The Dynein Gene Family in *Chlamydomonas reinhardtii*

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**ABSTRACT**

To correlate dynein heavy chain (*Dhc*) genes with flagellar mutations and gain insight into the function of specific dynein isoforms, we placed eight members of the *Dhc* gene family on the genetic map of *Chlamydomonas*. Using a PCR-based strategy, we cloned 11 *Dhc* genes from *Chlamydomonas*. Comparisons with other *Dhc* genes indicate that two clones correspond to genes encoding the alpha and beta heavy chains of the outer dynein arm. Alignment of the predicted amino acid sequences spanning the nucleotide binding site indicates that the remaining nine clones can be subdivided into three groups that are likely to include representatives of the inner-arm Dhc isoforms. Gene-specific probes reveal that each clone represents a single-copy gene that is expressed as a transcript of the appropriate size (>13 kb) sufficient to encode a high molecular weight Dhc polypeptide. The expression of all nine genes is upregulated in response to deflagellation, suggesting a role in axoneme assembly or motility. Restriction fragment length polymorphisms between divergent *C. reinhardtii* strains have been used to place each *Dhc* gene on the genetic map of Chlamydomonas. These studies lay the groundwork for correlating defects in different *Dhc* genes with specific flagellar mutations.

The presence of multiple dynein isoforms within the axoneme provides the cell with several potential mechanisms for affecting the flagellar waveform. Indeed, the study of flagellar mutations in *Chlamydomonas* has indicated that the outer and inner dynein arms have different functions in the generation of flagellar motility. Most mutants that lack the outer dynein arms swim with reduced beat frequencies (Mitchell and Rosenbaum 1985; Kamiya 1988), whereas mutants that lack one or more inner arm isoforms have aberrant waveforms or are completely paralyzed (Huang et al. 1979; Brokaw and Kamiya 1987). These observations indicate that although the outer arms add power to the flagellar beat, the inner dynein arms are both necessary and sufficient to generate the flagellar waveform (Brokaw and Kamiya 1987). Thus a critical step to understanding the mechanism of flagellar motility lies in elucidating the specific functions of the inner arm isoforms.

The complexity of inner arm composition also raises several important questions. First, what is the relationship between the multiple inner arm isoforms resolved by biochemical methods and numerous *Dhc* genes recently identified in several organisms by PCR procedures? Second, does each Dhc have a unique function or are some isoforms redundant? Finally, where are the multiple isoforms located in the flagellar axoneme, and how are they targeted to the appropriate site?

As a first step toward studying these questions, we have undertaken an extensive PCR screen to identify axonomal *Dhc* genes in *Chlamydomonas*. Although axonomal *Dhc* genes have recently been identified in other organisms (Asai et al. 1994; Gibbons et al. 1994; Rasmussen et al. 1994; Tanaka et al. 1995), *Chlamydomonas*...
has several unique advantages for the study of axonemal dyneins. First, Chlamydomonas is haploid, and thus it is very easy to isolate mutability mutations simply by screening for cells with altered swimming behaviors after mutagenesis. This strategy has identified mutations in >75 different flagellar loci (reviewed in Dutcher 1995). Second, the ease of culture and the highly ordered structural organization of the flagellar axoneme mean that it is often possible to correlate motility phenotypes with both polypeptide deficiencies and structural defects. Finally, recent advances in the development of molecular techniques, such as RFLP mapping (Ranum et al. 1988), transformation (Kindle 1990), and insertional mutagenesis (Tam and LeFebvre 1993), make it possible to correlate cloned genes with specific mutations. For instance, each of the outer arm Dhc genes (α, β, γ) has been linked to a specific outer arm mutation (Sakakibara et al. 1991; Porter et al. 1994; Wilkerson et al. 1994). However, there is still a large and growing collection of motility mutants for which the affected gene products have not yet been identified. These include several mutations that are known to disrupt the assembly of different inner arm isoforms (e.g., pf3, sup-pf3, suppf-5) (Huáng et al. 1979; Kamiya et al. 1991; Piperno et al. 1992, 1994; Porter et al. 1992; Kato et al. 1993; Gardiner et al. 1994), as well as several other mutations that interfere with flagellar assembly (Huáng et al. 1977; Adams et al. 1982; Harris 1989) and/or alter the ability of cells to undergo phototaxis (Hörst and Witman 1993; Pazour et al. 1995).

In this study, we report the identification and cloning of nine dynein-like sequences in Chlamydomonas. These sequences fall into three subgroups distinct from those associated with either cytoplasmic or outer arm dynein isoforms. Southern and Northern blot analyses suggest that the nine Dhc genes are the products of distinct genes whose expression is enhanced by deflagellation. RFLP mapping procedures have been used to place the Dhc clones on the genetic map of Chlamydomonas. Several of these Dhc genes map near loci that have previously been shown to be involved in flagellar function.

MATERIALS AND METHODS

PCR amplification of Dhc genes: For the initial PCR reactions, four degenerate oligonucleotide primers were synthesized based on regions of amino acid sequence conservation between the sea urchin beta axonemal dynein (Gibbons et al. 1991; Ogawa 1991) and Drosophila cytoplasmic dynein (Li et al. 1994). These primers correspond to amino acid sequences surrounding the predicted ATP hydrolytic site (Gibbons et al. 1991; Ogawa 1991) and were used previously to isolate a family of Dhc genes from Drosophila (Rasmussen et al. 1994). The specific amino acids and the nucleotide sequence of each primer is listed below, written 5' to 3'. Primer 1, a sense primer (ITPPLDR): AT(A/C/G/T)-AC(A/C/G/T)-CC(A/C/G/T)-CT(C/G/T)-AC(A/C/G/T)-GA(C/G/T)-CT(A/C/G). Primer 2, a sense primer (AGTGKTE): G(C/A/C/G/T)-GG(A/C/G/T)-AC(A/C/G/T)-GG(A/G/T)-AA(A/G)-AC(A/C/G/T)-GA. Primer 3, an antisense primer (GFDEFRN): C(G/T)-(A/G)-TT(A/G)-AA(A/C/T)-TC(A/G)-TC(A/G)-AA(A/G)-AA(A/G)-GA. Primer 4, an antisense primer (IFITMNP): GG(A/G)-TTG-AT(A/C/G/T)-GT(A/G/T)-AT(A/G)-AA(A/G/A)-AT. Oligonucleotide primers were purchased from Oligos, etc., (Wilsonville, OR) or synthesized on an Applied Biosystems 392 DNA synthesizer (Foster City, CA) and generously supplied by T. Haws, University of Minnesota.

To facilitate the recovery of all members of the dynein gene family, genomic DNA was used as the starting template for the PCR amplification. In this strategy, each dynein gene would be equally represented, regardless of the abundance of the corresponding mRNA. Genomic DNA (100–200 ng) prepared from a wild-type Chlamydomonas reinhardtii strain (197c) was added to 50–100 μl PCR reaction mixtures containing 0.2 mM dNTPs, 0.6–1.0 μM of each of two degenerate primers, 1X reaction buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton-X-100) and 0.025 U/μl Taq DNA polymerase (Promega Corp., Madison, WI). Following an initial 2-min denaturation step at 95°, the samples underwent 25 cycles of 95° for 2 min, 75° for 3 min, and 90° for 1 min, and then terminated with a single cycle of 95° for 2 min, 72° for 10 min.

The first rounds of PCR amplification utilized primers 1 and 4 in one reaction and primers 1 and 3 in a second reaction. Two microliters of amplified product then served as the starting template in a second round of reactions using internal primers. The first reaction was reamplified using primers 1 and 3 to yield a single product of ~230 bp. The second reaction was reamplified using primers 2 and 3 to yield two products of ~160 and ~300 bp. These products were recovered by ethanol-precipitation, resuspended in 20 μl TE, treated with T4 polynucleotide kinase and Klenow, and purified via low-melt agarose gels. The three fragments were then ligated separately into EcoRV-digested pBluescript KS (Stratagene, La Jolla, CA) and transformed into Escherichia coli DH5α (F') (Sambrook et al. 1989). Eighteen transformants were picked from each ligation and rescreened by PCR using primers 2 and 3. Plasmid DNA was then prepared from PCR positive transformants (Sambrook et al. 1989).

Forty-seven of the PCR positive clones were sequenced, and seven different dynein-related sequences were identified. Only one sequence (Dhc1) was identified in the 230-bp fragment, whereas three different sequences were identified in the 160-bp fragment (Dhc3 and the outer arm αDhc and βDhc genes). Three additional sequences were found in the 300-bp fragment (Dhc2, Dhc4, and Dhc5). All of the 300-bp products contained putative intron sequences in addition to dynein-related coding sequences.

To recover additional Dhc genes, a second PCR screen was performed at an annealing temperature of 59° using 10 μM of primer 1 and a modified version of primer 4 (5' NEW, antisense for FITMNP: CG(A/C/G/T)-GG(A/G/T)-TTG-AT(A/C/G/T)-GT(A/G/T)-AT(A/G/AA). Four PCR products ranging in size from ~400 to ~850 bp were identified, subcloned, and screened as described above. Sequence analyses of 21 PCR products yielded two new dynein-related sequences (Dhc6 and Dhc7) and longer versions of αDhc, βDhc, Dhc2, and Dhc3.

The number of times that each Dhc sequence was found among the different PCR products varied considerably for each Dhc gene; the final numbers are noted here in parentheses: αDhc (7), βDhc (15), Dhc1 (19), Dhc2 (19), Dhc3 (5), Dhc4 (1), Dhc5 (2), Dhc6 (6), and Dhc7 (2). We also failed to recover the yDhc sequence, although the degenerate primers used in these experiments would have been expected to amplify this gene (Wilkerson et al. 1994). These results indicate that the different PCR screens were not exhaustive, and that there may be additional Dhc genes present in the Chlamydomonas genome.

Isolation of genomic clones: To verify and extend each of
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the Dhc sequences, longer clones were obtained by screening a large insert genomic library (Figure 1). This library was constructed by R. SCHNELL (University of Minnesota) in XFIIXI (Stratagene, La Jolla, CA) using genomic DNA isolated from the C. reinhardtii wild-type strain 21gr, mt+ (SCHNELL and LEFEBVRE 1993). Approximately 50,000 pfu were plated on the E. coli strain LE392 and then screened by hybridization of 32P-labeled Dhc sequences to Magnagraph filter replicas (Micron Separations, Inc., Westboro, MA). Positive phage were plaque purified and rescreened two to three times by standard techniques (SAMBOOK et al. 1989). Phage DNA was isolated as described by CHISHOLM (1989), and insert DNA was released and mapped using the restriction enzymes NdeI, SadI, and SadI. DNA fragments encoding the putative ATP hydrolytic domain were identified by probing Southern blots of restriction digests with 32P-labeled PCR products or PCR primers tagged with terminal deoxynucleotidyl transferase (Gibco-BRL, Inc., Gaithersburg, MD) and a-32P-CTP. These fragments were then subcloned into a Bluescript II KS+ and sequenced using the appropriate primers. Complete double-stranded DNA sequence was obtained for the region encoding the hydrolytic domain were also subcloned and used to identify gene-specific probes for use in expression and mapping studies.

DNA sequencing and analysis: Double-stranded DNA templates were sequenced using either the PCR primers listed above or standard plasmid vector primers and Sequenase, version 2.0, according to manufacturer’s instructions (United States Biochemical, Cleveland, OH). Sequence data was assembled and analyzed using the Genetics Computer Group software, version 8 (Madison, WI) (DEVEREUX et al. 1984) on a Sun Microsystems Solaris 2.4 computer available through the Advanced Biosciences Computing Center (University of Minnesota) or the MacVector Sequence Analysis Software Package (International Biotechnologies, Inc., Rochester, NY) on a Macintosh microcomputer. Potential open reading frames were identified using the GCG program CodonPrefer, and a codon usage table compiled from the coding regions of several different Chlamydomonas nuclear sequences (SILFLOW et al. 1985; WILLIAMS et al. 1989; SAVEREIDE 1991; MITCHELL and BROWN 1994; ZHANG 1996; R. SCHNELL, personal communication). Potential splice donor and acceptor sequences were also identified based on splice junction consensus sequences found in these same Chlamydomonas nuclear genes.

Preparation of genomic DNA: Genomic DNA was isolated from three different wild-type Chlamydomonas strains that are polymorphic at the DNA sequence level, C. reinhardtii (137c), C. reinhardtii (S1-D2), and C. smithii (CC1373). Cells were grown in 5 liters of rich liquid media supplemented with additional potassium phosphate as described by WITMAN (1986). Cells were harvested in a Pellicon Cell Harvesting apparatus equipped with Durapore filter cassettes, resuspended in minimal media, and lysed overnight in 10 mM TrisHCl pH 7.6, 20 mM EDTA pH 8.0, 0.5% SDS, 1 mg/ml Pronase E at 50° with gentle agitation (JOHNSON and DUTCHER 1991). Ammonium acetate was added to a final concentration of 0.8 M, and the crude lysate was extracted once with phenol-chloroform. The aqueous phase was mixed with an equal volume of isopropanol to precipitate the genomic DNA. The pellet of DNA was washed twice with 70% ethanol and resuspended in 10 mM TrisHCl pH 8.0, 10 mM EDTA. The crude DNA preparation was then further purified by banding on CsCl gradients (SAMBOOK et al. 1989).

Southern blot analysis: DNA samples (3–5 µg per lane) were digested with a series of restriction enzymes (PvuII, PstI, EcoRI/Xbal, NdeI, SadI, Spal, HindIII, and Smal), separated on 0.8–1.0% agarose gels, and transferred overnight to nylon membranes (either Zetabind, Cuno, Inc., Meriden, CT or Magnagraph, Micron Separations, Inc., Westboro, MA) according to standard procedures (SAMBOOK et al. 1989). Zetabind membranes were washed 5 min in 2X SSC (1X SSC is 150 mM NaCl, 15 mM sodium citrate) at room temperature and air-dried, whereas Magnagraph membranes were washed in 5X SSC at 60°, baked in a vacuum oven at 80° for 1–2 hours, and then UV-crosslinked at a setting of 20,000 µjoules (Stratalinker, Stratagene, Inc., La Jolla, CA). Blots were prehybridized for a minimum of 4 hr at 65° in 6X SSPE (1X SSPE is 183 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA), 0.5% nonfat dried milk, 0.5% SDS, 50 mM Tris-HCl pH 7.5, 500 µg/ml salmon sperm DNA, and then hybridized overnight at 65° in the same solution plus 10% dextran sulfate and the appropriate 32P-labeled DNA probe. Blots were radiolabeled with α-32P-dCTP using the Prime-it II random primer labeling kit (Stratagene, Inc., La Jolla, CA) according to manufacturer’s instructions. Probe templates were then purified on SephadeG-50 spin columns, denatured, and added to hybridization solutions. Blots were washed twice with 2X SSC, 0.1% SDS at room temperature, then by three 30-min washes with 0.1X SSC, 0.1% SDS at 65° (high stringency conditions). Low stringency hybridizations and washes were carried out at 45°. Autoradiograms were exposed in the presence of enhancer screens (Dupont Cronex, Wilmington, DE) at ~80°. Blots were striped for reprobing according to manufacturer’s instructions (Zetabind) or by two washes in 0.2 M NaOH, 1% SDS at 42° for 20–25 min each (Magnagraph).

RNA preparation and Northern blot analysis: RNA for Northern blot analysis was prepared from wild-type Chlamydomonas (137c, mt+) cells both before and 45 min after deflagellation induced by pH shock (WITMAN et al. 1972; WILKERSON et al. 1994). Cells (2 × 10^7) were resuspended in 5 ml of minimal medium, added to 100 ml of lysis buffer (20 mM TrisHCl pH 8.0, 20 mM EDTA, 5% SDS and 1 mg/ml proteinase K) with gentle stirring, and incubated at room temperature for 2–4 hr. The lysate was then mixed with 10 ml of 3 M sodium acetate pH 5.2, followed by an equal volume of 1:1 phenol:chloroform and centrifuged at 5000 × g for 30 min. The aqueous phase was removed and mixed with an equal volume of isopropanol at room temperature to precipitate the RNA. After 15 min, the RNA was collected by centrifugation, washed with 80% ethanol, and resuspended in 5 ml of sterile dH2O. This solution was then mixed with 3 ml of 4 M LiCl and allowing to incubate at 4° overnight. The RNA was collected by centrifugation, and the supernatant containing contaminating DNA and carbohydrate was gently removed. The RNA pellet was dissolved in sterile dH2O. Sodium acetate was added to 0.3 M, and the RNA was reprecipitated by the addition of 2.5 volumes of ethanol for long term storage at ~80°.

For Northern blots, aliquots containing ~20 µg of total RNA were redissolved in sterile dH2O, mixed with sample buffer, and run on 1X MOPS, 18% formaldehyde, 0.75% agarose gels at 15–20 V overnight. Gels were washed briefly with 0.15 M NaCl, 0.05 M NaOH (one time for 5 min and one time at 10 min), neutralized with 0.15 M NaCl, 0.05 M Tris-HCl pH 8.0 (three times at 10 min), and transferred in 10X SSC by capillary action to a Zetabind membrane. After a 5-min wash in 5X SSC, the membranes were air-dried and then UV-crosslinked at a setting of 20,000 µjoules (Stratalinker, Stratagene, Inc., La Jolla, CA). Prehybridization and hybridization conditions were identical to those described above for Southern blots. Control experiments in which the Northern blots were hybridized with a probe for the ribosomal protein S14 gene (NELSON et al. 1994) confirmed that equal amounts of RNA were loaded in all lanes.

RNA preparation and reverse transcription (RT)-PCR anal-
ysis of dynein sequences: RNA was isolated from 2 \times 10^4 C. reinhardtii (197c) cells both before and 30 min after deflagellation using the guanidinium thiocyanate method (CHOMS-ZYNSKI and SACCHI 1987). To remove minor amounts of contaminating genomic DNA, total RNA was treated with RQI DNase (Promega Corp., Madison, WI), extracted with phenolchloroform and recovered by ethanol precipitation. cDNA was made from one microgram total RNA using AMV reverse transcriptase (Promega Corp.) with an antisense primer corresponding to the sequence encoding the amino acids ITMNPG, a highly conserved region present in all Chlamydomonas Dhc sequences identified thus far. The sequence of the RT primer is 5'-TA-G(C/G/A)CG-GGT-TATCAT-GGT-(G/C)A-3'. To control for any residual genomic DNA contamination, a parallel set of reactions was carried out in which the reverse transcriptase was omitted.

Two microliters of the resulting cDNA products were then used as the template in a PCR reaction containing a sense primer corresponding to conserved amino acids in the P-loop region and a gene-specific antisense primer corresponding to a region of sequence divergence 5' of the RT primer. Two different sense primers were used: a GKT-long primer corresponding to the amino acids TGKTETTK (5'-AC(G/C/T)-GGGAAGAC(G/C)-GAGACG3'). Two microliters of the resulting cDNA products were then used as the template in a PCR reaction containing a sense primer corresponding to the amino acids ITMNPG, a highly conserved region present in all Chlamydomonas Dhc sequences identified thus far. The sequence of the RT primer is 5'-TA-G(C/G/A)CG-GGT-TATCAT-GGT-(G/C)A-3'. To control for any residual genomic DNA contamination, a parallel set of reactions was carried out in which the reverse transcriptase was omitted.

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RESULTS

Cloning of dynein heavy chain (Dhc) genes in Chlamydomonas: Sequence analysis of axonemal and cytoplasmic Dhc genes in other organisms has identified several regions of amino acid homology (reviewed in Gibbons 1995). In particular, the region surrounding the first P-loop, which is thought to correspond to the primary ATP hydrolytic site, is highly conserved. Based on this conservation, five degenerate oligonucleotide primers were used in nested combinations to isolate PCR products from Chlamydomonas DNA ranging in size from 160 to 850 bp (see MATERIALS AND METHODS for details).

Sequence analysis of 68 subclones identified nine dynein-related sequences that contain the conserved ATP hydrolytic site. Two of the sequences correspond to the genes encoding the alpha and beta Dhcs of the outer dynein arm (Mitchell and Brown 1994), but the other sequences appear to represent seven different Dhc genes. To verify and extend these sequences, longer clones were recovered from a genomic library. For each gene, overlapping phage clones spanning 20–30kb of DNA around the region encoding the putative ATP hydrolytic site were isolated, subcloned, and partially sequenced (Figure 1). The predicted amino acid sequences through the hydrolytic domain of the Dhc clones are shown in Figure 2.

The genomic clones recovered for Dhc1–Dhc7 appear to contain small introns occurring at semi-conserved positions within the coding sequence. These introns were identified by sudden changes in codon bias and by the presence of Chlamydomonas consensus splice sites (see MATERIALS AND METHODS). To verify expression and confirm both the splice sites and the predicted amino acid sequences of the Dhc genes, the corresponding cDNA clones were amplified from total RNA using sequence specific primers (see Figure 2) and RT-PCR procedures (see below). RT-PCR products of the appropriate size were cloned, and individual colonies were sequenced. This analysis confirmed the splice sites and amino acid sequences shown in Figure 2.

Sequence analysis of the RT-PCR products also resulted in the recovery of two additional dynein-related sequences, Dhc8 and Dhc9. These two cDNAs were obtained from the pool of RT-PCR products amplified with the Dhc5 primer. Because Southern blot analysis suggests that the Dhc8 and Dhc9 sequences are derived from two additional Dhc genes (see below), the predicted amino acid sequences of these two clones are included in Figure 2.

Comparisons between the deduced amino acid sequences of the Chlamydomonas Dhc genes: Comparisons between the nine Dhc sequences recovered in the PCR screens and the three outer arm Dhc sequences identified by others (Mitchell and Brown 1994; Wirkerson et al. 1994) demonstrate that, within an 120 amino acid region surrounding the ATP hydrolytic site, the Chlamydomonas Dhc sequences are very highly conserved. For example, Dhc1–Dhc9 share 60–80% amino acid identity with the beta Dhc of the outer arm. However, further comparison between Dhc1–Dhc9 indicates that these sequences are even more closely related to one another than to the three Dhcs of the outer arm. As shown in Figure 3, the Dhc sequences can be divided into at least three subgroups. One group contains four genomic clones (Dhc2, Dhc4, Dhc5, and Dhc6) and the two cDNAs (Dhc8 and Dhc9). Interestingly, the four genomic clones in this group share a conserved exon splice site (see Figure 2). The Dhc3 and Dhc7 sequences represent a second group of closely related genes that also share another conserved exon splice site (see Figure 2). The Dhc1 sequence is similar to Dhc3 and Dhc7, but other evidence suggests that it may represent a third subclass of Dhc sequence (see below). Surprisingly, none of the Chlamydomonas sequences identified thus far shows significant homology to any of the cytoplasmic Dhc genes cloned in other organisms. These results suggest that the Dhc1–Dhc9 sequences correspond to structural genes for axonemal Dhcs and encode representatives of the 11, 12, and 13 inner arm isoforms identified by biochemical methods (Kagami and Kamiya 1992). Consistent with this interpretation, the Chlamydomonas Dhc sequences do share significant sequence homologies with axonemal Dhc sequences identified by expression analysis in sea urchin, Drosophila, Paramecium, and rat (Asai et al. 1994; Gibbons et al. 1994; Rasmussen et al. 1994; Tanaka et al. 1995, see DISCUSSION).

Size of the Dhc gene family in Chlamydomonas: To estimate the size of the Dhc gene family in Chlamydomonas, we performed genomic Southern blot analysis. Genomic DNA from two polymorphic Chlamydomonas strains was digested with a series of restriction enzymes, gel fractionated, blotted to reusable nylon membranes, and hybridized with each Dhc PCR product. Figure 4 illustrates the pattern of restriction fragments obtained with the enzymes SmaI, PstI, and SphI after hybridization at both high and low stringency with a 227-bp probe corresponding to a conserved region of the Dhc1 sequence. These enzymes do not cut the Dhc1 gene in the region represented by the probe. At high stringency (Figure 4, left panel), the Dhc1 probe hybridizes to one major band in each lane and approximately four or five minor bands. At lower stringency (Figure 4, right panel), however, the Dhc1 probe cross-hybridizes to at least 14 or 15 restriction fragments. We therefore stripped and reprobed this blot at high stringency with the other Dhc clones (data not shown). Using this strategy, we were able to identify the genomic restriction fragments corresponding to 11 Dhc genes (including the outer arm aDhc and bDhc genes). Our results suggest that the Chlamydomonas genome contains approximately 14–15 closely related Dhc genes, including at least three to four Dhc sequences that have not yet been identified (see Figure 4, right panel).
Expression of Dhc genes: Previous study has shown that large transcripts (>13 kb) encoding the outer arm Dhc are enriched in RNA preparations isolated from deflagellated cells (MITCHELL 1989; WILKERSON et al. 1994). To determine if the Dhc genes identified in this study show a similar pattern of transcript accumulation, we analyzed RNA isolated before and after deflagellation by both Northern blots and RT-PCR procedures.

For Dhc1–Dhc7, fragments ranging in size from 3 to 17 kb were cloned from a large insert genomic library (see Figure 1) and tested on Southern blots at high stringency to identify probes that are gene-specific. The gene-specific probes were then hybridized under identical conditions to Northern blots containing 20 μg of total RNA isolated before and 45 min after deflagellation induced by pH shock. As shown in Figure 5, each clone hybridized to a large transcript (>13 kb) that is enriched in RNA isolated from deflagellated cells.

The pattern of transcript accumulation is particularly interesting for two Dhc genes. Transcripts corresponding to the Dhc3 gene could only be detected after prolonged exposures and appear to be much less abundant than other Dhc transcripts (Figure 5). In addition, the Dhc4 probe hybridized with approximately equal intensity to two large transcripts that are both >13 kb (Figure 5). Whether these two transcripts represent alternatively spliced variants of the Dhc4 gene or incomplete mRNA processing of a single Dhc4 transcript is unknown. Regardless, all of the transcripts are large enough to encode Dhc polypeptides, and, as expected, all of the transcripts are upregulated upon deflagellation. These results, together with the analysis of the deduced amino acid sequences, suggest that the encoded Dhcs are involved in axoneme assembly and/or motility.

To verify that the signals observed on the Northern blots were due to specific hybridization with the transcript of interest and not cross-hybridization to other Dhc transcripts, we assayed for the presence of each sequence using the more sensitive procedure of RT-PCR. Total RNA was isolated from wild-type cells before and after deflagellation and then converted to cDNA using an antisense primer corresponding to the conserved amino acid sequence ITMNPG. This primer is degenerate and should therefore prime the synthesis...
The deduced amino acid sequences of 12 Dhc genes in the region surrounding the conserved ATP hydrolytic site, also known as the first P-loop, are shown here. The outer arm Dhc sequences (α, β, and γ) previously identified by Mitchell and Brown (1994) and Wilkerson et al. (1994) are included for comparison. In the consensus line below, capital letters indicate amino acid residues that are conserved in all family members. lowercase letters indicate those conserved in at least six of the clones, and periods indicate nonconserved residues. The positions of exon splice sites are shown by a single underline (_). Sequences used to design gene-specific primers are indicated by a double underline (___). Because the Dhc8 and Dhc9 sequences were recovered in the RT-PCR screen, the residues identified in lowercase correspond to the amino acids encoded by the PCR primers. These residues have not yet been confirmed by the sequence analysis of larger genomic clones. The Dhc1–Dhc9 sequences have been deposited in GenBank under the accession numbers U61364–U61372. The outer arm Dhc sequences are listed under the accession numbers L26049, U02963, and U15303, respectively.

**Identification of RFLPs:** The results of our expression studies suggest that Dhc1–Dhc9 play some role in axoneme assembly or motility. Because previous work in Chlamydomonas has identified >75 genetic loci that contain mutations which affect flagellar function (Har-
CrDhc4
CrDhc5
CrDhc2
CrDhc8
CrDhc6
CrDhc9
CrDhc3
CrDhc7
CrDhc1
CrbetaDhc
CrgammaDhc
CralphaDhc

FIGURE 3.—Diagrammatic alignment of the Dhc sequences. The Dhc sequences shown in Figure 2 were aligned using the GCG program Pileup. Dhc1–Dhc9 cluster into groups distinct from the outer arm Dhcs.

ris 1989; Dutcher 1995), we were interested in testing whether any of the Dhe sequences might be linked to any of the previously identified flagellar loci. However, before we could place the Dhc genes on the genetic map of Chlamydomonas, a specific RFLP had to be identified for each gene. A physical map of the Chlamydomonas genome had previously been constructed using two polymorphic strains, C. reinhardtii (137c) and C. smithii (Ranum et al. 1988). Because this map contains over 200 molecular and genetic markers (C. Silflow, personal communication), we first screened genomic DNA isolated from C. reinhardtii (137c) and C. smithii strains for the presence of RFLPs that could be used as molecular markers to map the Dhc genes. Unfortunately, RFLPs were infrequently observed between these two strains using the Dhc probes (data not shown). We therefore analyzed DNA from a third Chlamydomonas strain, S1-D2, for the presence of Dhc sequences. As shown in Figure 7, RFLPs were readily observed between these two strains using the Dhc probes (data not shown). We used these results to map the Dhc genes using tetrad progeny derived from crosses with the S1-D2 strain.

Construction of a RFLP map: Three strains of C. reinhardtii that carry 16 different genetic markers from 13 of the 17 known linkage groups were crossed to the wild-type strain, S1-D2. Eighteen tetrads were dissected from each cross, and the segregation of the different genetic markers in the resulting tetrad progeny were scored as previously described (see MATERIALS AND METHODS). Analysis of the pattern of segregation of the genetic markers indicated that each marker segregated 2:2, and that markers on different linkage groups as-sorted independently (Gross et al. 1988, and data not shown).

To provide coverage over the remainder of the Chlamydomonas genome, DNA was isolated from the tetrad progeny, digested with restriction enzymes known to reveal RFLPs, transferred to Southern blots, and hybridized with 26 different molecular markers. The pattern of segregation of each molecular marker was analyzed with respect to the genetic markers and the other molecular markers (data not shown but available upon request). A complete listing of the genetic and molecular markers used in this study and their positions on the different linkage groups is provided in Table 1.

Because we analyzed marker segregation in tetrads...
progeny, we were also able to estimate the approximate centromere distance for each marker by determining the frequency of tetratype tetrads with respect to the centromere-linked markers _ae17_ and _y1_ (see Table 1). This information allowed us to compare the centromere distances observed in the above crosses against the published data for crosses within _C. reinhardtii_ strains (HARRIS 1989; DUTCHER et al. 1991) or crosses between _C. reinhardtii_/_C. smithii_ strains (RANUM et al. 1988). The centromere distances shown in Table 1 are within 10 cM of previous estimates. The positions of the genetic and molecular markers are therefore consistent with other versions of the genetic map.

**Mapping of Dhc clones:** To place the _Dhc_ genes on the genetic map, the RFLPs identified in Figure 8 were used as molecular markers whose pattern of segregation in the tetrad progeny was compared to that of the other 42 genetic and molecular markers described in Table 1. Eight of the _Dhc_ genes show unique and consistent linkage to specific subsets of molecular and genetic markers (see Table 2). For each _Dhc_ clone, linkage was confirmed with at least two independent markers per linkage group. Only one _Dhc_ clone, _Dhc8_, failed to show close linkage to any of the markers tested and remains

**FIGURE 6.—RT-PCR analyses confirm that all _Dhc_ genes are expressed as mature transcripts.** One microgram of total RNA from deflagellated cells was converted to cDNA using an antisense primer corresponding to the conserved amino acid sequence ITMNPGY. Two microliters of the resulting cDNA product were then used as templates in a series of PCR reactions containing gene-specific primers that were chosen because they spanned regions of genomic DNA containing putative intron sequences (see Figure 2). The final reaction products were loaded on a 1.5% agarose gel and stained with ethidium bromide. Lane 1 contains the RT-PCR product amplified with two _Dhc1_ gene-specific primers. Lanes 2–7 contain the RT-PCR products amplified with a conserved sense primer corresponding to TGKTET(TK) and the appropriate gene-specific antisense primers for _Dhc2–Dhc7_, respectively. The major band in each lane was subcloned and sequenced to verify that the reaction product corresponded to the appropriate _Dhc_ gene. The _Dhc5_ reaction product (marked with *) contained two additional _Dhc_ sequences (see text).

**FIGURE 7.—Identification of RFLPs between two Chlamydomonas strains.** Genomic DNA from two different Chlamydomonas strains, 137c (lanes 1) and S1-D2 (lanes 2), was digested with the restriction enzymes EcoRI/XhoI, _SalI_, _SmaI_, and _SphI_, fractionated on a 0.8% agarose gel, blotted, and hybridized with an 6.2-kb fragment of the _Dhc3_ gene (p3E6.2). RFLPs are seen with all four enzymes.
The approximate map locations of the molecular markers used in this study have been established as described previously (see Ranum et al. 1988; Ranum 1989; Sakakibara et al. 1991; C. Silflow, personal communication, see Chlamydomonas database).

GP228 represents a collection of linked molecular markers that have not yet been assigned to a linkage group. Although recombination frequencies can differ in crosses between polymorphic strains, both the order of the markers and the map distances between markers are in good agreement with other versions of the map (Ranum et al. 1988; Gross et al. 1989; Harris 1989; Dutcher et al. 1991).

**TABLE 1**

Genetic and molecular markers used for RFLP mapping

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Arm</th>
<th>Test markers</th>
<th>( \text{ac} 17 ) distance (cM)</th>
<th>Centromere</th>
<th>( y_1 ) distance (cM)</th>
<th>Centromere</th>
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<tr>
<td></td>
<td></td>
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<td>PD:NPD:TT</td>
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<td>msl</td>
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<td>10</td>
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<td>4:2:10</td>
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<tr>
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<td>( \text{nie} 13 )</td>
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<tr>
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<td>1:1:7</td>
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<tr>
<td></td>
<td>Right</td>
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<tr>
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<td>7:6:11</td>
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<td>7:1</td>
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<tr>
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<tr>
<td></td>
<td>Left</td>
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<td>0:4:4</td>
<td>25</td>
<td>3:1:4</td>
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<tr>
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<td>4:2</td>
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<td>23</td>
<td>2:1:2</td>
<td>20</td>
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</table>

The approximate map locations of the molecular markers used in this study have been established as described previously (see Ranum et al. 1988; Ranum 1989; Sakakibara et al. 1991; C. Silflow, personal communication, see Chlamydomonas database). GP228 represents a collection of linked molecular markers that have not yet been assigned to a linkage group. Although recombination frequencies can differ in crosses between polymorphic strains, both the order of the markers and the map distances between markers are in good agreement with other versions of the map (Ranum et al. 1988; Gross et al. 1989; Harris 1989; Dutcher et al. 1991).
unmapped (data not shown). These results, together with the additional data on centromere distance, place eight of the \textit{Dhc} genes on six different linkage groups (Figure 9).

\textit{Dhc1} maps to the left arm of linkage group XII/XIII based on its tight linkage to the inner arm mutation pf9-2 (<3 cM) and the molecular marker known as \textit{Gulliver} element band d (\textit{Ranum} 1989; \textit{Porter} \textit{et al.} 1992). Consistent with this location, \textit{Dhc1} is a centromere distal marker, as evidenced by its high tetratype frequencies with respect to the centromere markers \textit{ac}17 and \textit{yl}. These data place the \textit{Dhc1} gene in the

\begin{table}
\centering
\caption{RFLP mapping of \textit{Dhc} genes}
\begin{tabular}{|l|l|l|l|l|l|l|}
\hline
Gene & Linkage group & Linkage data & Centromere distance & \\
\hline
\textit{Dhc1} & XII/XIII & \textit{pf}9 & 16:0:0 & <3.0 & \textit{ac}17 & 3:2:24 & 41 \\
& & Band d & 4:0:1 & 10 & \textit{yl} & 0:0:15 & 50 \\
\textit{Dhc2} & II & GP130 & 3:0:10 & 38 & \textit{ac}17 & 3:4:22 & 38 \\
& & GP366 & 6:0:10 & 31 & \textit{yl} & 2:2:12 & 37.5 \\
& & \textit{act}1 & 10:0:6 & 18.8 & & & \\
\textit{Dhc3} & VI & \textit{mt} & 10:0:12 & 27 & \textit{ac}17 & 11:5:9 & 18 \\
& & \textit{pc}18-13 & 19:0:9 & 16 & \textit{yl} & 5:3:6 & 21 \\
& & \textit{act}2 & 2:0:11 & 42 & & & \\
\textit{Dhc4} & II & GP130 & 6:0:9 & 30 & \textit{ac}17 & 9:1:17 & 13 \\
& & GP366 & 15:0:1 & 3.1 & \textit{yl} & 6:4:3 & 12 \\
& & \textit{act}11 & 14:0:3 & 8.8 & & & \\
& & \textit{Dhc2} & 12:1:14 & 33 & & & \\
\textit{Dhc5} & II & GP130 & 6:0:7 & 27 & \textit{ac}17 & 7:13:7 & 13 \\
& & GP366 & 14:0:1 & 3.3 & \textit{yl} & 8:4:3 & 10 \\
& & \textit{Dhc4} & 24:0:0 & <2.0 & & & \\
& & \textit{act}1 & 10:0:4 & 8.3 & & & \\
& & \textit{Dhc2} & 11:1:15 & 38 & & & \\
\textit{Dhc6} & V & \textit{PF1} & 14:0:5 & 13.2 & \textit{ac}17 & 10:7:12 & 21 \\
& & \textit{PF26} & 16:0:6 & 13.6 & \textit{yl} & 3:3:6 & 29 \\
\textit{Dhc7} & XV & \textit{TCR1-F} & 21:0:5 & 9.6 & \textit{ac}17 & 11:11:4 & 7.6 \\
& & \textit{ida}2 & 17:0:1 & 2.8 & \textit{yl} & 4:7:4 & 13 \\
\textit{Dhc8} & Unknown & & & & \textit{ac}17 & 11:11:3 & 6 \\
& & & & & \textit{yl} & 3:5:2 & 10 \\
\textit{Dhc9} & XVI/XVII & \textit{GP61} & 11:0:15 & 28.8 & \textit{ac}17 & 7:18:3 & 5.5 \\
& & \textit{yl} & 14:0:1 & 3.3 & \textit{yl} & 14:0:1 & 3.3 \\
\hline
\end{tabular}
\end{table}
vicinity of three closely linked flagellar loci, PF9 (IDA1), IDA4, and LF2, but cannot discriminate between these three loci as possible Dhc genes.

Dhc2 maps to the right arm of linkage group II, ~18.8 cM distal from the genetic marker act1. The only flagellar mutation that has been mapped to this region is pf18 (Ebersold et al. 1962). pf18 mutants lack the central pair microtubules, but no dynein defect has been described (Adams et al. 1981).

Two genes, Dhc4 and Dhc5, are closely linked to one another (~2 cM apart). They map to linkage group II, between the molecular marker GP366 and the genetic marker act1. These data place the Dhc4 and Dhc5 genes in the vicinity of three flagellar loci, LF1, PF12, and MIA1. The if1 mutation results in unusually long flagella (McVittie 1972a,b), the pf12 mutation in an abnormal swimming phenotype (Ebersold et al. 1962; McVittie 1972a), and the mial mutation in phototaxis defects (S. King and S. Dutcher, personal communication).

The Dhc3 gene maps to linkage group VI, between the mating type (mt) locus and the gene encoding a constitutively expressed transcript represented by the cDNA clone pcfl8-13 (Ranum et al. 1988). Analysis of recombinant tetrads for each marker on linkage group VI suggests that the Dhc3 gene is located on the left
arm and pcf8–13 is on the right. These data place the Dhc3 gene in the vicinity of the SHF1 locus. shf1 cells have short flagella and a slow swimming motility phenotype (JARVICK et al. 1984; KUCHKA and JARVICK 1987).

Dhc6 maps to the right arm of linkage group V, ~20 cM from two radial spoke genes, PF1 and PF26. No other flagellar mutations have been mapped to linkage group V.

Dhc7 maps to linkage group XV, within 3 cM of the inner arm locus IDA2. Analysis of 18 tetrads identified one tetraplete tetrad in which a recombination event was observed between the Dhc7 probe and the site of the ida2 mutation. These results would appear to suggest that Dhc7 and ida2 represent two different loci. However, because Chlamydomonas Dhc genes are typically 20–30 kb in size (MITCHELL 1989; MITCHELL and BROWN 1994), and the probe used in this experiment covers only a 5.2-kb central region of the Dhc7 gene, we cannot exclude the possibility that the tetraplete tetrad represents a cross-over event occurring within the Dhc7 gene, between the site of the ida2 mutation and the region represented by the 5.2-kb probe.

Dhc9 maps to linkage group XVI/XVII, based on linkage to the genetic marker y1 and the molecular marker GP61. No flagellar mutations have been mapped to this linkage group.

In summary, six Dhc genes are linked to genetic loci that have previously been shown to be involved in flagellar function; two Dhc genes map to regions of the genome where no flagellar mutation has yet been identified, and one Dhc gene remains unmapped.

**DISCUSSION**

In this study, we have identified nine Dhc genes whose expression is enhanced by deflagellation. Two of the Dhc genes characterized in this study were previously recovered as partial cDNAs in a PCR screen of a Chlamydomonas cDNA library (WILKERSON et al. 1994). All nine Dhc sequences are likely to be involved in axoneme assembly or motility, but they are clearly distinct from the outer arm Dhcs (MITCHELL and BROWN 1994; WILKERSON et al. 1994). However, only eight Dhc polypeptides have been identified as inner arm dynein isoforms (KAGAMI and KAMIYA 1992). These results suggest that some of the Dhc genes may encode additional inner arm Dhc isoforms that were not resolved in earlier studies. Alternatively, some of these Dhc sequences may be associated with other structures in the flagellar axoneme besides the inner dynein arms. Interestingly, several kinesin-related polypeptides have recently been identified as components of the central pair apparatus in Chlamydomonas (BERNSTEIN et al. 1994; FOX et al. 1994; JOHNSON et al. 1994), where they may play a role in central pair rotation. Another kinesin-related polypeptide is associated with outer doublet microtubules (FOX et al. 1994; WALThER et al. 1994) and basal bodies (VASHISHTHA et al. 1996), where it has been implicated in both intraflagellar transport and flagellar assembly (WALThER et al. 1994; KOZMINSKI et al. 1995; VASHISHTHA et al. 1996). It is therefore reasonable to suppose that some of the Dhc sequences identified in this study may play a role in axoneme assembly or motility distinct from that of an inner arm dynein.

Comparison of the Chlamydomonas Dhc sequences with Dhc sequences identified in other organisms suggests that we have recovered most, but not all members of the Chlamydomonas Dhc gene family. All nine of the Chlamydomonas sequences have closely related homologues in organisms as diverse as Drosophila, Paramecium, sea urchin, and rat (ASAI et al. 1994; GIBBONS et al. 1994; RASMUSSEN et al. 1994; TANAKA et al. 1995; see figure 10). The results of the Southern blot analysis further suggest that there are at least three or four additional Dhc genes (Figure 4). One of these genes corresponds to the gDhc gene of the outer dynein arm, which was not recovered in our PCR screen, but has previously been identified by expression cloning (WILKERSON et al. 1994). We have also not yet identified the Chlamydomonas homologue of the major cytoplasmic Dhc isoform (Figure 10). As the cytoplasmic Dhc sequence appears to be ubiquitous, even in organisms that do not assemble motile flagellar axonemes (KOONCE et al. 1992; ESHEL et al. 1993; LI et al. 1993; PLAMANN et al. 1994; XIANG et al. 1994; LYE et al. 1995), it seems probable that this gene is present in the Chlamydomonas genome and will likely be recovered in future PCR screens. Regardless, the 12 Dhc sequences identified thus far appear to represent the majority of the Dhc gene family in this organism.

Phylogenetic comparisons between Dhc sequences from different organisms suggest that the branch that contains the Chlamydomonas Dhc1 sequence represents one subdivision of the Dhc gene family (see GIBBONS et al. 1994; TANAKA et al. 1995; GIBBONS 1995). This branch includes the Drosophila Dhc98D sequence, the sea urchin Dhc4 and Dhc5C sequences, the rat Dhc2 and Dhc10 sequences, and the Paramecium Dhc5 sequence (see Figure 5 in TANAKA et al. 1995; and Figure 10, this study). Interestingly, the Chlamydomonas Dhc1 gene maps to a region of linkage group XII/XIII that contains three flagellar loci, LF2, PF9 and IDA4. If mutations result in the assembly of abnormally long flagella (McVITIE 1972a,b). Mutations at the PF9 locus (e.g., pf9, ida1, and pf30) disrupt the assembly of the 11 inner arm complex, which contains two Dhc polypeptides, 1-alpha and 1-beta (PIPERNO et al. 1990; KAGAMI et al. 1991; PORTER et al. 1992). ida4 mutations disrupt the assembly of three single-headed 12 inner arm subspecies (KAGAMI and KAMIYA 1992; MASTRONARDE et al. 1992). Recent work has demonstrated that the IDA4 locus encodes the structural gene for a 28 kD dynein light chain known as p28 (LEDIZET and PIPERNO, 1995). It is therefore plausible that the Dhc1 gene corresponds to either the LF2 locus or the PF9 locus and encodes either a Dhc involved in flagellar assembly or one of the two H inner...
arm Dhc. Indeed, recent work from our laboratory has confirmed that the PF9 locus is the Dhc1 structural gene (Myster et al. submitted for publication).

The remaining Chlamydomonas Dhc sequences (Dhc2–Dhc9) form two distinct but closely related groups and presumably include representatives of the I2 and I3 inner arm Dhcs. These sequences can be subdivided based on sequence similarities and the presence of conserved intron locations within their coding sequences. One group of sequences is represented by Dhc3 and Dhc7. These two genes, along with Dhc6, share an intron splice site at a conserved position just downstream from the region encoding the CFDEFNR consensus sequence. Dhc6 is also related to a second group of Dhc genes, Dhc2, Dhc4, and Dhc5. These four genes share another intron splice site located just upstream from the region encoding the CFDEFNR consensus sequence. Additional sequence information will be required to more fully understand the evolutionary relationships between the Dhc genes, but these preliminary observations suggest that Dhc2–Dhc7 are derived from one or two ancestral Dhc genes.

Analysis of Dhc transcripts by both Northern blots and RT-PCR procedures demonstrates that all of the Dhc genes identified in this study are upregulated in response to deflagellation. However, individual transcripts appear to vary in abundance, as evidenced by the relatively weak signals obtained with the Dhc3 probe (see Figures 5 and 6). Although we cannot exclude the possibility that the weaker signals on the blots are due to an abundance of intron sequences in the subclone used as the hybridization probe, Dhc3 may represent a Dhc isoform that is present in more limited amounts than the other Dhc polypeptides. The Dhc3 gene has also been mapped to the left arm of linkage group VI, between the mating type locus and its centromere. This position is based on the analysis of recombinant tetrads for the four markers tested on linkage group VI (e.g., mt, Dhc3, pcf8–13, and act2). As this region of the map is not densely populated with molecular markers (C. Silflow, personal communication), we are not able to estimate the location of Dhc3 more precisely. However, the slow swimming, short flagellar mutation shf 1 has also been mapped to this region (Jarvik et al. 1984).

![Figure 10](image-url)
Interestingly, previous work has suggested that certain Dhc isoforms are restricted to either the proximal or distal regions of the flagellar axoneme (Piperno and Ramins 1991; Gardner et al. 1994). A direct test of linkage between the Dhc3 gene and the shfl mutation will be an important next step toward identifying a possible function for the Dhc3 gene. For instance, Dhc3 could represent a low abundance Dhc isoform that is targeted to a specific region of the flagellar axoneme and is missing in shfl flagella.

Three closely related Dhc sequences, Dhc2, Dhc4, and Dhc5, all map to linkage group II. Dhc2 is linked to flfl, a central pair mutation that disrupts the assembly of 23 axonemal polypeptides (Adams et al. 1981; Dutcher et al. 1984). Whether this linkage is coincidental or suggestive of a previously unrecognized central pair-associated Dhc isoform remains to be determined. Dhc4 and Dhc5 are tightly linked to one another and map to a region that contains three closely linked flagellar loci, LFI, PF12, and MIA1. Whether any of these loci are good candidates for Dhc genes is also an open question. If1 mutations, like the if2 mutations described above, result in abnormally long flagella (McVittie 1972a,b). Limited biochemical analysis of if axonemes has not revealed any clear polypeptide defects, but Dhc5s have not been specifically examined (Barse 1987; P. Lefebvre, personal communication). If2 mutants swim with an aberrant motility phenotype (McVittie 1972a,b), but we have not uncovered any obvious inner arm dynein defects, either by FPLC analysis of dynein extracts or by ultrastructural analysis of if12 axonemes (Gardner, O'Tool, and Porter, unpublished results). mia1 mutants are defective in phototaxis, and preliminary results suggest that the phototaxis phenotype can be correlated with changes in the phosphorylation state of an inner arm dynein intermediate chain (S. King and S. Dutcher, personal communication). Given the close proximity between the two Dhc genes (Table 2) and the three flagellar loci (Harris 1989; S. King and S. Dutcher, personal communication), RFLP mapping of additional tetrad progeny is not a practical strategy for determining if any of these loci might be a Dhc gene. Future experiments to identify a Dhc locus may therefore require the rescue of a mutant phenotype by transformation with a wild-type copy of a Dhc gene.

The Dhc7 gene maps to linkage group XV, ~3 cM from the ida2 mutation. ida2 mutations, like the if9 (ida1) mutations described above, disrupt the assembly of the I1 inner arm complex (Kamya et al. 1991). However, the presence of a tetratype tetrad indicates that a recombination event has taken place between the site of the ida2 mutation and the 5.2-kb subclone used to map the Dhc7 gene. Whether this tetrad represents a recombination event occurring within a Dhc gene, such as has been observed previously with the outer arm beta Dhc gene (Porter et al. 1994), or alternatively, a recombination event between two different dynein related genes, is still unknown. We are currently trying to resolve this question by walking in both directions from the region encoding the predicted ATP binding site and probing the mapping filters with additional subclones of the Dhc7 gene.

The Dhc8 sequence is tightly linked to its centromere (Table 2), but appears unlinked to any of 46 genetic or molecular markers tested on the RFLP mapping filters in this study (data not shown). Because several of these markers are themselves centromere-linked (see Table 1), these results would appear to exclude linkage groups I–IV, VI–VIII, X–XI, XIV–XVI/XVII, and XIX as the possible locus of the Dhc8 gene. In addition, linkage groups IX and XII/XIII are represented by markers on both arms (Table 1) that are also unlinked to the Dhc8 clone. The Dhc8 gene may therefore be located on an unmarked region of linkage group V or XVIII, or alternatively identify a new linkage group in Chlamydomonas. Mapping of additional molecular markers will be required to further clarify the position of the Dhc8 gene.

Two of the Dhc genes (Dhc6 and Dhc9) map to regions of the genome where no flagellar mutation has yet been identified. These observations may mean that the functions of these Dhc genes are either subtle or redundant, and a specific Dhc mutation would simply not have been recovered in previous screens for flagellar mutants. Alternatively, these Dhc genes may perform some essential function that cannot be revealed by mutagenesis of a haploid organism. However, it is important to note that there are a large number of flagellar mutations that have not been definitively placed on the genetic map, and this number is growing rapidly with the advent of insertional mutagenesis procedures (Tam and Lefebvre 1993; Pazour et al. 1995).

In summary, we have identified a family of Dhc genes in Chlamydomonas that are highly homologus to Dhc genes in other organisms. Expression studies indicate that these genes appear to play a role in axoneme assembly or motility. Moreover, several of these genes map near previously identified flagellar loci. In the future, the isolation of “tagged” motility mutants that can be analyzed with these Dhc probes should identify Dhc mutations and clarify their relationship to the previously identified flagellar loci. In addition, the recovery of full-length genomic clones will also permit the identification of Dhc loci by the rescue of a mutant phenotype upon transformation with a wild-type copy of the appropriate Dhc gene.

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