

Sequence analysis of the *Chlamydomonas* alpha and beta dynein heavy chain genes

David R. Mitchell* and Kimberly S. Brown

Department of Anatomy and Cell Biology, and Program in Cell and Molecular Biology, SUNY Health Science Center, 750 E. Adams St, Syracuse, NY 13210, USA

*Author for correspondence

SUMMARY

We have sequenced genomic clones spanning the complete coding region of one heavy chain (beta) and the catalytic domain of a second (alpha) of the *Chlamydomonas reinhardtii* flagellar outer arm dynein ATPase. The beta heavy chain gene (*ODA-4* locus) spans 20 kb, is divided into at least 30 exons, and encodes a predicted 520 kDa protein. Comparison with sea urchin beta dynein sequences reveals homology that extends throughout both proteins. Over the most conserved central catalytic region, the *Chlamydomonas* alpha and beta chains are equally divergent from the sea urchin beta chain (64% and 65% similarity, respectively), whereas the *Chlamydomonas* gamma chain is more divergent from urchin beta (54% similarity). The four glycine-rich loops identified as potential nucleotide-binding

sites in other dynein heavy chains are also present in *Chlamydomonas* alpha and beta dyneins. Two of these four nucleotide-binding motifs are highly conserved among flagellar dyneins, but only the motif previously identified as the catalytic site in sea urchin dynein is highly conserved between flagellar and cytoplasmic dynein heavy chains. Predictions of secondary structure suggest that all dynein heavy chains possess three large domains, with the four nucleotide-binding consensus sequences located in a central 185 kDa domain that is bounded on both sides by regions that form multiple, short alpha-helical coiled-coils.

Key words: flagellar dynein, *Chlamydomonas*, dynein heavy chain

INTRODUCTION

Dyneins have been implicated in a variety of microtubule-associated movements, including vesicle transport, mitosis, cellular organization of membranous organelles, and flagellar motility (McIntosh and Porter, 1989; Porter and Johnson, 1989; Witman, 1992; Vallee, 1991; Corthésy-Theulaz et al., 1992). These large ATPase complexes typically consist of two or three ~500 kDa catalytic subunits, and as many as twelve smaller proteins. The mechanism of dynein force production remains poorly understood, in part because the large size of these molecules has prevented rapid application of the molecular approaches that have proven useful in other systems. Recent sequence analysis has shown that cytoplasmic and flagellar dynein heavy chains are similar to each other, but not to any other motor enzymes, and that cytoplasmic dynein heavy chains from *Dictyostelium* and rat brain are more similar to each other than either is to a sea urchin sperm flagellar dynein heavy chain (Gibbons et al., 1991; Ogawa, 1991; Koonce et al., 1992; Mikami et al., 1993). Dyneins clearly represent an entirely new class of ATPase motors, but sequence analysis of additional family members will be required to determine the full range of variability within this family, and to pinpoint conserved sequences and structures important to the force generating mechanism.

Based on the available sequences, all dyneins contain four

nucleotide-binding consensus sequences near the center of each molecule. These motifs conform to the nucleotide-binding A-site (P-loop) consensus (GXXGXGK, where G is glycine, K is lysine, and X indicates any amino acid), which forms a glycine-rich loop thought to bind to nucleotide phosphates in many adenine and guanine nucleotide metabolizing enzymes (Walker et al., 1982; Saraste et al., 1990). Biochemical studies with sea urchin dyneins suggest that only one of these sites, that closest to the N terminus, functions as a catalytically active ATPase (Gibbons et al., 1991). Functions of the remaining three sites have not been determined, but their comparatively limited sequence conservation, at least between flagellar and cytoplasmic dyneins, has left some doubt as to their significance.

Secondary structure predictions based on dynein heavy chain sequences have provided minimal correlations with the known tertiary structures of these molecules. Although dyneins appear in electron micrographs as globular particles attached to elongated tails (Goodenough and Heuser, 1984), unlike kinesin and sarcomeric myosin, dyneins lack extensive regions of primary sequence predicted to form rod-like secondary structures (Gibbons et al., 1991; Koonce et al., 1992). Based on biochemical analyses, however, models have emerged in which the N-terminal segment forms the tail and attaches to the load (outer doublet A-tubule for flagellar dyneins, vesicle for cytoplasmic dyneins), while the central segment spanning

the catalytic site forms the globular mechanochemical motor domain (Gibbons et al., 1991; Witman, 1989). Since the greatest differences between flagellar and cytoplasmic dynein heavy chain sequences are located in the N-terminal third of each molecule (Mikami et al., 1993), these differences might represent evolutionary adaptations that allow heavy chains to carry different loads. Until now, however, no other flagellar dynein heavy chain sequences were available to compare with urchin beta dynein, so that the divergence of this N-terminal region among flagellar dyneins, all of which attach to doublet microtubules, could not be determined.

To increase the number of flagellar dynein sequences available for comparisons, and to expand our ability to use site-directed mutagenesis and transformation to analyze flagellar dynein function, we have begun a sequence analysis of dynein heavy chain genes from the green alga *Chlamydomonas reinhardtii*. The ability to select mutations in dynein genes, and to re-introduce and express those genes through DNA-mediated transformation, have made *Chlamydomonas* a valuable organism for molecular studies of flagellar dynein structure and function. Most of the genetic and biochemical studies of *Chlamydomonas* dyneins have been directed at the outer row dynein arms, which contain alpha, beta, and gamma heavy chains, as well as two intermediate chains of 78 kDa and 70 kDa and several peptides of lower molecular mass (Piperno and Luck, 1979; Pfister et al., 1982). Mutations mapping to at least thirteen loci affect outer arm dynein assembly or function (Kamiya, 1988; Sakakibara et al., 1991), and among them are mutations that have been localized to the alpha heavy chain (*oda-11*; Sakakibara et al., 1991), beta heavy chain (*oda-4*; Kamiya, 1988), gamma heavy chain (*oda-2*; Wilkerson et al., 1994), and 70 kDa intermediate chain (*oda-6*; Mitchell and Kang, 1991) loci. All three outer arm heavy chains (Mitchell, 1989; Wilkerson et al., 1994) and both intermediate chains (Williams et al., 1986; Mitchell and Kang, 1991; King et al., 1992) have now been cloned, and molecular studies of intermediate chain structure and function have been reported (Mitchell and Kang, 1991, 1993; King et al., 1992). Similar studies with the catalytic subunits should be possible now that heavy chain sequence information is available (Wilkerson et al., 1994, and this paper).

Our sequencing efforts have been focused on the alpha and beta genes because phenotypes of mutations at each locus suggest that these two heavy chains have distinct roles in outer arm function. Whereas the *oda-11* mutation only blocks assembly of the alpha subunit, and has a barely perceptible effect on flagellar beat frequency (Sakakibara et al., 1991), most mutations in the beta dynein gene (*oda-4* locus) completely block assembly of outer row dynein arms, and reduce beat frequency by about 60% (Kamiya, 1988). Other less drastic beta chain mutations do not block outer arm assembly, but still reduce beat frequency by up to 55% (Sakakibara et al., 1993). The *sup-pf-1* mutation, which acts as a by-pass suppressor of dynein regulation by the radial spoke system, is also a beta dynein allele (Huang et al., 1982). Mutations that prevent assembly of radial spoke or central pair structures block motility, but *sup-pf-1* restores motility to these flagella without restoring the missing structures, suggesting that beta dynein is a specific target of regulatory signals from the central pair/radial spoke system. Although isolation of radial spoke mutant suppressors has generated multiple *sup-pf-1* alleles at

the beta dynein locus (as well as mutations at several other loci), no suppressors have been isolated at the alpha or gamma loci (Luck and Piperno, 1989; Huang et al., 1982; Piperno et al., 1992), further supporting a unique role for beta dynein in outer arm function.

Here we report the nucleotide and predicted amino acid sequence of the *Chlamydomonas* flagellar dynein beta heavy chain, and the most conserved central catalytic domain of the alpha heavy chain. Our results expand previous comparisons of flagellar and cytoplasmic dynein heavy chains to reveal sequences that may be common to all dynein motors and sequences that may subserve flagellar-specific functions. Further comparisons highlight regions that have diverged through speciation and through functional differentiation within an enzyme complex.

MATERIALS AND METHODS

Overlapping genomic clones spanning the complete beta dynein gene and part of the alpha dynein gene (Mitchell, 1989) were subcloned into phagmids pBluescript (Stratagene) or pEMBL (Dente et al., 1983), and each subcloned segment was sequenced from both orientations using a nested deletion strategy (Erase-a-base, Promega) and di-deoxy sequencing with Sequenase (United States Biochemicals) of rescued single-stranded DNA. Sequencing primers (Genosys) were used to complete some regions.

cDNA clones were selected from a *Chlamydomonas* gametic λ ZAP cDNA library (J. Woessner, Washington University) by hybridization screening with genomic beta clone B4.2a (Fig. 1) using standard methods (Sambrook et al., 1989). A single plaque-purified cDNA clone, cB10-1, was released as a plasmid by super-infection with helper phage and the insert was sequenced from both ends.

To correctly assign intron/exon junctions in genomic sequences, each reading frame was scanned for the presence of regions of codon bias typical of *Chlamydomonas* nuclear genes, using the GCG Codon-preference program and a codon usage table compiled with Codon-frequency from the coding regions of 35 different *Chlamydomonas* sequences, including the gene for another outer arm dynein subunit. This table is available from the authors on request.

To clarify ambiguous splice junctions in the beta dynein gene, RT-PCR (Kawasaki, 1990) was used to amplify selected regions of beta dynein mRNA. For these reactions, 10 μ g of total RNA isolated from deflagellated vegetative cells (Williams et al., 1986) was reverse-transcribed in a total reaction volume of 24.5 μ l containing 500 units of Superscript reverse transcriptase (BRL), 80 units RNasin (Promega Biotec), 100 pmole of random hexamers, 0.8 mM each deoxynucleotide, 3 mM MgCl₂, 8 mM DTT, 75 mM KCl and 50 mM Tris, pH 8.0, for 10 minutes at room temperature, 60 minutes at 42°C and 30 minutes at 52°C. After heat-inactivation for 10 minutes at 95°C, 10 μ l of the reverse transcriptase reaction was mixed with 5 μ l (50 pmole) of each gene-specific primer, 9 μ l 10 \times Taq polymerase buffer and 5 units Taq polymerase (Boehringer Mannheim Biochemicals) in a total volume of 100 μ l, and amplified in an MJ Research Minicycler with the following program: 2 minutes initial denaturation at 95°C, 20 cycles of 1 minute at 94°C, 1 minute at 65°C (decreasing by 0.5°C per cycle) and 1 minute at 72°C, and 15 cycles of 1 minute at 94°C, 1 minute at 50°C and 1 minute at 72°C followed by a final 10 minute extension at 72°C. Three primer pairs were designed to amplify sequences between nt 536 and 822, nt 3786 and 4338, and nt 6366 and 6848 of the beta dynein gene sequence. In each case a single amplification product was observed. Amplified fragments were cloned with a TA cloning kit (Invitrogen) and for each primer pair clones from at least two RT-PCR reactions were sequenced.

Sequence assembly and most sequence analysis employed the GCG

suite of molecular biology programs (Devereux et al., 1984). Regions of predicted coiled-coil secondary structure were computed with the program Newcoils (Lupas et al., 1991), transferred to a MacIntosh IICI, and plotted using Kaleidograph.

RESULTS

Nucleotide sequence

Fragments of the *Chlamydomonas* alpha and beta dynein genes were selected previously from a λgt11 expression library with monoclonal antibodies (Williams et al., 1986; Mitchell, 1989) and used to clone genomic regions spanning each gene (Mitchell, 1989). We have now determined the sequence of the clones diagrammed in Fig. 1, which span the entire beta gene (20258 bp) and the central catalytic domain of the alpha gene (10141 bp).

Because *Chlamydomonas* genes contain many introns, prediction of amino acid sequences required correct assignments of intron/exon junctions. Three criteria were used to assign splice junctions. First, each reading frame was scanned for the presence of regions of codon bias typical of *Chlamydomonas* nuclear genes, as described in Materials and Methods. Since *Chlamydomonas* has a well-recognized codon bias (Williams et al., 1989), and the dynein heavy chain genes retain this bias (Fig. 2), such crude exon determinations are simple and reliable. Second, splice donor and acceptor sequences were identified, based on splice junction consensus sequences compiled from other, fully characterized *Chlamydomonas* genes, and on the necessity to maintain the correct reading frame across exon boundaries. In most cases unambiguous choices could be made, and the resulting amino acid sequences could be aligned with corresponding sequences of other dynein heavy chains with no gaps at intron locations. Finally, for three beta dynein introns, the presence of multiple donor and/or acceptor sites required direct analysis of mRNA for correct splice junction assignment. Regions of beta dynein mRNA spanning each junction were reverse-transcribed, amplified with specific primers (indicated by inverted arrowheads in Fig.

1), and sequenced to identify the correct splice sites. The sequence of each fragment was identical to previously determined beta dynein genomic sequences except for the absence of introns. By comparing the sequence of cDNA clone cB10-1 with the corresponding genomic region, we found no introns in the 3' untranslated region; the presence and location of introns in the 5' untranslated region have not been determined. Protein coding regions confirmed directly by cDNA sequence are indicated by asterisks in Fig. 3.

A summary of the intron/exon structure, and other identified sequence features of each gene, is presented in Fig. 1. In all, 29 introns were identified within the beta dynein coding region, and 13 introns were present in the alpha dynein sequences analyzed. Intron sizes ranged from 67 to 432 nt (av. 199 nt). A beta dynein transcription initiation site has not been located, but is probably included in the sequenced region, since northern blot analyses show that adjacent clones extending 10 kb 5' to the sequenced region fail to hybridize to dynein mRNA (Mitchell, 1989). Because *Chlamydomonas* flagellar protein genes often lack a consensus TATA-like promoter element (Williams et al., 1989; Curry et al., 1992; Mitchell and Kang, 1993), we were unable to identify the beta dynein promoter or initiation site by inspection.

Assignment of the presumed translation start site for beta dynein at nt 536 was based on the presence of an ATG codon at the beginning of a coding segment (as determined with Codonpreference), and the absence of additional coding segments within several hundred nucleotides in the 5' direction. Another potential initiation site occurs at nt 554, but the surrounding sequence conforms less well to a translation initiation site consensus compiled from other *Chlamydomonas* nuclear gene transcripts. Beta dynein translation terminates at a TAA codon at nt 19520, which is followed by two copies of a *Chlamydomonas* cleavage and polyadenylation signal sequence, TGTAAG, at positions 20002 and 20049. These two sites occur within a 50 bp tandem duplication. The first of these sites is used in vivo, with poly A addition beginning 12 nt 3' to the TGTAAG signal in cDNA clone cB10-1.

Other notable nucleotide sequence features include direct

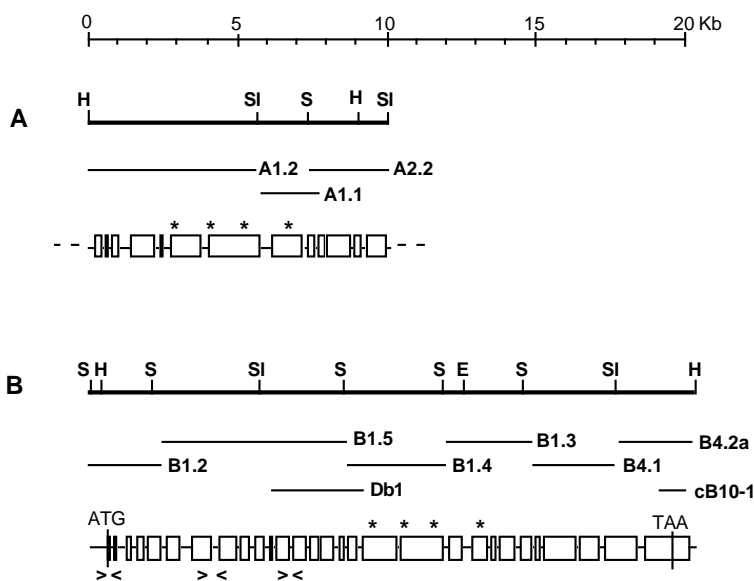


Fig. 1. Restriction maps of sequenced regions of the (A) alpha and (B) beta dynein loci are shown above lines that indicate the regions contained in each plasmid clone used for sequence determination, and representations of major sequence features of each gene. All clones are fragments of λEMBL4 genomic inserts except Db1, which was immunoselected from a λgt11 library (Williams et al., 1986), and cB10-1, which was selected from a λZAP cDNA library. Indicated nucleotide sequence features include exons (open boxes), sites of translation initiation (ATG) and termination (TAA), regions encoding the four nucleotide-binding consensus sequences (GK sites) of each protein (*), and the location of primers used for mRNA amplifications (arrowheads).

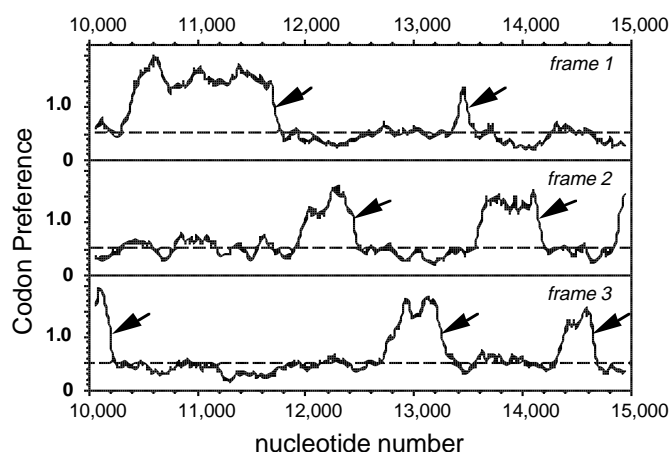


Fig. 2. Graphic output from the program Codonpreference demonstrating the identification of coding regions within the beta dynein sequence. For each coding frame, regions above the dashed line (arrows) span sequences with high coding probability. The program was run with a *Chlamydomonas*-specific codon usage table.

tandem repeats of 45 bp within an intron (nt 11759-11854), and of 20 bp located 5' to the coding region (nt 160-200) of beta dynein. A string of fifty tandem repeats of the dinucleotide GT were observed within another beta dynein intron (nt 12549-12639). The functional significance of these repetitive elements is not known, but genomic Southern blots probed with fragments containing the GT repeat identify a very large number of such elements in the *Chlamydomonas* genome (data not shown).

Sequences encoding nucleotide-binding consensus sites (GK sites; see below), marked with * in Fig. 1, occur at similar exon locations in alpha and beta genes. However, no exact conservation of intron locations could be seen when either nucleotide or amino acid sequences were aligned, even in regions where amino acid sequences were highly similar.

Predicted amino acid sequence

The predicted amino acid sequence of *Chlamydomonas* beta dynein (Fig. 3) spans 4568 amino acids and has a calculated molecular mass of 519,968. Although this calculated size is considerably larger than the size of 440 kDa determined by SDS-PAGE (King and Witman, 1987), similar discrepancies have been noted for other dynein heavy chains (Gibbons et al., 1991). A search for known functional motifs revealed four regions that conform to the nucleotide-binding consensus, GXXGXGK, of Walker et al. (1982). For simplicity, and to avoid the implication that all four are functional nucleotide- and/or phosphate-binding sites, we have designated these sites GK1-GK4. All four sites (underlined in Fig. 3) occur in the middle third of the sequence and correspond in location to similar sites previously identified in both cytoplasmic (Koonce et al., 1992; Mikami et al., 1993) and flagellar (Gibbons et al., 1991; Ogawa, 1991) dynein heavy chain sequences.

The sequence of genomic λ gt11 clone Db1, which was selected by screening an expression library with monoclonal antibody C11.13 (Mitchell and Rosenbaum, 1986), shows that the fusion protein recognized by this antibody contains the 32 amino acid sequence indicated by double underlines in Fig. 3.

The Db1 sequence continues into an intron and encodes an additional 43 amino acids before reaching a stop codon. When tryptic digests of salt-extracted dyneins were probed on western blots with C11.13 and with antibody 18betaC (King and Witman, 1988a), both antibodies reacted with a common 20 kDa fragment (data not shown). Our location for this epitope, approximately 125 kDa from the amino terminus, is consistent with previous maps of the location of the 18betaC epitope (King and Witman, 1988a).

Direct dot-matrix comparisons between this *Chlamydomonas* flagellar dynein and two previously sequenced dynein genes (Fig. 4) reveals similarity extending throughout the sequences. Comparison with a flagellar beta dynein from the sea urchin *Tripneustes gratilla* (Fig. 4A) shows that the greatest sequence divergence occurs within the N-terminal third of these proteins. A similar distribution of sequence divergence is revealed by a less stringent comparison with *Dictyostelium* cytoplasmic dynein (Fig. 4B). The C-terminal 700 amino acids of this cytoplasmic dynein retain only a low level of similarity, while the N-terminal 1200 amino acids show almost no significant similarity to flagellar heavy chains.

To provide a broader basis of comparison among dynein catalytic domains, a portion of the *Chlamydomonas* alpha dynein gene that encodes the most highly conserved central region was sequenced (Fig. 1A). Exons from this sequence encode a 2404 amino acid fragment of alpha dynein (Fig. 5) which also contains four recognizable GK sites, and is homologous to amino acids 1340-3710 of *Chlamydomonas* beta dynein. Comparisons of this domain of the *Chlamydomonas* alpha and beta chains with homologous regions of other dynein heavy chains using the GCG program Distances indicate that these two algal heavy chain catalytic regions are nearly equally divergent from other dyneins (Table 1). In particular, alpha and beta are equally divergent (within 1%) from the sea urchin beta chain, while the *Chlamydomonas* gamma chain is a more distant relative of urchin beta. Although the similarity between beta and sea urchin beta dynein is slightly higher than that between alpha and the sea urchin sequence, as confirmed by parsimony analysis, neither *Chlamydomonas* heavy chain can be designated a close homolog of this urchin subunit.

Alignment of the four GK sites of the *Chlamydomonas* alpha and beta heavy chains with the GK sites of a cytoplasmic dynein and sea urchin beta dynein (Fig. 6) shows that GK1 is

Table 1. Percent sequence similarity between central catalytic domains of dynein heavy chains

Chain	Cre a	Cre b	Cre g	Tgr b	Dicty
Cre a	—				
Cre b	60.4	—			
Cre g	52.0	53.1	—		
Tgr b	64.3	64.9	54.4	—	
Dicty	47.3	47.7	48.3	50.1	—
Rat	47.4	47.8	48.2	50.5	75.0

Pair-wise comparisons between homologous regions of each heavy chain were made with the GCG program Distances using default settings. Cre a, *Chlamydomonas reinhardtii* alpha dynein partial sequence, residues 1-2404; Cre b, *Chlamydomonas* beta dynein residues 1339-3708; Cre g, *Chlamydomonas* gamma dynein residues 1245-3640; Tgr b, *Tripneustes gratilla* beta dynein residues 1277-3631; Dicty, *Dictyostelium* cytoplasmic dynein residues 1378-3858; Rat, rat brain cytoplasmic dynein residues 1320-3775.

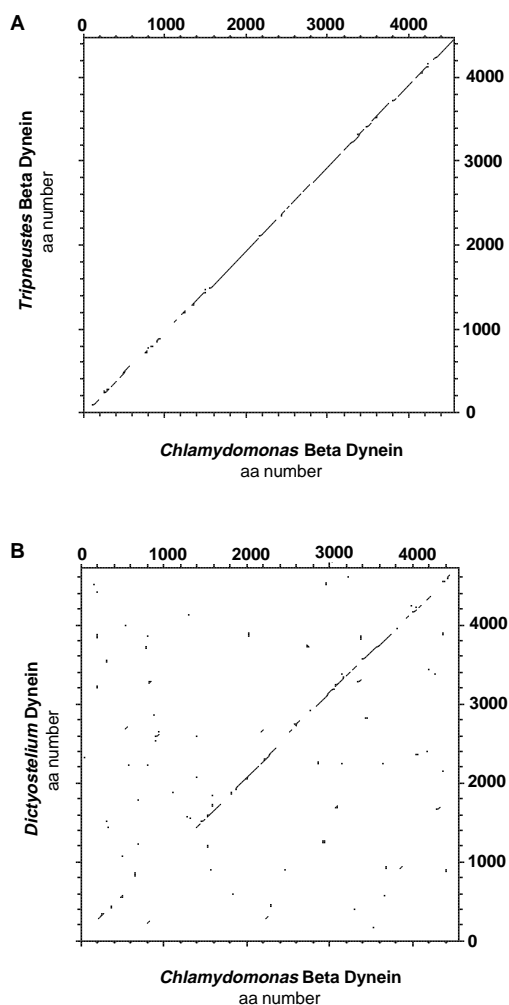


Fig. 4. Dot-matrix analyses of amino acid sequence similarities between (A) *Chlamydomonas* beta dynein and *Tripneustes* (sea urchin) beta dynein, and (B) *Chlamydomonas* beta dynein and *Dictyostelium* (slime mold) cytoplasmic dynein. Plots were generated using Compare with a window of 50 residues and a stringency setting of either 35 (A), or 22 (B). aa, amino acid.

the most highly conserved of these motifs. In addition to the 10 conserved residues around the GK1 site, two other completely conserved blocks of six and nine residues are found in this region, whereas no stretches of more than four consecutive conserved residues are found surrounding any of the other three GK sites. However, if comparison is limited to GK sites of the three flagellar dynein sequences, GK4 is seen to be nearly as conserved as GK1 (16 consecutive identical amino acids) whereas GK2 and GK3 are poorly conserved. Greatest divergence is seen in *Chlamydomonas* alpha dynein GK2, where the terminal lysine (K) has been replaced by an arginine (R).

We applied several computer programs that predict protein secondary structure to the *Chlamydomonas* beta heavy chain, and compared our predictions with those reported for other dynein heavy chains (Gibbons et al., 1991; Ogawa, 1991; Koonce et al., 1992), but in most cases were able to draw few conclusions about conserved heavy chain structures largely

due to problems of scale. However, when the alpha-helical coiled-coil predicting program Newcoils of Lupas et al. (1991) was used, major heavy chain domains were revealed. As seen in Fig. 7, several short regions with coiled-coil probabilities higher than 0.8 are evident in all four heavy chain sequences analyzed, and are clustered on either side of a central domain that contains the four GK sites (indicated by large arrowheads in Fig. 7). These peaks divide heavy chains into N-terminal, central, and C-terminal domains. Additional peaks of lower probability are scattered throughout the N-terminal 1800 residues, but few are seen near the C terminus. The overall similarity in peak distribution among all four dyneins analyzed extends in selected cases to the primary sequence level as well. When optimal alignments were created between sequences under each probability peak in Fig. 7 and the complete sequence of each of the other three heavy chains, using the GCG program Bestfit, probability peaks that are correspondingly numbered in Fig. 7 were aligned. Only peak 3 is present in all of the sequences analyzed, while peaks 1, 2, and 4 are each detected in two or three heavy chains. All four peaks appear in coiled-coil predictions of rat brain cytoplasmic dynein (Mikami et al., 1993).

DISCUSSION

Recent successes in the expression of cloned dynein genes in *Chlamydomonas* (Mitchell and Kang, 1991) make this a useful organism for molecular studies of dynein function, but sequence determination is a prerequisite to further heavy chain studies. Here we report the sequence of all of one and most of a second dynein heavy chain gene. Genes in this organism contain numerous small introns, as illustrated in Fig. 1, so that to extract protein coding information from our genomic sequences we have by necessity made assumptions about the correct placement of intron/exon boundaries. In most instances, only a single pair of splice junctions could be found that maintained the reading frame identified by codon bias scanning. In regions of the sequence where ambiguity remained, all located in the least-conserved amino-terminal domain of beta dynein, direct sequence analyses were performed on cDNA fragments amplified by RT-PCR. Alignments between the resulting predicted amino acid sequences and the sequences of other dynein heavy chains have not revealed any discrepancies that suggest incorrect exon identification, but we cannot rule out the possibility that some of the identified exons might be excluded from some molecules during transcript processing.

Flagellar dynein heavy chains

Direct comparison of the *Chlamydomonas* and sea urchin beta dynein sequences generates an overall identity of only 45%, indicating a level of sequence conservation far lower than previously observed for other motor proteins such as sarcomeric myosins (Goodson and Spudich, 1993; Cheney et al., 1993). One potential explanation might be that the nomenclature for sea urchin and *Chlamydomonas* dyneins is based on relative mobility on electrophoretic gels, not known structural or functional homology within the respective enzyme complexes. Sea urchin beta dynein could be more closely related to *Chlamydomonas* alpha or gamma dynein, rather than to *Chlamy-*

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1  ETVEDLVMKDVMDTAVLCELQFQDWRQTLSWSDIRTDIMEEGAKQFVKEVSKLHKVKRDEEDVFRGVDQVVKNFVLSVPLVADLRSPAMDRHRWEQLMATT 100
101 KMTFNVKDPNFKLDDLLALELHKFEVEEVEIVDRAQKEEKMEIAIRKLNDRTRVEVQFHRHKDYDVHTVKMAEEDFEALDNQVQVQGMIANRYMATFK 200
201 DEILGWQKLLNDVADVQIMAEIQRTWAYLESLEFHSEEVKKELPQATERFAAIDTEVKKVLRFPQQLKNCVSCCNREGLYANLETQERELEICKKALND 300
301 YMESKRRAFPRFYFVSSADLLDILSNGNPMRVQIHMNKCFQAINVRLDSEEVVGRPRKALGMESCVGIEYVFPSSLPLENKVEQYMNDIIAKMRNDV 400
401 RMVLKASVEDYPSKPRKDWLDFWPSQIILVNVQIYWCLEVEQAFTEMARGDKGAMSKYNEFQVKQLTKLIEVTRTDLSKPDQRKIMNMITIDAHSRDMVL 500
501 AGADQPDFSQWVQSLRSYWRDIDSCRIRICDASFYGYEYLGNGPRLVITPLTDRIYITATQACWLSLGTAPAGPAGTGKTETTKDLSAQLGKSVYVFN 600
601 CSPEMDYRTMGDIFKGLAASGSWGCDFENRNLVPEVLSVCSVQYKCVTDSQKKKTMPLGRGLEIKDGVKHPAVEHNSFIAADGVEMPLEEGETSAFITMN 700
701 PGYIGRAELPESLKAALFRPITVMPDRQLIMENMLMAEGFVEAKLAKKFAISLYLLEDDLSPQKHVDWGLRAIKSVLWVAGSLLRAEAGQVEADVLFRA 800
801 LRDFNIPKILAQDMVIFMGLLNDLDFGIDPFRKRDMFEFVIVSTIKDLGLTPEDDFVLRVVFSELLAIRHCVFVLMGPTGTGRTECYRVLAKAITKGCN 900
901 NPVNDYLKMTNKKKVVIRDINPKSISTYELYGQVQATREWKDGLLSYMRDVANMGPDDPKWLLDGLDANWIESMNSVMDDNRLTLPSNERIRVLP 1000
1001 HMKLIFEIRDLFATPATATRAGILYISEQQWHNMAMSWINRVKPYAERAKWKDPQLPCTWLREMPDKYIPPTLLEMKKSYSHTPLAQMNFISTLVN 1100
1101 IMEGLVKPENLSNKADQAMFEMVYFVAMIFWAGGGVLEKDGIPYRRNFKWFKQTWTTVKIPKGTVDYVFNPKTKQFQPMALVTDIDYDGSRPMSTV 1200
1201 FVPTAETSLSRFFLDMMVDRPKIMFVGGAGVGKTQLVKGKGLSNEEQISLSISFNFTDVSFQKVLSPLEKQAPAGINYGPPGTRKQLIYFVDDLNM 1300
1301 KLDLYETAMPISLIRQLHGWGHFDRAKLTPKNIINNTQYVACMNPAGSFIINPRQLRFLMFLAVDFPQDQSLMKIYGTFLQGHLLKFSSESIQDMGKTL 1400
1401 QAALALHDRVSTFRKTAIFNHYEFVTRHLANVQGLLMSPEAFNSPTKWKGLWHLHESERVYADRVLVSLDLDAYNKAATAIAKKYFSVADIDDYK 1500
1501 DPKPLIFCHFARGLADKAYDEVADYTSLYKTLTEALNEYNETNAAMDVLFEDAMKHVCRI SRIVSNP SGHALLVGVGSGKQSLARLAHICGYATQMI 1600
1601 VISGSYSMNNFKEDIQMYKRTGVKGEVGMFLFTDSQIVDERMLVYINDLLSSEIPDLFPQEDRDEIVNALRSETKSLGLLDTAENCWATFIQVKTNL 1700
1701 HVMFTASPVGENFRVRSQRFLATVSTVINDWFPWPESSLFSVAKRFLDEVDLGEDAVANAVVEFMPYSFQLVNKVSIKFRQERRNYTTPKTFLEL 1800
1801 LYKNVLAARKKANQDNTERLENGLHLKHKVQADVDILVEAKVKAVEVEHKVASANIFAEQVGEKEKVAENAAAQVEAEKCAVAKEVSEKQASCEKD 1900
1901 LAAAEPLVAEAMALETVTTKDLGEAKSLKPPGVDDITAVVYIILENNPKDKSQAAQKLMNVKDFLERVSKFSVIDAGQVARKTVDACRPLYALE 2000
2001 WFNREAIKGSAAAAGLCEWAVNIKYVDVVQVEVEPKRQGLAAANAKLEAVNTLAAVEEKVALLNAKVOLEQQYKEANDDKAAIRESERCQRKLELA 2100
2101 NRLINALASEGERWALTEQLRKSSEVLTGDMLLAAAFVSYAGPFTAKFRAHVIDDWILFLRERHMPMTEGITDPLKVLVDDALVAGWIREGLP SDPTSV 2200
2201 QNGTILTNSERWSLMDPQLQGIILWIKERESKNNLQVTRMGASNLMQVMERAI EAGHSVLENMGETIDAVLNP I ITRSTFKKGRSLYVKLGDKCEYK 2300
2301 NFRFLHLTKLSNPHYPEIQAEATTINFVTVEAGLEDQLLALVNNKERPDL EETKQLIQNTFTIKLKELEDGLLLKLSLTAEGDITEDVALIESLEDA 2400
2401 KRVS 2404

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Fig. 5. Predicted amino acid sequence of the central catalytic region of *Chlamydomonas* alpha dynein. Nucleotide-binding site consensus sequences are underlined. These sequences are available from GenBank under accession number L26049.

GK1

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Cre A RLVITPLTDRIYITATQACWLSLGTAPAGPAGTGKTETTKDLSAQLGKSVYVFNCSPEMDYRTMGDIFKGLAASGSWGCDFENR 547-631
Cre B CLCITPLTDRCFITLTAQARLVLGAPAGPAGTGKTETTKDLARALGICQYVFNCSQDMQYKAMGHTYKGLAQTAGWGCDFENR 1891-1975
Tgr B RLVITPLTDRCYITLTAQSLHVMGAPAGPAGTGKTETTKDLGRALGIMVYVFNCSQDMQYKSCGNIYKGLSQTGAWGCDFENR 1824-1908
Dicty RLVQTPLTDRCYLTLTAQLESRMGNPFPGAGTGKTEVTKALGSQLGRFVLVPCDEGFDLQAMSRIFVGLCQCGAWGCDFENR 1941-2025
-L--TPLTDR---T-TQ-----P-CPAGTGKTET-K-L--LG---VF-C---D-----GL---G-WGCDFENR

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GK2

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Cre A GLTPEDDFVLRVVFSELLAIRHCVFVLMGPTGTGRTECYRVLAKAITKGCNPNVNDYKMTNKKKVVIRDINPKSISTYELYGQV 850-934
Cre B GYQDDQFLKISHVRELFVVRWSVFLLAGAGCGKTAVWRLLRAQNSS.....GKTIYQAVNPKAVTRNELYGYL 2174-2245
Tgr B KLQAEFSVLKVVQLEELLAVRHSVFIIGNAGTGSQVLKVLNKYTSNM.....KRKPVLDLNPKAVTNDLFGII 2105-2176
Dicty HLVTQKQEWVEKILQLHLINLHGMVMPGSPGGKTTSEWVYLEAIEQV.....DNIKSEAHVMDPKAITKDQLFGLS 2243-2315
-----V--G--G--G-----K-----PK-----L-G--

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GK3

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Cre A FVPTAETSLSRFFLDMMVDRPKIMFVGGAGVGKTQLVKGKGLSNEEQISLSISFNFTDVSFQKVLSPLEKQAPAGINYGPPGTRKQLIYFVDDLNM 1200-1281
Cre B LFVPTVETTRLYFLDLSVSNKHAYFVNGTGTGKSAIMVKNLRNMDTMSFYITNMNSLSEAPALQVILEQPLE..KK.SGVR 2502-2583
Tgr B VLVHTNETTRVRFMDLLMERGRPVMLVGNAGLGKSVLVGDKLSNLGEDSM.VANVPFNYYTTSEMLQRVLEKPLE..KK.AGRN 2432-2512
Dicty VVPIPTVDTTRHVDVLHAWLSEHRPLILCGPPGSGKTMTLTSTLRAFPD..FEVVSLENSATTPELLLKTDFDHCEYKRTPSGET 2641-2723
---T--T-----G--G--G-----L-----E-----G--

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GK4

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Cre A LVLFEEDAMKHVCRI SRIVSNP SGHALLVGVGSGKQSLARLAHICGYATQMIVISGSYSMNNFKEDIQMYKRTGVKGEVGMFL 1548-1632
Cre B LVLFEQAMEHVTRIARIIDLPRGNAMLVGVGSGKQSLARLASIICGYEYVQISVSSYTGINDFKNELGLYRKAGTKGTPITPL 2851-2935
Tgr B LVLFEEDAMQHVCRINRILESPRGNALLVGVGSGKQSLARLASIYSSLEVFQITLRKGYGIPDLKLDLATVCMKAGLKNIGTVFL 2777-2861
Dicty LVLFEVLDHILRIDRVFROPQGHALLIGVSGGKSVLSRFVAMNGLSIYTIKVNNNYKSSDFDDDLRMLLKRAGCKEEKICFI 2983-3067
LVLFE---H--RI-R---P-G-A-L-GV-G-GK--L-R-----I---Y-----G-K----F-

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Fig. 6. Multiple sequence alignments of regions encompassing the four GK sites were created with Pileup. Sequences used were the *Chlamydomonas* alpha (Cre a; this study), *Chlamydomonas* beta (Cre b; this study), *Tripneustes* beta (Tgr b; Gibbons et al., 1991), and *Dictyostelium* cytoplasmic (Dicty; Koonce et al., 1992) dynein heavy chains. Residues conserved in all four sequences are displayed on a consensus line, and each GK site is underlined. Only GK1 is highly conserved among all four heavy chains, although GK4 is conserved among flagellar dyneins.

domonas beta dynein. Although analysis based on 100 amino acid sequences centered around the GK1 site of the urchin beta chain, several other sea urchin heavy chains (I. Gibbons, personal communication), the *Chlamydomonas* gamma chain sequence (Wilkerson et al., 1994) and our *Chlamydomonas* alpha and beta sequences suggest that the urchin and *Chlamy-*

domonas beta chains are indeed the most closely related (data not shown), this conclusion should not be taken as evidence that beta chains of *Chlamydomonas* flagella and sea urchin cilia are functionally equivalent. Comparisons based on a region spanning all four GK sites indicates that the *Chlamydomonas* alpha and beta chains are nearly equally divergent from sea

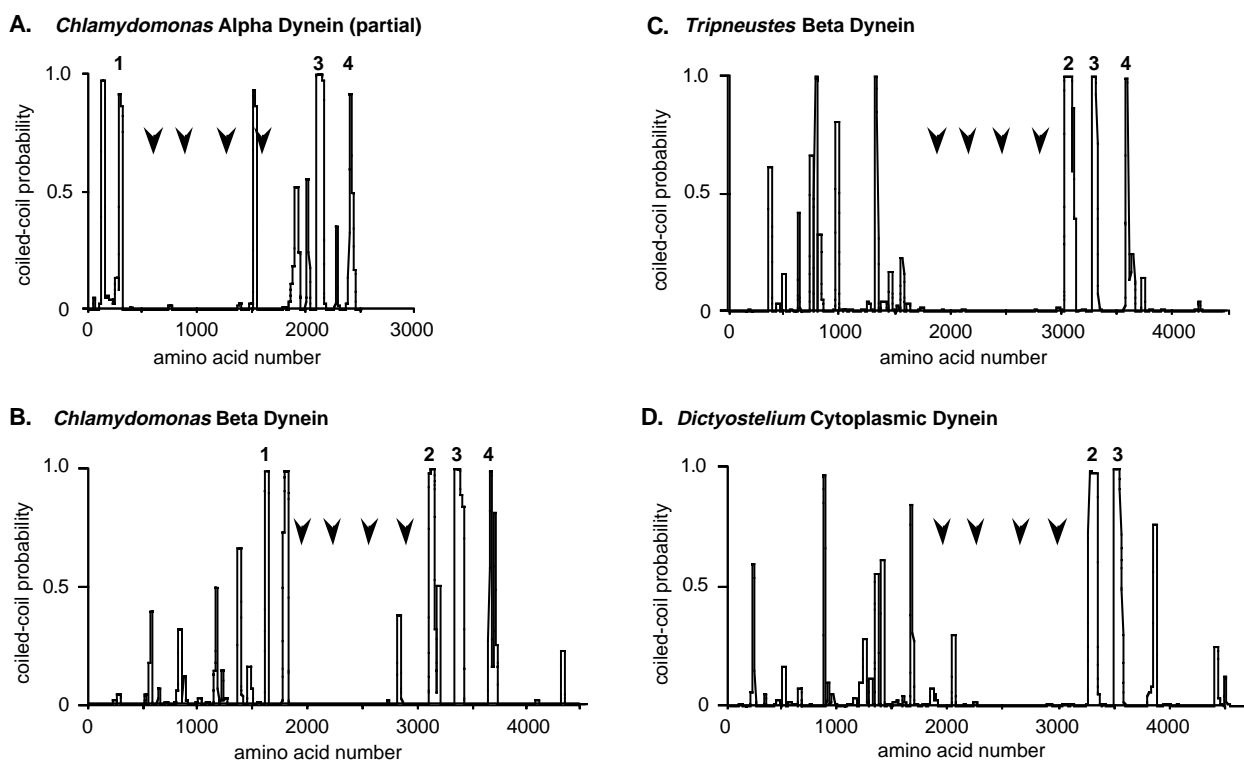


Fig. 7. For each sequence, the probability of forming regions of alpha helical coiled-coil structure was determined with the program Newcoils (Lupas et al., 1991) using a window of 28 residues. Peaks of high probability encoded by homologous regions in two or more sequences are numbered. Arrowheads indicate the locations of GK sites.

urchin beta dynein (Table 1). Functional studies of *Chlamydomonas* alpha and beta dynein mutants clearly show that these two heavy chains play very different roles in flagellar motility (Sakakibara et al., 1991, 1993), and until similar information is available for urchin alpha and beta chains no conclusions can be drawn concerning the functional equivalence of outer arm dynein heavy chains in these two species.

Relatively few regions are highly conserved among multiple dyneins. The most striking of these are two of the ATP-binding site consensus sequences, GK1 and GK4, which are identical over a region of 16-17 amino acids for all three flagellar heavy chains (Fig. 6). This high degree of conservation argues for an essential role for these sites. The urchin GK1 site has previously been identified as the probable phosphate-binding pocket for ATP catalysis and the site of vanadate photo-cleavage in the presence of magnesium and ATP (the V1 site) (Gibbons et al., 1991), and the V1 sites of *Chlamydomonas* outer arm heavy chains (King and Witman, 1988b, 1987) can also be approximately aligned with GK1 sites (this paper and Wilkerson et al., personal communication). The other site highly conserved among flagellar dyneins, GK4, may be a site of vanadate photo-cleavage in the presence of manganese (a V2 site) (Gibbons et al., 1987, 1991; Mocz et al., 1988) but the general conclusion that V2 cleavage always occurs at a GK site must be tested further. The urchin alpha and beta heavy chains (Gibbons et al., 1987) and the *Chlamydomonas* beta chain (King and Witman, 1987) each have a single V2 site, but the *Chlamydomonas* gamma chain has two V2 sites (King and Witman, 1988b) and the alpha chain has three (King and

Witman, 1987). Assuming that discrepancies between the size of heavy chains as estimated by gel electrophoresis and as calculated from the amino acid sequence can be applied to the size of vanadate photo-cleavage fragments, the *Chlamydomonas* alpha dynein V2a site corresponds very closely to GK3, the V2b site aligns with GK4, and the V2c site is located approximately 12 kDa C-terminal to GK4. However, based on similar calculations, the V2 site in beta dynein lies between GK3 and GK4, while the V2a and V2b sites in gamma dynein lie between GK2 and GK3, and between GK3 and GK4, respectively. Direct sequence analysis of cleavage products will thus be needed to identify clearly the location of V2 sites and determine whether or not they are coincident with GK motifs.

GK2 and GK3 are relatively poorly conserved among the flagellar sequences we analyzed. By comparison with the much greater degree of conservation typically found within other families of known catalytic sites, such poor conservation suggests that the role of these two central GK sites is no longer catalytic. Even so, both sites retain all of the features that define a typical phosphate-binding loop, including a group of four hydrophobic residues before the first glycine. Substitution of arginine for lysine in *Chlamydomonas* alpha dynein GK2 is the greatest deviation from the P-loop consensus. This variation is known to occur in active sites of phosphorylases, phospholipases, and protein kinases (Fry et al., 1986), so that a role for this least conserved GK site in some aspect of dynein mechanochemistry cannot be ruled out at the present time. A fifth GK site tentatively identified near the N terminus of one

sea urchin beta dynein (Gibbons et al., 1991) has no counterpart in any of the cytoplasmic or *Chlamydomonas* flagellar dynein sequences and probably lacks functional significance.

Secondary structure predictions based on our dynein sequences in general parallel the results of Gibbons et al. (1991) and Ogawa (1991) on sea urchin beta dyneins and suggest that dynein molecules can be broadly divided into three large domains. No extensive regions of rod-forming structure were identified, but the C-terminal and N-terminal domains have a somewhat greater alpha-helical content and, as illustrated in Fig. 7, contain isolated short regions of heptad hydrophobic periodicity that may participate in alpha helical coiled-coil interactions. A similar distribution of coiled-coil probability is seen in *Chlamydomonas* gamma dynein (Wilkerson et al., 1994). As the algorithm used to detect these regions cannot differentiate among regions of known parallel versus anti-parallel or double-helical versus triple-helical coiled-coil structures, the actual secondary structural characteristics of these regions remains speculative.

Flagellar versus cytoplasmic heavy chains

The distribution of sequence conservation among flagellar and cytoplasmic dynein heavy chains does not indicate the presence of large domains with specific flagellar versus cytoplasmic function (Koonce et al., 1992). Instead, there is an overall tendency for greater divergence within the N-terminal third of each protein (Fig. 4), which suggests that the more conserved C-terminal two thirds is involved in processes common to all dynein heavy chains, such as ATP-sensitive microtubule binding, ATP catalysis, and mechanochemistry, while the N-terminal domain is free to diverge and may provide functions specific to each heavy chain. Mutations that disrupt beta dynein (many alleles at the *oda-4* locus) prevent assembly of all outer arm dynein subunits (Kamiya, 1988), but preliminary analysis of a new allele, *oda-4-s7*, suggests that only the N-terminal 160 kDa (~35%) of the wild-type protein is required for outer arm assembly (Sakakibara et al., 1993). This N-terminal domain may therefore play a central role in assembly of outer arm dynein proteins onto doublet microtubules and may provide interaction sites for other outer arm proteins, such as the 70 kDa intermediate chain and 18 kDa light chain, that co-purify with beta dynein (Mitchell and Rosenbaum, 1986; Sakakibara et al., 1993).

The GK4 site in *Dictyostelium* cytoplasmic dynein ends in serine (GKS) (Koonce et al., 1992), and that of rat brain cytoplasmic dynein ends in threonine (GKT) (Mikami et al., 1993), the two most commonly occurring residues at this position in P-loop motifs (Saraste et al., 1990). In contrast, all presently known flagellar dynein GK4 sites end in glutamine (GKQ), a sequence not previously reported for a nucleotide-binding site. Along with the much greater overall sequence conservation of GK4 among flagellar dyneins than between flagellar and cytoplasmic dyneins (Fig. 6), this glutamine substitution suggests that GK4 has a specific flagellar function. Candidate functions might include coordination of outer arm dynein with inner arm dyneins, which appear to have a slower intrinsic microtubule translocation rate (Kurimoto and Kamiya, 1991), and temporospatial regulation of dynein activity during bend propagation (Omoto, 1991).

The sequence comparisons presented here highlight several regions of potential functional significance for dynein heavy

chains. Modification of GK sites and other motifs with site-directed reagents, through molecular analysis of dynein mutations, or through site-directed mutagenesis should eventually reveal the functional significance of these conserved regions and provide new insights into the mechanism of dynein force generation.

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