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Characterization of monoclonal antibodies against *Chlamydomonas* flagellar dyneins by high-resolution protein blotting

(axoneme/cilia/immunoblot/motility/nitrocellulose)

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ABSTRACT **Monoclonal antibodies that recognize individual polypeptides of the outer arm dyneins of *Chlamydomonas* flagella were obtained and used to study the structural relationships between the various polypeptides. Immunoblot analysis showed that the γ heavy chain of 12S dynein and the α and β heavy chains and M_r 69,000 intermediate chain of 18S dynein each contain immunoreactive sites not found in the other dynein chains or in any other axonemal protein. We also used these antibodies to investigate possible structural similarities between dynein polypeptides from *Chlamydomonas* and phylogenetically distant species. No crossreactivity was observed with antibodies against either the α, β, or γ heavy chains, demonstrating that each *Chlamydomonas* heavy chain has structural features distinct from those present in dyneins from the other species tested. However, one antibody against the M_r 69,000 polypeptide recognized an intermediate chain (M_r 76,000) of latent-activity dynein-1 from the sea urchin *Tripneustes gratilla*. This result provides further evidence that 18S dynein and latent-activity dynein-1 are related. In the course of the above studies, we modified existing procedures to achieve efficient transfer of high molecular weight proteins from NaDodSO$_4$/polyacrylamide gels to nitrocellulose sheets, and to detect small quantities of protein on nitrocellulose. Our modified procedure for staining total protein on nitrocellulose is rapid, inexpensive, and as sensitive as silver-staining of polyacrylamide gels. These methods should be useful to investigators working with small amounts of protein or requiring resolution of closely migrating polypeptides after transfer to nitrocellulose.**

Dynein is the name given to a class of ATPases associated with the arms of the outer-doublet microtubules of cilia and flagella. The outer arm of the green alga *Chlamydomonas* contains two distinct dyneins, termed 12S and 18S on the basis of their sedimentation coefficients, that have been highly purified and extensively characterized (1, 2). The 12S dynein is composed of a single heavy chain (γ chain) of $M_r \approx$ 330,000 and two smaller polypeptides of M_r 22,000 and 18,000; 18S dynein is a heteropolymer composed of α and β heavy chains ($M_r \approx$ 340,000), two intermediate chains (M_r 78,000 and 69,000), and eight light chains (M_r 7800–19,600). The α and β chains of 18S dynein and the γ chain of 12S dynein each contain a site of ATP binding and hydrolysis (3, 4).

To learn more about the structural relationships of the dynein polypeptides, we have generated a series of monoclonal antibodies specific for individual polypeptides of the *Chlamydomonas* 12S and 18S dyneins. Antibodies specific for the α, β, or γ heavy chains or the M_r 69,000 intermediate chain do not recognize other dynein chains or any other axonemal polypeptides. Thus, each of these dynein chains has unique structural properties that distinguish it from other chains. Because of the specificity of these antibodies, they should be very useful for localization of the individual polypeptides within the outer arm (5). In addition, one of our monoclonal antibodies specific for the M_r 69,000 intermediate chain of 18S dynein also recognizes an intermediate chain (M_r 76,000) of 21S latent-activity dynein-1 (LAD-1) from the sea urchin *Tripneustes gratilla*. This provides further evidence that *Chlamydomonas* 18S dynein and sea urchin 21S dynein are related (4).

The dynein heavy chains migrate very close to one another in NaDodSO$_4$/polyacrylamide gels and are well resolved only at very low protein loadings (2). Consequently, characterization of our antibodies by immunoblot analysis required (*i*) a reliable procedure for efficiently transferring high molecular weight polypeptides from NaDodSO$_4$/polyacrylamide gels to nitrocellulose sheets without loss of resolution and (*ii*) a sensitive method for detecting the transferred proteins on the nitrocellulose. The techniques that we have developed to fulfill these requirements were essential for unambiguous identification of the chains recognized by our antibodies and should be useful to a wide range of investigators needing to electrotransfer high molecular weight proteins or to detect nanogram amounts of protein on nitrocellulose.

MATERIALS AND METHODS

Preparation of Axonemes and Purification of Dynein. The isolation of flagella from *Chlamydomonas reinhardtii* (wild-type strain 1132D$^-$) by the dibucaine method, preparation of axonemes, extraction with 0.6 M KCl, and subsequent purification of 12S and 18S dyneins by sucrose gradient centrifugation and hydroxylapatite column chromatography were as described (2, 4).

Ram sperm tails were prepared from ejaculated sperm by modifications of methods described by Tash and Means (6) and Harrison (7). A pellet containing sperm tails and midpieces was treated with 0.05% Nonidet P-40 (BDH) in 30 mM Hepes, pH 7.5/5 mM MgSO$_4$/1 mM dithiothreitol/0.5 mM EDTA/25 mM KCl/1 mM phenylmethylsulfonyl fluoride to remove the membranes. The demembranated axonemes were then collected by centrifugation at 30,500 × *g* and resuspended in electrophoresis sample buffer.

Axonemes and latent-activity dynein-1 (LAD-1) from *T. gratilla* were the gift of W.-J. Y. Tang and I. Gibbons (Pacific Biomedical Research Center, University of Hawaii, Honolulu); axonemes and purified dynein (22S and 14S) from *Tetrahymena thermophila* were the gift of K. Johnson (Althouse Laboratory, Pennsylvania State University, University Park), and scallop gill cilia were the gift of W. Dentler (McCollum Laboratories, University of Kansas, Lawrence).

Production of Monoclonal Antibodies. Female BALB/c mice were immunized with hydroxylapatite-purified 12S or

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Abbreviations: LAD-1, latent-activity dynein-1; DNP, 2,4-dinitrophenyl; BSA, bovine serum albumin.

18S dynein. Initial subcutaneous injections of 50–250 μg of dynein in complete Freund's adjuvant were followed at 1–2 month intervals by further injections in adjuvant (subcutaneous) or in 0.2–0.3 M phosphate buffer (intraperitoneal). Mice were sacrificed 3–4 days after the last injection.

Our fusion procedure was essentially that described by Galfre and Milstein (8). Splenic lymphocytes and P3.NSI/1-Ag4-1 myeloma cells (9) were mixed at a ratio of 6:1 and fused with 37.5% polyethylene glycol (Koch-Lite 1000)/1.5% dimethyl sulfoxide (Me_2SO) in serum-free RPMI 1640 medium (GIBCO) supplemented with α-D-glucose (Sigma) at 2.5 g/liter. Fusion products were distributed into 24-well Falcon plates (Becton Dickinson Labware, Oxnard, CA) and grown for approximately 2 weeks in selective medium containing 20% fetal bovine serum (HyClone, Sterile Systems, Logan, UT), 2 mM glutamine, insulin (8 μg/ml), oxalacetic acid (132 μg/ml), sodium pyruvate (50 μg/ml), hypoxanthine (13.6 μg/ml), thymidine (19.3 μg/ml), and aminopterin (0.19 μg/ml) in glucose-supplemented RPMI 1640. All culture media routinely included penicillin (50 units/ml) and streptomycin (50 μg/ml) (GIBCO). Culture supernatants were assayed by either radioimmunoassay against native dynein or by the immunoblot procedure described below. Cells in positive wells were cloned by limiting dilution, adapted for growth at lower serum concentrations, and then recloned prior to the selection of a permanent stock culture.

Isotype determinations were made by double immunodiffusion against subtype-specific antisera (Litton Bionetics).

Gel Electrophoresis and Protein Blotting. The component polypeptides of isolated axonemes and purified dynein samples were separated by $NaDodSO_4$/PAGE in 0–2.4 M glycerol, 5–15% acrylamide gradient gels (2) by a modification of the procedure of Laemmli and Favre (10). The dynein heavy chains were resolved in 0–8 M urea, 3–5% acrylamide gradient gels with no $NaDodSO_4$ in either the stacking or separating gels (2). Gels were stained either with Coomassie brilliant blue R-250 (C.I. 42660) or by the silver-stain procedure of Merril *et al.* (11).

Electrophoretic conditions for the transfer of protein to nitrocellulose were modified from Towbin *et al.* (12). The transfer buffer contained 49.6 mM Tris, 384 mM glycine, 20% methanol, and 0.01% $NaDodSO_4$ (Bio-Rad). Transfers were performed in a TE 42 Transphor unit (Hoefer, San Francisco) at 0.4 A and 24–30 V for 1 hr and then at 1.5–2.0 A and 84 V for 18–22 hr.

The protein blots shown here were made using nitrocellulose with a pore size of 0.20 μm (BA-83; Schleicher & Schuell). In preliminary experiments, nitrocellulose of differing pore sizes was tested; BA-83 gave the best overall protein retention. In all experiments, Schleicher & Schuell 470 filter paper was used to cushion the gel/nitrocellulose sandwich; when TE46 paper (Hoefer) was substituted, unacceptably high background staining resulted.

Staining of Nitrocellulose Blots. The dinitrophenyl (DNP) immunostain procedure used for detecting protein on nitrocellulose sheets was extensively modified from that of Wojtkowiak *et al.* (13). In our experience, the times given below are the maximum required to obtain consistently dark bands against an essentially white background. All washes and antibody incubations were carried out at room temperature with gentle agitation.

Upon removal from the transfer apparatus, nitrocellulose sheets were air-dried and then washed for 10 min in two changes of Tris-buffered saline (Tris/NaCl: 10 mM Tris Cl, pH 7.25/0.15 M NaCl). Drying the nitrocellulose prior to immersion in Tris/NaCl was essential to minimize loss of protein from the sheet. After equilibration with 50 mM $NaHCO_3$ in 50% Me_2SO (two 5-min washes), protein was derivatized with 0.02–0.1% 1-fluoro-2,4-dinitrobenzene (Sigma) in $NaHCO_3/Me_2SO$ for 10 min. After further washes with $NaHCO_3/Me_2SO$ (three times for 3 min) and Tris/NaCl (twice for 5 min), the nitrocellulose was blocked with 1% horse serum* (HS; GIBCO) in Tris/NaCl for 20 min with two changes. Dinitrophenylated protein was subsequently detected by incubating the sheet for 1 hr with a 1:400 dilution in HS/Tris/NaCl of a rabbit antiserum raised against DNP-derivatized bovine serum albumin (DNP-BSA; Miles). After five 5-min washes with HS/Tris/NaCl, the sheet was incubated for 1 hr with peroxidase-conjugated sheep anti-rabbit IgG (Cappel Laboratories, Malvern, PA) at a dilution of 1:3000 in HS/Tris/NaCl. Following further washing with HS/Tris/NaCl (twice for 10 min), 0.5% Triton X-100/Tris/NaCl (once for 20 min), and 50 mM Tris Cl (pH 6.8) (three times for 3 min), the sheet was incubated with 20 ml of 0.3% 4-chloro-1-naphthol in methanol (made immediately prior to use), 100 μl of 30% H_2O_2 (Sigma), and 80 ml of Tris buffer for 5–10 min to develop a purple reaction product. The reaction was stopped by rinsing the sheet several times with distilled water.

Immediately after development, blots were photographed through a yellow filter using 35-mm Technical Pan film (Kodak 2415), which was developed with Kodak HC-110 (dilution B, 6 min).

To screen clones and characterize the monoclonal antibodies produced, air-dried nitrocellulose blots were blocked by two 10-min washes with HS/Tris/NaCl, and a thin strip was incubated with each hybridoma culture supernatant for 1 hr. After four washes with HS/Tris/NaCl, strips were incubated with peroxidase-conjugated goat anti-mouse IgG (Cappel Laboratories) at a dilution of 1:3000 for 1 hr. The final washes, color development, and photography were as described above for the DNP immunostain.

Some protein blots were stained with 0.1% amido black 10B (naphthol blue black, C.I. 20470; Sigma) in 45% methanol/2% acetic acid. The nitrocellulose was then destained with four changes of the methanol/acetic acid mixture, followed by a single wash with distilled water before a final wash with methanol/acetic acid.

RESULTS

Protein Blotting and Staining Sensitivity. Silver staining of polyacrylamide gels that had been subjected to electrophoretic transfer by the method described above showed that virtually all proteins, including high molecular weight proteins ($M_r > 300,000$), were removed from the gel. When the resulting blots were stained for total protein, the pattern of transferred proteins closely resembled that of silver-stained gels that had not been subjected to the transfer conditions (see Fig. 1). In contrast, gels from which proteins were transferred by the method of Towbin *et al.* (12) retained a considerable amount of protein (results not shown), and the corresponding blots were dramatically deficient in the high molecular weight proteins. Methanol in the transfer buffer retarded the elution of protein from the gel but was essential to retain eluted proteins on the nitrocellulose sheet. Therefore, to achieve efficient transfer, the buffer of Towbin *et al.* (12) was modified by adding 0.01% $NaDodSO_4$ and doubling the ionic strength to increase its conductivity.

A comparison of four techniques for detecting protein in polyacrylamide gels and on nitrocellulose blots is shown in Fig. 1. When lightly loaded gels or blots were stained with Coomassie brilliant blue or amido black, respectively, only the major axonemal components (tubulin and the dynein

*Horse serum instead of BSA was used in the blocking and wash buffers because (*i*) the primary antiserum, raised against DNP-derivatized BSA, might include contaminating anti-BSA antibodies that would contribute to background staining and (*ii*) horse serum is considerably less expensive than BSA.

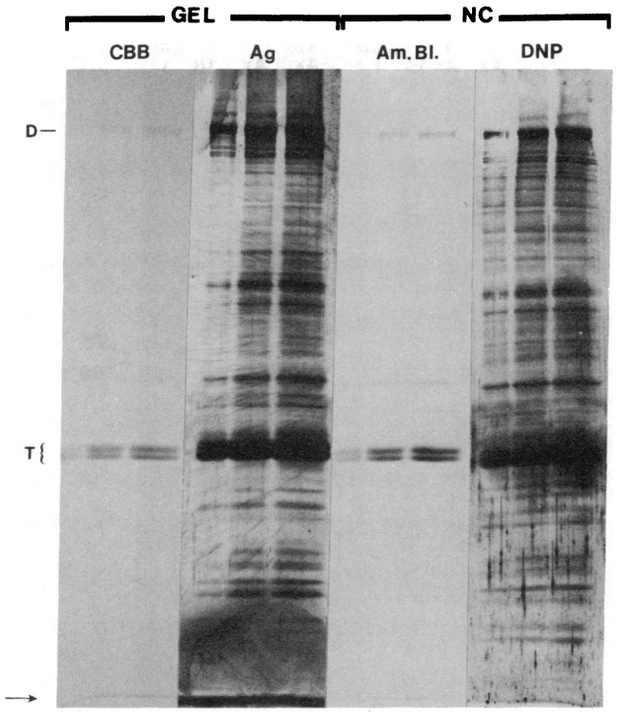

FIG. 1. Comparison of methods for staining polyacrylamide gels and nitrocellulose blots. Four sets of three lanes were loaded (from left to right) with 3, 10, and 17 μg of axonemal protein. The left half of the figure shows the 0–2.4 M glycerol, 5–15% acrylamide gradient gel stained with Coomassie brilliant blue (CBB) or silver (Ag). The two remaining panels show equivalent sets of lanes after transfer to nitrocellulose (NC) and staining with either amido black (Am.Bl.) or the DNP immunostain (DNP). The positions of the dynein heavy chains (D), tubulin (T), and the dye front (arrow) are indicated at left. The sensitivity of the DNP immunostain for minor bands on nitrocellulose is approximately equal to that of the silver stain for gels and considerably greater than amido black or Coomassie blue.

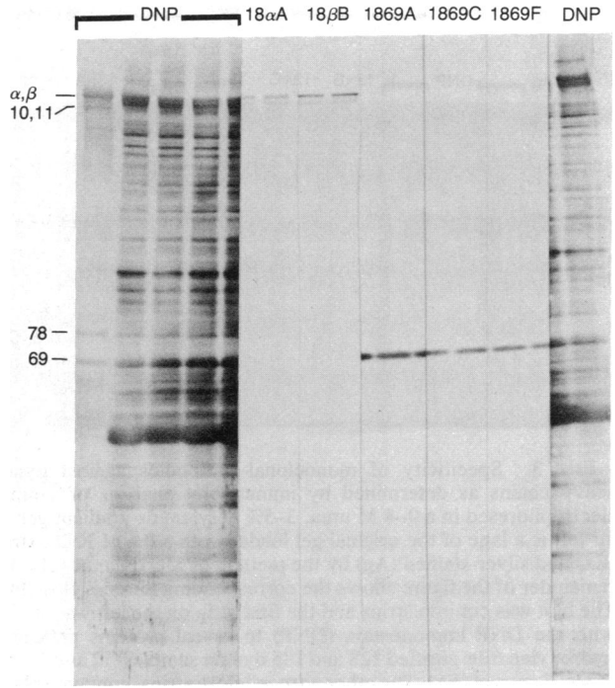

FIG. 2. Characterization of monoclonal antibodies against polypeptides of 18S dynein by immunoblot analysis of *Chlamydomonas* whole axonemal protein separated in a 0–2.4 M glycerol, 5–15% acrylamide gradient gel and transferred to nitrocellulose. The far left lane was loaded with hydroxylapatite-purified 18S dynein (≈2 μg). All remaining lanes were loaded with equal amounts (≈10 μg of protein) of purified axonemes. The blot was cut into strips and the first and last strips were stained with the DNP immunostain (DNP). The remaining strips each were incubated with the indicated antibody followed by peroxidase-conjugated goat anti-mouse IgG and color development with 4-chloro-1-naphthol. Antibodies against the M_r 69,000 intermediate chain did not crossreact with any other polypeptides. The α and β heavy chains are not resolved in this gel system, but antibodies against these chains clearly do not crossreact with intermediate or low molecular weight polypeptides. Band 10,11 consists of proteolytic products of the β and α chains (see text). By staining equivalently loaded lanes of the same nitrocellulose sheet for total protein or monoclonal antibody reactivity and then reassembling the sheet, it was possible to determine precisely which polypeptide reacted with the monoclonal antibody, despite small variations in the positions of bands across the width of the blot. Comparison of DNP immunostained and monoclonal antibody stained bands was further facilitated by cutting the lanes longitudinally in half and then staining one half for total protein and the other half for monoclonal antibody reactivity, as in lanes 5 and 13 from the left.

heavy chains) were revealed. Because the silver method for staining gels is 10- to 100-fold more sensitive than Coomassie blue (11), numerous minor axonemal polypeptides were visible in the silver-stained gel. The DNP immunostain for blots is comparable in sensitivity to the silver method for polyacrylamide gels and under some conditions can be much more sensitive (see Fig. 3).

Anti-Dynein Monoclonal Antibodies. Our monoclonal antibodies against components of 12S and 18S dyneins were derived from three separate fusions of mouse splenic lymphocytes with P3.NSI/1-Ag4-1 myeloma cells. In the first fusion, screening was performed using a solid-phase RIA with either KCl extract or purified 18S dynein as antigen. This screening procedure selected for clones secreting antibodies that reacted with native dynein. However, subsequent analysis revealed that many of these antibodies either did not recognize $NaDodSO_4$-denatured protein on nitrocellulose blots or they reacted with multiple axonemal polypeptides. In the two subsequent fusions, culture supernatants were screened directly by the immunoblot procedure. All antibodies discussed here are of the IgG1 isotype, except for 18βB† which is an IgG2a.

The immunoblot characterization of five monoclonal antibodies directed against 18S dynein is shown in Fig. 2. Whole axonemal protein was separated in a 5–15% acrylamide linear gradient gel; subsequent transfer to nitrocellulose and immunostaining revealed that antibodies 1869A, 1869C, and 1869F reacted specifically with the M_r 69,000 chain and antibodies 18αA and 18βB reacted with the heavy chains of 18S dynein. The antibodies against the M_r 69,000 chain did not crossreact with any other protein in the axoneme, and those against the heavy chains did not react with any low or intermediate molecular weight polypeptides.

Because the above gel system did not resolve the α and β chains of 18S dynein, more detailed analysis of antibodies against these chains and the γ chain of 12S dynein was performed after separating these polypeptides on a 0–8 M urea, 3–5% acrylamide gradient gel (Fig. 3). Comparison of the silver-stained gel and DNP-stained blot demonstrated that the closely spaced dynein bands remained resolved after transfer and that the two staining procedures have similar sensitivities for the detection of minor bands. The antibodies 12γB, 12γC, 18αA, 18βA, and 18βB were highly specific for their designated heavy chain; the epitope that each antibody

†The monoclonal antibodies described here are designated by a 12 or 18 (the dynein recognized), followed by α, β, γ, or 69 (the chain recognized) and finally a letter (A, B, etc.) to distinguish antibodies recognizing the same chain.

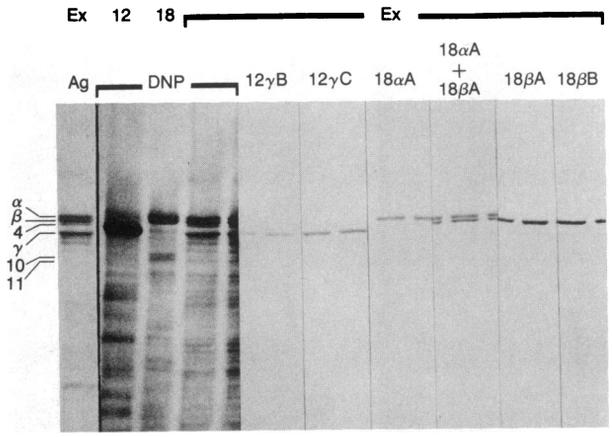

FIG. 3. Specificity of monoclonal antibodies against dynein heavy chains as determined by immunoblot analysis of samples electrophoresed in a 0–8 M urea, 3–5% acrylamide gradient gel. At far left is a lane of the original gel loaded with a 0.6 M KCl extract (Ex) and silver-stained (Ag) by the method of Merril *et al.* (11). The remainder of the figure shows the corresponding nitrocellulose blot. The blot was cut into strips and the first strip on the left was stained with the DNP immunostain (DNP) to reveal proteins present in hydroxylapatite-purified 12S and 18S dynein samples (12 and 18) and the KCl extract (Ex). The other nitrocellulose strips, containing lanes with identical samples of KCl extract, were incubated with the designated monoclonal antibody. The fifth strip of the blot was incubated with both 18αA and 18βA to confirm that each antibody recognized a different polypeptide. As also shown in Fig. 2, cutting blotted lanes longitudinally in half and either staining one half for total protein and the other for monoclonal antibody reactivity (lane 4 of blot) or staining each half with a different monoclonal antibody (lanes 9 and 11) and then reassembling the nitrocellulose sheet made it possible to identify unequivocally the reactive bands. Such precision was crucial for determining which of the closely migrating heavy chains was immunoreactive. Bands 10 and 11 are proteolytic fragments of the β and α chains, respectively. Band 4 is a component of the inner dynein arm (2).

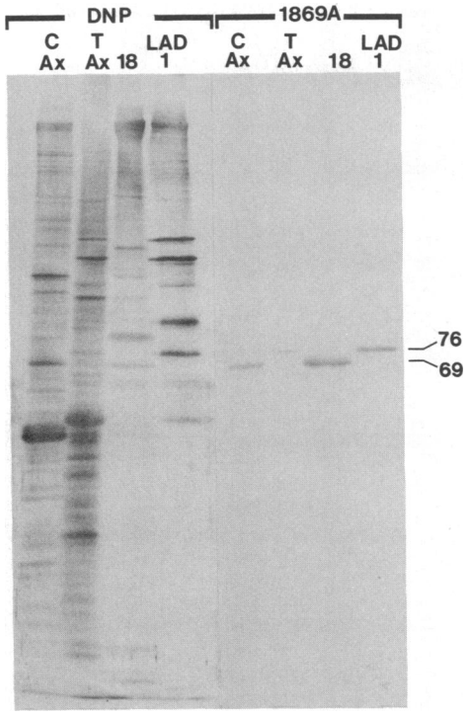

FIG. 4. Nitrocellulose blot of whole axonemes (Ax) from *Chlamydomonas* (C) and *Tripneustes* (T), and purified 18S and LAD-1 dyneins from the same species, respectively. In the first set of lanes protein was revealed with the DNP immunostain. The second set was incubated with antibody 1869A and stained to reveal antigen–antibody interaction. This monoclonal antibody, raised against the M_r 69,000 chain of *Chlamydomonas* 18S dynein, exhibits strong crossreactivity with the M_r 76,000 component of LAD-1.

recognized did not occur in other dynein polypeptides. Occasionally 18αA reacted with a second axonemal band (band 11) which has been shown to be a proteolytic fragment of the α chain (4). Interestingly, neither 18βA nor 18βB recognized the corresponding fragment (band 10) of the β chain.

Six of the antibodies (12γB, 18αA, 18βA, 18βB, 1869A, and 1869F) were used to test for crossreactivity between dyneins of different species by the immunoblot method. Antibody 1869A reacted strongly with the M_r 76,000 component of LAD-1 in both the purified dynein and the isolated axonemes of *Tripneustes* (Fig. 4). Similar blots stained with 1869F showed no reaction with any component of *Tripneustes* axonemes; this antibody therefore reacts with an epitope different from that recognized by 1869A. None of the antibodies described here recognized any components in axonemes prepared from scallop gill cilia or ram sperm or in purified 22S or 14S dyneins from cilia of *Tetrahymena*.

DISCUSSION

Since its introduction five years ago (12), protein blotting has become a widely used technique in cell biology (see reviews in refs. 14 and 15). With the development of sensitive silver-stain procedures for the detection of small amounts of proteins in polyacrylamide gels (11, 16), there arose a need for an equally sensitive method for detecting protein after transfer to nitrocellulose. Recently, several methods for detecting small quantities of protein on nitrocellulose have been reported (13, 17, 18). One of the most sensitive, versatile, and least complex of these procedures is that of Wojtkowiak *et al.* (13), in which primary amino groups are derivatized with dinitrofluorobenzene and the dinitrophenylated proteins are detected with commercially available antisera that react with DNP. We have modified this procedure to make it substantially more rapid, to eliminate background staining, and to lower the cost by a factor of about 20,‡ while retaining its very high sensitivity. Our modification of the DNP immunostain reveals minor components in complex mixtures of protein as sharply defined purple bands against an essentially white background (see for example the minor bands visible in Fig. 3), and its sensitivity is approximately 100-fold greater than that of amido black. In our experiments, we used a peroxidase-coupled secondary antibody; we anticipate that even greater sensitivity would be obtained with biotin-conjugated secondary antibody followed by avidin and biotinylated peroxidase (19) or with colloidal gold-conjugated secondary antibody followed by silver enhancement (20).

Comparison of equivalent nitrocellulose strips stained for total protein and for monoclonal antibody reactivity was necessary to identify conclusively a particular antigen; juxtaposition of the original silver-stained gel with blots stained with monoclonal antibodies was not sufficiently accurate to discriminate with confidence between the closely spaced dynein heavy chains. Furthermore, these polypeptides are well resolved only at very low protein loadings; loading the gel with enough protein to allow their detection with amido black after transfer to nitrocellulose resulted in coalescence of the α-, β-, and γ-chain bands. Consequently, the DNP immunostain was essential for unambiguous identification of the heavy chains recognized by the antibodies. This staining

‡As of March 1, 1985, the cost of staining 100 cm^2 of nitrocellulose was $2.48 by our method and $45.61 by the method of Wojtkowiak *et al.* (13).

procedure has wide applicability and should facilitate resolution on protein blots of any closely migrating polypeptides, including spectrin and fodrin (21), fibronectins (22), microtubule-associated proteins (23), and myosin isozymes (24).

In the present study, we observed crossreactivity between the M_r 69,000 intermediate chain of *Chlamydomonas* 18S dynein and the M_r 76,000 intermediate chain of LAD-1 from *Tripneustes*. The 18S dynein and LAD-1 previously have been shown to have several features in common: they are both heteropolymers composed of two distinct but closely migrating heavy chains and several intermediate and light chains (2, 25). Structural studies on isolated sea urchin LAD-1 (26) and *Chlamydomonas* 18S dynein (27) have revealed that both dyneins have two globular heads connected by a Y-shaped stalk. Biochemical studies have shown that both dyneins can be subfractionated into two distinct subunits, each of which has ATPase activity (4, 28). The fact that one of the intermediate chains of the two dyneins share a common antigenic determinant now lends further support to the hypothesis (4) that 18S dynein and LAD-1 are closely related. That specific sites are conserved over such a large evolutionary distance suggests that they may be of prime importance in the assembly and/or function of the outer dynein arm.

In contrast, our antibodies against the α, β, and γ chains of *Chlamydomonas* dyneins did not recognize any polypeptides in preparations of purified dynein from *Tripneustes* or *Tetrahymena* or in axonemes prepared from scallop gill cilia or ram sperm. All three chains therefore contain immunoreactive sites unique to *Chlamydomonas* dyneins. Further study will be necessary to determine the extent of evolutionary divergence of the heavy chains in different species.

Because each of the antibodies described here is specific for a single dynein polypeptide, they will be useful for determining the location of individual dynein chains within the outer arm. For example, we now have evidence, from the ultrastructural examination of thin-sectioned, immunogold-stained axonemes, that the epitopes recognized by both 12γA and 12γB are located on the external face of the outer arm (5). Further studies using immunoelectron microscopic methods capable of more precise localization should enable us to determine which chains, or regions of the chains, correspond to the different domains observed in the isolated dynein particles (27, 29) and the arms *in situ* (30, 31). The antibodies should also be important in elucidating the roles of the individual polypeptides and ATPases in the function and assembly of the outer arm.

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