A Myosin Heavy Chain-like Polypeptide Is Associated with the Nuclear Envelope in Higher Eukaryotic Cells

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Abstract. A high molecular weight polypeptide, identified as an ATPase subunit by direct ultraviolet photoaffinity labeling, has been shown to be a component of nuclear envelope-enriched fractions prepared from a variety of higher eukaryotes (Berrios, M., G. Blobel, and P. A. Fisher, 1983, *J. Biol. Chem.*, 258:4548-4555). In rat liver as well as *Drosophila melanogaster* embryos, this polypeptide appears to be a form of myosin heavy chain. This conclusion is based on both immunochemical and immunocytochemical data, as well as on the results of CNBr and chymotryptic peptide map analyses. In *Drosophila*, the identification of this myosin heavy chain-like polypeptide as a nuclear envelope component has been corroborated in situ by indirect immunofluorescence analyses using permeabilized whole cells, mechanically extruded nuclei, and cryosections obtained from a number of larval tissues. Localization appears to be restricted to the nuclear periphery in a manner similar to that observed for the nuclear lamins and the pore complex glycoprotein. Antibodies directed against the *Drosophila* nuclear envelope ATPase have also been shown to decorate mammalian and higher plant cell nuclei in situ. Implications for intracellular nuclear mobility and for nucleocytoplasmic exchange of macromolecules in vivo are discussed.

THE histochemical identification of an ATPase activity apparently associated with the nuclear pore complex of higher eukaryotes (Klein and Afzelius, 1966; Yasuzumi and Tsubo, 1966; Yasuzumi et al., 1967, 1968; Chardonnet and Dales, 1972) has led to considerable interest regarding the possible role(s) of this activity in providing the energy presumed necessary for nucleocytoplasmic exchange of macromolecules. Although subsequent histochemical analyses have demonstrated the presence of ATPase activities in other regions of the nucleus as well (Sikstrom et al., 1976; Vorbrodt and Maul, 1980; Fox et al., 1981), most biochemical studies have focused on attempts to identify and characterize ATPase activities specifically associated with the nuclear envelope (see Agutter and Richardson, 1980 for a review of these studies). These studies have been severely limited by the inability to solubilize the ATPase protein(s) in active form from the supramacromolecular structures with which they are apparently associated. It has been impossible, as a result, to purify and/or positively identify the enzyme polypeptide(s), or to attempt immunochemical or immunocytochemical analyses.

Studies from this laboratory have focused on an ATPase found associated with nuclear envelope-enriched fractions from both *Drosophila melanogaster* embryos, and a variety of vertebrate cell types (Berrios et al., 1983*a*, *b*). Through direct ultraviolet (UV) photoaffinity labeling experiments performed in conjunction with enzymologic analyses, we have been able to correlate the major ATPase activity of the *Drosophila* nuclear matrix-pore complex-lamina (NMPCL)[1] fraction with a single major polypeptide of ~188 kD (Berrios et al., 1983*a*).[2] A polypeptide of nearly identical molecular mass has been identified in similar photocross-linking experiments performed with nuclear envelope preparations obtained from the livers of chickens, guinea pigs, opposums, and rats. In each of these systems, including *Drosophila*, the photolabeled ATPase co-migrates on SDS polyacrylamide gels with an abundant glycoprotein also associated with higher eukaryotic nuclear structural protein subfractions (Berrios et al., 1983*b*). Through the use of chromatographic, immunochemical, and enzymologic techniques, we have

1. *Abbreviations used in this paper*: MSM, modified Shields' medium; NMPCL fraction, nuclear matrix-pore complex-lamina fraction; NPCL fraction, nuclear pore complex-lamina fraction.

2. In our initial SDS PAGE analyses and UV photolabeling studies of the *Drosophila* NMPCL fraction (Fisher et al., 1982; Berrios et al., 1983*a*, *b*), a molecular weight of 174,000 was assigned both to the NMPCL ATPase and to the putative nuclear pore complex glycoprotein. Recent work on the pore complex glycoprotein has resulted in a revision of this molecular weight estimate to 188,000 (Filson et al., 1985). A similarly revised estimate has now been made for the NMPCL ATPase. This revision has been made with explicit regard to the molecular weight of 190,000 reported for the rat liver nuclear pore complex glycoprotein (Gerace et al., 1982). It should be noted that this molecular weight is at the low end of the range of values commonly quoted for vertebrate skeletal muscle myosin heavy chains. Of significance in the present context is that skeletal muscle myosin heavy chain, the NMPCL ATPase, and the nuclear pore complex glycoprotein co-migrate on SDS polyacrylamide gels. Their apparent molecular weights should therefore be regarded as identical, regardless of the numerical value assigned.

been able to demonstrate that the ATPase polypeptide identified by UV photolabeling is distinct from this glycoprotein of nearly identical molecular mass (Berrios et al., 1983*b*; Filson et al., 1985). (This glycoprotein has been shown by immunoelectron microscopy to be a specific polypeptide component of the rat liver nuclear pore complex [Gerace et al., 1982]; immunocytology at the light microscopic level has confirmed localization of this glycoprotein to the nuclear envelope in *Drosophila* [Filson et al., 1985].)

The ability to specifically label the active site-containing polypeptide of the major NMPCL ATPase with α[^{32}P]ATP has allowed us to solubilize and purify it under harshly denaturing conditions and to obtain specific antibodies directed against the purified protein (Berrios et al., 1983*b*). In the present study, we have used these antibodies for immunochemical and immunocytochemical analyses. The results obtained demonstrate that this ATPase polypeptide is apparently a form of myosin heavy chain, and that it is localized to the nuclear envelope in situ.

Materials and Methods

Radiolabeled nucleotides and Triton X-100 were from New England Nuclear (Boston, MA). Specific IgG fractions were from Cappel Laboratories (Cochranville, PA). Monoclonal antibodies AGP-26 and AGP-78, directed against the putative nuclear pore complex glycoprotein obtained from *Drosophila* embryos, were ammonium sulfate purified from hybridoma tissue culture supernatants (Filson et al., 1985). DNAse I, *p*-toluenesulfonyl fluoride, phenylmethylsulfonyl fluoride, *N*-ethylmaleimide, 1-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK), *p*-phenylenediamine, cyanogen bromide, DL-dithiothreitol, 2-mercaptoethanol, polyoxyethylene sorbitan monolaureate (Tween 20), octylphenoxypolyethoxyethanol (Nonidet P-40), Fraction V-bovine serum albumin (BSA), chymotrypsin, and calf alkaline phosphatase were from Sigma Chemical Co. (St. Louis, MO). Protein A-Sepharose CL-4B was from Pharmacia Fine Chemicals (Piscataway, NJ). Hydroxylapatite Bio-Gel HTP was from Bio-Rad Laboratories (Richmond, CA). 5-Bromo-4-chloro-3-indolyl phosphate, *p*-toluidine salt, and *p*-nitro blue tetrazolium chloride were from United States Biochemical Corp. (Cleveland, OH). RNAse A (RAF grade) was from Worthington Biochemical Corp. (Freehold, NJ). Nitrocellulose was from Schleicher & Schuell, Inc. (Keene, NH). Paraformaldehyde, formic acid, and methylene bisacrylamide were from Fisher Scientific Co. (Springfield, NJ). Embedding medium for cryosectioning was from Lipshaw Mfg. Co. (Detroit, MI). SDS was from British Drug House (Poole, England). Acrylamide, X-Omat XAR x-ray film, Ektachrome ASA 400, and Tri-X pan ASA 400 films were from Eastman Kodak Co. (Rochester, NY). All other chemicals were obtained commercially, were of reagent grade, and were used without further purification.

Most of the methods have been previously described in detail (Fisher et al., 1982; Berrios et al., 1983*a*, *b*; Smith and Fisher, 1984). *Drosophila melanogaster* (Oregon R, P2 strain) were grown in mass culture and embryos were collected according to Allis et al. (1977). SDS PAGE according to Laemmli (1970), protein transfer from SDS gels onto nitrocellulose sheets (Western blots), and preparation of specific antisera were as previously described (Fisher et al., 1982). Blots were probed with antisera or specific IgG fractions, and bands of antibody reactivity were visualized according to Smith and Fisher (1984). Affinity purification of antibodies using antigens immobilized on nitrocellulose blots was also as described previously (Smith and Fisher, 1984). Immunoprecipitation was performed according to Chang et al. (1979). UV photoaffinity labeling of the *Drosophila* NMPCL ATPase was according to Berrios et al. (1983*a*).

Peptide Mapping of SDS-denatured Proteins

Cyanogen bromide digestion of SDS-denatured polypeptides was essentially according to Nikodem and Fresco (1979). Protein samples were denatured by boiling for 2–4 min in 2% (wt/vol) SDS, 20 mM dithiothreitol. They were then chilled briefly on ice and protein was precipitated by the addition of trichloroacetic acid (TCA) to a final concentration of 10% (wt/vol). The TCA precipitates were resolubilized in 80 μl of 88% (wt/wt) formic acid, and 100 μl of 20 mg/ml CNBr dissolved in 70% formic acid was added to each sample. Digestions were performed at 23°C for the times indicated in the various figures. CNBr digestions were stopped by the addition of 10 vol of cold water, followed by the addition of TCA to a final concentration of 10%. Protein precipitates were collected by centrifugation, resolubilized, and processed for SDS PAGE and Western blot analysis in the standard manner (Fisher et al., 1982; Smith and Fisher, 1984).

Chymotryptic mapping of SDS-denatured proteins was performed similarly and was essentially according to Cleveland et al. (1977). After TCA precipitation, samples were resolubilized at a final protein concentration of ~0.5 mg/ml in 100 mM Tris-HCl, pH 6.8, 0.5% SDS; boiled, cooled to 37°C, and digested with chymotrypsin at a final concentration of 40 μg per ml for the times indicated in the legend to Fig. 6. Chymotryptic incubations were terminated by the addition of an equal volume of 2% SDS, followed immediately by immersion in a boiling water bath. SDS PAGE and Western blot analyses were performed as above.

Indirect Immunofluorescence

Indirect immunofluorescence analyses were performed with permeabilized whole cells, extruded nuclei, cryosections, and permeabilized intact muscle fibers from *Drosophila melanogaster* third instar larvae, as well as with permeabilized COS-7 (SV40-transformed simian CV-1) tissue culture cells (Gluzman, 1981), and with mechanically disrupted onion cells. To obtain whole *Drosophila* cells, third instar larval salivary glands were dissected under a stereo microscope in modified Shields' Medium (MSM) (Shields et al., 1975) containing 18 mM $MgSO_4$, 5.0 mM $CaCl_2$, 40 mM KCl, 24 mM NaCl, 4.8 mM NaH_2PO_4, 4.3 mM $NaHCO_3$, 0.5% (vol/vol) Triton X-100, and 0.5% (vol/vol) Nonidet P-40. The glands were gently squashed between a clean microscope slide and a siliconized coverslip to spread the individual cells. To obtain fully extruded salivary gland nuclei, relatively more pressure was applied during the squashing procedure. Muscle fibers were dissected from the buccal apparatus of the larvae and squashed under similar conditions. After squashing, the coverslip was removed and the preparation fixed for 1–3 min in a freshly prepared mixture of 3% (wt/vol) paraformaldehyde in MSM. The slide was washed briefly in MSM and the first antibody, diluted in MSM as indicated in the individual figure legends, was applied to the specimen. First antibody incubations were for 30 min at 37°C in a humidified chamber. After incubation, the slide was washed for 10 min with three changes of fresh MSM. The second antibody, fluorochrome-conjugated goat anti-rabbit or goat anti-mouse IgG, was similarly diluted in MSM and incubated on the slide under the same conditions as those described for the first antibody at concentrations as reported previously (Fisher et al., 1982; Smith and Fisher, 1984). The slide was washed for 5–10 min with three changes of 560 mM NaCl, 10 mM KPO_4, pH 7.5, 0.1% (vol/vol) Triton X-100, 0.02% (wt/vol) SDS as previously described (Fisher et al., 1982). Fixed and permeabilized COS-7 cells were probed with antibodies and washed similarly, as were mechanically disrupted onion cells. Cryosections of *Drosophila* third instar larvae were prepared and stored at −70°C as described previously (Smith and Fisher, 1984). Upon removal from the freezer, they were thawed directly into fixative and then incubated for 10 min in MSM. Cryosections were probed with antibodies as described for whole cells and extruded nuclei. Before examination, a coverslip was mounted on each slide with a drop of 0.1% (wt/vol) *p*-phenylenediamine (Johnson and Araujo, 1981) in MSM without detergents; specimens were examined and photographed with a Leitz Ortholux II epifluorescence microscope equipped with an Orthomat W camera.

Extraction and Purification of Skeletal Muscle Myosin

Myosin was extracted from rat skeletal muscle according to LeStourgeon et al. (1975). *Drosophila* muscle myosin was extracted from adult flies and purified essentially according to the same procedure. (It is assumed that the bulk of myosin extracted in this way from adult flies is derived from striated muscle tissue in the legs and thoracic flight muscle.) Approximately 3 to 4 ml of packed adult flies were used for a standard preparation. The live flies were immobilized by chilling and were washed several times in 50 vol of ice cold buffer containing 120 mM NaCl, 0.1% Triton X-100. All subsequent procedures were performed at 4°C. The washed flies were collected by centrifugation at 1,000 *g* for 5 min and resuspended in ~10 vol of extraction buffer containing 1 M KCl, 20 mM Tris-HCl, pH 7.4, 2 mM *N*-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, 1 mM TPCK. The flies were homogenized with 10 strokes (tight pestle) in a Dounce homogenizer. The homogenate was filtered through a 120-μm Nitex screen and the filtrate was clarified by centrifugation, twice for 20 min at 20,000 *g*. The resulting supernatant was dialyzed overnight against two changes of 40 vol each, of buffer containing 50 mM KCl, 20 mM 2-mercaptoethanol, 20 mM

Tris-HCl, pH 7.4, and 10 mM EDTA. The actomyosin precipitate formed during dialysis was collected by centrifugation at 2,000 *g* for 10 min and the precipitate was resolubilized in 25 vol of 1 M KCl, 20 mM 2-mercaptoethanol, 20 mM Tris-HCl, pH 7.4. The dialysis step with precipitation and resolubilization was repeated twice and the final actomyosin pellet was either resolubilized and stored at −20°C in 600 mM KCl, 20 mM Tris-HCl, pH 6.8, 50% (vol/vol) glycerol, or was solubilized directly into 10% (wt/vol) SDS, 20 mM dithiothreitol.

Results

Antiserum Prepared against the Gel-purified 188-kD Drosophila NMPCL Polypeptides Is Specific for the 188-kD ATPase Identified by UV Photolabeling

One-dimensional SDS PAGE analyses of the *Drosophila* NMPCL fraction demonstrated the presence of a major band of protein at the 188-kD position (Fisher et al., 1982). An antiserum was prepared by excising the 188-kD region from the SDS gel, emulsifying the protein species along with the polyacrylamide in complete Freund's adjuvant, and injecting the emulsion into rabbits. The specificity of this antiserum was initially demonstrated by Western blot analyses and by immunoprecipitation. Western blot analyses of the eluate fractions obtained from SDS hydroxylapatite chromatography of the *Drosophila* NMPCL fraction demonstrated that the antiserum raised was specific for the 188-kD ATPase polypeptide (Fig. 1 *A*), identified as such in parallel experiments in which the NMPCL fraction was photolabeled with α[^{32}P]ATP before chromatography (Berrios et al., 1983*b*). The blot shown in Fig. 1 *A* was probed with 20 times the serum concentration typically used for optimal detection of the ATPase polypeptide on Western blots. This antiserum showed no detectable reactivity with the 188-kD nuclear pore complex glycoprotein, identified on a parallel blot probed with radiolabeled concanavalin A (Fig. 1 *B*) as previously

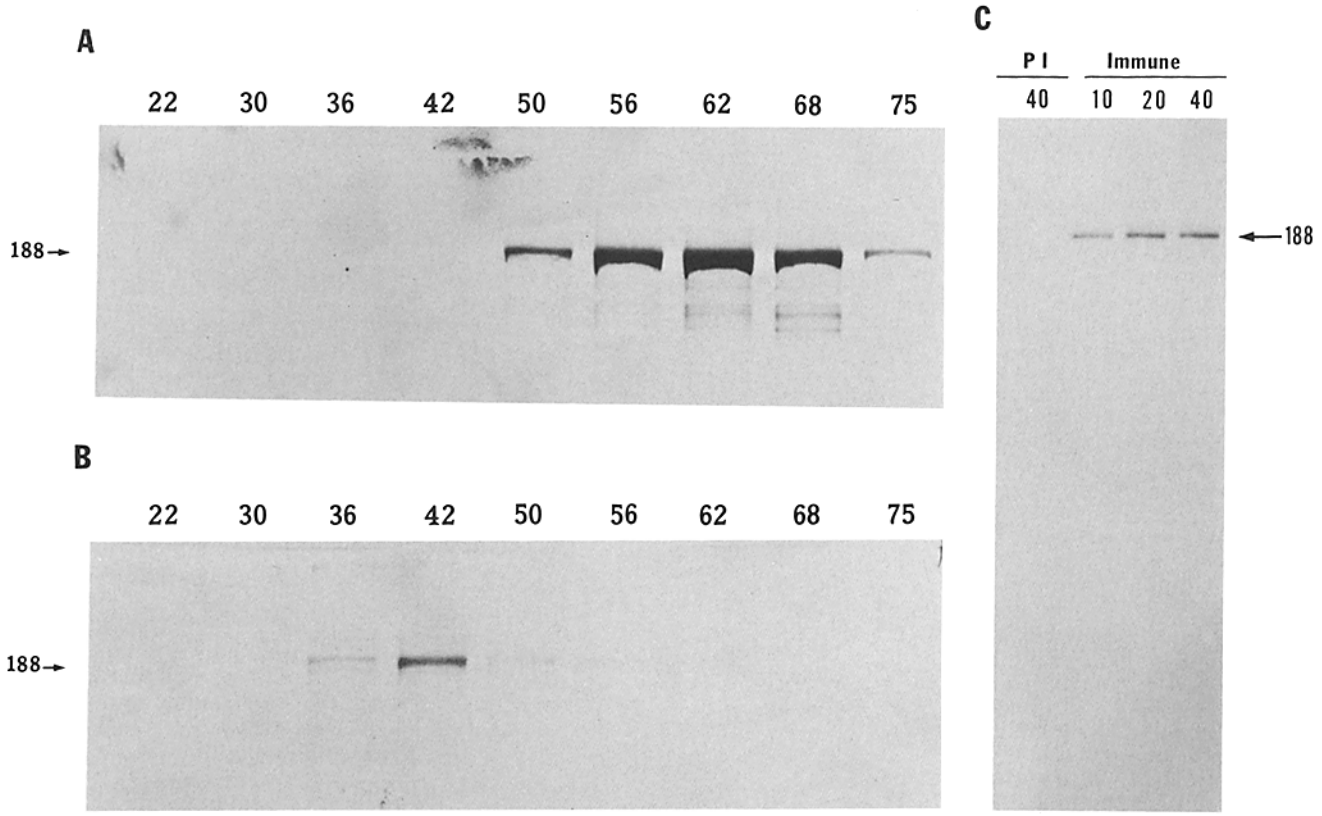

Figure 1. Antiserum prepared against the major 188-kD NMPCL gel band recognizes the 188-kD NMPCL ATPase. The *Drosophila* NMPCL fraction was boiled in SDS and chromatographed on an SDS hydroxylapatite column. (*A*) Aliquots of eluate fractions from the column as indicated above each lane were electrophoresed, blot transferred to nitrocellulose, and the blot was probed with antiserum raised against the 188-kD gel band at a final serum concentration of 1:50. Detection was with radiolabeled goat anti-rabbit IgG as previously described (Fisher et al., 1982). Fluorographic exposure was for 4 h at −70°C using preflashed Kodak XAR X-ray film. (*B*) An identical blot, prepared in parallel with that shown in *A*, was probed with radiolabeled concanavalin A to detect glycoprotein, also as previously described. Fluorography was for 20 h as in *A*. (Under conditions used, antibody staining is ~50–100-fold more sensitive than radiolabeled concanavalin A binding.) (*C*) Specific immunoprecipitation of the UV photolabeled *Drosophila* NMPCL ATPase polypeptide. The *Drosophila* NMPCL ATPase was photolabeled in the intact NMPCL fraction with α[^{32}P]ATP, solubilized by boiling in SDS, and further purified by SDS hydroxylapatite chromatography (Berrios et al., 1983*b*). The peak of antibody reactivity shown in *A* was exactly coincident with the peak of ^{32}P-labeled ATPase identified in this parallel experiment (Berrios et al., 1983*b*). Peak fractions were pooled and aliquots incubated with ammonium sulfate-purified IgG fractions obtained either from preimmune rabbits (*PI*) or from a rabbit immunized with 188-kD polypeptides SDS gel purified from the *Drosophila* NMPCL fraction (*Immune*). Amounts of IgG used are as indicated (μg) in the lane headings. Immunoprecipitation, SDS PAGE analysis, and fluorography of the gel were as described in Materials and Methods. The exposure shown was for 60 h as in *A*.

described (Fisher et al., 1982; Berrios et al., 1983*b*). The anti-ATPase antiserum was also effective at immunoprecipitating the photolabeled ATPase polypeptide (Fig. 1 *C*).

The 188-kD Drosophila NMPCL ATPase Is Immunochemically Homologous to Myosin Heavy Chain

It was noted in the course of our preliminary analyses that the photolabeled *Drosophila* NMPCL ATPase was nearly identical in one-dimensional SDS PAGE mobility with rat skeletal muscle myosin heavy chain, and was in fact, indistinguishable in SDS PAGE mobility from *Drosophila* muscle myosin heavy chain purified from adult flies (Fig. 2 *A*). Western blot analyses showed essentially complete immunochemical cross-reactivity between the *Drosophila* NMPCL ATPase and *Drosophila* muscle myosin heavy chain as well as limited cross-reactivity with rat muscle myosin heavy chain (Fig. 2 *B*). The immunochemical cross-reactivity observed with unfractionated antiserum (Fig. 2 *B*) was confirmed with IgG fractions that were affinity purified using either SDS hydroxylapatite-purified *Drosophila* NMPCL ATPase (Fig. 2 *C*) or SDS hydroxylapatite-purified *Drosophila* muscle myosin heavy chain (Fig. 2 *D*) as affinity ligands immobilized by blot transfer to nitrocellulose after SDS PAGE.

One-dimensional SDS PAGE Peptide Map Comparisons between the Drosophila NMPCL ATPase and Muscle Myosin Heavy Chain

Our investigation of immunochemical cross-reactivity between the *Drosophila* NMPCL ATPase and muscle myosin heavy chain was extended to include CNBr and chymotryptic peptide map analyses. Initially, *Drosophila* muscle myosin heavy chain was partially digested with CNBr and the digest was analyzed by SDS PAGE on polyacrylamide gradient gels. Coomassie Blue staining showed a complex pattern of fragments generated during the digestion; Western blot analysis of these same CNBr fragments with the anti-*Drosophila* NMPCL ATPase antiserum showed strong reactivity with the majority of these muscle myosin heavy chain fragments (data not shown). All fragments greater than or equal to approximately 15 kD were recognized.

A CNBr peptide map of the *Drosophila* NMPCL ATPase was generated under identical conditions and compared directly with the CNBr map of the *Drosophila* muscle myosin heavy chain. Coomassie Blue staining demonstrated that the two polypeptides were similar, but not identical in their CNBr maps (Fig. 3). A temporal analysis of CNBr treatment of the ATPase polypeptide is shown as indicated in the first five lanes of Fig. 3. The right lane of Fig. 3 shows a composite of the comparable time points used to generate the CNBr

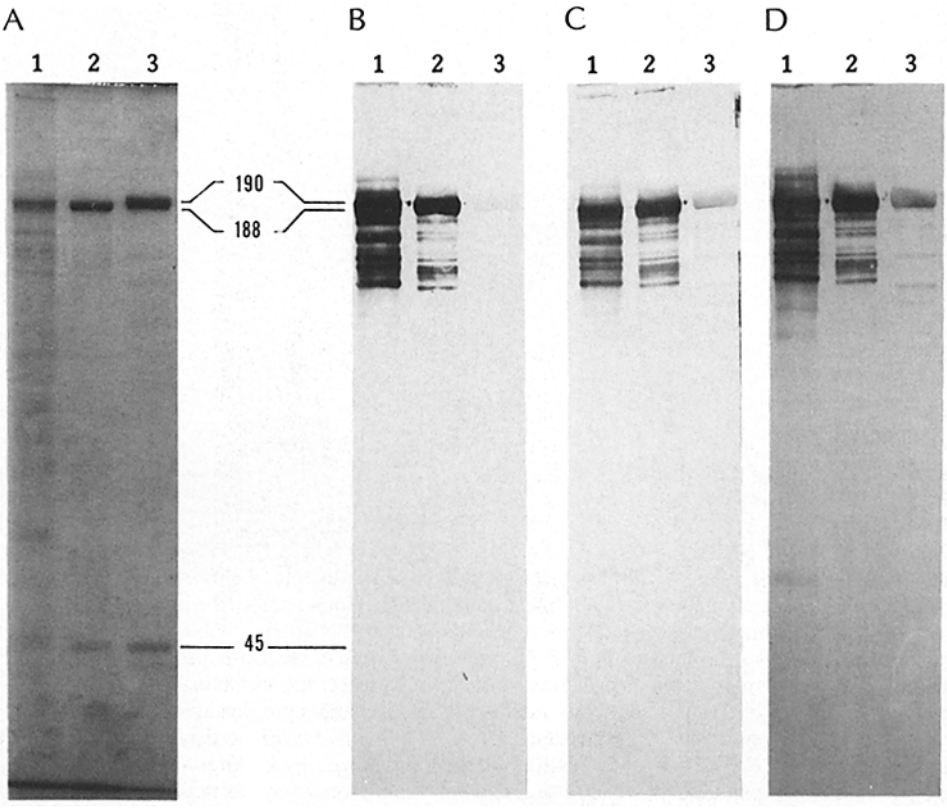

Figure 2. Immunochemical cross-reactivity between the *Drosophila* NMPCL ATPase and muscle myosin heavy chain. SDS hydroxylapatite-purified *Drosophila* NMPCL ATPase, *Drosophila* muscle myosin, and rat skeletal muscle myosin were electrophoresed in parallel lanes on an SDS 7% polyacrylamide gel. Approximately 2–3 μg of each protein fraction was loaded per lane. Lanes *1* were loaded with *Drosophila* NMPCL ATPase; lanes *2* were loaded with *Drosophila* muscle myosin; lanes *3* were loaded with rat skeletal muscle myosin. (*A*) Coomassie Blue-stained gel segment. (*B*) Western blot from a segment prepared in parallel with that shown in *A* and probed with unfractionated anti-188-kD *Drosophila* NMPCL polypeptides antiserum diluted 1:1,000. (*C*) Parallel Western blot probed with an equivalent amount of specific IgG affinity purified using SDS hydroxylapatite, SDS PAGE-purified *Drosophila* NMPCL ATPase as the affinity ligand. (*D*) Parallel Western blot probed with an equivalent amount of specific IgG affinity purified using SDS hydroxylapatite-purified, SDS PAGE-purified *Drosophila* muscle myosin heavy chain as the affinity ligand. Alkaline phosphatase staining of Western blots was for 5 min at room temperature. In addition to the major band in each lane at the 188–190-kD position, a complex pattern of apparent proteolytic breakdown products is observed. This has been confirmed using immunoaffinity-purified antibodies prepared from each of the major fragments (data not shown).

map of muscle myosin heavy chain. The similarity in CNBr maps between the NMPCL ATPase and muscle myosin heavy chain, as well as the broad immunocross-reactivity of the anti-ATPase antiserum was also demonstrated by Western blot analysis using the anti-*Drosophila* NMPCL ATPase antiserum as shown in Fig. 4. (The blot shown was made from an identical gel run in parallel to that shown in Fig. 3.) Here, too, the patterns are similar but non-identical. Most of the major fragments identified are common to both the ATPase and the muscle myosin heavy chain maps.

CNBr peptide map comparison of *Drosophila* muscle myosin heavy chain and NMPCL ATPase with the rat skeletal muscle myosin heavy chain is shown in Fig. 5. Lanes *1* and *2* show the highly similar but non-identical maps of the two different *Drosophila* polypeptides; lane *3* shows the largely dissimilar pattern obtained with rat muscle myosin heavy chain. The *Drosophila* muscle myosin heavy chain closely resembles the NMPCL ATPase from the same species, yet appears to have diverged considerably from the functionally homologous polypeptide obtained from a relatively distant organism.

Additional peptide-mapping data were obtained using chymotrypsin. Although limited by the amount of protein available and the sensitivity of the analysis, Coomassie Blue-stained gels showed similar but non-identical one-dimensional chymotryptic maps for both the *Drosophila* NMPCL ATPase and *Drosophila* muscle myosin heavy chain; comparable maps of rat skeletal muscle myosin heavy chain were distinctly different (data not shown). Western blot analyses of chymotryptic maps are shown in Fig. 6. Results shown in *A* were obtained with SDS hydroxylapatite-purified polypeptide fractions; results in *B* were obtained with un-

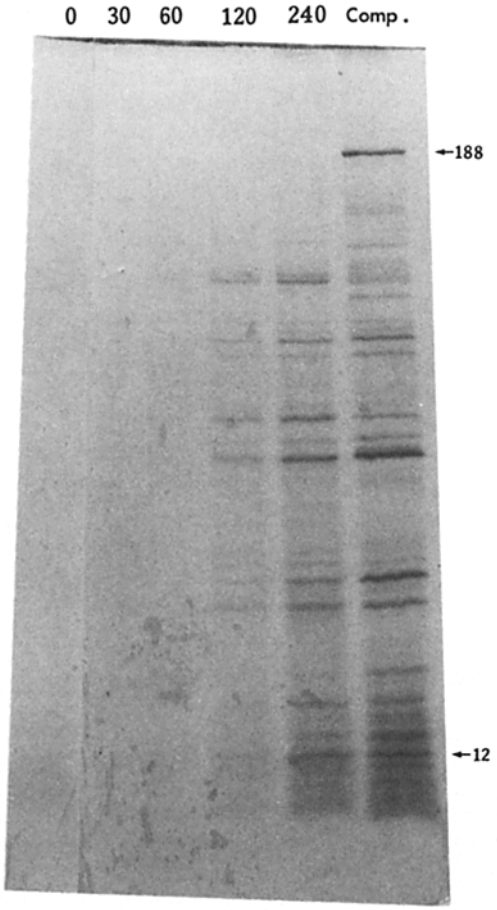

Figure 3. SDS PAGE analysis of the CNBr peptide map comparison between the *Drosophila* NMPCL ATPase and the *Drosophila* muscle myosin heavy chain. SDS hydroxylapatite-purified *Drosophila* NMPCL ATPase was digested with CNBr, and the CNBr fragments were electrophoresed on an SDS 7-15% polyacrylamide gradient gel (see Materials and Methods). The time of digestion of each aliquot is indicated in min above each gel lane. Approximate amounts of *Drosophila* NMPCL ATPase protein loaded in each gel lane were as follows. Lane *0*, 0.2 μg; lane *30*, 2 μg; lane *60*, 2 μg; lane *120*, 4 μg; lane *240*, 11 μg. The lane headings indicate the time of digestion in min. For purposes of direct comparison, a single lane, designated *Comp.*, was loaded with a pool of the CNBr fragments from the digestion of *Drosophila* muscle myosin. This pool contained 1 μg of the uncut heavy chain (*0* time); 3 μg from the 30-min time point; 5 μg from the 120-min time point; and 11 μg from the 240-min time point, of a comparable digestion to that shown for the NMPCL ATPase (protein from the 60-min time point was not included in the composite pool). The gel was stained with Coomassie Blue, destained, and photographed.

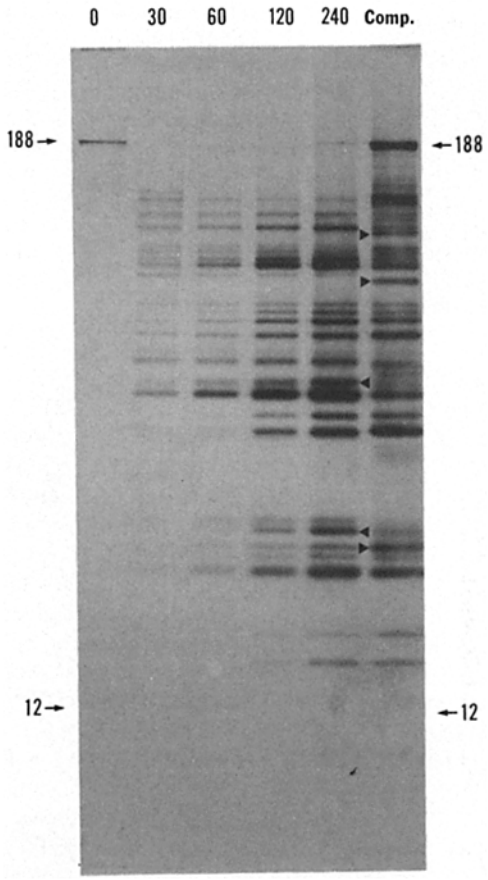

Figure 4. Western blot analysis of the CNBr peptide map comparison between the *Drosophila* NMPCL ATPase and *Drosophila* muscle myosin heavy chain. A parallel gel, loaded and run identically to that shown in Fig. 3, was blot transferred to nitrocellulose and the Western blot probed with anti-*Drosophila* NMPCL ATPase antiserum at 1:1,000. The blot was processed in the standard manner and was developed in the phosphatase stain reagents for 30 min at room temperature. Arrowheads between lanes *240* and *Comp.* designate some of the more prominent differences, either qualitative or quantitative, in the patterns obtained for the NMPCL ATPase and muscle myosin heavy chain.

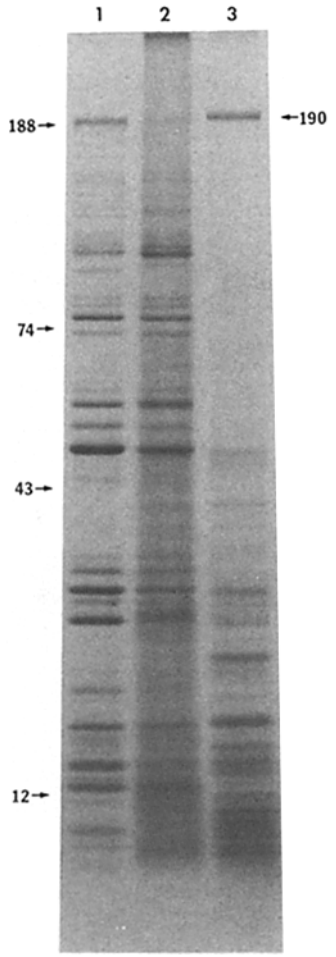

Figure 5. CNBr peptide map comparison of *Drosophila* muscle myosin heavy chain, *Drosophila* NMPCL ATPase, and rat muscle myosin heavy chain. SDS gradient PAGE analysis was as follows. Lane *1* was loaded with a composite fraction of CNBr fragments of *Drosophila* muscle myosin heavy chain exactly as in lane *Comp.* of Figs. 3 and 4. Lane *2* was loaded with a similar composite fraction generated by pooling fragments from the various times points of digestion of the *Drosophila* NMPCL ATPase also shown in Figs. 3 and 4. Only 0.2 μg of the uncut ATPase was used in order to conserve material. Lane *3* was loaded with a similar composite fraction generated by CNBr treatment of rat muscle myosin heavy chain exactly as was performed for the *Drosophila* muscle myosin heavy chain. The gel was stained with Coomassie Blue, destained, and photographed.

fractionated nuclei. All results shown pertain to *Drosophila* polypeptides. Fig. 6 *A*, lane *1*, shows the purified NMPCL ATPase before digestion with chymotrypsin; lane *2* shows the same material after a 16-min digestion. Lane *3* shows muscle myosin heavy chain after a 16-min digestion; lane *4* shows this material before digestion. Lanes *5* and *6* show NMPCL ATPase and muscle myosin heavy chain, respectively, digested for the same period of time at half the total protein concentration; half as much material was loaded on the gel. Fig. 6 *B*, lane *1*, shows a crude nuclear fraction before protease digestion; lane *2* shows the same material after an 8-min incubation with chymotrypsin at protein concentrations similar to those used in *A*, lanes *2* and *3*.

Overall, the chymotryptic maps for NMPCL ATPase and muscle myosin heavy chain, though complex, are quite similar; two sorts of differences are observed. Because of the fact that chymotryptic maps are generated enzymatically, they are inherently more difficult to control than is chemical cleavage with CNBr; trivial differences in the protein concentrations of the substrate polypeptides or in the amounts of minor contaminants can produce identifiable (and somewhat distracting) differences in patterns observed for a single polypeptide species. Differences of this sort are readily observed by comparing lanes *2* and *5* from *A* and lane *2* from *B*. These maps were generated from the same polypeptide either at different protein concentrations (Fig. 6 *A*, lane *2* vs. lane *5*) or with a different background of contaminating proteins (Fig. 6 *A*, lane *2* vs. Fig. 6 *B*, lane *2*).

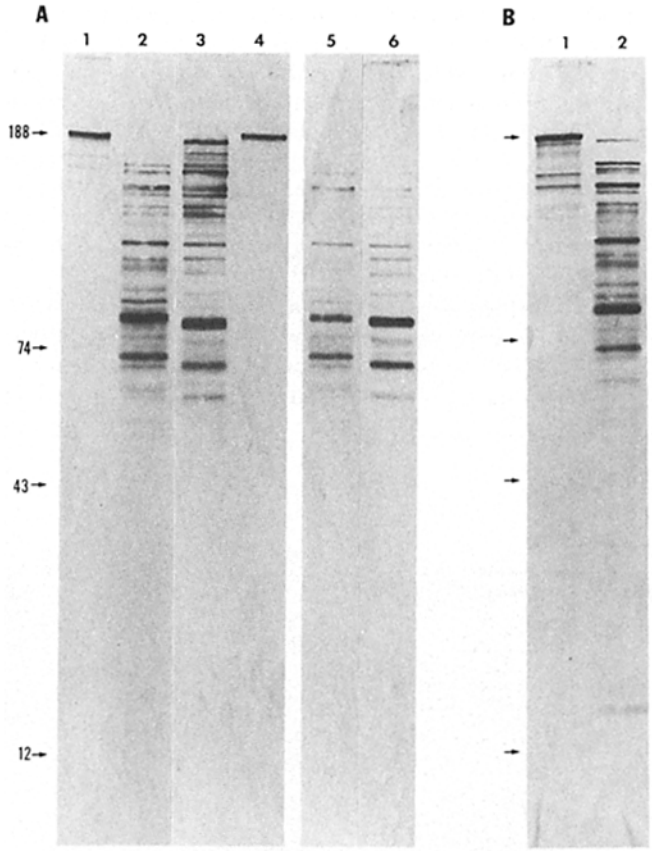

Figure 6. Chymotryptic peptide map comparisons of *Drosophila* NMPCL ATPase and *Drosophila* muscle myosin heavy chain. SDS gradient PAGE and Western blot analyses: blots were probed with anti-ATPase antiserum at 1:1,000, processed in the standard manner, and developed for 10–20 min in the phosphatase stain reagents. Arrows to the left of *A* and *B* designate the marker positions indicated with corresponding molecular masses to the left of *A*. (*A*) Lane *1* was loaded with 0.5 μg of uncut *Drosophila* ATPase; lane *2*, 5 μg of ATPase, digested with 40 μg/ml chymotrypsin for 16 min at 37°C at a final protein concentration of 500 μg/ml; lane *3*, 5 μg of *Drosophila* muscle myosin heavy chain, digested with chymotrypsin exactly as in lane *2*; lane *4*, 0.5 μg of uncut heavy chain; lane *5*, 2.5 μg of *Drosophila* NMPCL ATPase digested for 16 min at 37°C at a final protein concentration of 250 μg/ml with 40 μg/ml chymotrypsin; lane *6*, 2.5 μg of muscle myosin heavy chain treated exactly as described for ATPase in lane *5*. (*B*) Lane *1* was loaded with 100 μg of total nuclear protein, uncut; lane *2* was loaded with 500 μg of total nuclear protein, digested for 8 min at 37°C at a final protein concentration of 500 μg/ml, with 40 μg/ml chymotrypsin. Identical results to those shown in *A* and *B* were obtained with a variety of affinity-purified anti-ATPase antibody fractions (not shown).

In contrast with what appear to be technical artifacts, small but reproducible differences in the absolute mobility of a group of the major fragments common to both maps are consistently observed. This mobility shift is most apparent among the major fragments identifiable in the 50–80-kD portion of the map and may reflect either a small size difference in the different polypeptides from which these fragments were generated that was not well resolved in the high molecular weight region of the gel, or alternatively, the absence or inaccessibility of a single chymotryptic site in the NMPCL ATPase, leading to a ladder of partial cleavage

products all containing a small additional polypeptide segment. Differential phosphorylation has also been found to produce comparable mobility shifts in one-dimensional peptide maps for otherwise identical forms of the *Drosophila* lamins (Smith, D. E., and P. A. Fisher, manuscript in preparation).

Specificity of Affinity-purified Anti-Drosophila ATPase Antibodies

Before their use for immunocytochemical studies, affinity-purified anti-ATPase antibodies were characterized for cross-reactivity, both against the *Drosophila* NMPCL glycoprotein, and against crude organismal and whole nuclear extracts. Data shown in Fig. 1 demonstrate that the unfractionated anti-ATPase antiserum had no demonstrable reactivity with the nuclear pore complex glycoprotein; similar results were obtained with the affinity-purified antibodies. On the other hand, data published previously showed that although the anti-ATPase antiserum apparently recognized only a single polypeptide in the *Drosophila* NMPCL fraction, cross-reactivity was observed with a major cytoplasmic contaminant of ~45 kD (Fisher et al., 1982). We therefore tested the specificity of our affinity-purified antibodies in the context of a cell fractionation experiment as shown in Fig. 7. Equivalent amounts of crude homogenate (lanes *1*), postnuclear supernatant (lanes *2*), first nuclear wash supernatant (lanes *3*), second nuclear wash supernatant (lanes *4*), and

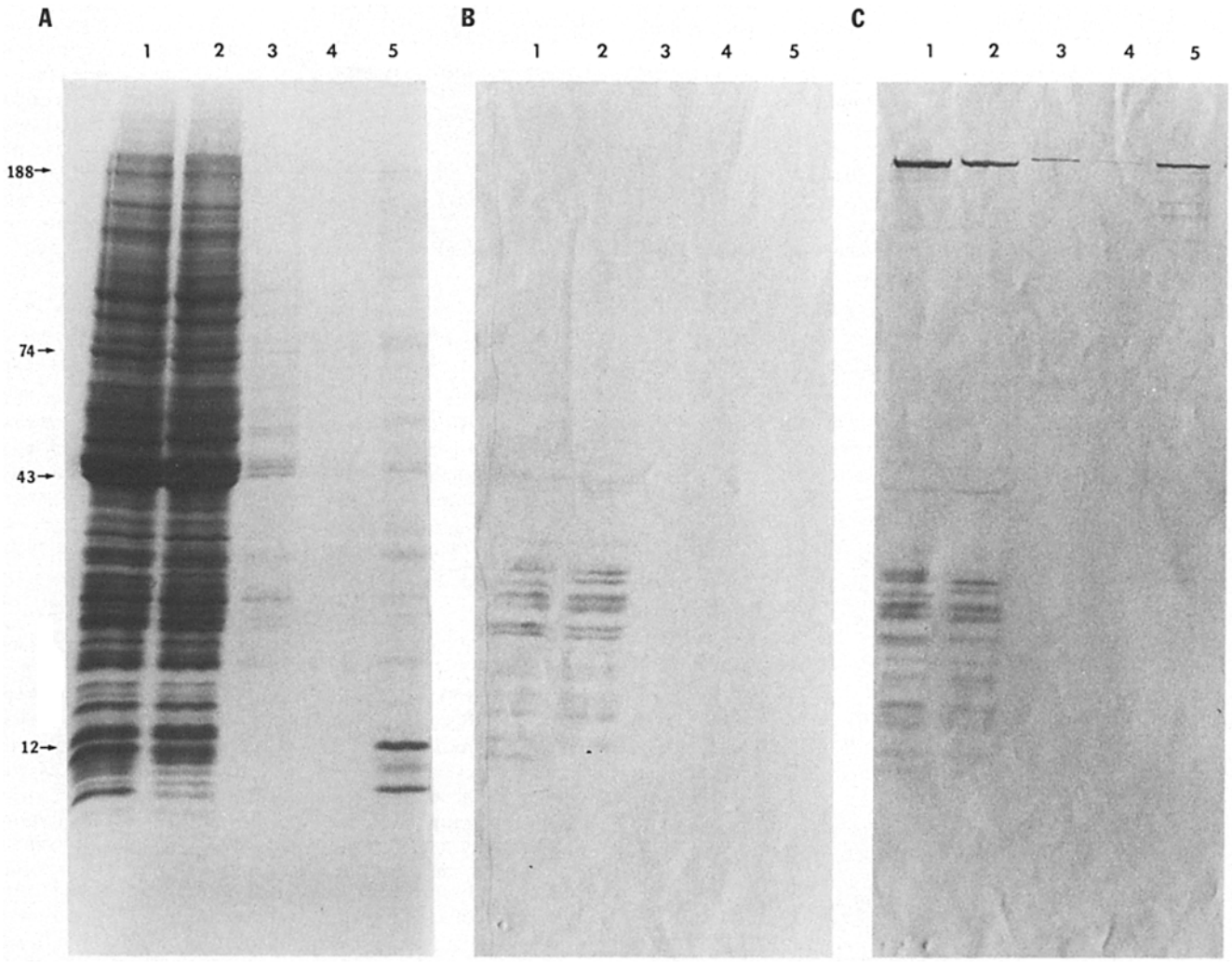

Figure 7. Specificity of affinity-purified anti-*Drosophila* NMPCL ATPase antibodies. *Drosophila* embryos were homogenized and nuclei purified according to standard procedures (Fisher et al., 1982), and aliquots of each fraction generated during the purification analyzed by SDS gradient PAGE. Equivalent amounts of each fraction, defined in terms of the total amount of embryo starting material, were electrophoresed in each lane. One unit is defined as the amount of material derived from 1 μl of packed embryos (~40–50 organisms) as previously reported (Fisher et al., 1982). Three parallel gel segments were loaded and electrophoresed identically; four units of respective fractions were loaded in each lane. Approximate amounts of protein are indicated as follows. Lanes *1*, 400 μg of filtered crude homogenate; lanes *2*, 380 μg of postnuclear supernatant; lanes *3*, 10 μg of first nuclear wash supernatant; lanes *4*, 2 μg of second nuclear wash supernatant; lanes *5*, 20 μg of purified nuclear protein. (*A*) Coomassie Blue-stained gel segment; (*B*) parallel segment blot transferred to nitrocellulose and probed with pre-immune serum at 1:1,000; (*C*) parallel segment blot transferred to nitrocellulose and probed with affinity-purified anti-*Drosophila* NMPCL ATPase IgG at a concentration of specific antibody equivalent to 1:100 anti-ATPase antiserum. Blots in *B* and *C* were processed routinely and developed in the phosphatase stain reagents for 30 min. Marker positions to the left of *A* apply to all three panels.

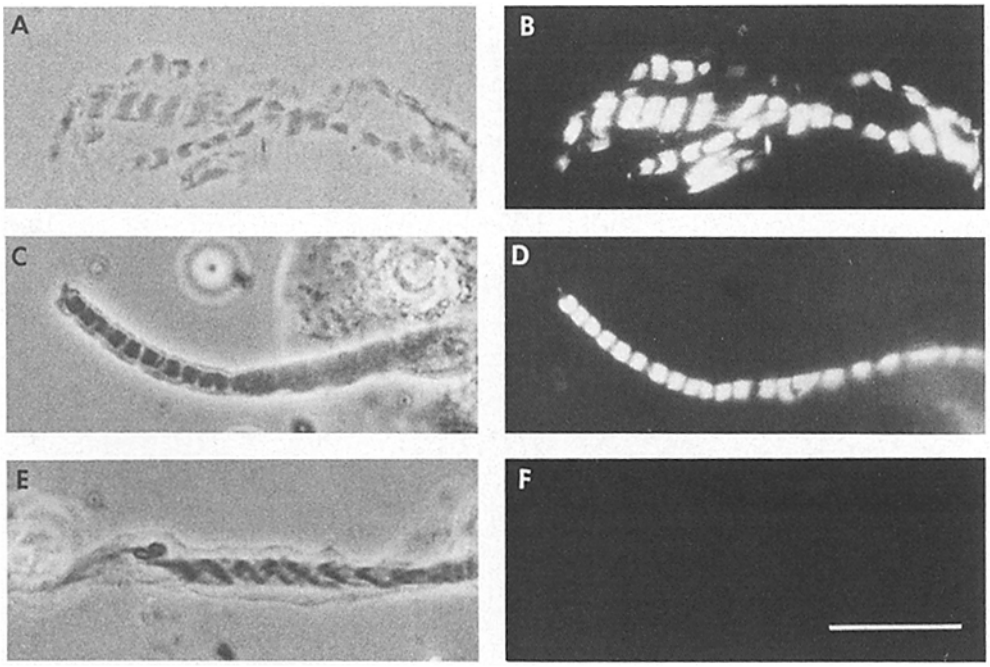

Figure 8. Affinity-purified anti-*Drosophila* NMPCL ATPase antibodies stain striated muscle. Muscle fibers were dissected from *Drosophila* larvae, fixed, and permeabilized as described in Materials and Methods. Phase-contrast (*A*, *C*, and *E*) and fluorescence (*B*, *D*, and *F*) micrographs of samples probed with anti-ATPase antibodies affinity purified using either the NMPCL ATPase polypeptide (*A* and *B*) or *Drosophila* muscle myosin heavy chain (*C* and *D*) as the specific affinity ligand. Affinity-purified IgG was at a specific antibody concentration equivalent to 1:100 anti-ATPase antiserum (based on quantitative Western blot comparison). The IgG was affinity purified exactly as described in the legend for Fig. 2. (*E* and *F*) Specimen was probed with preimmune serum at 1:100. Bar, 25 μm.

whole nuclear lysate (lanes *5*) were electrophoresed on an SDS polyacrylamide gradient gel. One segment was stained with Coomassie Blue (*A*); two parallel segments were blotted to nitrocellulose. The blot shown in *B* was probed with preimmune serum; nonspecific bands seen in lanes *1* and *2* were also seen in the absence of primary antibody (not shown). The blot shown in *C* with probed with affinity-purified anti-NMPCL ATPase IgG at an equivalent specific antibody concentration of 1:100 anti-ATPase antiserum (10 times the optimal concentration typically used). The same nonspecific bands seen in *B*, lanes *1* and *2*, are also observed in *C*, lanes *1* and *2*. The only specific bands observed in *B* are at the expected mobility position for NMPCL ATPase/myosin heavy chain, and minor proteolytic breakdown products thereof. The fractionation of this species is as would be anticipated. The nuclei are significantly enriched for the ATPase band, but a majority of apparently non-nuclear antigen is found in the postnuclear supernatant. (Controls probed with anti-lamin and anti-pore complex glycoprotein demonstrated that the postnuclear supernatant was substantially free of these specific nuclear markers [not shown]. This control has previously been published [Filson et al., 1985].) The major contaminating antibody species previously identified in the crude antiserum (Fisher et al., 1982) were quantitatively absent from the affinity-purified anti-ATPase IgG.[3]

Antibodies Directed against the Drosophila NMPCL ATPase Recognize Muscle Myosin In Situ

Indirect immunofluorescence analyses were used to further corroborate the identification of the *Drosophila* NMPCL ATPase as a form of myosin heavy chain. Three different anti-NMPCL ATPase antibody preparations were used to probe striated muscle tissue dissected from the buccal apparatus of the third instar larvae and smooth muscle tissue identified in cryosections of the larval digestive tract; only results obtained with striated muscle are shown. Unfractionated anti-ATPase antiserum (not shown), as well as IgG fractions affinity purified using either the NMPCL ATPase polypeptide (Fig. 8, *A* and *B*) or muscle myosin heavy chain (Fig. 8, *C* and *D*) as specific affinity ligands, were all found to stain both types of muscle specifically and intensely. The staining patterns obtained with the three different primary antibody fractions used were indistinguishable. In striated muscle specimens, the expected pattern of myosin thick filament (A band) staining of sarcomeres was observed (Warn et al., 1979). A non-immune IgG control was negative (Fig. 8, *E* and *F*).

Indirect Immunofluorescent Localization of the NMPCL ATPase in Drosophila Larval Nuclei

The intensity of immunofluorescent staining of muscle sarcomeres with anti-NMPCL ATPase IgG made it impossible to evaluate the possibility that there might also be relatively low level staining of muscle cell nuclei. However, nuclear staining was readily demonstrable using isolated nuclei, permeabilized whole cells, and cryosections obtained from nonmuscle tissues of *Drosophila* third instar larvae. These results are shown in Figs. 9–11. Initial results obtained with whole serum were confirmed with affinity-purified IgG prepared using either the SDS hydroxylapatite-purified *Drosophila* NMPCL ATPase polypeptide or *Drosophila* muscle myosin heavy chain as specific affinity ligands. (Before their use as affinity ligands, analyses of the SDS hydroxylapatite-purified NMPCL ATPase polypeptide and *Drosophila*

3. It should be noted that by using antibodies for immunocytochemistry that have been pre-selected based on Western blot reactivity, we exclude the possibility, a priori, that one type of antibody is responsible for the reactivity seen on blots whereas a second type of antibody that does not react well on blots is, on the other hand, more reactive with the "native" form of the antigen seen in situ and accounts for our immunofluorescence results.

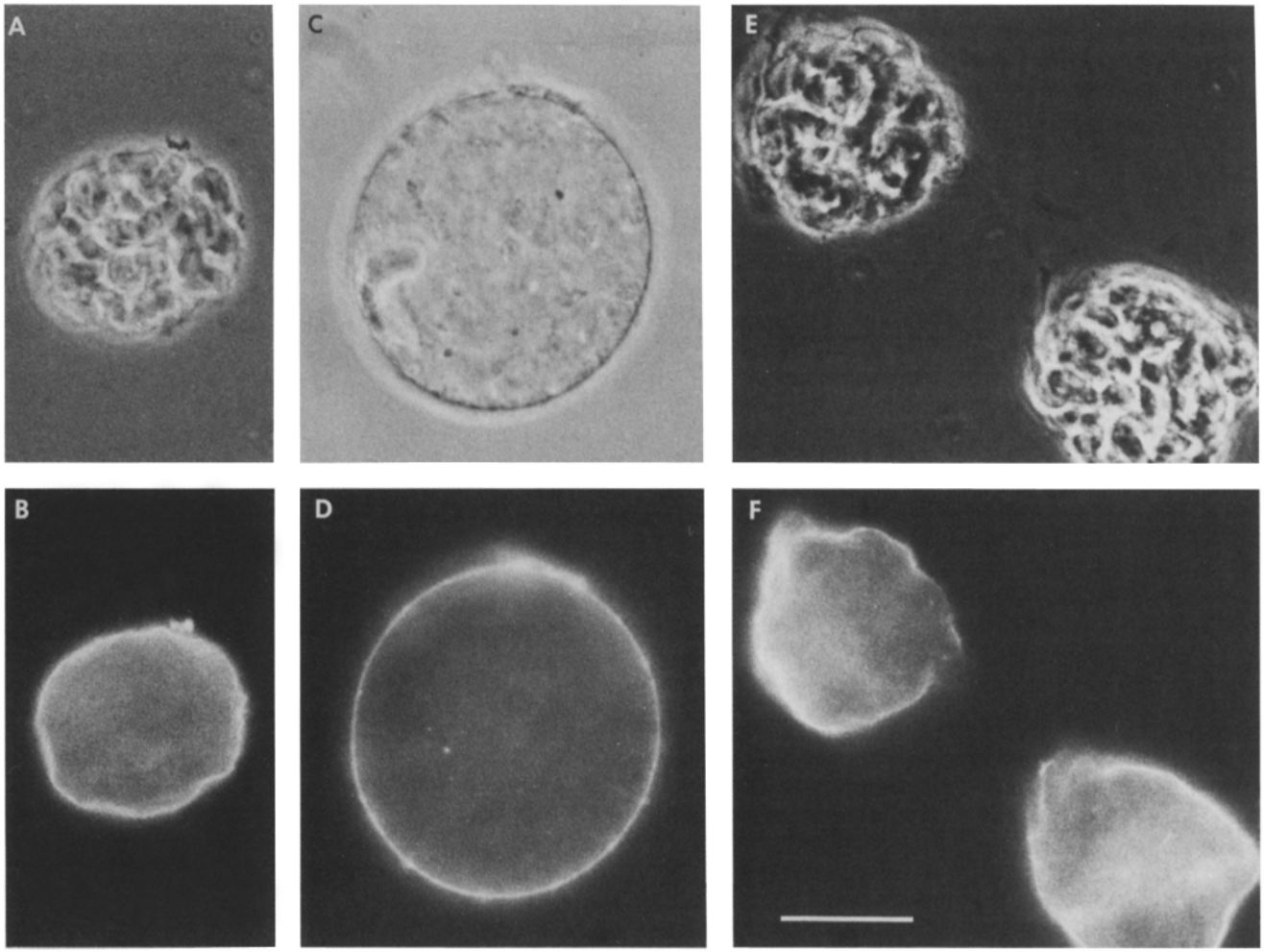

Figure 9. Indirect immunofluorescent localization of the NMPCL ATPase in extruded nuclei from *Drosophila* third instar larval salivary gland cells. Phase-contrast (*A* and *C*) and fluorescence (*B* and *D*) micrographs of extruded nuclei from early (*A* and *B*) and late (*C* and *D*) third instar larval salivary gland cells probed with affinity-purified anti-*Drosophila* NMPCL ATPase IgG at a titre of specific IgG equivalent to unfractionated antiserum at 1:100 (based on quantitative Western blot comparison). The IgG was affinity purified exactly as described in the legend for Fig. 2 *C*. Identical results were obtained with the unfractionated serum at 1:100, and with similarly calibrated, affinity-purified IgG prepared using muscle myosin heavy chain as the specific affinity ligand (prepared as in Fig. 2 *D*). Phase-contrast (*E*) and fluorescence (*F*) micrographs of extruded nuclei from early third instar larvae probed with monoclonal anti-nuclear pore complex glycoprotein antibodies AGP-26 and AGP-78 at approximately equivalent IgG concentrations to those in *A–D*, and as reported previously (Filson et al., 1985). Bar, 25 μm.

muscle myosin heavy chain with monoclonal antibodies AGP-26 and AGP-78 showed these protein fractions to be entirely free of contaminant 188-kD nuclear pore complex glycoprotein [data not shown].) Results obtained with all three anti-ATPase antibody fractions were identical. Only results obtained with IgG fractions affinity purified against the NMPCL ATPase polypeptide are shown except as indicated otherwise.

Staining of nuclei with anti-NMPCL ATPase IgG was most readily demonstrable with isolated nuclei manually extruded from *Drosophila* larval salivary gland cells (Fig. 9). A "rim" staining pattern was observed, consistent with specific localization of the NMPCL ATPase to the nuclear envelope. The staining pattern observed was identical with both early (Fig. 9, *A* and *B*) and late (Fig. 9, *C* and *D*) third instar larval nuclei and was indistinguishable from that observed using monoclonal antibodies directed against the 188-kD *Drosophila* nuclear pore complex glycoprotein (Fig. 9, *E* and *F*). A similar immunofluorescence staining pattern was observed when permeabilized whole *Drosophila* larval cells were probed with anti-NMPCL ATPase IgG fractions; nucleolar staining was also apparent (Fig. 10, *A* and *B*) but was not consistently observed in all cell types and preparations. Staining of permeabilized whole cells with monoclonal anti-nuclear pore complex glycoprotein antibodies is shown for comparison (Fig. 10, *C* and *D*). A non-immune control for the permeabilized whole cell experiments is shown in Fig. 10, *E* and *F*. The specific localization of the *Drosophila* NMPCL ATPase was confirmed using larval cryosections (Fig. 11). Immunofluorescent staining with anti-NMPCL ATPase IgG was largely restricted to the region of the nuclear envelope (Fig. 11, *A–D*). Weak immunofluorescent staining of the cytoplasm and relatively more intense staining in the region of the plasma membrane were also observed. The nuclear envelope staining shown in Fig. 11 was identical with that reported for the *Drosophila*

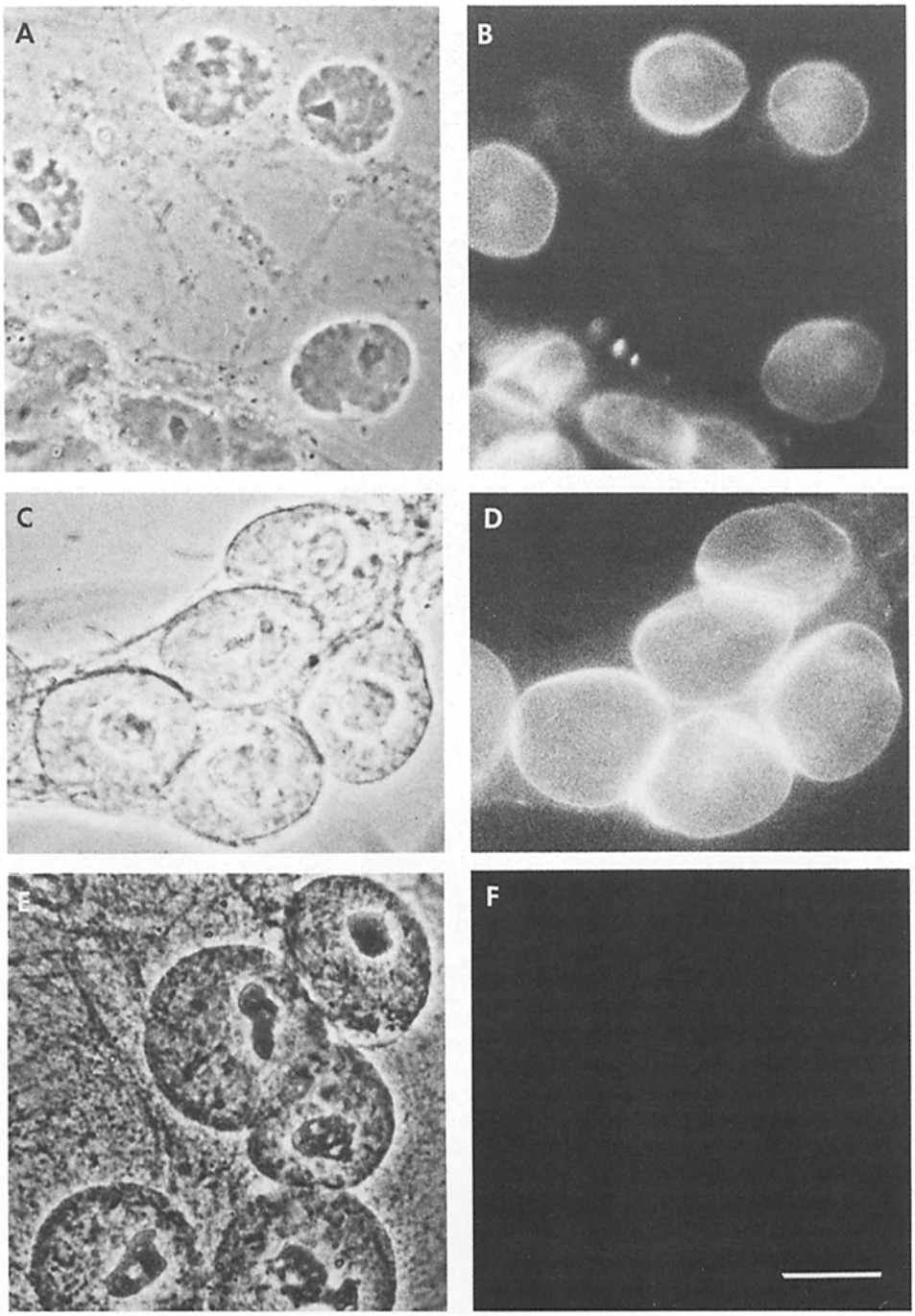

Figure 10. Indirect immunofluorescent localization of the NMPCL ATPase in permeabilized whole cells from *Drosophila* third instar larval salivary glands. Phase-contrast (*A*) and fluorescence (*B*) micrographs of permeabilized whole cells obtained using affinity-purified anti-*Drosophila* NMPCL ATPase IgG as in Fig. 9. Phase-contrast (*C*) and fluorescence (*D*) micrographs of permeabilized whole cells probed with monoclonal anti-nuclear pore complex glycoprotein antibodies AGP-26 and AGP-78 as in Fig. 9. Phase-contrast (*E*) and fluorescent (*F*) micrographs of permeabilized whole cells probed with nonimmune serum at 1:100. Bar, 25 μm.

nuclear pore complex glycoprotein (Filson et al., 1985) and for the nuclear lamins (Smith and Fisher, 1984).

The Drosophila NMPCL ATPase Is Immunochemically Homologous to the Major ATPase Identified by UV Photoaffinity Labeling of the Rat Liver Nuclear Pore Complex–Lamina Fraction

The *Drosophila* NMPCL ATPase had previously been shown to be nearly identical with a rat liver ATPase polypeptide, identified by UV photoaffinity labeling of the rat liver nuclear pore complex–lamina (NPCL) fraction, on the basis of both SDS PAGE mobility and elution from an SDS hydroxylapatite column (Berrios et al., 1983*a*, *b*). Immunochemical homology, determined by Western blot analysis, is shown in Fig. 12. Affinity-purified anti-rat NPCL ATPase IgG cross-reacted with the *Drosophila* NMPCL ATPase as well as with both the rat muscle myosin heavy chain and with the *Drosophila* muscle myosin heavy chain. We think it noteworthy that the cross-reactivity of the anti-rat NPCL ATPase antibodies with rat muscle myosin heavy chain, although readily demonstrable, was significantly weaker than had been observed between anti-*Drosophila* NMPCL ATPase antibodies and *Drosophila* muscle myosin heavy chain (Fig. 2). In fact, the anti-rat NPCL ATPase antibodies recognized *Drosophila* muscle myosin heavy chain nearly as well as they did rat muscle myosin heavy chain (Fig. 12). In reciprocal

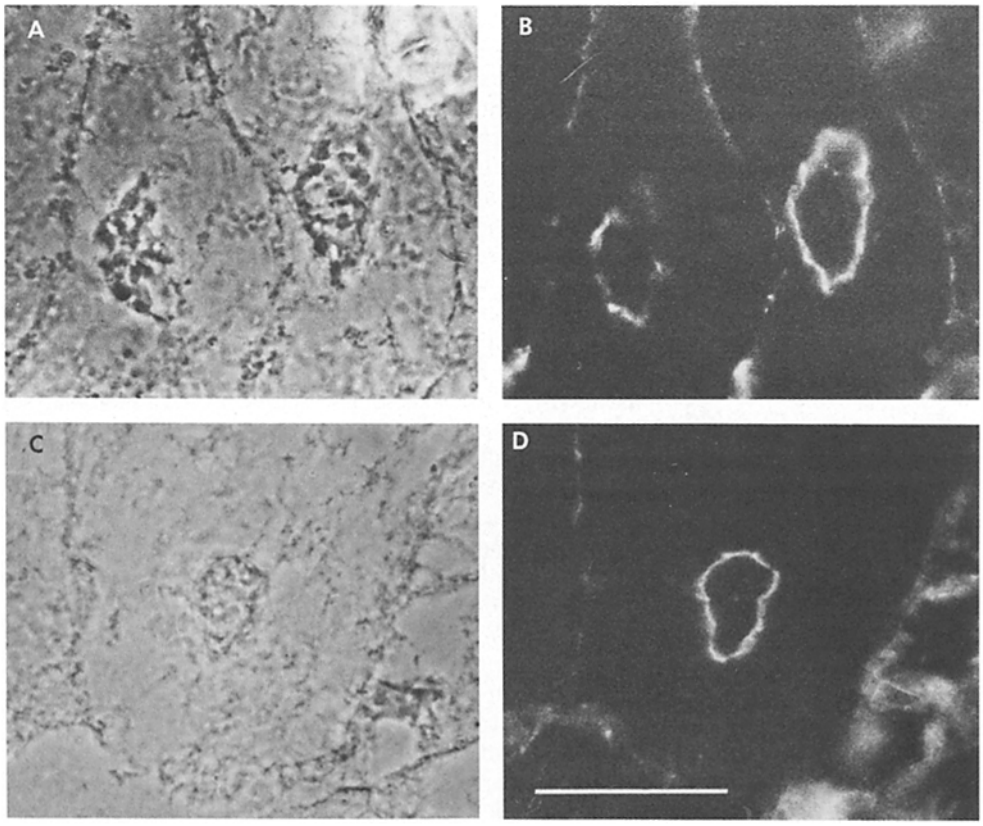

Figure 11. Indirect immunofluorescent localization of the NMPCL ATPase in cryosections of *Drosophila* third instar larval salivary glands. Phase-contrast (*A* and *C*) and fluorescence (*B* and *D*) micrographs of *Drosophila* larval cryosections probed with affinity-purified anti-*Drosophila* NMPCL ATPase IgG as in Fig. 9. Fields from two independent experiments are shown. Bar, 50 μm.

experiments, affinity-purified anti-*Drosophila* NMPCL ATPase antibodies also reacted with the rat NPCL ATPase considerably more effectively than with rat muscle myosin heavy chain (data not shown).

Indirect Immunofluorescence Localization of the NMPCL ATPase in Simian COS-7 Cells and Onion Cells

Affinity-purified anti-rat NPCL ATPase IgG was used to probe COS-7 cells that had been grown on coverslips, fixed, and permeabilized. Indirect immunofluorescence analyses demonstrated intense cytoplasmic staining in a pattern characteristic of cytoskeletal myosin (Fig. 13, *A* and *B*). Staining of nuclei, if any, was barely discernible. Immunofluorescent staining of the COS cell nuclei was readily observed, however, when specimens were probed with anti-*Drosophila* NMPCL ATPase antibodies (Fig. 13, *C* and *D*). Curiously, the anti-*Drosophila* NMPCL ATPase antibodies were relatively, quite reactive with the mammalian nuclei but only stained the cytoskeletal myosin weakly. Initial immunofluorescence experiments with higher plants were performed using onion cells that had been cut with a scalpel so as to incise their cell walls, and then further permeabilized in the same way as whole animal cells. Nuclear rim fluorescence was readily observed when these cells were probed with the anti-*Drosophila* NMPCL ATPase antiserum (Fig. 13, *E* and *F*). Similar results were obtained with affinity-purified IgG fractions (data not shown).

Discussion

A high molecular weight ATPase polypeptide, previously identified as a component of the *Drosophila* nuclear matrix-pore complex-lamina fraction (Berrios et al., 1983*a*) has been shown to be closely related to myosin heavy chain. Indirect immunofluorescence analyses of *Drosophila* salivary gland cryosections have demonstrated that this myosin heavy chain-like ATPase appears to be largely restricted to the nuclear envelope. These results confirm the impression obtained by examining extruded nuclei and permeabilized whole cells from a number of *Drosophila* tissues. Indirect immunofluorescence staining of these specimens with anti-ATPase antibodies showed a "rim" fluorescence pattern characteristic of antigens concentrated in the nuclear envelope. Anti-*Drosophila* NMPCL ATPase antibodies have also been used to decorate nuclei from mammalian tissue culture cells and from onions. A similar rim staining pattern was seen, at least with the onion cell nuclei. Thus, by the criteria of cell fractionation and in situ localization, it appears that myosin heavy chain or a myosin heavy chain-like molecule is a specific polypeptide component associated with the nuclear envelopes of higher animals and plants.[4]

A prevailing concern throughout these studies has been the possibility that the myosin heavy chain found associated with

4. The data in this paper pertain entirely to the identification of a myosin heavy chain-like polypeptide as a nuclear envelope component. It now appears that a 16-kD *Drosophila* NMPCL polypeptide described previously (Fisher et al., 1982) is immunochemically indistinguishable from an 18-kD *Drosophila* muscle myosin light chain. Immunofluorescence analysis of *Drosophila* skeletal muscle with the anti-16-kD polypeptide antiserum shows intense A band staining of sarcomeres similar to that seen with the anti-ATPase antibodies. Immunofluorescence analyses of larval cryosections indicate that this putative nuclear myosin light chain, like the NMPCL ATPase, is largely restricted to the nuclear envelope. A manuscript describing these results is in preparation.

Figure 12. Cross-reactivity between anti-rat NPCL ATPase IgG and rat muscle myosin heavy chain, *Drosophila* NMPCL ATPase, and *Drosophila* muscle myosin heavy chain. SDS hydroxyapatite-purified nuclear ATPase fractions from both rat liver and *Drosophila* embryos were electrophoresed in parallel with muscle myosin prepared from these same organisms on an SDS 7% polyacrylamide gel. Lane *1* was loaded with 2–3 μg of rat skeletal muscle myosin. Lane *2* was loaded with 0.2–0.3 μg of purified rat NPCL ATPase (the amount loaded was limited by availability of this material). Lane *3* was loaded with 2–3 μg of purified *Drosophila* NMPCL ATPase. Lane *4* was loaded with 2–3 μg of *Drosophila* muscle myosin heavy chain. The blot shown was probed with affinity-purified anti-rat liver NPCL ATPase IgG at a concentration of specific IgG equivalent to ~1:200 of the unfractionated antiserum. Blots were developed for 20 min in the phosphatase stain reagents.

isolated nuclei and nuclear structural protein subfractions represented a contaminant resulting from imperfect cell fractionation. This possibility cannot be completely excluded. However, the fact that in our initial UV photoaffinity labeling studies (Berrios et al., 1983*a*), a similar polypeptide was identifiable in nuclear envelope fractions prepared by a variety of methods and from several different organisms, argues against such an artifact. This argument has been strengthened by the in situ studies presented in this paper. Although redistribution of antigens during the processing and probing of samples for immunofluorescence analysis is difficult to rule out absolutely, we feel that the results obtained with larval cryosections are particularly compelling–these sections were cut from living tissue which had been flash-frozen in liquid nitrogen, and then fixed in either formaldehyde, paraformaldehyde, glutaraldehyde, or some combination of these fixatives as part of the thawing process. In this context, it is also relevant to note that the identification of a myosin heavy chain–like molecule as a nuclear polypeptide is not entirely novel (see, for example, Jockusch et al., 1973; LeStourgeon et al., 1975; Kuo et al., 1982). Our present results confirm these earlier observations.

In establishing the in situ localization of the NMPCL ATPase polypeptide, it was necessary to demonstrate as rigorously as possible, the specificity of the antibody fractions used for immunolocalization. This was particularly the case in that the nuclear pore complex glycoprotein, an established component of the nuclear envelope (Gerace et al., 1982; Filson et al., 1985), co-migrates with the NMPCL ATPase on one-dimensional SDS polyacrylamide gels. To assure that antibodies against the glycoprotein (or any other nuclear proteins for that matter) did not contaminate our anti-ATPase IgG, all analyses were repeated with several different affinity-purified IgG fractions prepared by immunoadsorption to either the *Drosophila* NMPCL ATPase or *Drosophila* muscle myosin heavy chain. Both of these polypeptides were highly purified and characterized before their use as affinity ligands. The apparent nonreactivity of our initial antiserum with the nuclear pore complex glycoprotein (Fig. 1), and even greater specificity of the affinity-purified IgG fractions as assessed by Western blot analyses both of crude organismal lysates and whole nuclear fractions (Fig. 7) as well as hydroxylapatite-purified polypeptides (see Results), argues against spurious results due to uncertain antibody specificity. Recently, we have undertaken to generate a library of monoclonal antibodies directed against the *Drosophila* NMPCL ATPase. The first two of these antibodies to be characterized show in situ immunofluorescence reactivity with *Drosophila* nuclei, as well as with both smooth and striated muscle, that is identical with that observed with the polyclonal IgG fractions used in this report (Berrios, M., unpublished observation).

Although all of our anti-*Drosophila* NMPCL ATPase antibodies stain *Drosophila* muscle tissue intensely, they show relatively little staining of cytoplasmic regions in nonmuscle cells. This may reflect a paucity of cytoskeletal myosin in the *Drosophila* cells and tissues examined to date. However, the anti-*Drosophila* NMPCL ATPase antibodies also stain nuclei from mammalian tissue culture cells relatively intensely, with little staining of cytoplasmic regions. In contrast, anti-rat liver NPCL ATPase antibodies stain cytoplasmic myosin in these cultured mammalian cells so intensely as to virtually obscure any nuclear staining that might be present. It seems, therefore, that the antigenic determinants accessible on the NMPCL ATPase in situ and recognized by the anti-*Drosophila* ATPase antibodies may not be accessible on cytoskeletal myosin; similarly, it may be that antibodies that recognize cytoskeletal myosin are relatively unreactive with the nuclear envelope ATPase in situ. This might explain, at least to some extent, the failure of other workers to note staining of nuclei with anti-myosin antibodies. This would also imply that the nuclear envelope ATPase polypeptide is distinguishable, at least immunocytochemically, from cytoplasmic myosin heavy chain. It remains to be determined whether or not the NMPCL ATPase represents a previously unidentified polypeptide species, or rather, is simply a cytoskeletal myosin heavy chain localized in the nuclear envelope.

In *Drosophila melanogaster*, only a single gene coding for myosin heavy chain has been found (Rozek and Davidson, 1983; Bernstein et al., 1983). The similarity in the peptide maps between the NMPCL ATPase and the muscle myosin heavy chain is consistent with this. However, this gene may actually encode several protein variants. Northern blot analyses have demonstrated the existence of at least three and perhaps four or more different transcripts deriving from the single myosin heavy chain gene (Rozek and Davidson, 1983; Bernstein et al., 1983); it seems possible that the differences observed in CNBr and chymotryptic map comparisons of the

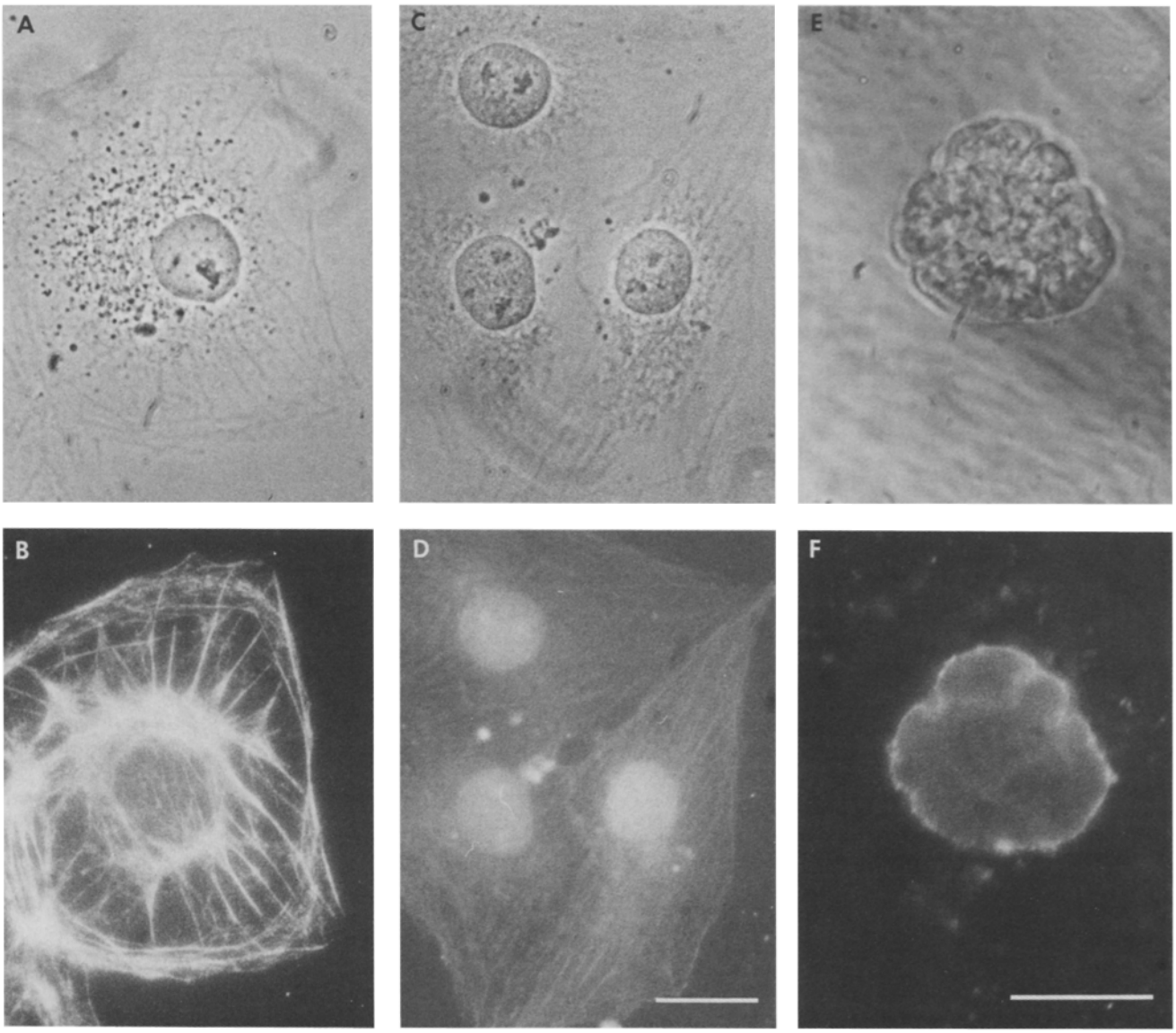

Figure 13. Indirect immunofluorescent localization of the NPCL/NMPCL ATPase in simian COS-7 cells and onion cells. Phase-contrast (*A*, *C*, and *E*) and fluorescence (*B*, *D*, and *F*) micrographs. Fixed and permeabilized COS-7 cells were probed with affinity-purified anti-rat NPCL ATPase IgG (*A* and *B*) or affinity-purified anti-*Drosophila* NMPCL ATPase IgG (*C* and *D*) at approximately equivalent concentrations based on relative Western blot reactivity with species-homologous antigens, and for the anti-*Drosophila* ATPase IgG, exactly as in Figs. 9–11. Mechanically disrupted onion cells were probed with anti-*Drosophila* NMPCL ATPase antiserum diluted at 1:100 (*E* and *F*). Bar in *D* (*A–D*), 25 μm. Bar in *F* (*E* and *F*), 25 μm.

NMPCL ATPase with the muscle myosin heavy chain reflect differences in primary protein sequence that result from translation of different transcripts.

In contrast with *Drosophila*, preliminary CNBr peptide map comparisons between rat muscle myosin heavy chain and the rat NPCL ATPase reveal largely, though not entirely dissimilar patterns (data not shown). Immunochemical comparisons of the rat liver ATPase with rat skeletal muscle myosin heavy chain have also shown relatively poor homology. In fact, the cross-reactivity between the rat NPCL ATPase polypeptide and skeletal muscle myosin heavy chain is comparable to the cross-reactivity between the rat NPCL ATPase and the *Drosophila* NMPCL ATPase (or for that matter, the *Drosophila* muscle myosin heavy chain). Given the evidence for multiple myosin heavy chain genes in mammals (see, for example, Whalen et al., 1982), it seems probable that the rat NPCL ATPase is encoded by a gene distinct from those coding for skeletal muscle myosin heavy chains.

The results that have been presented in this paper are of a largely descriptive nature. It is therefore impossible to attach any certain functional significance to our observations. At least one role that could reasonably be ascribed to myosin localized in the nuclear envelope is in conferring mobility to nuclei. A number of developmental processes, particularly in *Drosophila*, involve wholesale nuclear migrations (Zalokar and Erk, 1976) for which a contractile apparatus might be necessary. The ability of myosin-coated beads to actually traverse actin cables in vitro (Sheetz and Spudich, 1983) makes plausible the notion that a similar process may account for nuclear mobility in vivo.

It has previously been proposed that a contractile apparatus may similarly be required for the nucleocytoplasmic ex-

change of macromolecules in vivo (LeStourgeon, 1978). Although a number of mechanisms might be envisioned, detailed speculation seems, in most respects, premature. There is perhaps one exception. In examining current models of nuclear pore complex ultrastructure (Unwin and Milligan, 1982), we have noted that the so-called annular subunits of both the nuclear and cytoplasmic faces of the nuclear pore complex are each nearly identical in size with the head of the native myosin molecule. The tail of the molecule is of sufficient length and flexibility to span the nuclear envelope and form the lumenal walls of the nuclear pore. Mechanisms whereby the energy of ATP hydrolysis in the myosin head could be coupled to the propulsion of macromolecules through the pore may also be proposed. In its simplest form, this model might therefore posit that there are eight myosin molecules in octagonal array with their heads facing the cytoplasm and their tails pointing radially inward and down toward the nucleus. These myosin molecules would be arranged tail-to-tail with eight myosin molecules of opposite orientation; that is, with their heads on the nucleoplasmic side of the nuclear envelope and with their tails pointing radially inward and up toward the cytoplasm. The bidirectional transport of macromolecules through the pore could be accomplished by a peristaltic wave of contraction generated by ATP hydrolysis in the myosin heads at the margins of the nuclear pore complex. A similar, though structurally less explicit hypothesis has previously been put forth by LeStourgeon (1978). The recent report of Schindler and Jaing (1986) lends direct experimental support to this and related hypotheses. Alternative hypotheses remain to be tested.

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