Molecular Cloning and Expression of Flagellar Radial Spoke and Dynein Genes of *Chlamydomonas*

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Abstract. Several flagellar dynein ATPase and radial spokehead genes have been isolated from a *Chlamydomonas* genomic expression library in λgt11. The library was probed with polyclonal and monoclonal antibodies raised against purified flagellar polypeptides, and recombinant phage giving positive signals were cloned. In vitro translation of mRNAs hybrid-selected by the cloned sequences from whole cell RNA provided confirmation of identity for three of the four clones. Evidence supporting the identification of the fourth, which encodes a dynein heavy chain, was provided by antibody selection; the fusion protein produced by this clone selected heavy chain–specific antibodies from a complex polyclonal antiserum recognizing many dynein determinants. One of the radial spoke sequences isolated here is of particular interest because it encodes the wild-type allele of a locus which was defined previously by temperature-sensitive paralyzed flagella mutation *pf*-26$_{ts}$ (Huang, B., G. Piperno, Z. Ramanis, and D. J. L. Luck, 1981, *J. Cell Biol.*, 88:80–88). The cloned sequence was used to hybrid-select mRNA from mutant *pf*-26$_{ts}$ cells, and when translated in vitro, the selected mRNA produced a mutant spokehead polypeptide with an altered electrophoretic mobility. This confirms that the *pf*-26$_{ts}$ mutation alters the primary structure of a radial spokehead polypeptide. To quantify spokehead and dynein mRNAs during flagellar regeneration, all of the cloned sequences were used as hybridization probes in RNA dot experiments. Levels increased rapidly and coordinately after deflagellation, peaked 3–10-fold above nondeflagellated controls, and then returned to control values within 2 h. This accumulation pattern was similar to that of flagellar α-tubulin mRNA.

GENETIC analysis of the eucaryotic flagellum has taken place almost exclusively in the biflagellate alga *Chlamydomonas* (for review see reference 20). The flagellar substructures best characterized are the radial spokes and dynein arms. These multi-peptide complexes are attached in longitudinal rows along the outer doublet microtubules of the axoneme. The ATPase-containing dynein arms cross-bridge adjacent outer doublet microtubules and slide them past one another, generating the basic bending force of flagellar motility (40). Radial spokes, which extend inward and interact with the central pair complex at the flagellar axis, regulate the pattern of flagellar bending, producing the asymmetrical ciliary beating stroke (2, 3, 14).

Genetic analysis and biochemical fractionation of the flagellum in *Chlamydomonas* has allowed the identification of radial spoke (13, 28, 29) and dynein arm (12) polypeptides, and provided methods for their partial purification (27, 29). The radial spoke is composed of at least seventeen polypeptides ranging in size from 124 to 20 kD, all of which fail to assemble into the flagella of spokeless mutant *pf*-14 (13). A genetically defined subset of five of these polypeptides form the globular spokehead complex, which is located at the distal end of the radial spoke, directly adjacent to the central microtubule pair. These five radial spoke components are missing from the flagella of spokeheadless mutant *pf*-1. In addition, biochemical evidence indicates a sixth spoke polypeptide may also contribute to the spokehead structure, or may serve as a link between the spokehead and the basal stalklike portion of the radial spoke. This polypeptide is released from the spoke stalk along with the five genetically defined spokehead components when axonemes are dialyzed into a low salt solution (29).

Genetic analysis of the radial spoke has uncovered six distinct loci affecting its assembly and function (13). Paralyzed flagella mutation *pf*-26$_{ts}$ is mapped to one of these loci. *pf*-26$_{ts}$ cells assemble axonemes that have wild-type morphology, but analysis of axonemal polypeptides by two-dimensional gel electrophoresis indicates that the primary structure of one of the five spokehead polypeptides, radial spoke component 6, is altered by the mutation. The wild-type 6 polypeptide is completely absent, and novel polypeptide 6* appears to replace it. This new flagellar component has a lower apparent molecular weight than wild-type 6 protein, and a more basic isoelectric point. These differences are not the result of a secondary modification that is blocked in the mutant cells, as was demonstrated by the co-expression of 6 and 6* in wild-type/*pf*-26$_{ts}$ dikaryons (13).

The dynein outer arm, one of the two types of dynein arms which form distinct rows along each outer doublet microtubule, is composed of at least 14 polypeptides, all of which

are missing from the flagella of dynein outer armless mutants *pf*-13 and *pf*-22 (12). These polypeptides include three heavy chains, α, β, and γ (ranging from 330 to 300 kD), two chains of intermediate molecular weight (86 and 73 kD), and at least nine additional lower molecular weight polypeptides (from 19 to 15 kD) (12, 27). Biochemical evidence supports and extends these results. The dynein outer arm can be removed from the axoneme by high salt extraction and separated into 18S and 12S ATPase-containing particles on sucrose gradients. The 18S particle includes the α- and β-heavy chains, the two intermediate molecular weight polypeptides, and eight low molecular weight polypeptides (27, 30). The 12S particle contains the γ-heavy chain and at least one low molecular weight polypeptide (27, 30).

Four loci affecting the outer dynein arm have been identified (12, 14, 24), but only the locus defined by mutation sup_{pf}-1 has been tentatively linked to a gene product, the β-heavy chain (14). sup_{pf}-1 was isolated as an intergenic suppressor mutation restoring flagellar activity to paralyzed radial spoke or central pair mutants, and correlated with it is an alteration in β chain electrophoretic mobility.

Isolation of genes encoding the dynein β-heavy chain and spoke component 6 would be a first step in the molecular genetic analysis of flagellar mutations affecting these loci. In addition, these genes would be useful molecular probes for the analysis of flagellar gene expression during biogenesis of this organelle; to date, the tubulin genes have been the only identified flagellar sequences available for these studies (23, 39).

To clone radial spoke and dynein genes we have constructed a *Chlamydomonas* genomic expression library in λgt11 and screened it with antibodies raised against radial spoke and dynein polypeptides. We have isolated two radial spoke genes and two dynein genes, including loci that encode radial spoke component 6 and the dynein β heavy chain. The identities of these sequences are confirmed by hybridization selection and antibody selection experiments. In hybridization selection experiments with radial spoke mutant *pf*-26_{ts}, the cloned genomic sequence for wild-type spokehead polypeptide 6 specifically selected an mRNA encoding the mutant 6* polypeptide, thus confirming that the *pf*-26_{ts} mutation alters the primary structure of spokehead component 6. Finally, we have used the cloned genomic fragments as hybridization probes in RNA dot experiments to quantify radial spoke and dynein mRNA abundance changes during flagellar regeneration.

Materials and Methods

Chlamydomonas and Bacterial Strains

Chlamydomonas mutant strains *pf*-1 and *pf*-26_{ts} (both mt+) have been characterized previously and were obtained from the *Chlamydomonas* Genetics Center (Duke University, Durham, NC). Mutant *pf*-28 has been previously described (24). Preparations from wild-type cells used strain 21gr (mt+).

Bacterial strains BNN97, Y1088, and Y1090 were obtained from the American Type Culture Collection (Rockville, MD).

Isolation and Fractionation of Flagella

Procedures were essentially as previously described (24). Dynein, extracted from axonemes with 0.6 M KCl, was dialyzed against a low salt solution and, after centrifugation to remove precipitated nondynein protein, was either layered onto a 0–20% sucrose gradient or frozen at −70°C until prepared for one- or two-dimensional gel electrophoresis. The pelleted high salt extracted axonemes were resuspended and dialyzed against a low salt buffer (29) for 6 h at 4°C to release radial spokehead polypeptides. After dialysis the axonemes were pelleted at 35,000 *g* for 30 min, and the low salt soluble supernatant enriched for spokehead polypeptides was stored at −70°C.

In preparation for two-dimensional gel electrophoresis, the dialyzed crude dynein fraction was diluted directly into two-dimensional gel sample buffer (25). The radial spokehead fraction was prepared by acetone precipitation, resuspension in 1 vol of 0.2% SDS, and then addition of 4 vol of complete two-dimensional gel sample buffer.

Gel Electrophoresis

Two-dimensional gel electrophoresis was as described by O'Farrel (25), with minor modifications (22). Ampholines with pH ranges 3–10, 5–7, and 6–4 were purchased from Serva Fine Biochemicals Inc. (Garden City Park, NY) and mixed in the ratio of 1:3:1. Second dimension SDS gels were 9% acrylamide and buffered with Tris-glycine (16). One-dimensional SDS PAGE was carried out on gels either identical to the second dimension gels just described, or on 4–16% acrylamide gradient gels (24). Gels were stained with Coomassie Blue and, for in vitro translated samples, treated with autofluor (National Diagnostics, Inc., Somerville, NJ), dried, and exposed at −70°C to Kodak XAR-5 film. Molecular weight standards were myosin (205,000), phosphorylase B (97,500), bovine serum albumin (BSA) (66,000), and soybean trypsin inhibitor (20,100).

Immunization

Two-dimensional gels loaded with 400 μg of wild-type spokehead fraction were stained with Coomassie Blue, destained, and equilibrated with water. Spokehead polypeptides 1, 4, and 6 were excised, and the gel fragments were minced and lyophilized. After rehydration in physiological saline (0.9% NaCl), the gel fragments were macerated by multiple passages through a syringe, and then emulsified in an equal volume of either Freund's complete adjuvant for initial injections, or Freund's incomplete adjuvant for all subsequent injections. Rabbit A was injected subcutaneously at multiple sites with ~70 μg of combined spokehead proteins 4 and 6 once a week for four consecutive weeks, and then bled during week 5. Rabbit B was injected with 150 μg of spokehead protein 1 on weeks 1, 2, and 4, and then bled during week 5.

Sucrose gradient fractions containing 18S dynein proteins were dialyzed against phosphate-buffered saline, boiled, and mixed with Freund's adjuvant before injection into BALB/c mice. After 3 wk, animals were boosted with 18S dynein in incomplete Freund's adjuvant and antiserum was collected. The animal that produced the antiserum for antibody selection experiments was not used to generate the monoclonal antibodies for library screening.

Immunoblotting

Western blots with ^{125}I-protein A (Amersham Corp., Arlington Heights, IL) as secondary probe were done essentially as described (6), except that filters were stained with Amido black before they were probed with 200-fold dilutions of crude rabbit antisera.

Affinity Purification of Radial Spokehead Antibodies

Wild-type radial spokehead fraction (7 mg) was dialyzed into 0.5 M NaCl buffered with 0.1 M $NaHCO_2$ (pH 8.3), clarified by centrifugation, and concentrated by ultrafiltration (PM10, Amicon Corp., Danvers, MA) to 2 ml. The concentrated spokehead fraction was bound to 1.2 ml of CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) following the procedures recommended by the manufacturer. After packing, the column was used to affinity-purify spokehead antibodies from crude serum as described (9).

Isolation of Nuclear DNA

Wild-type vegetative *Chlamydomonas* were grown in acetate-supplemented medium I (36), concentrated with a Pelicon filter (Millipore Corp., Bedford, MA), the cells washed, and then DNA was isolated essentially as described by Rochaix (32). Nuclear DNA peaks from CsCl gradients were pooled, dialyzed extensively against a solution containing 10 mM Tris, 1 mM EDTA, pH 8.0, and concentrated by ethanol precipitation.

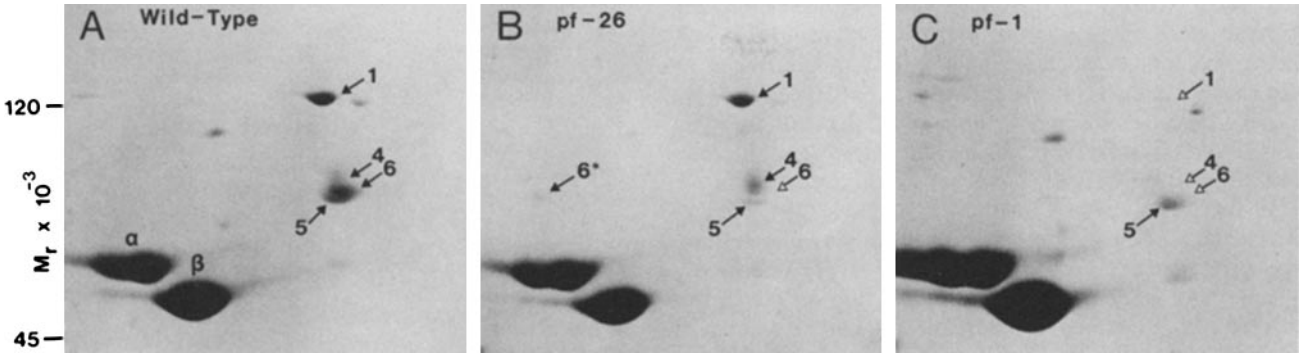

Figure 1. Coomassie Blue-stained gels of wild-type, *pf*-26$_{ts}$, and *pf*-1 flagellar spokehead fractions. Radial spokehead polypeptides were released from axonemes by dialysis against a low salt solution, concentrated, and then run on two-dimensional gels. Only a portion of each gel is shown. Gels are oriented with the acidic polypeptides on the right. Radial spoke components are labeled by number; tubulins are labeled α and β. Open arrowheads indicate positions of spokehead polypeptides absent from mutant fractions. (*A*) Wild-type spokehead fraction. (*B*) Mutant *pf*-26$_{ts}$ spokehead fraction; polypeptide 6 is absent and mutant polypeptide 6* is present but at greatly reduced amounts relative to the other radial spoke components. (*C*) Mutant *pf*-1 spokehead fraction; spokehead polypeptides 1, 4, and 6 are absent. Polypeptides 2 and 3, which are components of the spoke stalk, are not prominent in the spokehead fraction and are not labeled.

Construction and Screening of the Chlamydomonas Expression Library

All enzymes used in these procedures were purchased from New England Biolabs (Beverly, MA) except where noted. The genomic expression library was constructed using procedures modified from those described by Snyder and Davis (41). *Chlamydomonas* nuclear DNA was sheared by sonication (Branson Sonic Power Co., Danbury, CT) to an average length of 5 kb, and 120 μg was methylated in a 1-ml reaction containing 50 mM Tris (pH 7.5), 1 mM EDTA, 80 μM *S*-adenosyl-L-methionine, and 900 U of Eco RI methylase (1 h, 37°C). The reaction was stopped by heating to 70°C for 20 min; dATP, dCTP, dGTP, and dTTP were added to final concentrations of 100 μM each, $MgCl_2$ to 10 mM, and the fragments were "blunt ended" with 120 U of T4 polymerase (37°C, 15 min). After phenol extraction and ethanol precipitation, the fragments were ligated (T4 ligase, International Biotechnologies, Inc., New Haven, CT) to phosphorylated Eco RI linkers (Collaborative Research, Inc., Waltham, MA). Eco RI sticky ends were generated by digestion with Eco RI, and the DNA was size fractionated on a 1% agarose gel. Fragments from 2 to 10 kb were electroeluted, mixed with carrier tRNA, phenol-extracted, ethanol-precipitated, and resuspended in 10 mM Tris, 1 mM EDTA, pH 8.0. The size-fractionated genomic fragments (5 μg) were ligated to Eco RI-restricted dephosphorylated (calf intestinal phosphatase, Boehringer Mannheim Biochemicals, Indianapolis, IN) λgt11 phage arms (17 μg) in a 50-μl reaction. After ligation the DNA was packaged in vitro following protocol II of Maniatis et al. (21), and the primary library containing 7 × 10^6 independent recombinants was amplified on strain Y1088.

The amplified library was screened at a density of 5 × 10^4 plaque-forming units per 15-cm plate, as described (41), with the following modifications. Filters were blocked and incubated with antibody in 150 mM NaCl and 50 mM Tris, pH 8.0 (TBS)[1] containing 3% BSA, and rinsed after exposure to primary and secondary antibodies with two 10-min TBS washes separated by a very brief rinse in TBS with 0.05% Nonidet P-40. Antibody reactions were detected using secondary affinity-purified goat anti-rabbit peroxidase (Bio-Rad Laboratories, Richmond, CA) for screens with rabbit antisera, and affinity-purified goat anti-mouse peroxidase (Bio-Rad Laboratories) for the screens with mouse monoclonal antibodies, both developed with 4-chloro-1-naphthol as the chromophore.

Phage that gave positive signals were cloned, their DNA prepared, and the genomic inserts excised with Eco RI and subcloned into the Eco RI site of plasmid SP65 by standard methods.

Dynein Antibody Selection by Expressed Proteins

Proteins expressed by cloned phage were bound to nitrocellulose filters and used to affinity-purify antibodies from a polyclonal antiserum. Phage were plated on *E. coli* strain Y1090 at 2 × 10^4 plaque-forming units per 10-cm plate. After 3 h at 42°C, plates were overlaid with isopropyl β-D-thiogalactopyranoside-soaked filters and incubated for 6 h at 37°C. Filters were rinsed in TBS, blocked in TBS/3% BSA, incubated overnight at 4°C in mouse anti-dynein serum diluted 1:100 in TBS/3% BSA, and rinsed (3 × 10 min) in TBS/0.05% Nonidet P-40. Bound antibodies were eluted by pipeting 1 ml of 0.1 M glycine (pH 3.0) over each filter, plaque side up in a petri dish, for 1 min. The eluate was neutralized with 0.02 ml 1 M Tris (pH 9.0) and diluted with 1 ml TBS/3% BSA.

Hybridization Selection

Procedures for binding DNA to nitrocellulose and for hybridization to RNA were essentially as described (21). Hybridization was in a volume of 300 μl and contained 1 mg of whole cell RNA isolated from *Chlamydomonas* 25 min after mechanical deflagellation. Selected RNA was translated in vitro in the rabbit reticulocyte lysate system (26), and the translation products were prepared for two-dimensional gel electrophoresis as previously described (19).

RNA Isolation

Whole cell *Chlamydomonas* RNA used in hybrid selection experiments was isolated from gametes or vegetative cells by the method of Dobberstein et al. (7) with modifications (37). RNA was separated from DNA by centrifugation through a cushion of $CsCl_2$ (8). Vegetative cells were grown in medium I (36), concentrated, and mechanically deflagellated (35). Gametes were prepared as described (15).

For RNA dot hybridization experiments, whole cell RNA was isolated by a similar but abbreviated procedure (1).

RNA Dot Hybridization

RNA dot analysis was performed as previously described (37).

Results

Preparation of Antibodies to Radial Spokehead Polypeptides

Spokehead components were identified on gels by comparing fractions from wild-type flagella with fractions from flagella of mutant *pf*-1, which lacks the spokehead structure, and mutant *pf*-26$_{ts}$, which assembles a radial spoke with normal appearance, but apparently has an alteration in spokehead component 6 primary structure. Using the nomenclature of Huang et al. (13), radial spoke protein 1 migrates at 123 kD,

1. *Abbreviation used in this paper*: TBS, 150 mM NaCl and 50 mM Tris, pH 8.0.

and spoke components 4, 6, and 5 migrate in overlapping spots with descending apparent molecular weights, 74, 67, and 65 kD, on our gel system (Fig. 1 *A*). As expected, polypeptides 1, 4, and 6, all components of the spokehead, are missing from spokeheadless mutant *pf*-1 (Fig. 1 *C*). Polypeptide 5, which may link the spokehead to the spoke stalk, fractionates with the spokehead polypeptides, but is present in *pf*-1 flagella (Fig. 1 *C*) as previously described (29). The relative positions of components 4 and 6 were verified by comparing spokehead fractions from wild-type and *pf*-26_{ts} flagella. Component 6 is missing from the *pf*-26_{ts} spokehead fraction, and there is an unstained gap on the gel at its normal position between polypeptides 4 and 5 (Fig. 1, compare *A* and *B*).

Interestingly, also present in the *pf*-26_{ts} spokehead fraction is the novel protein 6*, which runs in the region above the α-tubulin spot (13), but is at low levels relative to the other spokehead polypeptides and is therefore hard to visualize by protein stain in Fig. 1 *B*. This may be due to anomalous fractionation of the mutant polypeptide. Its presence in the *pf*-26_{ts} spokehead fraction is clearly demonstrated by immunoblot analysis (see below).

Antiserum raised by injecting rabbit A with polypeptides 4 and 6 was characterized by immunoblot analysis in which spokehead fraction from wild-type or *pf*-26_{ts} flagella were run on two-dimensional gels, transferred to nitrocellulose, and then probed with crude serum. Antibodies reacted with the region occupied by the overlapping 4, 6, and 5 spots on the wild-type immunoblot (Fig. 2, compare *A* and *B*). This antiserum reacts much more strongly with component 6 than to the other two radial spoke components in this cluster as revealed by the immunoblot of mutant *pf*-26_{ts} spokehead fraction. Even though mutant polypeptide 6* is barely visible by protein stain (Fig. 2 *C*), in the immunoblot the serum produces a much stronger signal to it than to spoke components 4 or 5 (Fig. 2 *D*).

Antiserum from rabbit B, immunized with spokehead protein 1, gives a strong reaction to this polypeptide (Fig. 2, compare *E* and *F*).

Initial screenings of the expression library with crude antiserum resulted in the isolation of "positive" clones later found to react strongly with preimmune serum. These reactions could not be removed by absorbing the antisera against a lysate prepared from λgt11 lysogen BNN97, or from Y1090 lawn cells used for screening. To remove antibodies recognizing nonspokehead epitopes, the antisera were affinity-purified on a column constructed by coupling wild-type radial spokehead fraction to CNBr-activated Sepharose 4B.

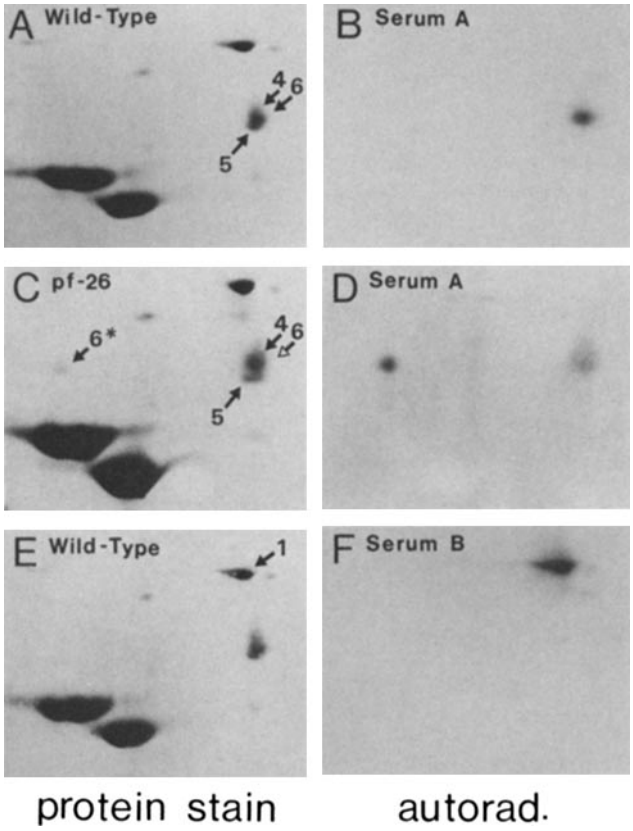

Figure 2. Immunoblot analysis of antibodies against flagellar spokehead polypeptides. Radial spokehead fractions isolated from wild-type and mutant *pf*-26_{ts} flagella were separated on two-dimensional gels, transferred to nitrocellulose, and stained with Amido black to reveal proteins (left panels). The same filters were then incubated with antiserum raised in rabbits against gel-purified spokehead polypeptides, and antibody binding was detected by subsequent incubation with ^{125}I-protein A and autoradiography (right panels). The portion shown of each filter and autoradiograph corresponds to that shown in Fig. 1. Spokehead components are labeled; open arrowhead indicates absence of wild-type spokehead component 6 from mutant *pf*-26_{ts} spokehead fraction, and 6* indicates position of the mutant *pf*-26_{ts} spokehead component. (*A*) Protein stain of wild-type spokehead fraction; (*B*) corresponding autoradiograph after reaction with antiserum A. (*C*) Protein stain of mutant *pf*-26_{ts} spokehead fraction; (*D*) corresponding autoradiograph after reaction with antiserum A. (*E*) Protein stain of wild-type spokehead fraction; (*F*) corresponding autoradiograph after reaction with antiserum B. Antiserum A reacts strongly with wild-type spokehead component 6 (*B*) and mutant component 6* (*D*); it also reacts weakly against spokehead component 4 and possibly 5 (*D*). Antiserum B reacts strongly against component 1 (*F*).

Cloning of Radial Spokehead Sequences

Approximately 5 × 10^6 λgt11 recombinants containing *Chlamydomonas* genomic inserts were screened with affinity-purified antibodies from rabbit A, which reacts strongly with spokehead component 6. Three positive recombinants were isolated; preimmune serum failed to react with plaques of any of these clones. The genomic inserts of all three, RSa1, RSa2, and RSa3, were subcloned into the Eco R1 site of plasmid SP65. Restriction analysis showed that the RSa1 and RSa2 inserts are identical, while RSa3 is of different length, but overlaps the others. A restriction map of clone RSa1, which was used in hybrid selection experiments described below, is included in Fig. 3.

A screen of 2 × 10^6 recombinants with affinity-purified polyclonal antibodies from rabbit B, recognizing spokehead component 1, yielded eight positive clones, none of which reacted with preimmune serum. The genomic inserts of two of these positive recombinants, RSb1 and RSb2, were subcloned into SP65, restriction mapped, and found to have overlapping inserts. A restriction map of clone RSb1, used in hybrid selection experiments (see below), is shown in Fig. 3.

Cloning of Sequences Encoding Dynein Polypeptides

A pool of three mouse monoclonal antibodies, each reacting with a different polypeptide of the dynein outer arm, was

used to screen 4 × 10^6 λgt11 recombinants. The monoclonal pool consisted of antibodies 2.14, 11.4, and 11.13, which have been previously characterized (Mitchell, D. R., and J. L. Rosenbaum, manuscript submitted for publication). Antibody 2.14 binds the outer dynein arm α heavy chain, 11.13 binds the β heavy chain, and 11.4 reacts with the intermediate molecular weight 73-kD polypeptide. Positive clones were isolated and then tested separately against the individual antibodies. The anti-β reacted with three clones, Db1, Db2, and Db3, and the anti-73 kD with four clones, Da1, Da2, Da3, and Da4. Several additional clones reacted with the anti-α; these will be described elsewhere. None of the clones reacted with more than one antibody.

The inserts from all three putative β-heavy chain clones were subcloned into SP65. Restriction maps of two, Db1 and Db2, were identical. The map of the third, Db3, showed no similarities to the other two. Interestingly, the Db3 λgt11 plaques showed a much weaker reaction with the anti-β chain antibody than those of clones Db1 and Db2. Fig. 3 includes a restriction map of clone Db1, which was used in antibody selection experiments.

Of the four putative 73-kD clones, two were subcloned into SP65. Restriction analysis of genomic inserts Da1 and Da2 indicate these are overlapping clones. A map of Da1, used in antibody selection and hybridization selection experiments, is shown in Fig. 3.

Confirmation of Gene Identity by Hybridization Selection

Putative spokehead clones RSa1 and RSb1 and dynein clone Da1 were used to select mRNA from whole cell RNA isolated during flagellar regeneration. The selected mRNAs were translated in vitro in the presence of [^{35}S]methionine and run on one-dimensional and two-dimensional polyacrylamide gels. Lanes *D* and *E* of Fig. 4 show the translation products of RNA selected by SP65 recombinant plasmids containing genomic inserts RSa1 and RSb1, respectively. Single additional bands with apparent molecular weights of 67 (lane *D*) and 123 kD (lane *E*) are present when compared with the translation products of SP65 vector-selected control RNA (lane *C*). To verify the identity of these additional bands, the radioactively labeled translation products were also mixed with unlabeled spokehead fraction and run on two-dimensional gels. Comparison of Coomassie Blue staining (Fig. 5 *A*), which reveals the unlabeled polypeptides of the spokehead fraction, and the autoradiograph of the same gel (Fig. 5 *B*), shows that the additional polypeptide labeled in the translation of RSa1-selected mRNA co-migrates with spokehead component 6. The α- and β-tubulin also present in this autoradiograph can be seen clearly in a longer autoradiographic exposure of the translation products of SP65 vector-selected control mRNA (Fig. 5 *H*), indicating tubulin mRNA is not specifically selected by the RSa1 genomic insert. Nonspecific selection of tubulin is not surprising, as tubulin mRNAs are among the most abundant mRNAs in regenerating cells as seen in the translation products of whole cell regenerating RNA (Fig. 4, lane *A*).

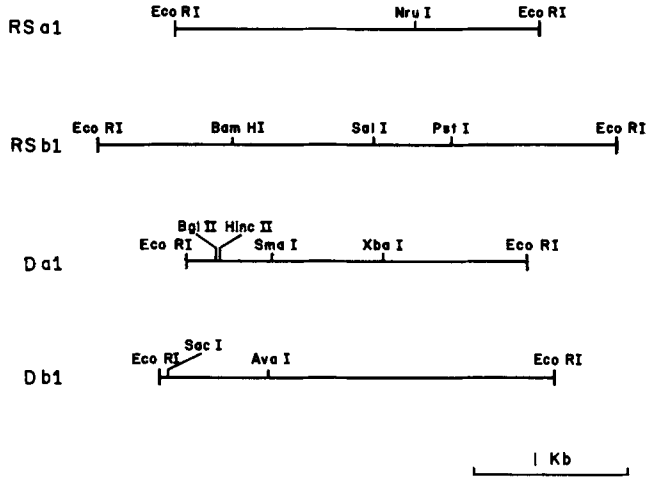

Figure 3. Restriction maps of genomic inserts from positive λgt11 recombinants. λgt11 positives isolated with radial spokehead and outer dynein arm antibodies were cloned, and their genomic inserts were subcloned into vector SP65 and restriction mapped. Genomic inserts RSa1 and RSb1 were isolated from positive phage clones reacting with radial spokehead antiserum A (strong reaction to spokehead polypeptide 6) and antiserum B (strong reaction to spokehead polypeptide 1), respectively. Da1 and Db1 were isolated with individual monoclonal antibodies recognizing the 73-kD polypeptide and the β heavy chain of the outer dynein arm, respectively. The enzymes that did not cut the individual inserts are: for RSa1, Bam HI, Bgl II, Cla I, Hind III, Kpn I, Sph I, and Xba I; for RSb1, Bgl II, Cla I, Hind III, Kpn I, and Nru I; for Da1, Bam HI, Cla I, Hind III, Kpn I, and Nru I; and for Db1, Bam HI, Hinc II, Hind III, Sal I, Sma I, and Xba I.

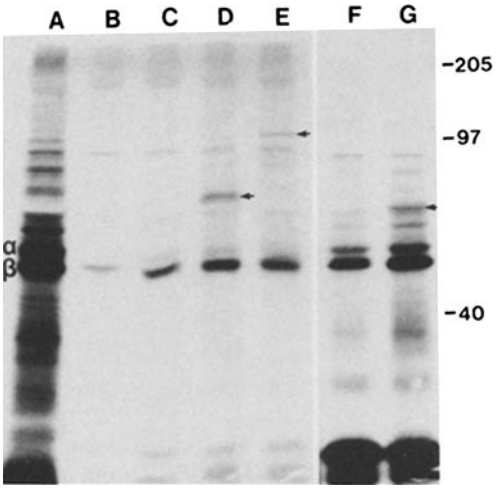

Figure 4. In vitro translation of RNA isolated by hybridization selection. Genomic inserts from positive λgt11 recombinants were subcloned into plasmid vector SP65, the plasmids bound to nitrocellulose filters, and hybridized to whole cell RNA which was isolated during flagellar regeneration. The hybridized RNA was eluted and translated in the rabbit reticulocyte lysate system. The radioactively labeled translation products were separated on an SDS-acrylamide (9%) gel and autoradiographed. Lane *A*, translation products of the whole cell RNA used for the hybridizations; *B*, endogenous translation products of the reticulocyte lysate produced in the absence of any added *Chlamydomonas* RNA; *C*, *D*, and *E*, translation products of RNA selected by vector SP65, SP65 containing genomic insert RSa1, and SP65 containing genomic insert RSb1, respectively. Lanes *F* and *G* are from a separate experiment and show translation products from RNA selected by vector SP65, and SP65 containing genomic insert Da1, respectively. Genomic inserts RSa1, RSb1, and Da1 specifically hybridized to mRNAs encoding single polypeptides with apparent molecular weights of 67, 123, and 73 kD, respectively (*arrows*). α- and β-Tubulin are prominent translation products of whole cell RNA isolated during flagellar regeneration, and are labeled in lane *A*.

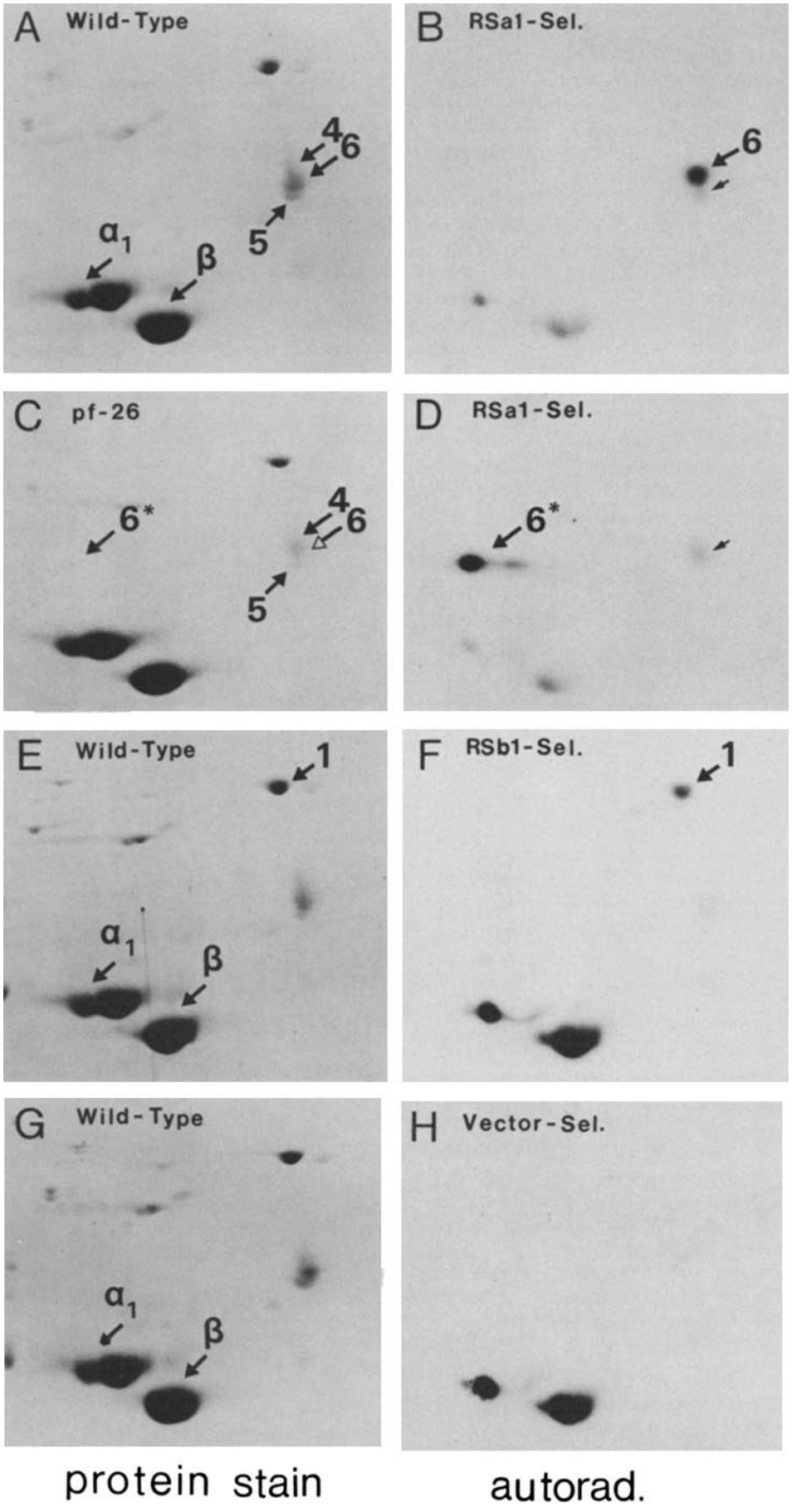

Figure 5. Coelectrophoresis of unlabeled spokehead proteins with radioactively labeled in vitro translation products from RSa1 and RSb1 hybrid-selected RNA. To verify that the 67 and 123-kD polypeptides present in the in vitro translation products of RSa1- and RSb1-selected RNA (Fig. 4) are spokehead polypeptides, the radioactively labeled translation products were mixed with unlabeled spokehead fraction, which served as carrier, and run on two-dimensional gels. In addition, clone RSa1 was used to hybrid-select RNA from spokehead mutant *pf*-26_{ts} whole cell RNA, and the in vitro translation products were run on a gel with *pf*-26_{ts} spokehead fraction as carrier. After Coomassie Blue staining to visualize the spokehead polypeptides (left panels), gels were autoradiographed to reveal the radioactively labeled translation products of the hybrid-selected mRNAs (right panels). The portion shown for each gel and autoradiograph corresponds to the region included in Fig. 1. Spokehead components (numbers) and tubulins (α and β) are labeled; open arrowhead indicates the absence of wild-type spokehead component 6 from mutant *pf*-26_{ts} spokehead fraction, and 6* indicates position of the mutant *pf*-26_{ts} spokehead component. Small arrows indicate a minor amount of a labeled polypeptide apparently co-migrating with spokehead component 5 in the RSa1-selected RNA translation products. (*A*) Protein stain of wild-type spokehead fraction; (*B*) corresponding autoradiograph showing translation products of RNA hybrid-selected by clone RSa1 from wild-type whole cell RNA. (*C*) Protein stain of *pf*-26_{ts} spokehead fraction; (*D*) corresponding autoradiograph showing translation products of RNA hybrid-selected by clone RSa1 from mutant *pf*-26_{ts} whole cell RNA. (*E*) Protein stain of wild-type spokehead fraction; (*F*) corresponding autoradiograph showing translation products of RNA hybrid-selected by clone RSb1. (*G*) Protein stain of wild-type spokehead fraction; (*H*) corresponding autoradiograph showing translation products of SP65 vector-selected RNA. Genomic insert RSa1 hybridizes strongly to RNA encod-

Further confirmation that genomic insert RSa1 encodes component 6 is provided by a similar hybridization selection experiment performed with whole cell RNA isolated from mutant *pf*-26$_{ts}$. The translation products were mixed with unlabeled *pf*-26$_{ts}$ spokehead fraction and run on a two-dimensional gel. Comparison of Coomassie Blue stain (Fig. 5 *C*) and autoradiograph (Fig. 5 *D*) reveals that the selected mRNA encodes mutant flagellar protein 6*. In addition to demonstrating that clone RSa1 encodes wild-type 6 locus, the selection of mRNA encoding 6* is positive evidence confirming that mutation *pf*-26$_{ts}$ alters the primary structure of component 6.

Interestingly, the long autoradiographic exposures shown in Fig. 5, *B* and *D* reveal that the translation products of both wild-type and *pf*-26$_{ts}$ RSa1-selected RNA include, in addition to the predominant 6 and 6* polypeptides, a small amount of a polypeptide apparently co-migrating with spokehead component 5 (small arrows). Although at present we cannot account for this result, it may reflect a weak cross-hybridization between the fragment of the radial spokehead component 6 gene we have isolated here and mRNA-encoding spoke component 5.

Two-dimensional gels loaded with in vitro translation products from RSb1 hybridization selection indicate this clone encodes spokehead polypeptide 1 (Fig. 5, compare *E* and *F*). Because very long exposures were needed to visualize labeled polypeptide 1, the tubulins are more intense in this autoradiograph and the comparably exposed vector control (Fig. 5 *H*).

Confirmation that genomic insert Da1 encodes the 73-kD dynein outer arm polypeptide was provided by similar hybridization selection experiments. The Da1 selected mRNA encodes a 73-kD polypeptide (Fig. 4 *G*), which was absent from the vector-selected control (Fig. 4 *F*). Analysis on two-dimensional gels shows that the 73-kD in vitro translation product (Fig. 6 *A*) co-migrates with a 73-kD protein which is present in dynein fractions of wild-type flagella (Fig. 6 *C*) but absent from dynein fractions of outer armless mutant *pf*-28 (Fig. 6 *D*). The tubulins present in the autoradiograph are not specifically selected by insert Da1, as indicated by their presence in the vector-selected control (Fig. 6 *B*).

Confirmation of Gene Identity by Antibody Selection

We could not hybrid-select and translate in vitro an mRNA encoding the dynein β–heavy chain. This may be due to technical difficulties introduced by the large size of the mRNA encoding this 325-kD polypeptide. To provide evidence supporting the identity of this genomic insert, we used the protein expressed by the recombinant λgt11 clone Db1 in antibody selection experiments. The 73-kD dynein clone (Da1), confirmed already by mRNA hybridization selection, was used as a positive control. Clones Da1 and Db1 were initially selected for their ability to express the single determinants recognized by each monoclonal antibody. The presence of bona fide dynein heavy chain and intermediate chain determinants on these expressed products was tested with a polyclonal mouse antiserum directed against a wide range of antigenic determinants on several proteins of the 18S dynein ATPase complex. The antibodies specifically binding to the expressed proteins of the individual cloned phage were affinity-purified and their specificity was then tested against a blot of total 18S dynein proteins.

Comparison of the Coomassie Blue–stained gel of 18S dynein proteins (Fig. 7 *A*) with the antibody reaction of the polyclonal antiserum (Fig. 7 *B*) shows that this serum recognizes determinants on the 18S α–heavy chain, β–heavy chain, and 73-kD intermediate chain. Weak reactivity toward the 83-kD intermediate chain is also present. Antibodies selected by expression products of uncloned phage, which contain random genomic inserts, do not recognize any 18S dynein proteins (Fig. 7 *C*), while antibodies selected by the expression product of clone Db1 specifically recognize the 325-kD β–heavy chain (Fig. 7 *D*). Likewise, antibodies

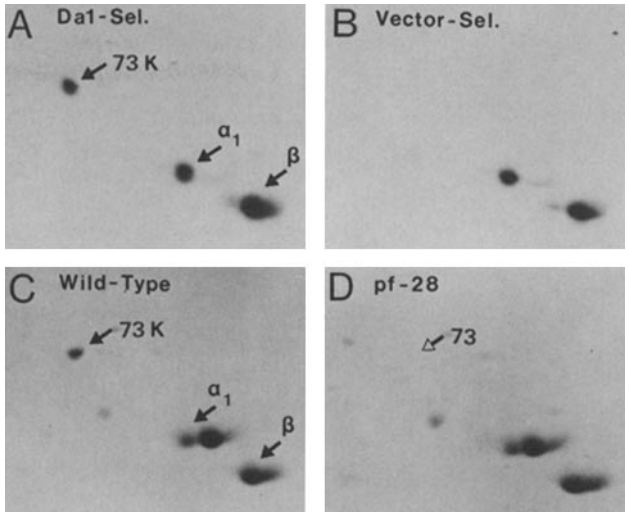

Figure 6. Coelectrophoresis of unlabeled dynein fraction with radioactively labeled in vitro translation products from Da1 hybrid-selected RNA. To verify that the 73-kD polypeptide present in the in vitro translation products of Da1-selected RNA (Fig. 4) is the 73-kD polypeptide of the outer dynein arm, the radioactively labeled translation products were mixed with an unlabeled dynein fraction prepared from wild-type axonemes, run on a two-dimensional gel, and autoradiographed. The position of the dynein outer arm 73-kD polypeptide on our gel system was identified by comparison of Coomassie Blue–stained gels loaded with dynein fractions from wild-type cells and dynein outer armless mutant *pf*-28. Only a portion of the autoradiographs and Coomassie Blue–stained gels are shown; this area overlaps the region shown in Fig. 1, but extends further toward the basic end of the gel. The outer dynein arm 73-kD polypeptide and the tubulins (α_1 and β) are labeled; open arrowhead indicates the absence of the dynein outer arm 73-kD polypeptide from the *pf*-28 dynein fraction. (*A* and *B*) Autoradiographs showing in vitro translation products of RNA hybrid-selected by SP65 containing genomic insert Da1 (*A*), and vector SP65 with no inserted sequence (*B*); RNA encoding a 73-kD polypeptide hybridizes specifically to genomic insert Da1. (*C* and *D*) Coomassie Blue stain of gels loaded with wild-type and *pf*-28 dynein fractions, respectively; the outer dynein arm 73-kD polypeptide is identified by its absence in the *pf*-28 fraction. The 73-kD in vitro translation product from the hybridization selection experiment and the outer dynein arm 73-kD polypeptide migrate in the same position on the two-dimensional gels (compare *A* and *C*), confirming the identity of the in vitro translation product.

ing both the wild type 6 (*B*) and mutant 6* (*D*) spokehead components. Genomic insert RSb1 hybridizes specifically to RNA encoding spokehead component 1 (*F*). Tubulins present in both the RSa1-selected and RSb1-selected translation products are also present in the SP65 vector-selected control (*H*).

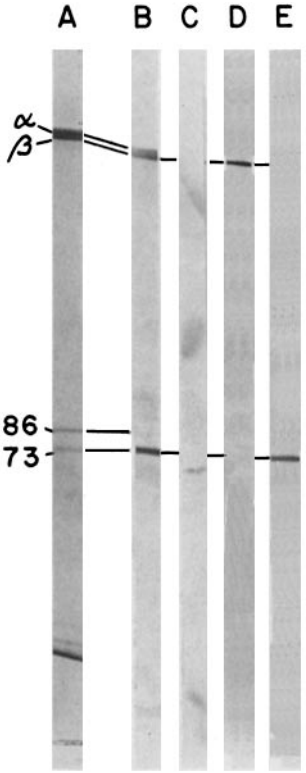

Figure 7. Antibody selection by dynein clones. Fusion proteins produced by λgt11 clones were used to affinity-purify antibodies from a polyclonal anti-dynein serum. The specificity of the selected antibodies was tested against a Western blot of 18S dynein. (*A*) SDS urea gel (acrylamide gradient, 4–16%) of 18S dynein stained with Coomassie Blue. (*B–E*) Western strips of the same gel stained with mouse anti-dynein and peroxidase-labeled goat anti-mouse antibodies. (*B*) Anti-dynein serum (unselected) reacts with the two heavy chains and two intermediate chains of 18S dynein. (*C*) Antibodies selected with uncloned phage show no specific reaction. (*D*) Antibodies selected with clone Dbl react specifically with the β-heavy chain. (*E*) Antibodies selected with clone Dal react specifically with the 73-kD intermediate chain.

selected by the Dal expression product recognize the 73-kD protein on Western blots (Fig. 7 *E*). No additional reactivities were seen when these affinity-selected antibodies were reacted with Western blots of total flagellar proteins (data not shown).

Regulation of Spokehead and Dynein mRNAs

In light of in vivo labeling studies, which show increases in radial spoke and dynein polypeptide synthesis in response to deflagellation (18, 31), and evidence for different kinetic classes of mRNA accumulation during flagellar regeneration (37), it was of interest to directly measure spokehead and dynein mRNA accumulation patterns in response to deflagellation. Whole cell RNA was prepared from vegetative wild-type *Chlamydomonas* before and at various times after deflagellation, and equal amounts of the RNA were dotted onto nitrocellulose and probed with nick-translated plasmids containing the genomic inserts RSa1, RSb1, Da1, and Db1. The levels of two additional mRNAs were measured as controls in this experiment: α-tubulin mRNA, which has been shown to accumulate in response to deflagellation (39), and an unidentified constitutive mRNA, which remains at the same level during flagellar regeneration (37). cDNA probes for the α-tubulin and constitutive mRNAs were clones pcf 4-2 and pcf 2-40 of Schloss et al. (37), respectively. Autoradiographic exposures of the dots are shown in Fig. 8 *A*, and quantitation of hybridization by scintillation counting is shown in Fig. 8 *B*. The radial spokehead mRNAs and the dynein mRNAs were induced rapidly by deflagellation showing significant increases by 15 min after deflagellation. The kinetics of accumulation were similar for the radial spokehead and dynein sequences. All four mRNAs reached peaks in abundance 3–7-fold over predeflagellation levels at ~30 min after flagellar excision, slightly before the α-tubulin peak which occurred at 45 min. The radial spokehead and dynein mRNAs then returned to predeflagellation levels by 80 min, and were followed by α-tubulin mRNA which declined more gradually, reaching predeflagellation levels by 100 min.

Discussion

We have described the molecular cloning of genes encoding several radial spokehead and several dynein outer arm polypeptides using a *Chlamydomonas* genomic expression library. These clones are the first identified nontubulin flagellar sequences that have been isolated, and have allowed the quantitation of their corresponding mRNAs during flagellar regeneration. In addition, one of the radial spokehead sequences and probably one of the dynein sequences overlap genetic loci where flagellar mutations have been previously mapped. Isolation of these clones, which can be used as molecular probes to clone the mutant sequences, is therefore a first step in the molecular genetic analysis of flagellar assembly and function.

We constructed a large genomic expression library because its usefulness is not limited to the isolation of flagellar sequences, as might have been the case with a smaller cDNA library made from mRNA isolated during regrowth of amputated flagella. A genomic expression library has been successfully used to isolate yeast nuclear genes (42) and, although the *Chlamydomonas* genome is approximately seven times the size of the yeast genome (11, 17), it is small enough to make screening a genomic library feasible.

The library was successfully screened with both affinity-purified polyclonal antibodies and single monoclonal antibodies. Polyclonal antibodies were affinity-purified after it was discovered that crude antisera reacted with many plaques that were also recognized specifically by preimmune serum. These erroneous "positive" reactions could not be eliminated by preadsorbing antisera with lysates from λgt11 lysogen BNN97 or Y1090 plating cells. Those phage that were checked contained DNA inserts, so it is possible that preimmune sera recognized determinants on a number of different nonspokehead *Chlamydomonas* polypeptides encoded by these sequences. Although radial spokehead clones could theoretically have been identified by a differential screen with immune and preimmune serum, we chose to purify antibodies on a spokehead affinity column, and found that this eliminated the erroneous "positive" reactions, and allowed the isolation of two radial spokehead sequences.

Single monoclonal antibodies were also effective probes and were used to isolate two dynein sequences. The staining of positive plaques was weaker than when using polyclonal antisera, and when compared to the strong polyclonal antisera against spokehead polypeptide 1, monoclonal antibodies yielded fewer positives per recombinant phage screened. Positives were nonetheless isolated without screening the entire library, indicating that the library complexity is probably sufficient to allow expression of any single determinant of interest encoded in the nuclear genome. This bodes well for future screenings with single monoclonal antibodies that recognize peptide determinants.

The identities of three of the four cloned genomic inserts were verified by positive hybridization selection experiments. As expected, translation of the selected mRNAs in

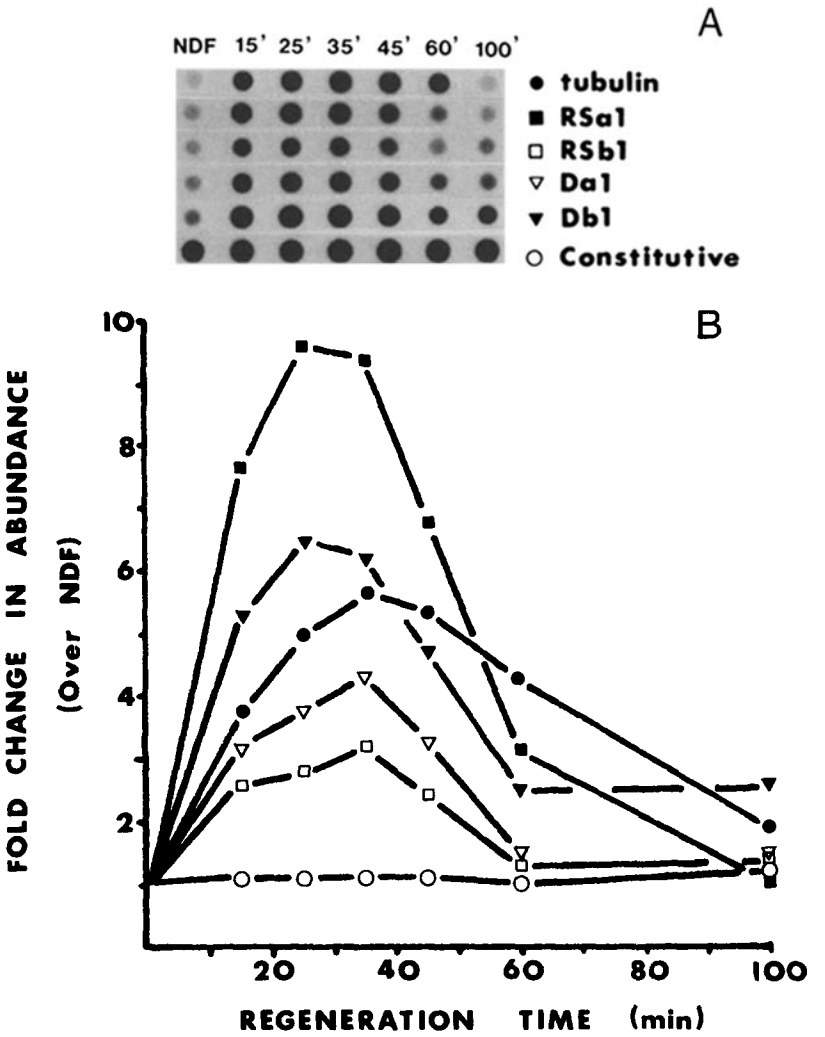

Figure 8. Accumulation of spokehead and dynein mRNA during regeneration of amputated flagella. Whole cell RNA was prepared from vegetative wild-type *Chlamydomonas* before and at various times after deflagellation, and then dotted onto nitrocellulose. Dots were probed with nick-translated plasmids containing spokehead genomic sequences RSa1 and RSb1, dynein genomic sequences Da1 and Db1, and for comparison, plasmids containing an α-tubulin cDNA sequence and an unidentified cDNA sequence corresponding to a constitutive mRNA. After autoradiographic exposure (*A*) dots were quantitated by scintillation counting (*B*). Spokehead and dynein mRNAs increased in abundance rapidly after deflagellation, peaked, and then returned to predeflagellation levels within ~2 h. This accumulation pattern differed slightly from that of α-tubulin mRNA, which peaked slightly later, and then declined more gradually to predeflagellation levels. Flagella regrew to full length by 90 min in this experiment.

vitro produced the appropriate spokehead and dynein polypeptides. Of particular interest, however, was the hybrid-selection of an mRNA encoding altered spokehead component 6* from radial spoke mutant *pf*-26$_{ts}$. This result verifies the fact that the wild-type sequence we have cloned overlaps the locus defined by the temperature-sensitive *pf*-26$_{ts}$ mutation, and simultaneously confirms that this mutation directly alters the primary structure of spokehead component 6. The alteration in primary structure was previously suggested by Huang et al. (13), based on the lack of a wild-type component 6 in *pf*-26$_{ts}$ flagella and the appearance of the novel polypeptide 6*. In addition, these authors ruled out the possibility that polypeptides 6 and 6* differ by a secondary posttranslational modification by showing that they were coexpressed in wild-type/*pf*-26$_{ts}$ dikaryons. Now that the wild-type component 6 gene has been isolated, it is being used as a hybridization probe to clone the mutant *pf*-26$_{ts}$ allele. This should allow a molecular genetic characterization of the mutation, which appears to have a temperature-dependent effect on the assembly and function of the radial spoke (13).

Isolation of the gene that encodes spokehead component 6 is also significant because it can be used in attempts to rescue the mutant *pf*-26$_{ts}$ strain back to wild-type flagellar motility by DNA-mediated transformation. Such transformation with a homologous *Chlamydomonas* gene has not yet been reported and may improve the low rates of transformation previously attained in *Chlamydomonas* (10, 33, 34). The ability to clone identified radial spoke genes using antibody probes may also allow this type of experiment to be performed with other radial spoke mutant strains such as *pf*-14 and *pf*-24, for which the deficient radial spoke components have been identified (13).

The hybrid-selection experiment, which confirmed the identity of the spokehead component 6 gene, also revealed that a small amount of mRNA apparently encoding spokehead component 5 was specifically selected along with the component 6 mRNA. This may be the result of cross-hybridization between the sequences encoding components 5 and 6. It is also possible that the genes for these proteins are very closely linked, and that the genomic fragment encoding component 6 also includes a small part of the component 5 gene. There is no genetic evidence suggesting this possibility, as mutations affecting spoke component 5 have not been reported. There is, however, genetic evidence suggesting close linkage between the loci encoding spokehead components 4 and 6. Revertant analysis indicates mutation *pf*-1 affects the component 4 locus, and mutations *pf*-1 and *pf*-26$_{ts}$ have not yet been separated by a crossover event (13). The two mutations complement in diploid dikaryons,

however, suggesting that they affect separate loci (13). The interesting possibility that the genes encoding spokehead components 4, 5, and 6 form a tightly linked cluster may be answered as soon as large genomic fragments including the component 6 gene are isolated and used in additional hybridization selection experiments. Such a linkage between genes encoding polypeptides that contribute to the same flagellar substructure and migrate in a tight constellation on a two-dimensional gel would be intriguing, and may allow inferences into the evolution of the radial spoke genes.

Evidence supporting gene identity for the putative dynein β heavy chain clone, which was isolated with a single monoclonal antibody, was provided by an antibody selection experiment. Antibodies binding the expression product produced by the recombinant phage reacted monospecifically with dynein β-heavy chain, and not with any other flagellar polypeptides on immunoblots. These results do not rule out the possibility that the insert encodes a nondynein protein fragment that fortuitously shares an antigenic determinant with the dynein β-heavy chain polypeptide. Although hybridization selection experiments were unsuccessful for the putative dynein β-heavy chain sequence, probably due to the technical difficulty of working with the very large mRNA necessary to encode a protein of this size, RNA dot hybridization revealed mRNA abundance changes during flagellar regeneration similar to those of the other identified flagellar mRNAs. The antibody selection results coupled with the regulation data provide strong evidence supporting the identification of this clone as a portion of the dynein β-heavy chain gene.

The isolation of a dynein β-heavy chain sequence is particularly important because a mutation affecting the dynein β-heavy chain electrophoretic mobility has been isolated by Huang et al. (14). This mutation, sup_{pf}-1, was recovered as a suppressor mutation that restores flagellar motility to mutants lacking either radial spokes or the central pair complex. Eventual molecular cloning and sequencing of the entire wild-type and mutant alleles, now feasible using the wild-type sequence isolated here as a molecular probe, may therefore allow identification of a region on the dynein β-heavy chain which functions as a regulatory link between the force-producing dynein outer arm and the radial spoke/central pair system.

Expression of Radial Spoke and Dynein Genes

The cloning of several different radial spokehead and dynein sequences has allowed measurement of mRNA accumulation patterns for these genes during regrowth of amputated flagella. The coordinate mRNA accumulation curves peaked slightly earlier than that of tubulin mRNA, and then declined more rapidly to basal levels. This observation is interesting in light of data reported by Remillard and Witman (31) on synthesis of radial spoke proteins in vivo. These authors found that synthesis of several different radial spoke components peaked coordinately and then declined well before peaks in synthesis of the tubulins. Our data show that several different radial spoke and dynein mRNAs accumulate with coordinate kinetics, and this kinetic pattern differs only slightly from that of tubulin mRNA; they do not show a dramatically earlier peak for the radial spoke and dynein mRNAs relative to that of α-tubulin mRNA. An understanding of the significance of these qualitative differences in the mRNA accumulation and protein synthesis patterns must await further experiments designed specifically to address this issue.

With the exception of tubulin gene expression, for which cloned gene sequences are available as molecular probes (23, 39), previous analysis of flagellar mRNA accumulation relied upon in vitro translation of mRNA isolated during regeneration (19), and RNA dot analysis using unidentified cDNA probes likely to encode flagellar polypeptides (37). Both studies indicate that a transient accumulation of flagellar mRNAs occurs during flagellar regeneration. Interestingly, reminiscent of the existence of several different kinetic patterns at the level of flagellar protein synthesis (18, 31), RNA dot analysis with the unidentified cDNAs revealed a number of different mRNAs whose accumulation patterns fell into several distinct kinetic classes (37). One of these classes has an mRNA accumulation pattern very similar to the one reported here for radial spoke and dynein mRNAs.

Before this report the only identified flagellar sequences that had been molecularly cloned in *Chlamydomonas* were the two α- and two β-tubulin genes (23, 39). Transcripts from all four genes appear to accumulate coordinately during flagellar regeneration (5, 39, 43), and sequence comparisons among these genes have revealed several conserved 5′ flanking regions (4) as well as an intriguing homology among all the genes in one of their intervening sequences (38, 43). These conserved sequences are potential control regions for the induction of tubulin mRNA synthesis during flagellar regeneration, but because tubulin is used by the cell in organelles other than the flagellum, such as the mitotic spindle, there is a possibility that these sequences function to regulate tubulin gene expression during cellular events other than the production of new flagella. The significance of these homologies to gene regulation during flagellar regrowth may be clarified if any of them are discovered in completely unrelated flagellar genes. Use of the sequences isolated here as molecular probes should allow recovery of full length radial spoke and dynein genomic clones. Comparison of nucleotide sequences with those published for the tubulin genes may then permit identification of sequences important in gene regulation during the flagellar regeneration response.

In conclusion, we have cloned the first nontubulin flagellar sequences by using antibodies raised against purified flagellar polypeptides to screen a *Chlamydomonas* genomic expression library. One of the radial spokes and probably one of the dynein sequences overlap loci where flagellar mutations have been isolated. This is a first step in the molecular genetic analysis of these mutations, and also sets the stage for transformation of *Chlamydomonas* using a homologous gene. In addition, these clones have allowed analysis of radial spoke and dynein gene expression during regrowth of amputated flagella, and future sequence comparisons with the tubulin genes may allow identification of regions specifically involved in regulation of flagellar genes during the biogenesis of this organelle. Finally, we anticipate these cloned flagellar sequences will also be useful molecular probes for studying radial spoke and dynein gene expression and organization in other organisms.

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Note Added in Proof. Additional evidence indicating that genomic insert Dbl encodes part of the 325-kD dynein β-heavy chain was recently provided by Northern blot analysis; insert Dbl hybridizes to a single mRNA, ~11 kb, which is of sufficient length to encode this polypeptide. Characterizations of the anti-dynein monoclonal antibodies 2.14, 11.4, and 11.13 are being published elsewhere (Mitchell, D. R., and J. L. Rosenbaum, 1986, *Cell Motility and the Cytoskeleton*, in press).

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