Dynein from *Dictyostelium*: Primary Structure Comparisons Between a Cytoplasmic Motor Enzyme and Flagellar Dynein

Michael P. Koonce,* Paula M. Grissom, and J. Richard McIntosh

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309

Abstract. We report here the cloning and sequencing of a cytoplasmic dynein heavy chain gene from the cellular slime mold *Dictyostelium discoideum*. Using a combination of approaches, we have isolated 14,318 bp of DNA sequence which contains an open-reading frame of 4,725 amino acids. The deduced molecular weight of the polypeptide predicted by this reading frame is 538,482 D. Overall, the polypeptide sequence is 51% similar and 28% identical to the recently published sequences of the β-dynein heavy chain from sea urchin flagella (Gibbons, I. R., B. H. Gibbons, G. Mocz, and D. J. Asai. 1991. *Nature (Lond.)*. 352: 640–643; Ogawa, K. 1991. *Nature (Lond.)*. 352:643–645). It contains four GXXXXGKT/S motifs that form part of a consensus sequence for ATP-binding domains; these motifs are clustered near the middle of the polypeptide. The distribution of the regions sharing sequence similarity between the *Dictyostelium* and sea urchin heavy chain polypeptides suggests that the amino termini of dyneins may contain domains that specify axonemal or cytoplasmic functions.

DYNEIN is a high molecular weight, microtubule-associated, mechanochemical ATPase (reviewed in Warner et al., 1989). In cilia and flagella, axonemal dynein slides adjacent doublet microtubules over one another to generate a bending motion. Cytoplasmic forms of dynein have recently been identified in a wide variety of cell types, including some that lack cilia or flagella (reviewed in McIntosh and Porter, 1989; Vallee and Shpetner, 1990). Structural features of axonemal and cytoplasmic dyneins are quite similar. Both are very large complexes composed of multiple heavy, intermediate, and light chains. Both of the heavy chains undergo a photocleavage reaction with UV light in the presence of ADP and vanadate ions. Nonetheless, the functional aspects of the two types of dynein are believed to be quite distinct. Not only are they localized to different regions of the cytoplasm, but some biochemical differences in nucleoside triphosphatase activity, microtubule binding, and rates of microtubule gliding between the two dynein forms have been reported (Grissom et al., 1992; Foltz and Asai, 1988; Porter et al., 1988).

Like kinesin, cytoplasmic dynein is thought to act as a motor for vesicle transport (Schroer and Sheetz, 1991; McIntosh and Porter, 1989; Vallee and Shpetner, 1990), and cytoplasmic dynein has been immunolocalized to both spindle microtubules and to the kinetochore regions of mitotic chromosomes (Pfarr et al., 1990; Steuer et al., 1990, Wordeman et al., 1991). Because the kinetochore is an important site of mitotic force production (reviewed in Mitchison, 1988; McIntosh and Pfarr, 1991; Sawin and Scholey, 1991), dynein's localization suggests that it may contribute to chromosome movements. There is not yet, however, any direct functional evidence regarding the roles of cytoplasmic dynein in cell behaviors.

The cellular slime mold *Dictyostelium discoideum* contains at least one isoform of cytoplasmic dynein, which we have previously purified and characterized biochemically (Koonce and McIntosh, 1990). Using an antiserum against the heavy chain of this dynein as an entering wedge, we cloned and sequenced a gene which encodes the high molecular weight polypeptide of cytoplasmic dynein from *Dictyostelium*. The deduced protein sequence of this heavy chain identifies regions of both high and low similarity to the sequence of sea urchin flagellar β-dynein heavy chain (Gibbons et al., 1991; Ogawa, 1991), suggesting the locations of common functional domains as well as regions that are perhaps specialized for axonemal or cytoplasmic roles.

Materials and Methods

Unless otherwise indicated, the molecular methods were performed as described by Sambrook et al. (1989).

cDNA Cloning

Approximately 600,000 plaques from a *Dictyostelium* cDNA expression library (Clontech Inc., Palo Alto, CA) were screened with a polyclonal antiserum raised against the dynein heavy chain (Koonce and McIntosh, 1990). This screen identified one 4.0-kb clone that was characterized in some detail before it was used to isolate additional sequences (see Fig. 2). Restriction fragments from both the 5′ and 3′ ends of this initial clone were used to rescreen the library by hybridization, resulting in the identification of three additional clones that confirm the 5′ end of the coding sequence, and one

Please address all correspondence to Michael P. Koonce, Department of Molecular, Cellular, and Developmental Biology, Campus Box 347, University of Colorado, Boulder, Colorado 80309.

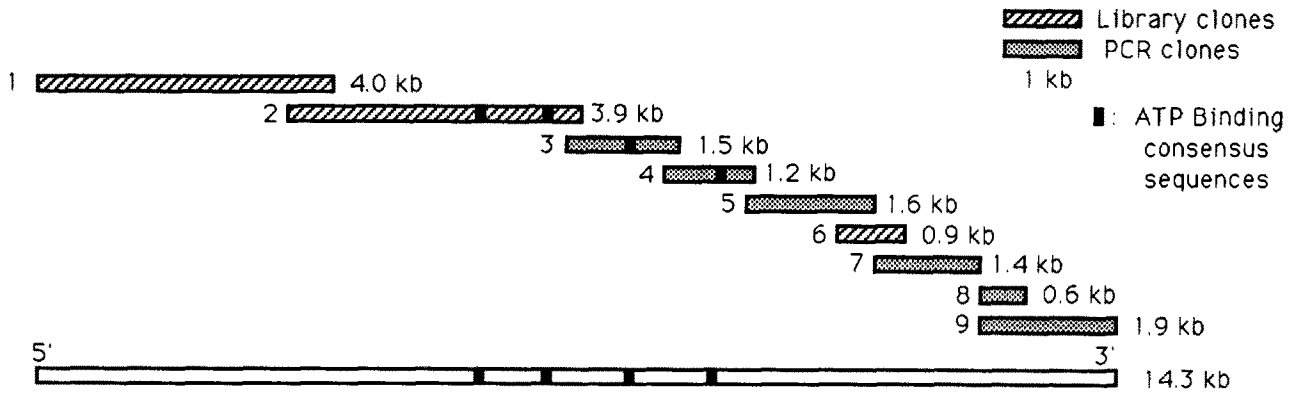

Figure 1. Dictyostelium dynein cloning summary. The clones are numbered in their order of isolation, but only the clones used to compile the final sequence are represented here. Nucleotide numbers 1–7,347 and 10,727–11,568 were derived from the Clontech cDNA library (clones 1, 2, and 6). Nucleotide numbers 7,073–12,937 were derived from the genomic PCR walk (clones 3–5, 7 and 8). The final clone (9, nucleotide 12,404–14,318) was derived from the PCR amplification of reverse transcribed RNA.

clone extending the open-reading frame 3.3 kb in the 3′ direction. Subsequent hybridization screens using this new 3′ end of the sequence failed to identify clones containing any additional sequence in either the Clontech or in three additional cDNA libraries, so an alternative strategy based on the polymerase chain reaction (PCR)[1] was adopted to walk to the 3′ end of the gene. Several of the resulting PCR clones were also used to screen the cDNA libraries, but only one positive plaque (clone 6) had an insert large enough to warrant additional characterization.

PCR Cloning

A detailed genomic restriction map was constructed using Southern blots probed with sequence from the 3′ end of the known dynein gene fragment. This map allowed us to identify enzyme sites in the 3′ direction that were ~1.5 kb apart and we used these sites to asymetrically cut and size select genomic DNA. Ligation of this DNA into a predicted orientation in a plasmid vector then gave us known DNA sequence at the 3′ end with which to construct a specific downstream primer for PCR amplification.

For each PCR clone, 200 μg of genomic DNA was digested with appropriate enzymes and electrophoresed on an agarose gel. The region of the gel whose *Rf* would correspond to the fragment of interest was excised and the DNA was glass purified. This DNA was then ligated to an equimolar amount of a vector (pGEM 7Zf, Promega Corp., Madison, WI) that had been digested with the appropriate restriction endonucleases. One-fourth of this ligation reaction was added to a standard 100-μl PCR cocktail containing 20 pmol of an upstream (gene-specific) primer, and 20 pmol of a downstream (vector-specific) primer based on either the T7 or SP6 RNA polymerase binding sequences flanking the polylinker site. 35 cycles of amplification were performed using standard conditions in a thermocycler (Perkin-Elmer Corp., Norwalk, CT). The entire reaction product was electrophoresed on an agarose gel; products of the predicted size were excised, their ends were blunted with Klenow, and they were subcloned into the SmaI site of pGEM. The presence of predicted internal restriction sites, the identity of sequence at the region overlapping the previous clone, and the degree of sequence similarity to the published flagellar dynein sequences were the criteria used to identify positive products.

DNA Sequencing and Sequence Analysis

Double-stranded DNA sequencing was performed using the dideoxy chain termination method (Sanger et al., 1977) and Sequenase 2.0 (US Biochemicals Inc., Cleveland, OH). For most clones, nested deletions were made with exonuclease III using the Erase-a-base kit from Promega Inc. Two clones were sequenced by subcloning overlapping restriction fragments into a plasmid vector. For each PCR step, two independent clones were sequenced in their entirety. Sequence discrepancies between the PCR clones were resolved by sequencing those regions in a third independent clone, using specific primers.

1. *Abbreviations used in this paper*: PCR, polymerase chain reaction.

Sequence assembly and initial characterization was performed using DNA Strider. Subsequent DNA and protein analysis was performed using the UWGCG sequence analysis package (Devereux et al., 1984).

Northern and Southern Blot Analysis

For Northern blot analysis, 20 μg of total RNA was electrophoresed on a formaldehyde-denaturing agarose gel and transferred to nitrocellulose. Blots were probed with ^{32}P-labeled clones in 50% formamide, 4× SSC, 50 mM potassium phosphate (pH 6.5), 0.1% SDS, 5× Denhardt's solution, and 0.4 mg/ml carrier DNA at 42°C overnight, and then washed five times with 0.1× SSC, and 0.1% SDS at 65°C (see Fig. 2 *A*). Initial Southern blots were probed with ^{32}P-labeled DNA in 50% formamide, 6× SSC, 0.1% SDS, 5× Denhardt's solution, and 0.5 mg/ml carrier DNA at 42°C overnight, and then washed five times with 0.1× SSC, and 0.1% SDS at 65°C (see Fig. 2 *B*). Subsequent Southern blots were probed using the ECL Direct Labeling Kit following the manufacturer's high stringency conditions (Amersham Corp., Arlington Heights, IL).

Antibody Production and Western Blotting

Clone 1 (see Fig. 1) was subcloned into the proper orientation and reading frame of the pET 5 expression vector and protein was expressed in the lysogenic *Escherichia coli* host BL21(DE3) (Studier et al., 1990). Induced *E. coli* lysates were electrophoretically separated on slab gels, and the region containing the 150-kD–expressed polypeptide was excised and used to immunize two New Zealand white rabbits (described in Koonce and McIntosh, 1990). Each animal produced a high titer antiserum that specifically recognized the dynein heavy chain in *Dictyostelium*.

Dictyostelium protein preparation, electrophoresis, and immunoblotting was performed as described by Koonce and McIntosh (1990).

Results

Using an antiserum directed against the dynein heavy chain, we screened a cDNA expression library to identify and isolate an initial, partial clone from the heavy chain gene (clone 1). Additional clones were identified using restriction fragments from either end of clone 1 to reprobe the library by hybridization and by the PCR-based strategy, described in the Materials and Methods, to walk in the 3′ direction along genomic DNA. A summary of the clones used to compile the final sequence is presented in Fig. 1.

Clone 1 contains the first in-frame AUG translational start codon of the open-reading frame. Three additional, independent, clones isolated from the cDNA library also contained the same open-reading frame, as well as two in-frame stop

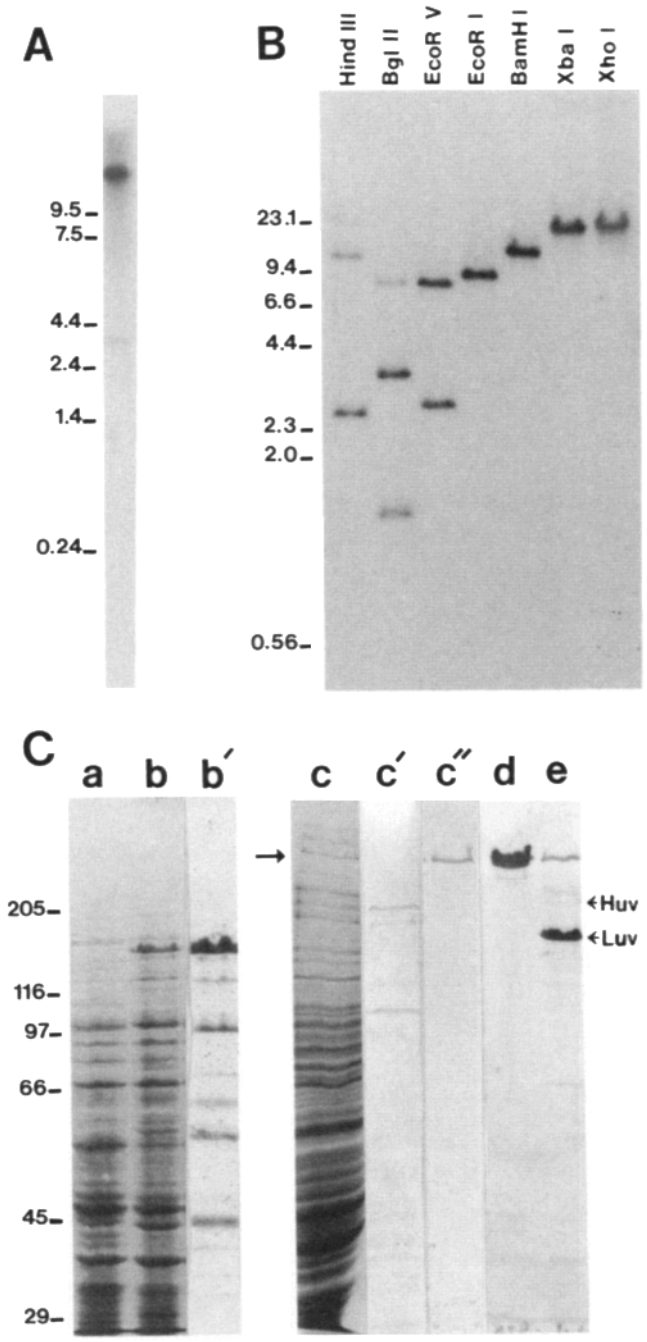

Figure 2. Characterization of the initial cDNA (clone 1). (*A*) Northern blot of *Dictyostelium* total RNA probed with clone 1. (*B*) Southern blot of *Dictyostelium* DNA digested with the enzymes listed on top of the respective lanes. HindIII cuts once in the insert (the faint 23-kb band is due to incomplete digestion) and BglII cuts twice. Two closely spaced EcoRV sites are present in this clone, but the 50-bp internal fragment can not be seen on this blot. (*C*) Immunoblots demonstrating antibody cross-reactivity. Lanes *a* and *b* show Coomassie blue-stained gel lanes of uninduced and induced cultures of the *E coli* containing the expression vector, lane *b′* shows an immunoblot of a sample equivalent to lane *b*, probed with heavy chain-purified antibodies from the original antiserum (Koonce and McIntosh, 1990). The cross-reactive bands beneath the prominent 150-kD band do not appear in blots of uninduced *E. coli*, and probably represent proteolytic fragments of the expressed polypeptide. Lane *c* shows a Coomassie blue-stained gel of a high-speed supernatant from *Dictyostelium*. The *arrow* marks the position of the dynein heavy chain. Lanes *c′* and *c″* show immunoblot replicas of lane *c* probed with the preimmune (1:1,000 dilution, *c′*) and immune serum (1:5,000 dilution, *c″*) from one rabbit immunized with the fusion protein. The second rabbit gave a very similar result. Lanes *d* and *e* show immunoblots with antibodies raised against the clone 1 fusion protein used to stain a blot of ATP extract from a microtubule affinity preparation of *Dictyostelium* protein. Lane *d* shows an unirradiated sample; lane *e* shows a sample that has been irradiated with UV light in the presence of vanadate and ATP. The positions of the cleavage fragments (*Huv* and *Luv*) are indicated.

codons just 5′ of the AUG described above. The putative start methionine is within a consensus sequence which is characteristic of eukaryotes in general (Kozak, 1991), and *Dictyostelium* in particular (Steel and Jacobson, 1991). The final clone (clone 9) was amplified from first-strand cDNA made from *Dictyostelium* RNA by reverse transcription with an anchored oligo dT-primer. It contains three in-frame stop codons, four putative polyadenylation signals, and a long poly(A) tail.

Several properties of the initial clone were analyzed to determine whether it encoded a dynein-like gene product (Fig. 2). Northern blots demonstrated that this clone hybridizes to a ~14-kb RNA (Fig. 2 *A*), a size expected for the heavy chain message. A genomic Southern blot probed with clone 1 suggested that this is a single copy gene in *Dictyostelium* (Fig. 2 *B*). Clone 1 was expressed in bacteria and the resulting polypeptide was used to immunize rabbits. These antisera reacted strongly with the heavy chain from *Dictyostelium* (Fig. 2 *C*), but, unlike the original antiserum, they recognized epitopes found only on the lower molecular weight polypeptide defined by cleavage with UV light, ATP, and vanadate (the "LUV" polypeptide) (Gibbons et al., 1987; Koonce and McIntosh, 1990).

The derived amino acid sequence of the *Dictyostelium* dynein heavy chain gene is shown in Fig. 3. The predicted M_r of this polypeptide is 538,482 D. Like the flagellar β-dynein heavy chains, *Dictyostelium* dynein contains four GXXXXGKT/S sequences clustered in the middle third of the polypeptide. This motif represents the most conserved part of a consensus sequence for ATP-binding domains (Walker et al., 1982; Fry et al., 1986). In contrast to the flagellar dynein, however, the *Dictyostelium* sequence does not contain a fifth consensus site near its NH_2-terminus. This may reflect a functional difference between these two types of dynein.

An alignment of the predicted amino acid sequences for *Dictyostelium* and sea urchin dynein is shown in Fig. 4. Using the UWGCG program BESTFIT (Devereux et al., 1984) with a gap weight of 3.0 and a length weight of 0.1, these two sequences are 51% similar and 28% identical over their entire lengths. However, as is evident in Fig. 4, this similarity is not uniformly distributed. The amino terminal 1,500-amino acids show regions of marginal similarity (19% identical), while the central region and the carboxy terminal 1,500-amino acids are 32% and 29% identical, respectively. The region of lowest sequence similarity occurs at the amino terminal 100–200 amino acids. Antibodies so far raised to the NH_2-terminal region of *Dictyostelium* dynein do not cross-react with other species of cytoplasmic or axonemal dyneins, further defining its individuality (data not shown).

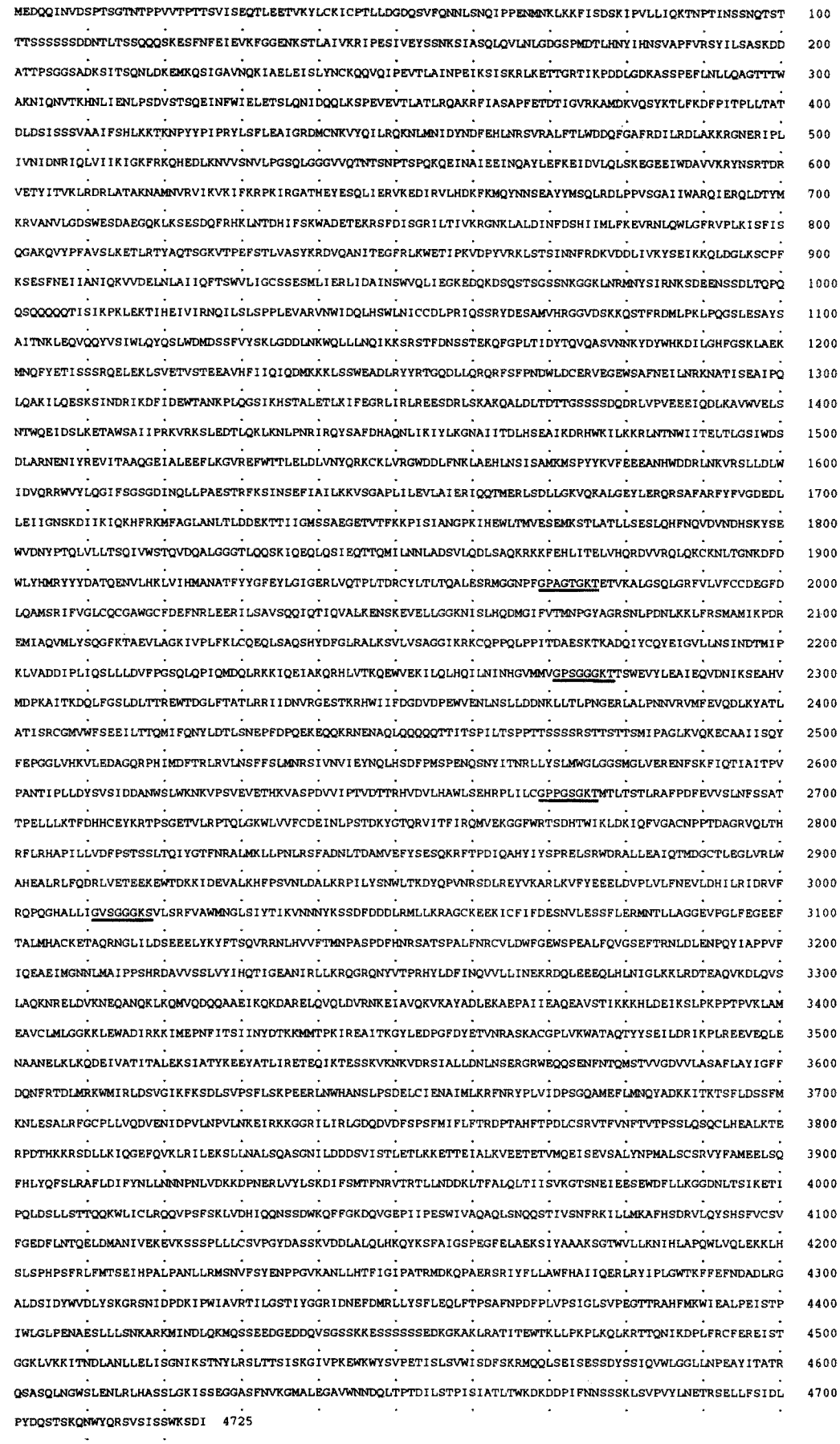

Figure 3. Deduced amino acid sequence of the cytoplasmic dynein heavy chain from *Dictyostelium.* The positions of the four putative ATP-binding consensus sequences are underlined.

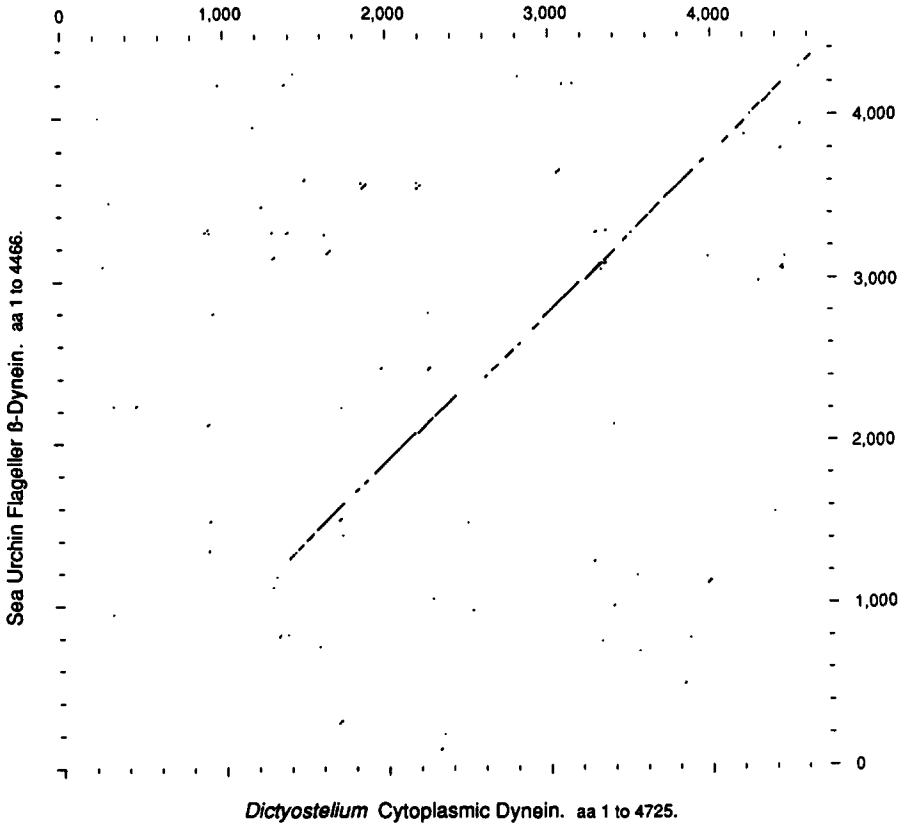

Figure 4. Dot matrix comparison of sequence similarity between the 4,725-amino acid *Dictyostelium* dynein sequence and the 4,466-amino acid sea urchin β heavy chain flagellar sequence from Gibbons et al. (1991). This diagram was constructed with the UWGCG program COMPARE, using a window size of 50 and a stringency of 25. Decreasing the stringency of the comparisons did not further highlight similarities between the two NH_2 termini.

The region of highest similarity between the *Dictyostelium* and sea urchin dynein sequences is in and around the putative ATP-binding consensus sequence GPAGTGKT, which shows 54% identity over 235 amino acids (*Dictyo* aa 1,924–2,159 vs. urchin aa 1,806–2,041). We have expressed a 115-amino acid peptide centered over this ATP-binding motif in *E. coli*. An antiserum raised against this peptide reacts well with dyneins from *Dictyostelium*, a mammalian cell line (HeLa), and sea urchin sperm flagella (data not shown), suggesting that this region is highly conserved among dyneins.

Fig. 5 shows direct comparisons between the two dynein sequences from the regions within and surrounding the four "segment 1" consensus sequences for ATP binding (Fry et al., 1986). Gibbons et al. (1991), have suggested that the GPAGTGKT site is part of the principal hydrolytic ATP-binding site of dynein. Our data, which identify both this motif and its surroundings as regions of high sequence identity to flagellar dynein, support this assertion.

A secondary structure predicted for the *Dictyostelium* dynein sequence is presented in Fig. 6 *A*. Given the size of the amino acid sequence, these results are hard to display, and given the inherent uncertainty in the algorithms that pre-

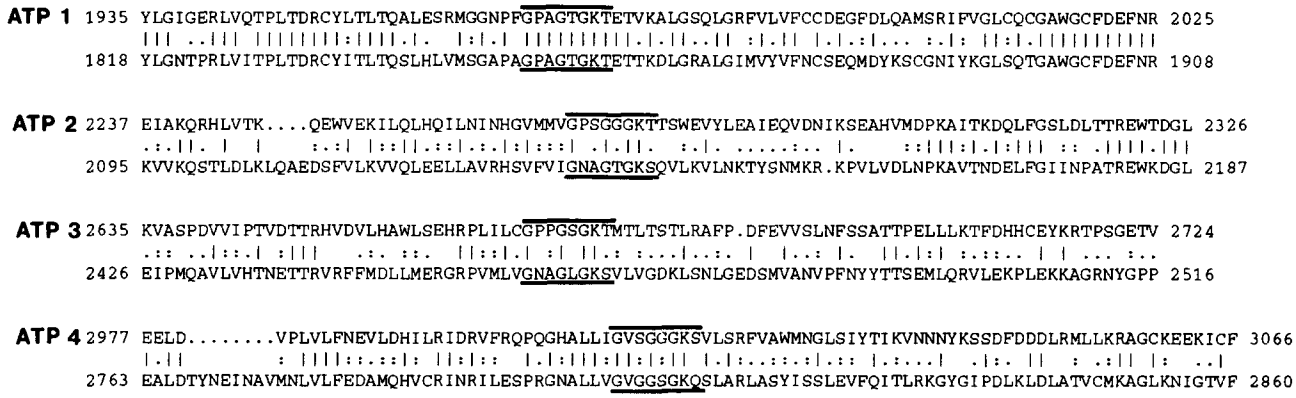

Figure 5. Cytoplasmic and flagellar dynein sequence comparisons of the four regions in and around the putative GXXXXGKT/S ATP-binding consensus sequences. The *Dictyostelium* sequence is shown on the upper line of the comparisons, and the sea urchin β-dynein sequence of Gibbons et al. (1991) is on the lower line. The sequence shown around the first *Dictyostelium* consensus sequence (*ATP 1*) is 64% identical to the corresponding region in the sea urchin sequence; for the second, third, and fourth consensus sequences, the identity is 36, 24, and 37%, respectively.

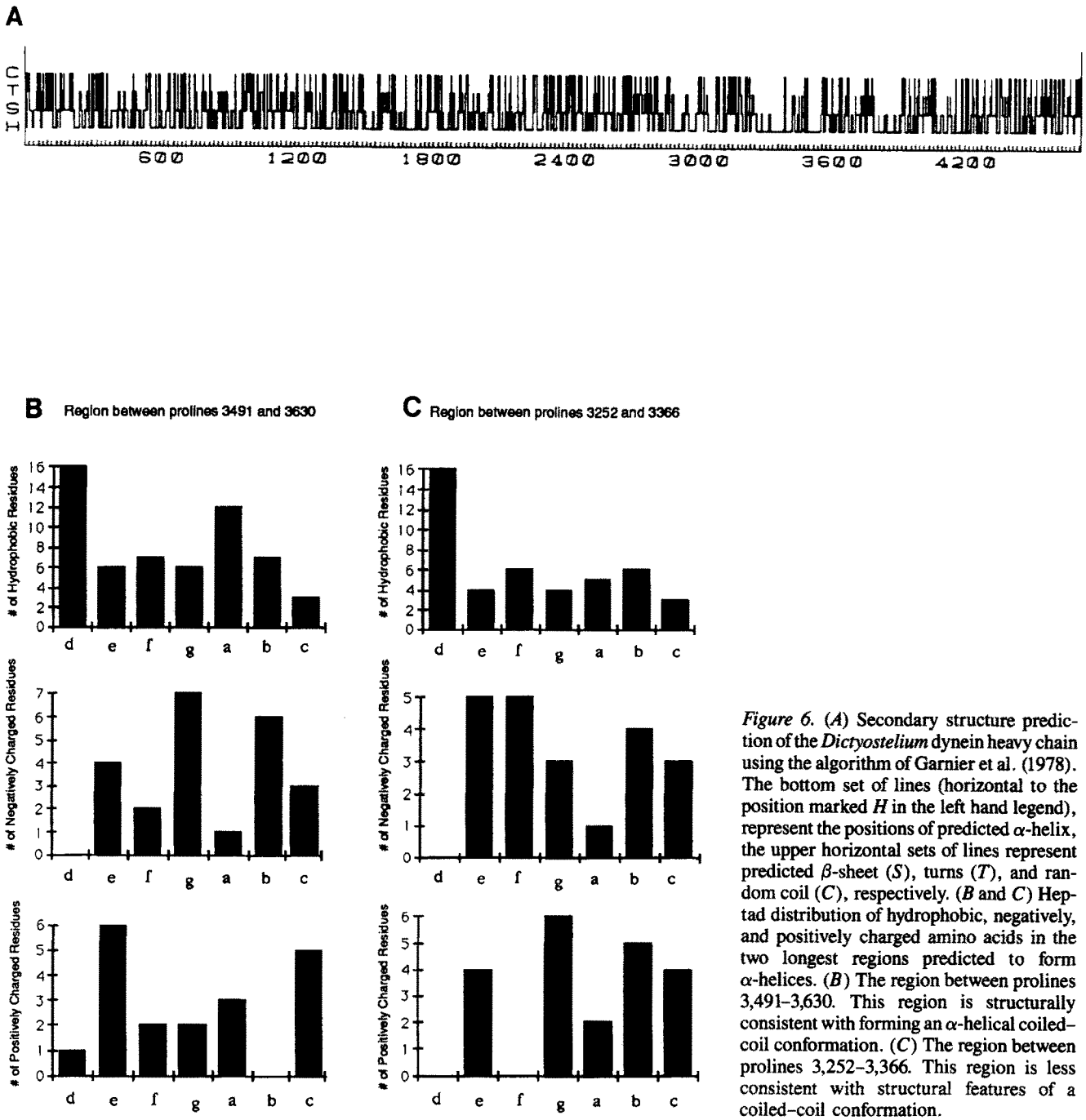

Figure 6. (*A*) Secondary structure prediction of the *Dictyostelium* dynein heavy chain using the algorithm of Garnier et al. (1978). The bottom set of lines (horizontal to the position marked *H* in the left hand legend), represent the positions of predicted α-helix, the upper horizontal sets of lines represent predicted β-sheet (*S*), turns (*T*), and random coil (*C*), respectively. (*B* and *C*) Heptad distribution of hydrophobic, negatively, and positively charged amino acids in the two longest regions predicted to form α-helices. (*B*) The region between prolines 3,491–3,630. This region is structurally consistent with forming an α-helical coiled-coil conformation. (*C*) The region between prolines 3,252–3,366. This region is less consistent with structural features of a coiled-coil conformation.

dict structure, they are admittedly difficult to interpret. Nonetheless, the structural information shown in Fig. 6 *A* is consistent with a mostly globular polypeptide conformation, an observation consistent with most electron microscopic analyses of purified dynein (Warner et al., 1989), and with the structural predictions of the β-dynein heavy chain sequence (Gibbons et al., 1991; Ogawa, 1991).

The two longest regions of predicted α-helix in the *Dictyostelium* sequence occur between prolines 3,252–3,366 (114 amino acids) and prolines 3,491–3,630 (139 amino acids). Because the intact dynein molecule is a complex of several tightly associated polypeptides, and because coiled-coil interactions between α-helices are a fairly common motif in the formation of protein complexes, we tested whether these two regions of predicted α-helix might show diagnostic features of coiled-coil interactions (Fig. 6, *B* and *C*). The "second" region of α-helix (amino acids 3,492–3,629) contains a heptapeptide repeat pattern with an enrichment of hydrophobic amino acid residues at positions *a* and *d*, as well as the periodicity of positively and negatively charged amino acids that is characteristic of a coiled-coil structural conformation (McLachlan and Karn, 1983). (Fig. 6 *B*). The "first" region of α-helix (amino acids 3,253–3,365) shows a strong peak of hydrophobic amino acid residues at position *d*, but there is no second enrichment of hydrophobic residues within the 7-amino acid repeat pattern (Fig. 6 *c*). In addition,

there is no obvious periodicity of the positively and negatively charged residues.

While these types of analyses do not rigorously demonstrate the presence or absence of a coiled-coil structural conformation, at least they provide a suggestion for a more detailed molecular characterization.

Discussion

Using a combination of immunological, DNA hybridization, and PCR-based techniques, we have isolated a gene sequence that encodes a dynein heavy chain from the cellular slime mold *Dictyostelium*. All the portions of this sequence so far tested hybridize to a ~14-kb RNA on Northern blots, and under high stringency conditions for Southern blotting, they recognize a single copy gene in the *Dictyostelium* genome. Bacterially expressed protein for two separate regions of this sequence have elicited antibodies in rabbits that bind with high avidity to the native dynein heavy chain. This sequence contains an open-reading frame of 4,725 amino acids, which shows substantial similarity to the flagellar β-dynein heavy chain (Gibbons et al., 1991; Ogawa, 1991).

Because part of our cloning strategy involved a multistep PCR walk along genomic DNA, we were concerned about two possible artifacts: jumping between related dynein sequences, and including sequence from introns. Three kinds of data dispel the first concern: (*a*) whenever adjacent clones overlapped, they contained identical nucleotide sequence; (*b*) all clones, except numbers 8 and 9, were used to probe Southern blots, and, under high stringency conditions, all blots were consistent with a single copy gene in this organism (data not shown); and (*c*) all the restriction sites identified by genomic Southern blotting were found in their predicted order and spacing in the final sequence. In addition, preliminary Southern blot analysis under low stringency conditions suggests that *Dictyostelium* contains only a single dynein gene (data not shown). The concern about introns can also be dismissed. In *Dictyostelium*, introns are believed to be small (~100 bp), infrequent, highly A/T rich (95%), and bounded by conserved splice sites (Kimmel and Firtel, 1982); the clones presented here do not contain sequence with these characteristics.

Because of the small size of the *Dictyostelium* genome (~50,000 kb; Kimmel and Firtel, 1982), the scarcity of introns in this organism, and the various levels of selection in the procedure, the PCR-based technique we have adapted was a rather quick and efficient method to walk in a given direction along a chromosome. One might expect this procedure to be a useful alternative in cloning the 5′ and 3′ nontranscribed regions of genes in organisms with genomes similar to *Dictyostelium*.

With the exception of the two flagellar dyneins (Gibbons et al., 1991; Ogawa, 1991), the sequence reported here shows no substantial similarity to any other proteins or nucleic acids in the GenBank, NBRF, or SwissProt databases, including detailed comparisons we have made with myosin (Warrick et al., 1986), kinesin (Yang et al., 1989), NCD (Endow et al., 1990; McDonald and Goldstein, 1990), and dynamin (Obar et al., 1990). The COOH-terminal two-thirds of the flagellar and cytoplasmic dynein heavy chains are rather similar (32% identical), suggesting that this region comprises the essential structural and mechanochemical domains of a dynein molecule. In contrast, the low degree of similarity at the amino terminus and the first one-third of the coding sequence, suggests that this region may define some of the important functional differences between cytoplasmic and axonemal dyneins, such as sites for vesicle or kinetochore binding, or for the ATP-insensitive binding to microtubules which is characteristic of the axonemal forms (Warner et al., 1989; Gibbons, 1988; Vallee and Shpetner, 1990).

Both biochemical and ultrastructural data suggest that cytoplasmic and flagellar dyneins comprise two (or three) heavy chains and various smaller molecular weight polypeptides (Warner et al., 1989; McIntosh and Porter, 1989; Vallee et al., 1988). The heavy chains of two other mechanochemical proteins myosin II and kinesin, form dimers, at least in part, through coiled-coil interactions in their rod domains (McLachlan and Karn, 1983; de Cuevas et al., 1992). Whether the dynein heavy chains associate through a similar interaction is not known. A prediction of secondary structural features in the *Dictyostelium* sequence suggests that there are only two regions of α-helix greater than 100 amino acids, both following the fourth ATP-binding consensus sequence. Both of these regions show some degree of similarity (~17% identity) to a portion of the rod domain of several myosin heavy chains and to several regions within the kinesin rod domain. Amino acid heptad repeats that are characteristic of coiled-coil structure (McLachlan and Karn, 1983) can also be found within at least one of these regions. A similar structural motif in approximately the same position has been reported for the flagellar β-dynein sequences (Gibbons et al., 1991; Ogawa, 1991). This region may reflect a conserved feature of dynein, and might be important for the tertiary and/or quarternary structure of the native molecule, either by providing some three-dimensional folding information or through association with another heavy chain or conserved accessory protein.

We are now working with this dynein sequence in an attempt to map important functional domains and to develop an understanding of dynein's role in mitosis or other cellular motile processes.

A number of people provided invaluable technical expertise. In particular, we wish to thank Tom Hays, John Tamkun, George Golumbeski, and Marc Perry for their help in answering our infinite questions. Rex Chisholm, Michiel Van Lookeren Champagne, and Richard Kessin kindly provided cDNA libraries. We also thank all the members of the McIntosh Lab, in particular Corey Nislow, Vivian Lombillo, Eugeni Vaisberg, and Bonnie Neighbors for their help and support.

This work was supported in part by grants from the National Institutes of Health (GM36663 to J. R. McIntosh) and by the Damon Runyon-Walter Winchell Cancer Research Foundation (DRG-980 to M. P. Koonce). The sequence data reported here (which represents 0.03% of the *Dictyostelium* genome) are available from EMBL/GenBank/DDJB under accession number Z15124.

Received for publication 3 September 1992 and in revised form 18 September 1992.

References

de Cuevas, M., T. Tao, and L. S. B. Goldstein. 1992. Evidence that the stalk of *Drosophila* kinesin heavy chain is an α-helical coiled coil. *J. Cell Biol.* 116:957–965.

Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acid Res.* 12:387–395.

Endow, S. A., S. Henikoff, and L. Soler-Niedziela. 1990. Mediation of meiotic and early mitotic chromosome segregation in *Drosophila* by a protein related to kinesin. *Nature (Lond.).* 345:81–83.

Foltz, K.R., and D. J. Asai. 1988. Ionic strength-dependent isoforms of sea urchin egg dynein. *J. Biol. Chem.* 263:2878–2883.

Fry, D. C., S. A. Kuby, and A. S. Mildvan. 1986. ATP binding site of adenylate kinase: Mechanistic implications of its homology with *ras*-encoded p21, F_1-ATPase, and other nucleotide-binding proteins. *Proc. Natl. Acad. Sci. USA.* 83:907–911.

Garnier, J., D. J. Osgunthorpe, and B. Robson. 1978. Analysis of the accuracy of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* 120:97–120.

Gibbons, I. R. 1988. Dynein ATPases as microtubule motors. *J. Biol. Chem.* 263:15837–15840.

Gibbons, I. R., A. Lee-Eiford, G. Mocz, A. Phillipson, W.-J. Y. Tang, and B. H. Gibbons. 1987. Photosensitized cleavage of dynein heavy chains. *J. Biol. Chem.* 262:2780–2786.

Gibbons, I. R., B. H. Gibbons, G. Mocz, and D. J. Asai. 1991. Multiple nucleotide-binding sites in the sequence of dynein β heavy chain. *Nature (Lond.).* 352:640–643.

Grissom, P. M., M. E. Porter, and J. R. McIntosh. 1992. Two distinct isoforms of sea urchin egg dynein. *Cell Motil. Cytoskel.* 21:281–292.

Kimmel, A. R., and R. A. Firtel. 1982. The organization and expression of the *Dictyostelium* genome. In The Development of *Dictyostelium discoideum.* W. F. Loomis editor. Academic Press, New York. 233–324.

Koonce, M. P., and J. R. McIntosh. 1990. Identification and immunolocalization of cytoplasmic dynein in *Dictyostelium. Cell Motil. Cytoskel.* 15:51–62.

Kozak, M. 1991. An analysis of vertebrate mRNA sequences: Intimations of translational control. *J. Cell Biol.* 115:887–903.

McDonald, H. B., and L. S. B. Goldstein. 1990. Identification and characterization of a gene encoding a kinesin-like protein in *Drosophila. Cell.* 61:991–1000.

McLachlan, A. D., and J. Karn. 1983. Periodic features in the amino acid sequence of nematode myosin rod. *J. Moll Biol.* 164:605–626.

McIntosh, J. R., and C. M. Pfarr. 1991. Mitotic motors. *J. Cell Biol.* 115:577–585.

McIntosh, J. R., and M. E. Porter. 1989. Enzymes of microtubule-dependent motility. *J. Biol. Chem.* 264:6001–6004.

Mitchison, T. J. 1988. Microtubule dynamics and kinetochore function in mitosis. *Annu. Rev. Cell Biol.* 4:527–550.

Obar, R. A., C. A. Collins, J. A. Hammarback, H. S. Shpetner, and R. B. Vallee. 1990. Molecular cloning of the microtubule-associated mechanochemical enzyme dynamin reveals homology with a new family of GTP-binding proteins. *Nature (Lond.).* 347:256–261.

Ogawa, K. 1991. Four ATP binding sites in the midrange of the β heavy chain. *Nature (Lond.).* 352:643–645.

Pfarr, C. M., M. Coue, P. M. Grissom, T. S. Hays, M. E. Porter, and J. R. McIntosh. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature (Lond.).* 345:263–265.

Porter, M. E., P. M. Grissom, J. M. Scholey, E. D. Salmon, and J. R. McIntosh. 1988. Dynein isoforms in sea urchin eggs. *J. Biol. Chem.* 263:6575–6581.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger, F., S. Miklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463–5467.

Sawin, K. E., and J. M. Scholey. 1991. Motor proteins in cell division. *Trends Cell Biol.* 1:122–129.

Schroer, T. A., and M. P. Sheetz. 1991. Functions of microtubule-based motors. *Annu. Rev. Physiol.* 53:629–652.

Steel, L.F., and A. Jacobson. 1991. Sequence elements that affect mRNA translational activity in developing *Dictyostelium* cells. *Dev. Genet.* 12:98–103.

Steuer, E. R., L. Wordeman, T. A. Schroer, and M. P. Sheetz. 1990. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature (Lond.).* 345:266–268.

Studier, F. W., A. H. Rosenerg, J. J. Dunn, and J. W. Dubendorf. 1990. Use of T7 RNA polymerase to direct the expression of cloned genes. *Methods Enzymol.* 185:60–89.

Vallee, R. B., and H. S. Shpetner. 1990. Motor proteins of cytoplasmic microtubules. *Annu. Rev. Biochem.* 59:909–923.

Vallee, R. B., J. S. Wall, B. M. Paschal, and H. S. Shpetner. 1988. Microtubule-associated protein 1C from brain is a two-headed cytosolic dynein. *Nature (Lond.).* 332:561–563.

Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold *EMBO (Eur. Mol. Biol. Organ). J.* 1:945–951.

Warner, F. D., P. Satire, and I. R. Gibbons, editors. 1989. Cell Movement. Vol. 1. Alan R. Liss, New York. 337 pp.

Warrick, H. M., A. De Lozanne, L. Leinwand, and J. A. Spudich. 1986. Conserved protein domains in a myosin heavy chain gene from *Dictyostelium discoideum. Proc. Natl. Acad. Sci. USA* 83:9433–9437.

Wordeman, L. G., E. Steuer, M. P. Sheetz, and T. J. Mitchison. 1991. Chemical subdomains within the kinetochore domain of isolated CHO mitotic chromosomes. *J. Cell Biol.* 114:285–294.

Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analysis. *Cell.* 56:879–889.