmuscle myosin heavy chain in *Drosophila* K$_c$ cells. Both have highly similar one-dimensional chymotryptic peptide maps as detected by immunoreactivity with a common antiserum.

The hypothesis that myosin is a major participant in the architecture of the nuclear pore complex leads to a number of functional predictions. The most obvious is that the hydrolysis of ATP required for transport through the pore (6–9) might be catalyzed by the ATPase activity residing in the annular subunits (i.e., myosin heads) of the pore complex. Another is that myosin itself may contain the binding site(s) necessary for karyophilic signal recognition (see, for example, ref. 31). Others have had considerable success using either wheat germ agglutinin (see, for example, ref. 32) or monoclonal antibodies (33) to block specific transport of karyophilic proteins through nuclear pores, presumably by binding to p62 and related pore complex constituents. These results substantiate the role that p62 plays in pore complex function. It remains to be determined whether analogous studies performed with anti-myosin antibodies can be used similarly to test explicit functional predictions of our hypothesis.

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