Isolation and Characterization of a Proteinaceous Subnuclear Fraction Composed of Nuclear Matrix, Peripheral Lamina, and Nuclear Pore Complexes from Embryos of *Drosophila melanogaster*

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ABSTRACT   Morphologically intact nuclei have been prepared from embryos of *Drosophila melanogaster* by a simple and rapid procedure. These nuclei have been further treated with high concentrations of DNase I and RNase A followed by sequential extraction with 2% Triton X-100 and 1 M NaCl to produce a structurally and biochemically distinct preparation designated *Drosophila* subnuclear fraction I (DSNF-I). As seen by phase-contrast microscopy, DSNF-I is composed of material which closely resembles unfractionated nuclei; residual internal nuclear structures including nucleolar remnants are clearly visible. By transmission electron microscopy, nuclear lamina, pore complexes, and a nuclear matrix are similarly identified. Biochemically, DSNF-I is composed almost entirely of protein (>93%). SDS PAGE analysis reveals several major polypeptides; species at 174,000, 74,000, and 42,000 predominate. A polypeptide coincident with the Coomassie Blue–stainable 174-kdalton band has been shown by a novel technique of lectin affinity labeling to be a glycoprotein; a glycoprotein of similar or identical molecular weight has been found to be a component of nuclear envelope fractions isolated from the livers of rats, guinea pigs, opossums, and chickens. Antisera against several of the polypeptides in DSNF-I have been obtained from rabbits, and all of them show only little or no cross-reactivity with *Drosophila* cytoplasmic fractions. Initial results of immunocytochemical studies, while failing to positively localize either the 174- or 16-kdalton polypeptides, demonstrate a nuclear localization of the 74-kdalton antigen in all of several interphase cell types obtained from both *Drosophila* embryos and third-instar larvae.

In recent years, considerable attention has been focused on the characterization of various internal structural elements found ubiquitously in eucaryotic cells. A particularly intriguing subset of these investigations has dealt with the cell nucleus. Largely as a result of such work, it is now reasonable to conceptually subdivide the nucleus into two distinct structural domains, the nuclear periphery composed of the lamina and its associated pore complexes (for current reviews of the nuclear envelope which discuss these structures, see references 1–5), and the more recently described internal nuclear matrix (see review by Shaper et al. [6]).

Work on the characterization of the nuclear lamina and associated pore complexes has been performed primarily with rat liver (7–15), avian erythrocytes (12, 16–18), and with various *Xenopus* tissues (particularly the oocyte) (19). After appropriate preparative procedures, a subnuclear fraction has been obtained from these organisms which appears to be composed exclusively of the peripheral lamina and pore complexes. Biochemical analyses of these fractions have shown them to contain three major polypeptides with molecular weights between 60,000 and 80,000, and these have been designated lamins A, B, and C (10). Further, a major high molecular weight polypeptide (150,000–200,000) has also been observed and, in the case of the rat liver nuclear pore complex–lamina fraction, PAS (periodic acid–Schiff) staining has shown this polypeptide to be a glycoprotein (20; Gerace and Blobel, unpublished observation). In addition to the major species, all pore complex–lamina fractions characterized thus far contain numerous, quantitatively minor polypeptides making up at least 50–60% of the total protein found in these fractions. As yet, these minor

components have not been studied to any significant extent.

In apparent contrast to the above results, several workers, using preparative procedures similar to those employed in the isolation of nuclear pore complex–lamina fractions, have been able to demonstrate, in addition to peripheral structures, a meshwork located internally and designated the nuclear matrix (21–33). This meshwork has been found in organisms ranging from *Tetrahymena* (24, 27, 30) and *Physarum* (31), on the one hand, to human HeLa cells (26, 33), on the other; most of the reports published to date have dealt with mammalian cell nuclei. Although several workers have reported an intimate association between the nuclear matrix and hnRNA (28, 30, 33), it seems clear at present that significant amounts of nucleic acid are not required for the integrity of the isolated matrix structure (see, e.g., 25, 33).

Despite the extensive characterization outlined above, there are as yet no definitive results regarding the specific polypeptide composition of the nuclear matrix. SDS PAGE analyses of the rat liver nuclear matrix (25) as well as the nuclear matrix from cultured 3T6 cells (34) reveal a polypeptide composition that is strikingly similar to that observed upon comparable analyses of pore complex–lamina fractions (see above). However, given that immunocytochemical localization studies demonstrate lamins A, B, and C to be exclusively peripheral, both in fixed material and isolated nuclei (10) as well as in frozen sections (15), it seems unlikely that the lamins are themselves major components of the internal matrix.

Because of the successful past application of immunochemical and immunocytochemical approaches to problems of nuclear structure (10, 11, 15) and because of the difficulty experienced in raising antibodies to the major pore complex–lamina antigens in rabbits (10), we decided to attempt further study of nuclear substructure with an organism evolutionarily distant from mammalian sources of immunoglobulin. Although any of the lower eucaryotes would have served this purpose, our ongoing interest in the mechanisms of mitotic disassembly of the nuclear envelope (10, 11) precluded the use of a species that undergoes the so-called closed type of mitosis characteristic of lower eucaryotes (for review, see reference 4). An animal that fulfills both basic criteria and offers innumerable other advantages (see Discussion) is *Drosophila melanogaster*. In the present report, we describe a simple, rapid, and efficient procedure for the purification of nuclei from *Drosophila* embryos and for the subsequent generation of a subnuclear fraction that closely resembles nuclear matrix preparations obtained from vertebrates. This fraction has been characterized with respect to its polypeptide composition, and comparisons with rat liver subnuclear fractions, both biochemical and immunochemical, have been performed.

MATERIALS AND METHODS

DNase I, RNase A, and soybean trypsin inhibitor were obtained from Worthington Biochemical Corp. (Freehold, NJ); β-galactosidase and phosphorylase *a* were from Boehringer Mannheim Biochemicals (Indianapolis, IN); crystallized and lyophilized BSA (bovine serum albumin), catalase, ovalbumin, bovine hemoglobin, and cytochrome *c* were from Sigma Chemical Co. (St. Louis, MO); specific IgG fractions were from Cappel Laboratories Inc. (Cochranville, PA); Con A (concanavalin A) was from Miles-Yeda (Rehovot, Israel); Fraction V BSA was from Reheis Chemical Company (Phoenix, AZ). [^{14}C]formaldehyde, [^{3}H]thymidine, and [^{3}H]uridine were from New England Nuclear (Boston, MA). Nitrocellulose was from Schleicher & Schuell, Inc. (Keene, NH). SDS was from BDH Chemicals (Poole, England); acrylamide and methylene bis-acrylamide were from Eastman Kodak (Rochester, NY). Nonsterile goat serum was from Rockland, Inc. (Gilbertsville, PA). Opossums were the generous gift of Dr. J. M. Bedford (Cornell University Medical School, New York). Osmium tetroxide, 8% glutar-

aldehyde, Walton's lead aspartate, lead nitrate, uranyl acetate, Spurr low viscosity epoxy resin, and sodium cacodylate were from Polysciences, Inc. (Warrington, PA). Paraformaldehyde and HPLC grade acetone were from Fisher Scientific Co. (Pittsburgh, PA); Epon 812 was from Ladd Research Industries, Inc. (Burlington, VT). All other reagents obtained commercially were of reagent grade and were used without further purification.

Rearing of Drosophila melanogaster in Mass Culture

Drosophila melanogaster (Oregon R, P2 strain) were grown in mass culture and embryos were collected essentially according to Allis et al. (35). Embryos were either used immediately or rapidly frozen in liquid N$_2$ and stored at −70°C. Embryos used for all studies were between 4 and 20 h old.

Preparation of Nuclei from Embryos of Drosophila melanogaster

Drosophila embryos were fractionated essentially according to the procedure of Elgin and Hood (36) with modifications as follows (this fractionation is summarized in Fig. 1). All work was done at 4°C except as indicated, and all quantities expressed in numbers of volumes refer to the original volume of embryos used in the preparation. 1 ml of packed embryos contains ~4–5 × 10^4 individual organisms. Washed, dechorionated embryos (fresh or freshly thawed) were suspended in 10 vol of Extraction Buffer containing 50 mM Tris-Cl, pH 7.5, 50 mM NaCl, 5 mM MgCl$_2$, 250 mM sucrose, 1 mM PMSF (phenylmethylsulfonyl fluoride), 1 mM TPCK (L-tosylamide-2-phenylethyl chloromethyl ketone), and 2.5 mM NEM (N-ethyl maleimide). The suspended embryos were Dounce-homogenized (four strokes, tight pestle) and filtered through two layers of 120-μm nylon mesh to produce a filtered crude homogenate (FCH). The FCH was centrifuged at 1,000 g for 10 min; the postnuclear supernatant (PNS) was removed and the crude nuclear pellet was resuspended by gentle vortexing in 5 vol of Extraction Buffer. Centrifugation was repeated as above, the supernatant (WS-1) was removed, and the nuclei were again resuspended in 5 vol of Extraction Buffer. The nuclear suspension was centrifuged (10 min, 1,000 g) and the

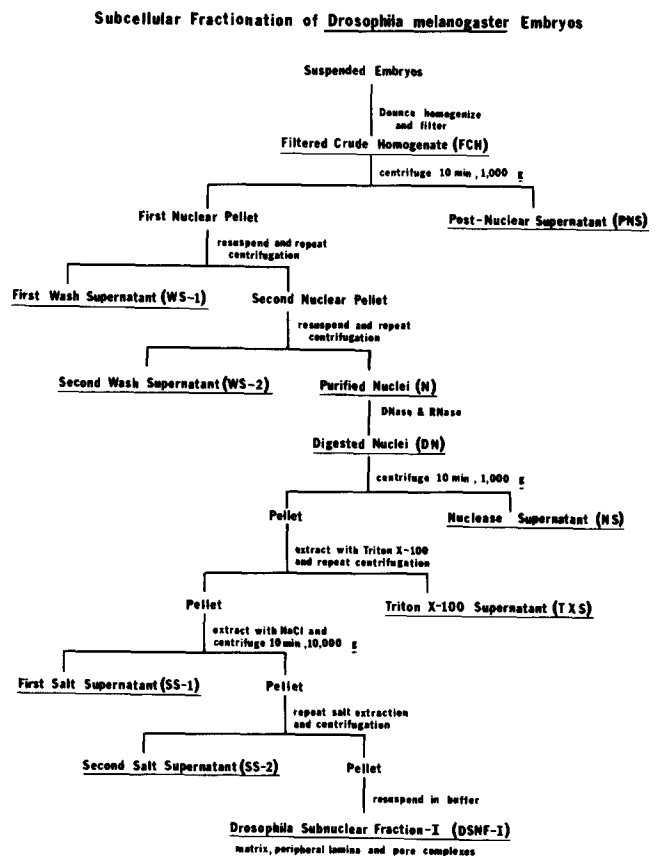## Subcellular Fractionation of Drosophila melanogaster Embryos

FIGURE 1 Flow diagram showing the subcellular fractionation of *Drosophila melanogaster* embryos. The details of the fractionation are as described in Materials and Methods.

supernatant (WS-2) was removed. The final nuclear pellet (N) was suspended by gentle vortexing in 1 vol of 20 mM Tris-Cl, pH 7.5, 5 mM MgCl₂.

Subfractionation of Drosophila melanogaster Nuclei

The purified nuclei were incubated at a final concentration of 10 μg/ml DNase I and 8 μg/ml RNase A for 15 min at 37°C; >50% of the total nucleic acid was rendered cold-TCA(trichloroacetic acid)-soluble by this digestion. The digested nuclei (DN) were centrifuged for 10 min at 1,000 g, the nuclease supernatant (NS) was removed, and the pellet was resuspended in 0.9 vol of 290 mM sucrose, 10 mM Tris-Cl, pH 7.5, and 0.1 mM MgCl₂. After resuspension, 0.1 vol of 20% (vol/vol) Triton X-100 was added and the suspension was allowed to incubate on ice for 10 min; it was then centrifuged for 10 min at 1,000 g and the supernatant (TXS) was removed. The pellet was suspended in 0.5 vol of 100 mM Tris-Cl, pH 7.5, 290 mM sucrose, 0.1 mM MgCl₂, followed by the addition of 0.5 vol of 2 M NaCl. After a 10-min incubation on ice, the suspension was centrifuged for 10 min at 10,000 g and the supernatant (SS-1) was removed. The resuspension, addition of 2 M NaCl, incubation on ice, and centrifugation were repeated; the supernatant (SS-2) was removed and the final pellet thus obtained was designated *Drosophila* subnuclear fraction I (DSNF-I). A quantitative summary of our purification protocol is shown in Table I and described in Results.

Preparation of the Rat Liver Nuclear Pore Complex–Lamina (RNPCL) Fraction

The RNPCL fraction was prepared from adult rat livers essentially as previously described (9), with modifications as follows. Only a single DNase I digestion step was used, RNase A was included at the same concentration as the DNase, and the incubation was carried out for 15 min at 37°C. After the initial extraction with 1 M NaCl, a second identical salt extraction was performed to ensure complete removal of the histone proteins.

Preparation of Nuclear Envelope Fractions from Guinea Pig, Opossum, and Chicken Livers

Nuclei were prepared from the livers of guinea pigs, opossums, and baby chickens and digested with nucleases, essentially as described above for rat livers. After nuclease digestion, the nuclear envelopes (NE) were collected by centrifugation and used as such; detergent and salt extractions were not performed.

Biochemical Composition of Drosophila Subcellular Fractions

Protein was determined according to Schaffner and Weissman (37). Nucleic acid compositions were determined by first solubilizing samples in SDS and cold-TCA-precipitating them to remove cold-acid-soluble nucleotide. After several washes in cold-TCA (5% wt/vol), the pellets were resolubilized in 0.1 M Na₂HPO₄, 0.1% (wt/vol) SDS and treated with NaOH to hydrolyze RNA. RNA was determined by measuring alkali-labile OD₂₆₀ after subsequent cold-TCA precipitation of DNA and protein. DNA was determined by measuring hot-TCA-soluble OD₂₆₀ after prior alkali treatment to remove RNA. Conditions for alkali treatment (0.3 N NaOH for 60 min at 60°C) and hot-TCA treatment (5% (wt/vol) TCA for 20 min at 90°C) were established using trace amounts of in vivo ³H-labeled nucleic acids; quantitative solubilization of RNA without loss of TCA-precipitable DNA was observed after alkali treatment; quantitative solubilization of DNA was observed after hot acid treatment. Absorbance measurements were converted into micrograms of nucleic acid by assuming an average nucleotide molecular weight of 330 and an average nucleotide molar extinction coefficient of 10⁴ at 260 nm.

SDS PAGE

SDS PAGE was performed essentially according to Laemmli (38) with modifications as follows. Gradient separating gels, 7.5–15% (wt/vol) acrylamide, were stabilized during polymerization by a 0–18% (wt/vol) sucrose gradient. Stacking gels (5% acrylamide) contained 60 mM Tris-Cl, pH 6.8; 0.1% (wt/vol) SDS and 15% (wt/vol) sucrose. The electrophoresis buffer was 380 mM glycine, 50 mM Tris, 0.1% (wt/vol) SDS. Unless otherwise indicated, sample preparation was as follows. Samples were diluted into Load Buffer containing 60 mM Tris-Cl, pH 6.8, 2% (wt/vol) SDS, 20 mM dithiothreitol (DTT), heated to 90°C for 3 min and chilled; 0.20 vol of cold 100% (wt/vol) TCA was added and the resulting precipitates were collected by centrifugation. After the supernatants were re-

moved, residual acid was neutralized by the addition of 7 μl of 2 M Tris and samples were resolubilized in 75 μl of Load Buffer. After complete resolubilization, samples were again heated to 90°C for 3 min, cooled, and made 100 mM in iodoacetamide. Samples were incubated for 30 min in the dark at 23°C, after which they were loaded onto the gels and electrophoresed. Gels were stained with Coomassie Blue and destained essentially according to Weber and Osborn (39). Standard proteins were treated in the manner described for all other samples and were as follows: β-galactosidase (116,000), phosphorylase *a* (94,000), BSA (66,000), catalase (60,000), IgG heavy chain (52,000), ovalbumin (43,500), DNase I (31,000), IgG light chain (25,000), soybean trypsin inhibitor (21,500), RNase A (13,700), and cytochrome *c* (12,400).

Production of Specific Antisera

Antibodies to specific polypeptides in the DSNF-I fraction were raised in female New Zealand White rabbits as follows. DSNF-I from 6 ml of *Drosophila* embryos (9.8 mg total protein) was prepared as described above and electrophoresed on two preparative (2-mm thick, 250-mm long, 350-mm wide, no wells) 7.5–15% (wt/vol) polyacrylamide gradient gels. Gels were stained and destained as described above and dried by vacuum onto Whatman 3 MM filter paper. Bands of interest were excised from the stained, dried gels; the strips of acrylamide were rehydrated in H₂O, and the filter paper backing was removed. The rehydrated gel strips were then homogenized with a motor-driven Teflon pestle in ~5 ml of Na₂HPO₄; excess liquid was removed from the fully hydrated polyacrylamide homogenate (final volume, 5–6 ml) and an equal volume of complete Freund's adjuvant was added. The adjuvant and homogenized gel were emulsified using two interconnected syringes, and the entire emulsion (10–12 ml) was injected into six subcutaneous sites along the flanks of each animal. This initial injection was followed at 14 d by a second injection, identical in all respects to the first. Animals were bled weekly beginning on day 21 and boosted monthly with gel-purified antigen, eluted into Na₂HPO₄ and emulsified in incomplete Freund's adjuvant. Booster injections were given intramuscularly and contained amounts of antigen comparable to the primary injections.

Antiserum against the entire RNPCL fraction was prepared in a female New Zealand White rabbit as follows. The RNPCL fraction was denatured and prepared as described above for SDS PAGE except that the final TCA precipitate was resolubilized in 100 mM Na₂HPO₄. Approximately 20 mg of this material was emulsified in an equal volume of complete Freund's adjuvant (final volume, 6 ml) and was injected into the animal both subcutaneously (six sites, both flanks) and intramuscularly (six sites, both hind legs). An identical injection was repeated after 14 d and the animal was bled weekly beginning on day 21.

In Vitro ¹⁴C-Labeling of Proteins with Retention of Biological Activity

The reductive methylation procedure of Jentoft and Dearborn (40) was used to covalently [¹⁴C]methylate goat anti–rabbit IgG and Con A under conditions which did not perturb the biological activity of these protein molecules. Reactions were from 12 to 24 h at 23°C; final protein concentrations were between 10 and 15 mg/ml; phosphate buffer, pH 7.5, was used in all cases; NaCNBH₃ was at a final concentration of 20 mM and reactions were initiated by the addition of [¹⁴C]formaldehyde to a final concentration of 200 μCi/ml (~4 mM). Between 50 and 75% of the added label was covalently linked to protein such that a final specific activity of ~15,000 cpm/μg of final product was achieved. Labeled proteins were used without subsequent purification and were stable for at least 8 wk when frozen at −20°C.

Blot Transfer of Proteins from SDS Polyacrylamide Gradient Gels to Sheets of Nitrocellulose

Gel blots were set up essentially according to Southern (41) with modifications as follows. The transfer buffer used was electrophoresis buffer without the SDS. Gels were set up for blot transfer immediately after completion of electrophoresis; no intervening washes of the gel were employed. Gels were allowed to blot for 24–72 h; representative nitrocellulose blots were stained with amido black and destained according to Schaffner and Weissman (37). The residual gel was stained and destained as above. Transfer efficiency was between 50 and 100% as quantitated both from stain results and by the use of in vitro ¹⁴C-labeled protein fractions; transfer efficiency was independent of polypeptide molecular weight. Comparison of amido black-stained blots with parallel Coomassie Blue-stained gels showed that there was no detectable loss of resolution during the transfer procedure and that all bands visible on the stained gel were discernible on the nitrocellulose blot.

Antibody Identification of Antigens on Nitrocellulose Blots

All work was performed at 23°C. All volumes expressed are for a 20 cm² (1 × 20 cm) strip of nitrocellulose and were increased proportionately for larger blots. After transfer of proteins to the nitrocellulose (see above), blots were incubated for 6–12 h in a solution of 140 mM NaCl, 10 mM KPO₄, pH 7.5, 10 mg/ml BSA, Fraction V. After this, blots were either used immediately or air-dried and stored indefinitely. When dried blots were employed, they were rehydrated with water immediately before use. The specific antisera (10–500 μl depending on the titer) were diluted in 140 mM NaCl, 10 mM KPO₄, pH 7.5, 10 mg/ml BSA, Fraction V, 0.1% (vol/vol) Triton X-100, 0.02% (wt/vol) SDS (Ab Buffer). In general, 1–2 ml of first antibody solution (serum plus Ab Buffer) was used per strip. Incubation of first antibody solution with the nitrocellulose blot was for 12–24 h in a Seal-N-Save (Sears) bag; the nitrocellulose was then washed in the bag with 2.5–5 ml of Ab Buffer, three times for 30 min each. After the final wash, 1 ml of Ab Buffer containing 400,000–500,000 cpm of the appropriate ¹⁴C-labeled IgG second antibody was added to the bag and incubated with the strip overnight (12–16 h). The bag was then drained and the nitrocellulose strip removed and washed for 2 h in multiple changes of 560 mM NaCl, 10 mM KPO₄, pH 7.5, 0.1% (vol/vol) Triton X-100, 0.02% (wt/vol) SDS (total volume for five strips, 1–2 liters). The washed strips were dried at 80°C for 10 min, dipped into a solution of 10% (wt/vol) PPO in toluene, dried at 23°C, and fluorographed at −70°C using preflashed film essentially as described (42–44). In all experiments performed, preimmune and mock-immune serum controls were negative. Film types and exposure times were as indicated in the individual figure legends. Preliminary attempts to use blots for antibody probing which had first been stained with amido black (37; see above) were not successful.

Nitrocellulose Spot Assay for the Quantitative Titration of Crude Antisera

To screen multiple samples of our crude antisera rapidly and simultaneously, a nitrocellulose spot assay was developed and performed as follows. Antigens to be adsorbed to nitrocellulose were first denatured by heating to 90°C for 30 min in a solution containing 2% (wt/vol) SDS. Protein was then acid-precipitated with a final concentration of 10% (wt/vol) TCA, resolubilized in 0.5 M NaPO₄, pH 6.8, 0.2% (wt/vol) SDS, and diluted 10-fold with water. The resolubilized, diluted protein solution was incubated for 12–24 h in a Seal-N-Save bag with sheets of nitrocellulose that had been prewashed as for blot transfer of proteins from gels. For a 100-cm² piece of nitrocellulose, a total of 2–5 mg of unfractionated antigen protein in a final volume of 10 ml was routinely used. Representative strips of antigen-treated nitrocellulose were stained with amido black (37) to ensure uniform adsorption of the protein, and the remaining nitrocellulose was then treated with BSA in buffer as described for the treatment of blots after transfer of proteins from polyacrylamide gels. The blocks of nitrocellulose were air-dried and stored at 23°C for up to 3 mo without apparent detriment.

Before use, dried pieces of nitrocellulose were thoroughly rehydrated in H₂O, and the excess H₂O was blotted onto filter paper. 1 μl of each serum to be screened was then spotted directly onto the nitrocellulose and allowed to air-dry. In practice, as many as 50 samples could be spotted in one sitting with no observable difference in duplicates spotted first and last, respectively. After completion of the spotting procedure, the nitrocellulose sheets were processed as described above for the antibody probes of gel blots, beginning with three half-hour washes in Ab Buffer. Spots were localized by fluorography and quantitated after excision (with a small hole-puncher) by direct scintillation counting in a standard nonaqueous cocktail. Under these conditions, binding of antibody to the nitrocellulose was antigen dependent, was negligible for preimmune or mock-immune sera, and was linear with dilution of the initial antiserum (dilutions performed in preimmune serum) over a >10-fold range.

Identification of Glycoproteins on Nitrocellulose Blots with ¹⁴C-Labeled Con A

After transfer, blots (either stained or unstained) were washed for 6–12 h in a solution of 10 mg/ml hemoglobin, 50 mM Tris-Cl, pH 7.5, 140 mM NaCl. Strips were then incubated for 1–2 h in 5 ml of buffer containing 10 mg/ml hemoglobin, 140 mM NaCl, 50 mM Tris-Cl, pH 7.5, 1 mM MgCl₂, 2.5 mM CaCl₂ (Con A Buffer) and, where indicated, 0.5 M α-methyl mannoside. The bags were drained and 1–2 ml of Con A Buffer (with or without α-methyl mannoside) containing 400,000–500,000 cpm of ¹⁴C-labeled Con A was added to each bag. Incubations with the labeled Con A were carried out for 6–12 h, after which the strips were washed, dried, and fluorographed as described above for antibody identification of antigens bound to nitrocellulose. Unlike the antibody-antigen interaction, Con

A binding to nitrocellulose-immobilized glycoproteins was not significantly perturbed by prior staining of the nitrocellulose blots with amido black.

Electron Microscopy

Electron microscopy was performed using standard techniques. Samples were fixed either in suspension or as pellets in modified Karnovsky fixative (45), 3.1% (wt/vol) glutaraldehyde, 4.1% (wt/vol) paraformaldehyde, 60 mM Na cacodylate, pH 7.5. Samples were postfixed in 1% (wt/vol) OsO₄ in buffer as above and stained with Walton's lead aspartate for 45 min at 60°C (46). They were then dehydrated in acetone, embedded in either Epon 812 or Spurr low viscosity epoxy resin (47), and sectioned onto uncoated copper grids (300 mesh). Grids were stained with 5% (wt/vol) aqueous uranyl acetate and examined in a Jeol-100 CX transmission electron microscope.

Indirect Immunofluorescence

Preparations of Drosophila salivary gland cells, neuronal ganglion cells, and cells from various other third-instar larval tissues were obtained, fixed with formaldehyde, and processed for immunofluorescence staining essentially as described (48), except that Triton X-100 was omitted from the initial dissection buffer and acetic acid was included at a final concentration of 1% (vol/vol) in the ethanol wash step. Drosophila embryo squashes were prepared and processed identically after gentle rupture of dechorionated embryos. Incubations with the primary antiserum were carried out for 30 min at 37°C in a humidified chamber; dilutions of the primary antiserum were made in the Ab Buffer identical to that used for probing nitrocellulose blots (see above). Slides were washed three times for 3 min each in Ab Buffer and then incubated for 30 min at 37°C with fluoroscein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG. Before use, the FITC-IgG was reconstituted with water to its original volume as prescribed by the manufacturer (Cappel Laboratories), passed over a Sepharose column to which total extracts of both Drosophila embryos and third-instar larvae had been covalently coupled, and diluted 1:250 in Ab Buffer. After incubation with FITC-IgG, slides were washed three times for 3 min each in a solution containing 560 mM NaCl, 10 mM KPO₄, pH 7.5, 0.1% (vol/vol) Triton X-100, 0.02% (wt/vol) SDS. A cover slip was then mounted and the stained specimens were examined and photographed with a Zeiss fluorescence microscope.

RESULTS

Morphologic Characterization of Drosophila Subcellular Fractions

To establish the purity of our Drosophila nuclei as well as to determine the morphologic composition of the various subnuclear fractions generated during our procedure, samples of material obtained at each step in the purification were examined by both phase-contrast light microscopy and transmission electron microscopy. The results of these studies are shown in Fig. 2. In Fig. 2A, a phase-contrast micrograph of DSNF-I is shown. Although in terms of other aspects of gross morphology they are essentially identical to the nuclei from which they were derived, in contrast to the other Drosophila nuclear and subnuclear fractions examined, the nuclear remnants seen in DSNF-I exhibit only minimal refractility. The inset in Fig. 2A shows a higher magnification view of DSNF-I. Panels B–E in Fig. 2, show the results of transmission electron microscopy. Fig. 2B shows a representative field of our purified nuclear preparation examined before any nuclease digestion or subfractionation. Particularly noteworthy is the absence of any distinct nonnuclear contamination as well as the relatively intact nature of the nuclei that are observed. Inner and outer nuclear membranes as well as numerous ribosomes attached to the outer nuclear membrane are clearly seen at higher magnification (not shown). Examination of these same nuclei after nuclease digestion revealed few if any differences (not shown). After Triton X-100 extraction, internal nuclear morphology was similarly unperturbed but both inner and outer membranes and ribosomes were absent (also not shown). In contrast to nuclease digestion and detergent extraction, after extraction
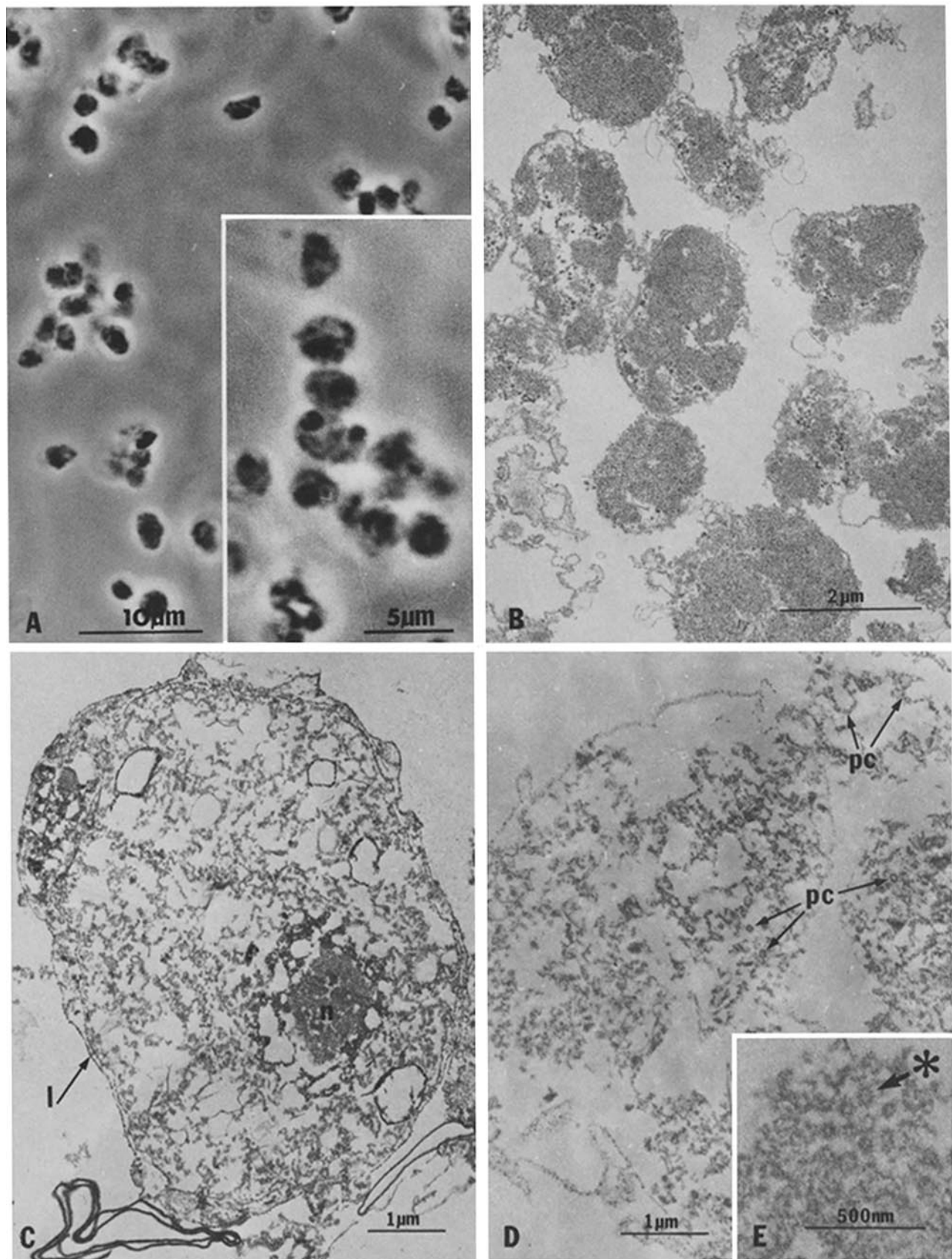
FIGURE 2   (A) Phase-contrast micrograph of DSNF-I. × 2,240; *inset*, DSNF-I. × 3,680. (B– E) Examination of *Drosophila* subcellular fractions by transmission electron microscopy. (B) Purified nuclei (N), × 13,500. (C) DSNF-I; lamina (l) and nucleolar residue (n) are as indicated. × 14,300. (D) DSNF-I; pore complexes (pc) are as indicated. × 16,000. (E) Higher magnification (× 44,000) view of pore complexes; asterisk denotes pore complex with preservation of apparent subunit structure. (Note: by phase-contrast microscopy the matrix structures seen in DSNF-I are similar in size to the nuclei from which they were derived. However, after preparation for electron microscopy, the matrix structures appear considerably expanded. Thus, the discrepancy in size between nuclei in panel B vs. the matrix structures in panels C and D is due primarily to an apparent expansion of the matrix.)

with 1M NaCl much of the electron-dense internal nuclear material is lost, leaving behind a meshwork characteristic of nuclear matrix preparations from a variety of sources (21–33). Two representative examples of DSNF-I preparations are shown in Fig. 2C and D. Clearly discernible as indicated in Fig. 2C are a peripheral nuclear lamina, substantial diffuse internal structure, and a readily identifiable nucleolar remnant. Pore complexes are not abundantly visible on this section. In Fig. 2D, a second example of DSNF-I is shown and, here again, both peripheral lamina as well as substantial amounts of internal material are demonstrated. In addition, numerous pore complexes are seen as indicated. In this section, no nucleolar remnants are visible. Fig. 2E shows a high magnification view of a grazing section through several pore complexes. As indicated, subunit structure is readily apparent, although the material is not sufficiently well preserved to rigorously establish the classical 8-fold symmetry (see references 2 and 4 for review).

Biochemical Composition of Drosophila Subcellular Fractions

To obtain a quantitative perspective on the relationship of DSNF-I to the embryos from which it was derived and, further, to assess the potential role of nucleic acids in contributing to the nuclear matrix structures seen in DSNF-I, we performed determinations of protein, DNA, RNA, and cold-acid-soluble nucleotide on each of the fractions generated in our purification procedure. The results of these measurements are shown in Table I. Fraction abbreviations are as indicated in Materials and Methods.

The first point to be made from the data in Table I concerns the recovery of total cellular nucleic acid in our purified nuclear fraction. Approximately 80–90% of the total embryo DNA is recovered with the purified nuclei, whereas only about 6% of the total RNA is similarly retained. Also noteworthy are the relative amounts of total protein in DSNF-I, the purified nuclei and the FCH. DSNF-I constitutes almost 40% of the total nuclear protein and slightly more than 2% of the total embryo protein. These values are on the order of 20- to 40-fold greater than the ratio of RNPCL to rat liver nuclear protein and total

TABLE I

Subcellular Fractionation of Drosophila melanogaster Embryos

Fraction*	Protein‡	DNA‡	RNA‡	Cold-acid-soluble nucleotide‡
	mg	*µg*	*µg*	*µg*
FCH	71.8	625	8,440	—
PNS	69.4	93	7,168	—
WS-1	0.28	71	274	—
WS-2	0.07	0	163	—
N	4.13	578	536	454
DN	4.06	313	213	1,071
NS	0.19	20	19	539
TXS	0.25	19	19	94
SS-1	2.03	222	119	163
SS-2	0.09	18	26	36
DSNF-I	1.64	11	29	80
	(93.2%)§	(0.6%)§	(1.6%)§	(4.5%)§

* Fraction abbreviations are as defined in Materials and Methods and Fig. 1.
‡ All values refer to quantities obtained from 1 ml of frozen embryos. A typical preparation was started with 50 ml. (There are ~4–5 × 10⁴ embryo/ml.)
§ Numbers in parentheses represent the relative percent compositions of each of the DSNF-I components.

rat liver cellular protein. The final point to be made regarding the data in Table I relates to the overall composition of DSNF-I. From the results and calculated percent compositions shown, it is clear that protein constitutes by far the single major component of DSNF-I. Of the remaining mass, cold-acid-precipitable nucleic acid (minimum length, 5–10 nucleotides) constitutes only a minor fraction, with the rest (~70%) being cold-acid-soluble (<5–10 nucleotides in length). In light of their overall quantitative insignificance, it thus seems unlikely that the nucleic acid fragments present in DSNF-I play a primary structural role.

Characterization of DSNF-I by SDS PAGE

To obtain a detailed understanding of the polypeptide composition of the Drosophila subcellular fractions generated by our purification procedure, an aliquot of each fraction was denatured and electrophoresed on an SDS polyacrylamide gel; the results of this analysis are shown in Fig. 3. From these data it can be seen that DSNF-I (lanes 11–14) contains three major polypeptides (indicated on the right-hand side of Fig. 3 by the larger arrows) as well as several relatively minor species (one of which has also been indicated; smaller arrow). Molecular weights of 174,000, 74,000, and 42,000 for the three predominant polypeptides and 16,000 for the minor band indicated have been calculated on the basis of the mobilities of the standard proteins shown. (It should be noted that in the case of the 174-kdalton polypeptide this is a rather imprecise estimate given the limited range of our molecular weight standards.) Qualitatively identical polypeptide patterns have been obtained for preparations of DSNF-I generated under a variety of extraction conditions. In the initial Extraction Buffer, the absence of protease inhibitors or NaCl, the use of 25 mM MgCl₂ plus 10 mM EDTA, and the substitution of 2.5 mM CaCl₂ for 5 mM MgCl₂ are all without effect on the final product. Similarly, DSNF-I prepared from fresh rather than frozen embryos, or from nuclei which, in addition to the purification procedure described above (Materials and Methods), were also pelleted by ultracentrifugation through 2.2 M sucrose before subfractionation, is essentially identical to that shown in Fig. 3. Thus, the polypeptide pattern shown in Fig. 3, lanes 11–14 is obtained reproducibly, both from one preparation to another and over a wide range of fractionation conditions.

SDS PAGE examination of earlier fractions in the purification of DSNF-I (lanes 1–10) demonstrates results that are qualitatively consistent with the quantitative assessment of protein composition shown in Table I. Lanes 1–4 of Fig. 3 simply document the polypeptide composition of the fractions generated in our nuclear isolation procedure. Lane 5 of Fig. 3 shows the gel pattern obtained from an aliquot of purified nuclei; lane 6 represents a similar aliquot of this same fraction immediately after nuclease digestion (i.e., without any subsequent purification). Comparison of lanes 5 and 6 demonstrates the apparent lack of degradation of nuclear polypeptides during the course of the 37°C nuclease incubation. In addition, consistent with the fact that almost 40% of the total nuclear protein is found in DSNF-I, all of the major DSNF-I polypeptides can be readily identified in the unfractionated nuclei. Lanes 7 and 8, showing the nuclease and detergent supernatants (NS and TXS), respectively, contain only few species and in minor amounts. In contrast, the 1 M salt supernatant (SS-1; lane 9) contains essentially all of the histone proteins and several other polypeptides as well. Of the major DSNF-I
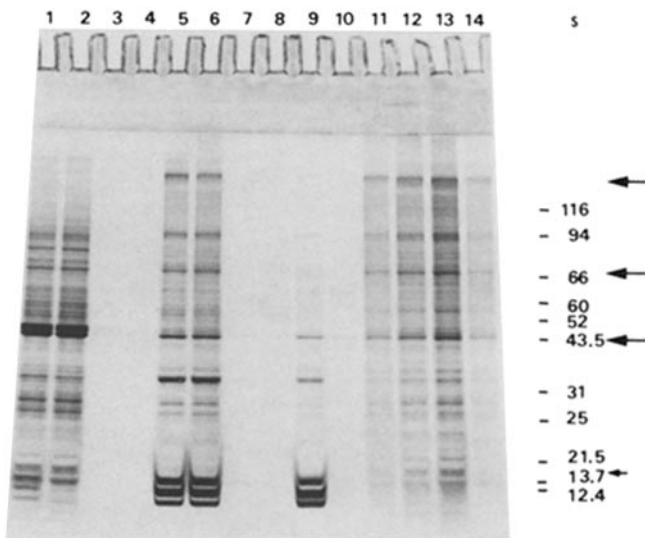
FIGURE 3 SDS PAGE analysis of *Drosophila* subcellular fractions. *Drosophila* subcellular fractions were prepared and subjected to SDS PAGE as described (Materials and Methods). Samples and amounts loaded were as follows: 1 U is defined as the amount of material derived from 1 μl of intact embryos (40–50 individual organisms). Fraction abbreviations are as defined (Materials and Methods). Lane *1*, FCH, 1.5 U; lane *2*, PNS, 1.5 U; lane *3*, WS-1, 1.5 U; lane *4*, WS-2, 1.5 U; lane *5*, N, 15 U; lane *6*, DN, 15 U; lane *7*, NS, 15 U; lane *8*, TXS, 15 U; lane *9*, SS-1, 15 U; lane *10*, SS-2, 15 U; lanes *11–14*, DSNF-I, 15, 30, 45, and 15 U, respectively. Positions of standard proteins electrophoresed on the same gel are as indicated (*s*); large arrows demarcate the 174-, 74-, and 42-kdalton DSNF-I polypeptides; a smaller arrow indicates the position of the 16-kdalton species. Note that the mobility of the 174-kdalton DSNF-I band is anomalously increased as increasing amounts are loaded on the gel.

polypeptides, it is noteworthy that substantial amounts of the 42-kdalton peptide and relatively lesser amounts of the 174- and 74-kdalton species are routinely observed in the SS-1 fraction. Although the partitioning of the 42-kdalton polypeptide into SS-1 does seem quite substantial and reproducible, the appearance of the other two bands in SS-1 is much more variable. In preparations made in the absence of protease inhibitors, there is a substantial increase in the amount of both the 74- and 174-kdalton species in SS-1 whereas simply increasing the centrifugation time for the salt extraction step greatly reduces the SS-1 concentration of these DSNF-I polypeptides. Thus, it is our tentative conclusion that the appearance of the 74- and 174-kdalton polypeptides in lane 9 results from fragmentation of DSNF-I during preparation and does not reflect a true solubilization of this material. As shown in lane 10, relatively little additional protein is extracted into SS-2 and thus, as was concluded for the nuclear fraction (see above), DSNF-I may be considered to represent a stable subcellular fraction of *Drosophila* embryos.

Production of Antibodies to Specific DSNF-I Polypeptides and to the Total RNPCL Fraction

To generate the immunologic reagents required for detailed characterization of DSNF-I polypeptides, we attempted to raise antibodies in rabbits to the 174-, 74-, and 16-kdalton species isolated from one-dimensional gels as well as to the entire RNPCL fraction; the results of these efforts are shown in Figs. 4 and 5. Fig. 4 demonstrates the time-course of the rabbits' immune response to each of the four antigens injected. Fig. 4*A*

is a fluorogram taken from our standard nitrocellulose spot screening assay; Fig. 4*B* shows these same results quantitatively. The injection schedule is indicated on the abscissa. In the *inset* of Fig. 4*B*, the linearity of the spot assay with respect to serum dilution is shown, as is the dependence of the response on the specific antigen which is bound to the nitrocellulose.

Immunochemical Characterization of *Drosophila* Subcellular Fractions with Respect to DSNF-I Antigens

The availability of anti-DSNF-I antisera allowed us to examine the various subcellular fractions generated during our purification for the presence of the respective DSNF-I polypeptides. Such an analysis seemed particularly imperative in light of the fact that, by SDS PAGE mobility alone, two of the major DSNF-I antigens (42- and 74-kdalton) could not be distinguished from major cytoplasmic (PNS) species of similar mobility. Therefore, primarily to determine whether the 74-kdalton polypeptide in DSNF-I was simply a residue of the 74-kdalton species seen in the PNS, we probed nitrocellulose blots of the SDS polyacrylamide gels run on the DSNF-I, PNS, and FCH material with each of the three available antisera. The results of this experiment are shown in Fig. 5.

In Fig. 5*A*, a stained gel block and parallel stained blot are shown. The transfer of all three lanes was essentially quantitative based on the absence of stainable protein in the residual gel fragments and the recovery from the nitrocellulose of ^{14}C-labeled standard proteins transferred in parallel. Blots prepared in parallel with the one in Fig. 5*A* and probed with antiserum as indicated are shown in Fig. 5*B*. Several points are demonstrated. In all three cases, the respective antisera show a high degree of polypeptide specificity. Further, the DSNF-I polypeptides appear to be highly enriched in DSNF-I relative to both the FCH and PNS. Most important, the major 74-kdalton polypeptide in DSNF-I is immunologically distinct from the 74-kdalton polypeptide in the PNS.

On the short fluorographic exposures of the blots shown (Fig. 5*B*), it is barely possible to detect the 174-kdalton DSNF-I polypeptide in the FCH fraction (indicated by the arrow). Fig. 5*C* shows a longer exposure of the same blot, clearly demonstrating the presence of the 174-kdalton polypeptide in the FCH and its essential absence in the PNS. Similar results were obtained with the 74-kdalton polypeptide (not shown) although the somewhat lesser amounts of 74-kdalton material in all of the fractions make a truly quantitative interpretation impossible. In the case of the 16-kdalton polypeptide, there is not sufficient material to detect this species in either the FCH or PNS. Finally, it should be noted that the anti-174-kdalton antiserum cross-reacts with a 42-kdalton polypeptide present in both FCH and PNS but not detectable in DSNF-I. Although there are a number of plausible explanations for this phenomenon, at present we have no specific insight into the actual mechanisms involved.

Immunochemical Comparison of DSNF-I with the RNPCL Fraction

To examine the cross-reactivity between DSNF-I and RNPCL antigens, nitrocellulose blots were prepared from SDS polyacrylamide gels on which samples of both DSNF-I and the RNPCL fraction had been electrophoresed; several strips were obtained, each containing both the rat and *Drosophila* fractions in adjacent positions. Fig. 6*A* shows both a section of gel containing one pair of wells routinely stained with Coo-

A

■ ● ▲ ◆

1
23
31
43
47
56
61
67

1
21
23
28
35
43
47

B

Antibody Titer (CPM x 10⁻³)

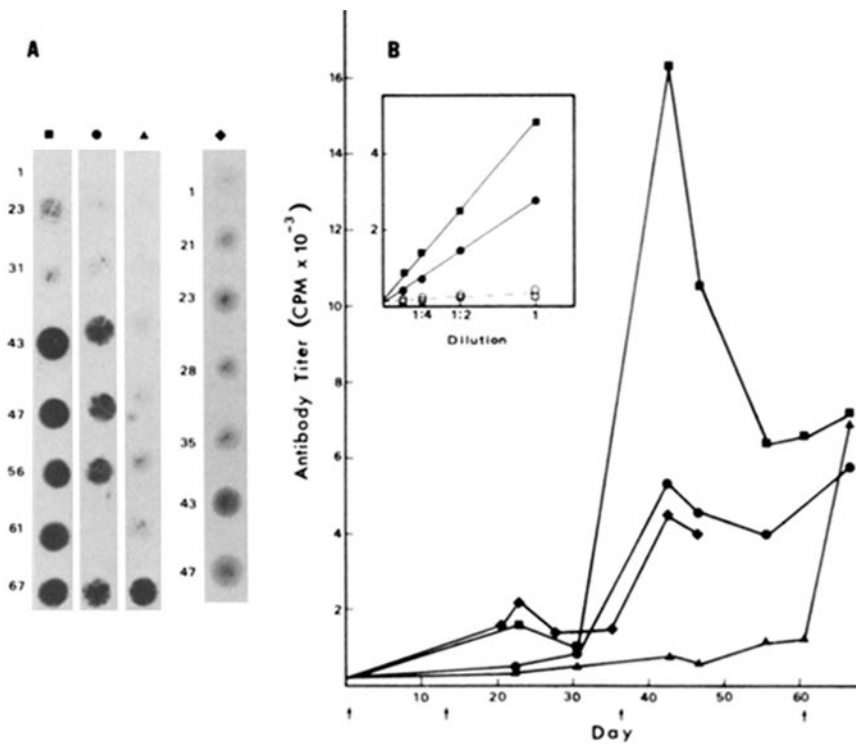(inset) Dilution: 1:4 1:2 1

10 20 30 40 50 60

Day

FIGURE 4 Titration of rabbit antisera using a nitrocellulose-based spot assay. Spot assays were performed as described in Materials and Methods. (A) Fluorogram showing response of rabbits to 174- (■), 74- (●), and 16-kdalton (▲) DSNF-I polypeptides and to the total RNPCL fraction (◆). Fluorography was carried out for 6 h on DuPont Cronex film. Numbers to the left of the strips indicate the day of the bleed. (B) Spots shown in panel A were excised and counted in a liquid scintillation counter; symbols are as in A. Arrows along the abscissa indicate injections of antigen. (Inset) Sera from rabbits injected with the 174- (■, □) and 74-kdalton (●, ○) DSNF-I polypeptides were diluted in preimmune serum as indicated and used to probe pieces of nitrocellulose to which either DSNF-I (■, ●) or RNPCL (□, ○) had been adsorbed. Spots were localized as in A and quantitated as in B.
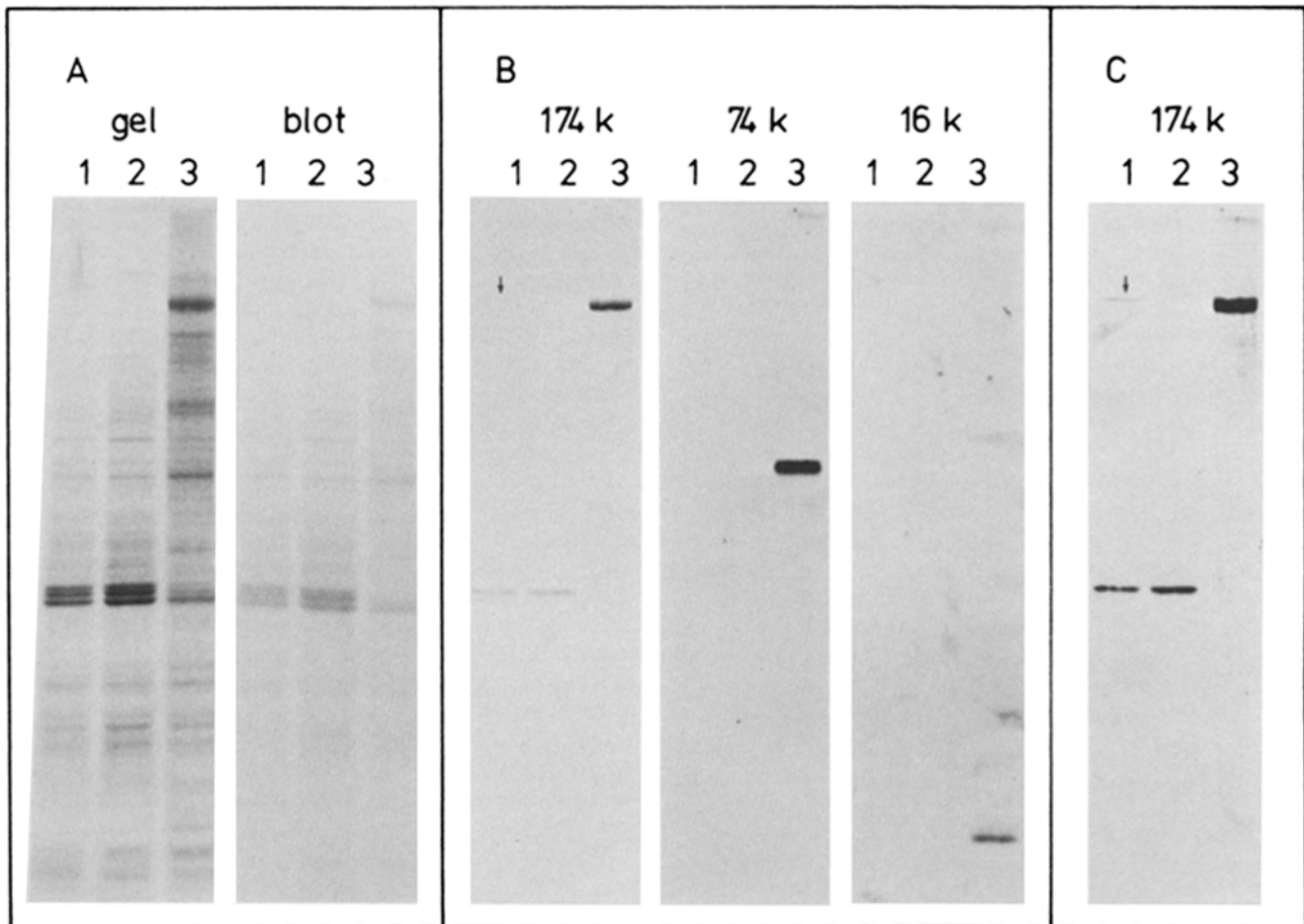

A   gel   blot
1 2 3   1 2 3

B   174 k   74 k   16 k
1 2 3   1 2 3   1 2 3

C   174 k
1 2 3

FIGURE 5 Immunochemical characterization of Drosophila subcellular fractions. FCH, PNS, and DSNF-I were prepared, electrophoresed, and blot-transferred to nitrocellulose as described (Materials and Methods). A total of four blots were prepared from four parallel segments of gel; a fifth segment of gel was not blotted but was stained with Coomassie Blue. In all panels, lane 1 was loaded with 50 μg (0.70 U) of FCH, lane 2 with 50 μg (0.72 U) of PNS, and lane 3 with 50 μg (30 U) of DSNF-I. (A) Coomassie Blue–stained gel and parallel amido black–stained blot, both as indicated. (B) Each of three parallel blots was probed (Materials and Methods) with antiserum raised against the 174- (bleed of day 23), 74- (day 23), or 16-kdalton (day 56) DSNF-I polypeptides; 0.4 ml of serum was used for each. Fluorography was done for 2 h (174 k), 6 h (74 k), or 16 h (16 k) on Kodak XRP film. The arrow on the 174-kdalton blot indicates the position of a faint 174-kdalton band present in the FCH. (C) Long exposure (16 h, Kodak XRP film) of the 174-kdalton blot shown in B. The 174-kdalton band in the FCH is indicated as in B.

massie Blue and an amido black–stained nitrocellulose blot obtained from a parallel strip of gel. Within the limits of detection of the two staining techniques, it can be seen that all of the bands on the Coomassie Blue–stained gel are similarly identifiable on the blot.

Four additional blots, made in parallel with the one shown in Fig. 6A, were challenged with our available antisera under conditions of relative antibody excess; the results are as shown in Fig. 6B and as follows. Consistent with the low level of cross-reactivity between the anti-DSNF-I polypeptide antisera and the total rat liver nuclear antigen as shown in the *inset* in Fig. 4B, there was little or no reactivity of these antisera with any of the individual RNPCL polypeptides displayed on the blot (data not shown). In contrast, the blot shown in Fig. 6B
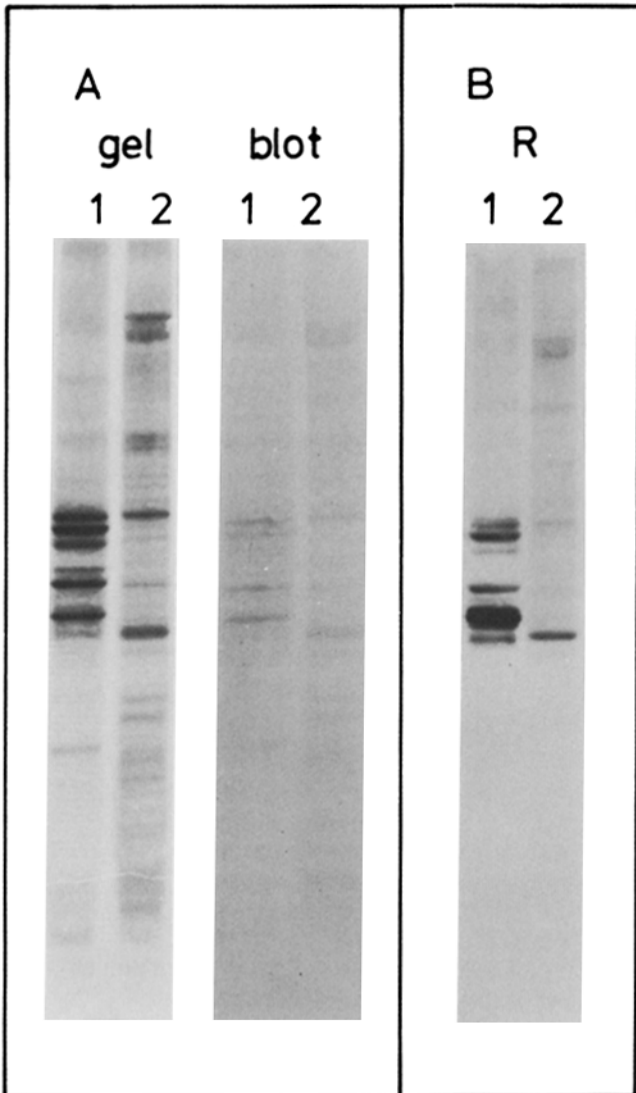

FIGURE 6 Immunochemical comparison of DSNF-I with RNPCL. DSNF-I and RNPCL were prepared, electrophoresed, and blot-transferred to nitrocellulose as described (Materials and Methods). An additional segment of gel was not blotted but was stained with Coomassie Blue. In both panels, the lanes labeled 1 were loaded with RNPCL (430 U) and the lanes labeled 2 were loaded with DSNF-I (36 U). *(A)* Coomassie Blue–stained gel and parallel amido black–stained blot, both as indicated. *(B)* The blot was probed with antiserum raised against the entire RNPCL fraction (bleed of day 23). Specific procedures were as described in Materials and Methods; 1 ml of serum used. Fluorography was done for 3 h on Kodak XRP film.

was probed, as indicated, with an antiserum raised against the entire RNPCL fraction; as above, experiments were performed in antibody excess with respect to the homologous RNPCL antigens. It can be seen from the blot that most of the polypeptides in the RNPCL fraction have been labeled using this antiserum and, furthermore, that this antiserum also contains antibodies that cross-react at a low level with the majority of the DSNF-I polypeptides. Thus, despite the evolutionary distance and the overt morphologic differences between DSNF-I and the RNPCL fraction, the results in Fig. 6B clearly suggest some significant degree of broad immunochemical homology between the two fractions.

Identification of Glycoproteins in DSNF-I, RNPCL, and Subnuclear Fractions from Guinea Pigs, Opossums, and Chickens

It had previously been shown by PAS staining (20; Gerace and Blobel, unpublished observation) that the major high molecular weight polypeptide seen in the RNPCL fraction (10) was a glycoprotein. To investigate the glycoprotein content of our *Drosophila* subnuclear fractions, we transferred the DSNF-I and RNPCL polypeptides to sheets of nitrocellulose and probed these blots with ^{14}C-labeled Con A (see Materials and Methods). The results of this analysis are shown in Fig. 7A. In both DSNF-I (lane *1*) and RNPCL (lane *3*), major species are identified using this technique, the most prominent of which in DSNF-I is exactly coincident with the 174-kdalton polypeptide seen by Coomassie Blue staining of parallel gel lanes and amido black staining of blots (not shown). Further, this 174-kdalton glycoprotein is virtually coincident with the previously identified RNPCL glycoprotein. Lanes *2* and *4* of Fig. 7A demonstrate the results of an identical experiment performed in the presence of 0.5 M α-methyl mannoside. As can be seen, essentially all binding of labeled Con A to the nitrocellulose blot is blocked by the presence of the sugar. (Note that, for the experiments shown in Fig. 7A, ~10 times as much rat liver material (based on original OD_{260} equivalents) was used relative to DSNF-I.) In addition to the data presented in Fig. 7A, the existence of a 174-kdalton glycoprotein in DSNF-I has been demonstrated by specificity of binding of in vitro ^{14}C-labeled (40) DSNF-I to columns of Con A–Sepharose and by *in situ* probe of Coomassie Blue–stained SDS polyacrylamide gels with ^{14}C-labeled Con A essentially according to Burridge (49) (not shown). Both approaches give results which corroborate the conclusions drawn from Fig. 7A.

Using the same technique of Con A labeling, we were able to examine nuclear material from several additional organisms. The results of these experiments are shown in Fig. 7B. As can be seen, the nuclear envelope fractions from the several organisms examined all showed a major glycoprotein similar or identical in mobility to the 174-kdalton glycoprotein of DSNF-I. As in Fig. 7A, odd-numbered lanes show results obtained in the absence of α-methyl mannoside, and even-numbered lanes are from experiments in which the sugar was included at 0.5 M.[1]

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[1] Although in DSNF-I, the major 174-kdalton Coomassie Blue– (and amido black–)stainable band is exactly coincident in mobility with the Con A binding polypeptide identified on nitrocellulose blots, the possibility that this reflects fortuitous comigration of two (or more) different species must be considered. At present, preliminary results of two-dimensional polypeptide analyses of DSNF-I suggest that such is in fact the case; thus a relatively minor component of the 174-kdalton SDS gel band is apparently responsible for the bulk or all of the ^{14}C-
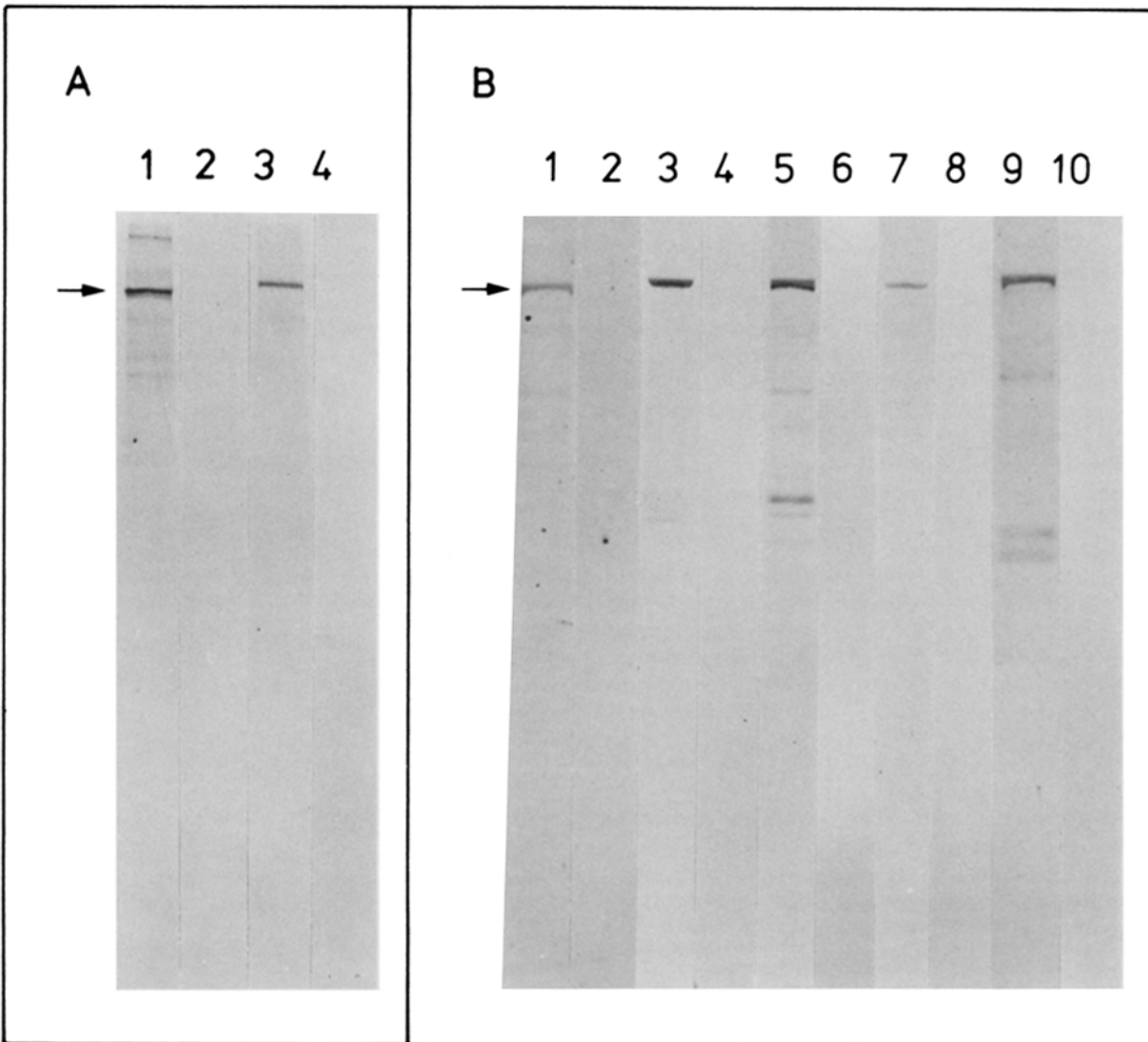
FIGURE 7 Con A affinity labeling of subnuclear fractions. For purposes of comparison, units (U) of subnuclear fractions from all tissue sources are defined as the amount of material derived from 0.06 OD$_{260}$ unit of the respective SDS-lysed nuclei; for *Drosophila* subnuclear fractions, the unit so defined is equivalent to the volumetric unit defined in the legend of Fig. 3. *(A)* DSNF-I, 40 U per lane, and RNPCL, 400 U per lane, were prepared, electrophoresed, blot-transferred to nitrocellulose, and probed with ^{14}C-labeled Con A as described (Materials and Methods). Lanes *1* and *2*, DSNF-I; lanes *3* and *4*, RNPCL. Lanes *2* and *4* were probed in the presence of 0.5 M α-methyl mannoside; lanes *1* and *3* were probed in its absence. Fluorography was done for 110 h on Kodak XRP film. The position of the 174-kdalton DSNF-I polypeptide on the amido black–stained blot is indicated by the arrow on the left. *(B)* The DN fractions from *Drosophila* (lanes *1* and *2*), chicken liver (lanes *3* and *4*), opossum liver (lanes *5* and *6*), rat liver (lanes *7* and *8*), and guinea pig liver (lanes *9* and *10*) were prepared, electrophoresed (30 U of each fraction per lane), blot-transferred, and probed with ^{14}C-labeled Con A. Lanes *2, 4, 6, 8*, and *10*, 0.5 M α-methyl mannoside present; lanes *1, 3, 5, 7*, and *9*, no α-methyl mannoside present. Fluorography was done for 22 h on Kodak XAR film. Arrow on the left indicates the position of the 174-kdalton DSNF-I polypeptide.

Localization of DSNF-I Antigens by Indirect Immunofluorescence

The three anti-DSNF-I antisera characterized above and a fourth antiserum raised against the entire DSNF-I material were used to attempt *in situ* localization of their respective antigens; squashes of developmentally heterogeneous mixtures

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labeled Con A binding observed after a single dimension of electrophoresis. Antibody recognition, on the other hand, appears to involve the major Coomassie Blue–stainable species exclusively. The relevance of these findings to the high molecular weight polypeptides of nuclear envelope fractions derived from other organisms remains to be determined.

of *Drosophila* embryos as well as several tissues obtained from third-instar larvae were challenged. The results of these experiments are as described below and as shown in Fig. 8. When antisera raised against the 174- and 16-kdalton antigens were used, essentially negative results were obtained with all cell types challenged (data not shown). Specifically, there was no evidence of either perinuclear or intranuclear staining, and polytene chromosomes were not labeled. (Weak cytoplasmic staining was observed with the anti-174-kdalton antiserum and is perhaps attributable to the soluble 42-kdalton polypeptide found to cross-react with this serum [see Fig. 5].)

In contrast to the above, when the same tissues were challenged with highly diluted (1:200) anti-74-kdalton antiserum, the results shown in Fig. 8 were obtained. As can be seen, for
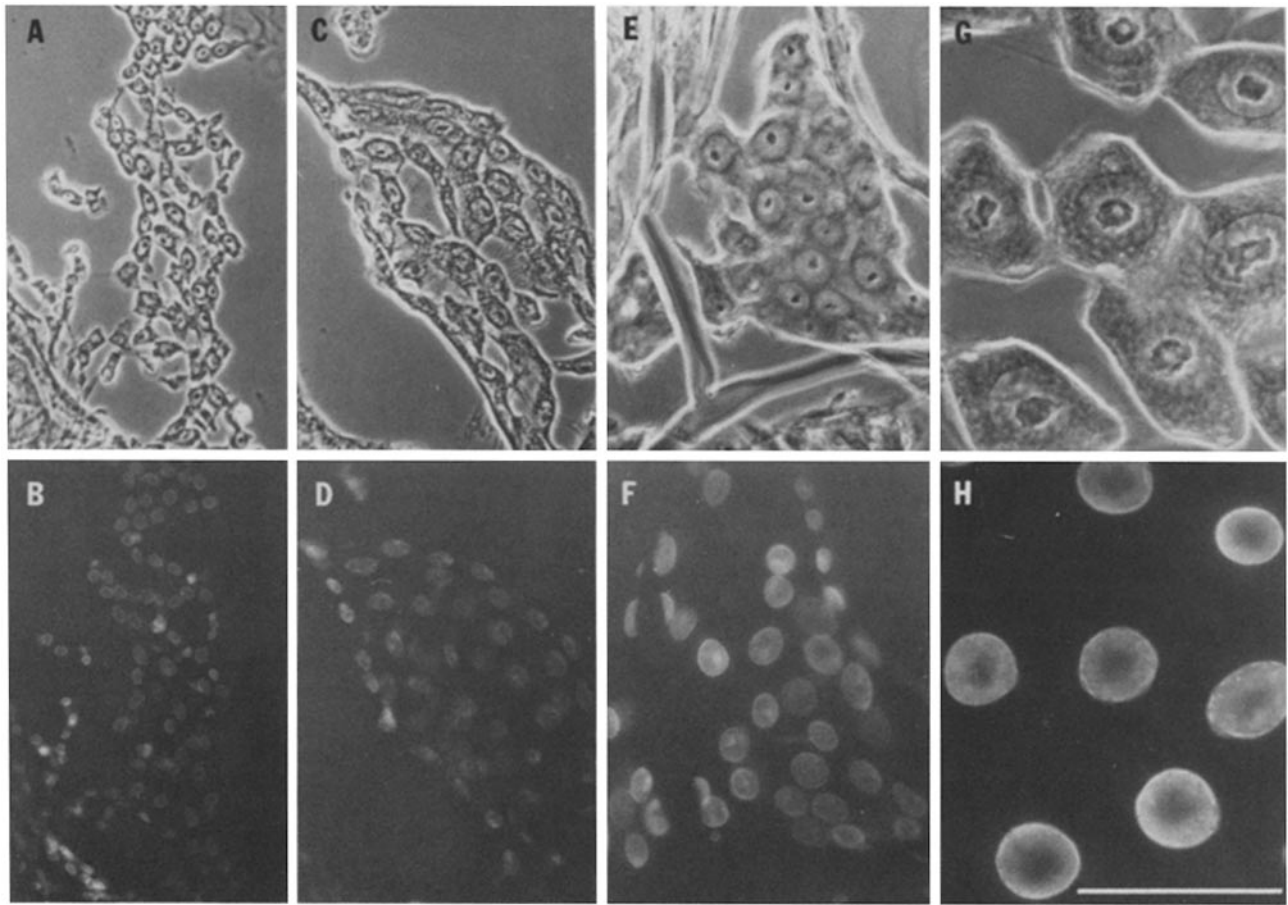
FIGURE 8   Indirect immunofluorescence localization of the 74-kdalton DSNF-I antigen. Specimens were obtained from either *Drosophila* embryos (*A–D*) or early third-instar larvae (*E–H*). *E* and *F* show material anatomically identified as neural ganglion cells; *G* and *H* show salivary gland cells. Both phase-contrast (upper panels) and fluorescence (lower panels) micrographs are shown. The anti-74-kdalton antiserum used was from the bleed of day 43 (see Fig. 4) and was diluted 1:200 before use. Bar, 25 μm. × 1,100.

all cell and tissue types examined, there is an intense and qualitatively similar pattern of nuclear fluorescence observed, with little or no cytoplasmic staining. Further, although a relatively more intensely stained nuclear rim is demonstrable (consistent with peripheral localization of antigen), there also appear to be areas of increased fluorescence intranuclearly. Finally, as with the anti-174-kdalton and anti-16-kdalton antisera, the polytene chromosomes of the larval salivary gland are not recognized by the anti-74-kdalton antibodies, either *in situ* (Fig. 8 *G* and *H*) or in isolated chromosomal spreads (data not shown).

When identical immunofluorescence experiments were performed with a high-titer antiserum raised against the entire DSNF-I material, results qualitatively similar to those shown in Fig. 8 were obtained (data not shown). Specifically, intense nuclear fluorescence was easily demonstrated and, despite the fact that this antiserum recognized virtually every DSNF-I polypeptide identifiable on SDS polyacrylamide gels, there was no detectable staining of larval polytene chromosomes.

DISCUSSION

We have presented results detailing a rapid and efficient method for the purification of nuclei from embryos of *Drosophila melanogaster* and have further described procedures for the subfractionation of these nuclei to generate material morphologically similar to the nuclear matrix preparations ob-

tained from a variety of eucaryotic tissues (21–33). While the isolation of the nuclear matrix is by no means a novel accomplishment, there are only a few reports in the current literature which describe the preparation of this subcellular fraction from invertebrate sources; to date, all of those papers have dealt exclusively with lower eucaryotes (24, 27, 30, 31). Thus, to our knowledge, the present work on *Drosophila* constitutes the first definitive demonstration of an isolable nuclear matrix in a higher eucaryotic invertebrate.

The choice of *Drosophila melanogaster* as an organism with which to pursue the detailed molecular investigation of nuclear structure and function seems, for a variety of reasons, a particularly suitable one. As described in Results, the procedures for subcellular fractionation of *Drosophila* embryos and, specifically, for the generation of a nuclear matrix preparation in high yield are both simple and rapid. Starting with 50 ml of frozen embryos, it is possible to prepare >80 mg of DSNF-I, a fraction composed of nuclear matrix, peripheral lamina, and pore complexes, in as little as 4 h. Quite unlike its mammalian counterparts (see, e.g., 25, 32, 50), the *Drosophila* nuclear matrix so obtained is remarkably stable and can withstand a variety of mechanical stresses; resuspensions of DSNF-I pellets by Dounce homogenization or gentle vortexing have been performed routinely without any obvious ill effects on the morphology of the matrix fraction. Further, it has recently been demonstrated that digestion of rat liver nuclei with RNase before high-salt extraction or subcellular fractionation of rat

liver in the presence of NEM results in a dramatic destabilization of nuclear matrix structure (50); both of these chemical manipulations are part of our standard fractionation of *Drosophila* embryos and, in contrast, appear to have no adverse effect on the integrity of the DSNF-I matrix. Although we have no data directly pertinent to an understanding of this unusual resilience, from a biochemical standpoint, the structural stability of DSNF-I should greatly facilitate further, more detailed investigations.

Another of the more immediate advantages of working with *Drosophila* as a tissue source has been the relative ease with which large quantities of highly specific rabbit antisera have been obtained. Thus, we have been able to raise high-titer antibodies to polypeptide species throughout the common molecular weight range (16,000–174,000) and, similarly, we have been able to elicit responses to relatively minor as well as major polypeptides. Most recently, by simply injecting the entire DSNF-I into a single animal, we have been able to obtain an antiserum that recognizes virtually every DSNF-I polypeptide identifiable on SDS polyacrylamide gels (data not shown). From this library of immunoglobulins, it should be relatively straightforward to selectively immunoadsorb out components specific for any of the several antigens of interest in DSNF-I.

Using the above antisera, it has been possible to demonstrate that by the criterion of cell fractionation, the 174-, 74-, and 16-kdalton polypeptides are all predominantly nuclear proteins (Fig. 5), thus providing important confirmation of morphological and biochemical data regarding the effectiveness of our preparative procedures. Further, the relatively high degree of specificity exhibited by our unfractionated antisera argues against the occurence of significant proteolytic degradation during the course of our purification. This point is particularly important in light of the problems with proteolysis that have plagued previous studies of vertebrate nuclear structure (17, 51; Gerace and Blobel, unpublished observations).

The nuclear matrix fraction from *Drosophila melanogaster* embryos appears to contain only a single major polypeptide (74-kdalton) in the size range of the three predominant polypeptides found in most vertebrate pore complex–lamina and matrix preparations. Further, relative to comparable preparations from a variety of vertebrate species, DSNF-I contains a substantially greater amount of a high molecular weight polypeptide migrating on our gels with an apparent molecular weight of 174,000. Although the significance of these qualitative and quantitative differences in polypeptide composition is at present uncertain, it should be noted that the predominance of these two specific DSNF-I polypeptides (74- and 174-kdalton) is strikingly similar to the SDS gel pattern observed upon analysis of a pore complex–lamina preparation obtained from the oocyte (but not the liver) of the vertebrate *Xenopus*. The possible developmental implications of this apparent similarity between embryos of *Drosophila melanogaster* and *Xenopus* oocytes suggests a potentially fruitful area of future investigation that may readily be pursued in the *Drosophila* system.

One clear finding that has emerged from the present work on *Drosophila* concerns the positive identification of a high molecular weight glycoprotein as a common component of DSNF-I, RNPCL, and nuclear envelope preparations from guinea pigs, opossums, and chickens. While there is as yet no specific information regarding the precise biological function of this ubiquitous high molecular weight polypeptide, the fact that it is a glycoprotein suggests an interaction with the inner nuclear membrane. Immunocytochemical studies will be required in order to address this question directly.

As alluded to above, crucial questions regarding the in vivo significance of the operationally defined nuclear matrix polypeptides may initially be approached most directly by the use of a number of immunocytochemical techniques. In this regard, our initial results of indirect immunofluorescence staining are particularly encouraging. We have been able to demonstrate predominantly or exclusively nuclear localization of the 74-kdalton antigen in a wide variety of histologically and developmentally distinguishable *Drosophila* cell types. Further, the pattern of fluorescence observed, while clearly consistent with peripheral localization of antigen, appears to suggest an intranuclear distribution as well. Staining of sections through *Drosophila* nuclei will be required to rigorously confirm these preliminary conclusions and, in the event that such studies are confirmatory, fractionation of the antiserum by immunoadsorption to two-dimensionally purified antigen polypeptides will be necessary to establish the precise molecular components involved. Such studies should be feasible, given currently available technology.

In light of the essentially negative immunofluorescence results obtained with antisera directed against both the 174- and 16-kdalton antigens, it is impossible to offer any insight regarding their localization in vivo other than that provided by cell fractionation studies (Fig. 5). From a technical standpoint, there are numerous possible explanations for such completely negative immunofluorescence results (see, e.g., 52, 53), and these are currently under investigation. For similarly technical reasons, the nonreactivity of all of our antisera with the larval polytene chromosomes must be interpreted with caution. However, given the demonstrable accessibility of polytene chromosomes to immunofluorescence staining reagents in specimens prepared in the same way as those used for the present study (48, 52) and the relative ease with which antibodies to *Drosophila* chromosomal proteins have been obtained in the past (see, e.g., 48, 53), it seems unlikely that such a generally negative result would have been observed if *Drosophila* chromosomal proteins were a significant contaminant of DSNF-I. As with our attempts to localize the 174- and 16-kdalton antigens, we are currently examining the effects of various technical modifications on our inability to detect operationally defined matrix polypeptides associated with the *Drosophila* chromosomes.

Finally, it should be noted that other workers, using monoclonal antibodies, have recently identified an 80-kdalton polypeptide in extracts of *Drosophila* nuclei obtained from Kc tissue culture cells which appears to localize in the same way as the 74-kdalton DSNF-I antigen (52). Although the biochemical significance of this result remains to be determined, it clearly demonstrates the potential applicability of monoclonal antibodies to problems of nuclear structure; it seems likely that, in the future, monoclonal antibodies will be a valuable and perhaps necessary adjunct to studies performed primarily with polyclonal reagents.

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