The 78,000 M_r Intermediate Chain of *Chlamydomonas* Outer Arm Dynein Is a WD-repeat Protein Required for Arm Assembly

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Abstract. We have isolated and sequenced a full-length cDNA clone encoding the 78,000 M_r intermediate chain (IC78) of the *Chlamydomonas* outer arm dynein. This protein previously was shown to be located at the base of the solubilized dynein particle and to interact with α tubulin in situ, suggesting that it may be involved in binding the outer arm to the doublet microtubule. The sequence predicts a polypeptide of 683 amino acids having a mass of 76.5 kD. Sequence comparison indicates that IC78 is homologous to the 69,000 M_r intermediate chain (IC69) of *Chlamydomonas* outer arm dynein and to the 74,000 M_r intermediate chain (IC74) of cytoplasmic dynein. The similarity between the chains is greatest in their COOH-terminal halves; the NH_2-terminal halves are highly divergent. The COOH-terminal half of IC78 contains six short imperfect repeats, termed WD repeats, that are thought to be involved in protein–protein interactions. Although not previously reported, these repeated elements also are present in IC69 and IC74. Using the IC78 cDNA as a probe, we screened a group of slow-swimming insertional mutants and identified one which has a large insertion in the IC78 gene and seven in which the IC78 gene is completely deleted. Electron microscopy of three of these IC78 mutants revealed that each is missing the outer arm, indicating that IC78 is essential for arm assembly or attachment to the outer doublet. Restriction fragment length polymorphism mapping places the IC78 gene on the left arm of chromosome XII/XIII, at or near the mutation *oda9*, which also causes loss of the outer arm. Mutants with defects in the IC78 gene do not complement the *oda9* mutation in stable diploids, strongly suggesting that *ODA9* is the structural gene for IC78.

DYNEINS are molecular motors involved in various types of microtubule-based motility. These motors are currently divided into two major groups: axonemal and cytoplasmic dyneins. Axonemal dyneins (reviewed by Witman, 1992; Witman et al., 1994) are essential for flagellar and ciliary beating, while cytoplasmic dyneins (reviewed by Bloom, 1992; Holzbauer et al., 1994) are involved in spindle positioning and the movement of membranous organelles toward the "minus" ends of microtubules. Axonemal dyneins can be divided further into inner and outer arm dyneins. Inner arm dyneins probably are responsible for bend initiation and maintenance of the angle of propagating bends, while the outer arm dynein provides as much as four-fifths of the beating force (Brokaw, 1994). Both axonemal and cytoplasmic dyneins have an ATP-sensitive microtubule-binding site that interacts transiently with microtubules during force production. Outer arm dynein and at least some inner arm dyneins have a second structural microtubule-binding site that serves to anchor the arm to the A-tubule of the outer doublet. In contrast, cytoplasmic dynein interacts with membranous vesicles and kinetochores (Pfarr et al., 1990; Steuer et al., 1990), and so must contain a binding site either for a vesicle or kinetochore component or a protein capable of binding these structures.

The most well-characterized dynein with regard to structural binding is the outer arm dynein of *Chlamydomonas reinhardtii*. *Chlamydomonas* outer arm dynein contains three different heavy chains, each of which has a mass in excess of 500 kD, two intermediate chains (ICs)[1] of 78,000 M_r (IC78) and 69,000 M_r (IC69), and 10 light chains (LCs) ranging from 8,000 to 22,000 M_r (Piperno and Luck, 1979; Huang et al., 1979; Pfister et al., 1982; King and Witman, 1989). Each heavy chain contains a globular head domain and a flexible stem domain (Witman et al., 1983). The head

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1. *Abbreviations used in this paper*: DHC, dynein heavy chain; IC, intermediate chain; LC, light chain; RFLP, restriction fragment length polymorphism; TRP, tetratricopeptide.

contains at least one ATP-hydrolytic site and is presumed to bind transiently to the B-tubule of the outer doublet during force production, whereas the stem extends to the base of the soluble dynein particle and appears to interact with the stems of the other dynein heavy chains (DHCs) to hold the whole complex together. The two intermediate chains and several of the light chains are associated with each other in a discrete complex (Mitchell and Rosenbaum, 1986; King et al., 1991) that is located at the base of the stems (King and Witman, 1990). Protein cross-linking studies indicate that one of the components of this complex, IC78, is in direct contact with α tubulin in the axoneme (King et al., 1991). This suggests that IC78 may be involved in anchoring the outer arm to the outer doublet.

To learn more about the function of IC78, we now have sequenced a full-length cDNA clone encoding it. The deduced amino acid sequence reveals that it is homologous to IC69 and also to the 74,000 M_r intermediate chain (IC74) of cytoplasmic dynein. Southern blot analysis using the cloned cDNA has enabled us to identify several new cell lines, generated by insertional mutagenesis, in which the IC78 gene is either completely deleted or is disrupted by an insertion. Electron microscopy indicates that the axonemes of these cell lines lack outer arms. Therefore, IC78 is essential for outer arm assembly or attachment to the outer doublet microtubule. Restriction fragment length polymorphism analysis maps the IC78 gene near or to a previously described mutation (*oda9*) which results in loss of the outer arms (Kamiya, 1988). The new cell lines in which the IC78 gene is disrupted or deleted do not complement *oda9* in stable diploids, indicating that the primary defect in *oda9* may be a mutation in the gene encoding IC78. Elsewhere, we demonstrate that IC78 translated in vitro is a bona fide microtubule-binding protein (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication). Taken together, these results provide strong support for the hypothesis that IC78 plays an important role in the structural binding of the outer-arm dynein to the A-tubule of the outer doublet.

Materials and Methods

Strains

Strains used included *Chlamydomonas reinhardtii* strains CC-124 (1132D "wild-type"; *nit1*; *nit2*; *agg1*; *mt*−); *nit1*-305 (*nit1*-305; *NIT2*; *mt*+); g1 (*nit1*; *NIT2*; *agg1*; *mt*+; derivative of crosses between CC-124 and *nit1*-305, selected for ease of transformation); CC-2244 (*oda9*; *nit1*; *nit2*; *mt*+); CC-2245 (*oda9*; *nit1*; *nit2*; *mt*−); CC-2229 (*oda1*; *nit1*; *nit2*; *mt*−); B214 (*ac17*; *nit1*; *NIT2*; *agg1*; *mt*−); CC-1952 (S1-D2 "wild-type", *mt*−). A *C. smithii* strain (CC-1373) also was used. ("CC" numbers are catalog numbers of the *Chlamydomonas* Genetics Center, Duke University, Durham, NC.)

Isolation and Partial Direct Amino Acid Sequencing of IC78

Axonemes isolated from 1132D cells as previously described (Witman, 1986) were extracted with 0.6 M NaCl to remove the inner and outer dynein arms (King et al., 1986). The resulting extract was then subjected to sucrose density gradient centrifugation to purify the $\alpha\beta$ dimer of the outer arm dynein. IC78 was separated from other proteins in this complex by SDS-PAGE and transferred to a PVDF membrane. This PVDF replica was stained with amido black, the portion containing IC78 excised, and the bound IC78 directly sequenced in a sequencer (model 477a; Applied Biosystems, Foster City, OR). A similar strip of PVDF containing IC78 was digested with 40 mg/ml CnBr in 70% formic acid for 16 h. The CnBr solution was removed and the strip twice extracted at 37°C for 16 h with a solution containing 70% isopropanol and 1% TFA. The CnBr and isopropanol solutions were combined and dried under vacuum. These peptides were separated by SDS-PAGE and transferred to PVDF. This blot was stained with amido black and a peptide excised and sequenced as above.

Cloning and Sequencing of IC78

Chlamydomonas cells were deflagellated by pH shock (Witman et al., 1972) and allowed to regenerate their flagella for 30 min to induce synthesis of flagellar proteins. Total RNA was isolated from these cells and poly A^+ mRNA was prepared by oligo-dT cellulose chromatography. This mRNA was used to construct a cDNA library in the vector λZAP II using the protocol provided by the manufacturer. Clones containing IC78 sequences were identified by screening with an oligonucleotide designed using the sequence of the internal CnBr fragment. Duplicate plaque lifts were hybridized with the labeled oligonucleotide at 37°C in 6× SSC, 1% SDS and 5× Denhardt's solution for 48 h. Washing consisted of subsequent incubations in 6× SSC and 1% SDS at 22, 37, and 45°C. Filters were autoradiographed after washes at each temperature and plaques showing strong signals at 45°C were removed and plaque purified. The cDNA inserts in the purified phage were isolated by helper phage excision. Insert sizes were determined by digesting the resulting plasmids with EcoRI and XhoI. Exonuclease III–nested deletions were generated from the largest insert with the Erase-a-Base kit (Promega Biotec, Madison, WI), and sequenced using the 7-deaza Sequenase kit (United States Biochemical, Cleveland, OH). Northern and Southern blots were performed as previously described (Wilkerson et al., 1994).

To obtain a genomic clone containing IC78 sequence, an IC78 cDNA clone (pc78k3) was used to screen a λFIX II library (kindly provided by Rogene Schnell, University of Minnesota, Minneapolis, MN) prepared from DNA of the *Chlamydomonas* strain *21gr*. The resulting genomic clone contained an insert of 9 kb.

Insertional Mutagenesis

The *C. reinhardtii* strain g1, which has a mutant nitrate reductase allele (*nit1*) and a wild-type *NIT2* allele, was transformed with the plasmid pGP505, which contains the wild-type *NIT1* allele (Fernandez et al., 1989), and plated on selective medium containing only NO_3 as a nitrogen source. Colonies expressing the wild-type allele of *NIT1* were screened for slow swimming. From 2978 colonies screened, 24 independently isolated cell lines with reduced swimming speed were obtained. DNA was isolated from these slow-swimmers and used to prepare Southern blots, which were then probed with the IC78 cDNA or with clones encoding portions of the α, β, or γ DHCs or IC69 of *Chlamydomonas* outer arm dynein.

Linkage Analysis

A strain (CC-2244) carrying a mutation at the *ODA9* locus was mated to the distantly related strain CC-1952. Tetrads were dissected and DNA was isolated from the products of 15 complete tetrads. These DNAs were digested with BamHI and used to prepare Southern blots, which were then probed with the cDNA clone pc78k3 encoding IC78. Map distances were calculated from the formula ([NPD + 0.5T]/[PD + NPD + T]) × 100 = map distance (Harris, 1989).

Complementation in Diploids

V24 and V27 cells, in which the IC78 gene is disrupted or deleted, respectively, were crossed to B214 (*ac17*; *nit1*; *mt*−) cells to obtain slow-swimming *mt*+ haploid products that required acetate for growth and could use NO_3 as a nitrogen source. These cells then were mated to CC-2245 (*oda9*; *nit1*; *nit2*; *mt*−) or CC-2229 (*oda1*; *nit1*; *nit2*; *mt*−) cells in low ionic strength nitrogen-free medium (Sager and Granick, 1954; medium I minus nitrate [M−N] diluted 5×) supplemented with 0.1% sodium acetate. The mated cells were washed three times in M−N medium to which had been added 0.0375% KNO_3 (M−N+KNO_3), plated immediately onto M−N+KNO_3 agar, and then subjected to a 15 h light/9 h dark regime. The absence of acetate and the presence of nitrate as the sole nitrogen source permitted the growth of diploids and meiotic progeny but did not allow the haploid parental cells to survive. After 7-10 d single colonies were transferred to M−N+KNO_3 liquid medium and scored for motility. Restriction fragment length polymorphism (RFLP) analysis using a 1.6-kb XbaI fragment of λ phage QK7, which is tightly linked to the mating-type locus (Ferris and Goodenough, 1994; and Ferris, P., personal communication), permitted unequivocal identification of true stable diploids as those which contained both mating types.

Computational Analysis

The GCG suite of programs (Devereux et al., 1984) was used for sequence assembly, dot plot comparisons and protein structure predictions. The ALIMAT and FILTER programs (Vingron and Argos, 1991) were used to generate filtered dot plots. The program NEWCOILS (Lupas et al., 1991) was used to predict regions of coiled-coil structure. The BLAST program (Altschul et al., 1990) was used to search the database for related sequences.

In Vitro Transcription and Translation

T3 RNA polymerase was used to generate synthetic RNA from the plasmid pc78k3 which had been digested with XhoI. This RNA was translated in an in vitro reticulocyte lysate system (Promega Biotec) using [^{35}S]methionine to label the proteins.

Electron Microscopy

Chlamydomonas cells were fixed in glutaraldehyde as described previously (Hoops and Witman, 1983), embedded in 4% low melting temperature agarose (SeaPrep; FMC Corp. Bioproducts, Rockland, ME), washed three times for 10 min in 0.1 M cacodylate buffer (pH 7.2), postfixed for 1 h with 1% OsO_4 in 50 mM cacodylate buffer, washed again with buffer followed by deionized water, stained en bloc with 2% aqueous uranyl acetate, rinsed, dehydrated, and embedded in Poly Bed 812 (Polysciences, Inc., Warrington, PA).

Results

Cloning and Sequence of IC78

As a first step in furthering our understanding of outer arm dynein intermediate chain structure and function, we cloned and sequenced an IC78 cDNA. One of the simplest and most direct methods for isolating a cDNA clone for a specific protein is to obtain partial protein sequence and use this sequence to design an oligonucleotide probe based on the codon usage of the organism under study. Because it is possible to obtain large amounts of pure outer-arm dynein from *Chlamydomonas* and to readily resolve the IC78 polypeptide from the other outer-arm dynein subunits by SDS-PAGE, we chose this method to clone an IC78 cDNA. This approach was additionally facilitated by the very restricted codon usage of *Chlamydomonas*, which allowed the design of oligonucleotide probes having only a small amount of degeneracy.

NH_2-terminal sequence was determined directly from purified IC78 (see Materials and Methods); internal sequence was obtained from a CnBr fragment. The NH_2-terminal and internal sequences are shown in Table I. The oligonucleotide probe SA-1 (5′-[G/A]TCGTTCAG[G/A]TCGAAGTTCAT-3′) was designed from the peptide sequence MNFDLND obtained from the internal CnBr fragment. This oligonucleotide was used to screen a cDNA library prepared from RNA isolated from *Chlamydomonas* cells in the process of regenerating their flagella. Three positive plaques were found and purified. The insert from each of these phage was excised by digestion with EcoRI and XhoI, which were used in the original library construction. The cDNA clone pc78k3 was found to have the largest insert with a length of 2.65 kb. This size is consistent with the expected size of a mRNA encoding a protein of 78,000 M_r. Hybridization of this cDNA probe to Southern and northern blots revealed that this sequence occurs once in the *Chlamydomonas* genome and that the mRNA and the cDNA clone are of approximately the same size (Fig. 1). To check the identity of this cDNA we used the clone pc78k3 to produce a synthetic mRNA and translated it in an in vitro translation system. The major translation product was immunoprecipitated with the monoclonal antibody 1878A (Fig. 2), which is specific for IC78 (King et al., 1986). Because the antibody precipitation is independent of the cloning method, these results confirmed that the cDNA clone isolated using the oligonucleotide SA-1 encodes IC78. The size of the translation product was identical to that of IC78, suggesting that the cDNA clone contains the entire coding region.

Using convenient restriction enzyme sites and exonuclease III–generated nested deletions, we obtained the complete sequence, in both directions, of the clone pc78k3. This sequence is shown in Fig. 3. The first ATG is located at nucleotide position 153. The next 18 amino acids correspond to the NH_2-terminal peptide sequence obtained directly from IC78, confirming that this is indeed the translation initiation site. Because this NH_2-terminal sequence was not used to obtain the cDNA clone, its presence in the sequence predicted by the clone also rigorously establishes the identity of this clone. At nucleotide 1827 is an amino acid sequence that matches the peptide sequence obtained from the CnBr fragment. In-frame stop codons are present at nucleotides 2202 and 2205. At nucleotide 2608 there is a consensus poly A addition signal (TGTAAA) and at nucleotide 2626 the poly A tail is found. Therefore, this clone contains the entire cod-

Table I.

IC78 sequences obtained by direct protein sequencing	
NH_2-terminal sequence	PALSPAKKGTDKGKTGK
Internal sequence	NFDLNDSIGDVXXA

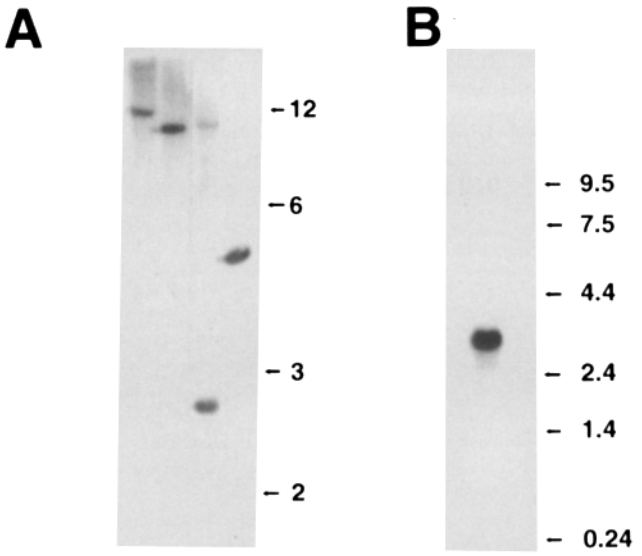

Figure 1. (*A*) Southern blot of *Chlamydomonas* DNA probed with the IC78 cDNA clone pc78k3. Each lane was loaded with 5 μg DNA cleaved with, from left to right, BamHI, KpnI, SmaI, or PvuII. A single band was detected in each lane except for the SmaI digest, where two bands were expected. (*B*) Northern blot prepared with 10 μg of polyA$^+$ RNA isolated from *Chlamydomonas* cells that were regenerating their flagella. The probe pc78k3 hybridized with a single band of 3.1 kb. Size standards (kb) are shown to the right of each blot.

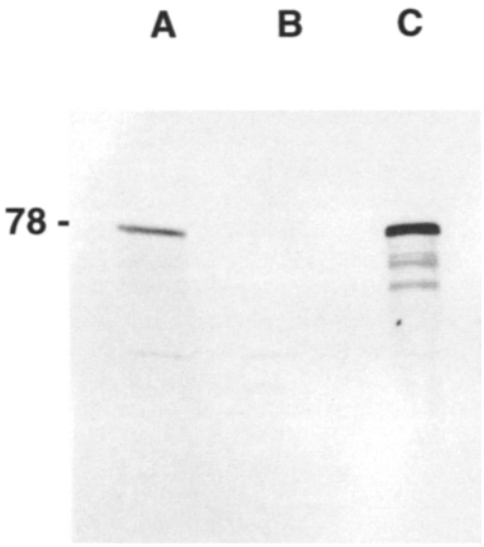

Figure 2. In vitro translation of synthetic RNA prepared from pc78k3 and immunoprecipitation of the product with an IC78-specific monoclonal antibody. Lane *A* of the autoradiograph shows the product obtained when RNA prepared from pc78k3 (see Materials and Methods) was translated in vitro in a reticulocyte lysate system. Lane *B* shows what remained in the supernatant after immunoprecipitation, and lane *C* shows the pellet obtained by immunoprecipitation with monoclonal antibody 1878A (King et al., 1985). The product of the in vitro translation was completely removed from the lysate by the immunoprecipitation.

ing region of IC78. The protein is predicted to contain 683 amino acids and to have a mass of 76.5 kD, in good agreement with the size estimated by SDS-PAGE.

An examination of the predicted secondary structure of the IC78 sequence using the NEWCOILS program does not show any regions of extended α-helix, and no segments show a strong propensity to form coiled-coil structures. The NH_2-terminal 25 amino acids form an extremely hydrophilic region that is predicted to be a random coil and is separated from the rest of the protein by a region rich in glutamine and proline. The REPEAT program revealed that the COOH-terminal half of the molecule contains five imperfect repeats; visual inspection revealed a sixth (Fig. 4). These repeats are similar to the WD repeats found in many proteins and believed to be involved in protein-protein interaction (van der Voorn and Ploegh, 1992; Neer et al., 1994; and see Discussion).

Homologous Proteins

Comparing the IC78 nucleotide sequence to the sequences in the non-redundant PDB+SwissProt+SPupdate+PIR+GenPept+Genupdate database using the BLAST program revealed three sequences that showed significant similarity to IC78. Two of these were IC69 of the *Chlamydomonas* outer arm dynein (Mitchell and Kang, 1991) and IC74 of rat cytoplasmic dynein (Paschal et al., 1992). Fig. 5 shows dot plot comparisons of the IC69, IC74, and IC78 sequences and a filtered dot plot which shows those sequences common between all three ICs. Clearly, the COOH-terminal halves of these sequences share the most similarity, and the NH_2-terminal halves are the most divergent. It previously was shown that the cytoplasmic dynein IC was homologous to IC69. The present finding that IC78 is homologous to IC69, even though the two chains appear to have different functions (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication), indicates that there was a gene duplication followed by divergent evolution of function. The fact that IC78 is homologous to IC69 and IC74 also allows a finer dissection of the sequences conserved between these three chains. A close examination of a three-way alignment of these sequences (Fig. 6) shows that several positions (e.g., positions 374 to 376, 395 to 404, and 516 to 521 in Fig. 6, corresponding to residues 353 to 355, 374 to 383 and 494 to 498 in the IC78 sequence shown in Fig. 3) contain a high proportion of residues that are identical in all three chains. In addition, there are numerous instances of residues that are identical in two of the chains but not the third, indicating that the divergence of the two outer-arm IC sequences occurred near the time of the divergence of cytoplasmic and axonemal ICs. Although not previously recognized, IC69 and IC74 also each contain five repeat elements which closely match the WD consensus sequence (Fig. 4 *A*); a sixth repeat in each

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1     CACGAGATTGTGTTCACATCACATTACACGTAGTAACAATTTAGGATATCGATTTGCTAGTTTACATGGCACTGGTTCCCTAGGGCGCGACGCCCTAACCGTCTCGTTGGCCCTTTCACG
121   CCATCTTAATAGCCTTAGAATAGCGAGGAGCCATGCCGGCGCTCAGCCCCGCCAAGAAGGGGACAGACAAGGGCAAGACCGGCAAGAAGACTGGCAAGCAGGAGCAAAATGCACAAGACT
                                  M  P  A  L  S  P  A  K  K  G  T  D  K  G  K  T  G  K  K  T  G  K  Q  E  Q  N  A  Q  D   29
241   ACATCCCGCCTCCCCCGCCTATGCCGGGCGATGAGGCATTTGCTATGCCCATCCGTGAAATCGTTAAGCCTGACAACCAGCTCTGGCTGTCGGAGGCGGACCTCAATGAGGAGGTGGCAA
      Y  I  P  P  P  P  P  M  P  G  D  E  A  F  A  M  P  I  R  E  I  V  K  P  D  N  Q  L  W  L  S  E  A  D  L  N  E  E  V  A   69
361   AGATGCTGACAGCCAACAACCCAGCAGCGCCAAAGAACATCGTCCGATTCAACATGAAGGACAAAGTCTTTAAGCTGGAGCCCATGGTAGAGCAGACAGTGGTTCACTACGCTACGGATG
      K  M  L  T  A  N  N  P  A  A  P  K  N  I  V  R  F  N  M  K  D  K  V  F  K  L  E  P  M  V  E  Q  T  V  V  H  Y  A  T  D  109
481   GCTGGCTGCTGCATAAGAGCTCGGATGAGGCGAAGCGGCAGATGGACATGGAGAAGATGGAGCAGGAGGCCAGTGCACGGTTCCAGGCGGACATCGACCGCGCTAGCCACGAGCATAAAG
      G  W  L  L  H  K  S  S  D  E  A  K  R  Q  M  D  M  E  K  M  E  Q  E  A  S  A  R  F  Q  A  D  I  D  R  A  S  H  E  H  K  149
601   ACCATGGGGACGTGGAGCCCCCGGATGACTCGCGCCAGCTGCGCAACCAGTTCAACTTCAGCGAGCGGGCAGCGCAGACCCTCAACTACCCGCTGCGGGACCGGGAGACGTTCACGGAAC
      D  H  G  D  V  E  P  P  D  D  S  R  Q  L  R  N  Q  F  N  F  S  E  R  A  A  Q  T  L  N  Y  P  L  R  D  R  E  T  F  T  E  189
721   CGCCGCCGACAGCGACCGTCTCGGGTGCCTGCACACAGTGGGAGATCTACGACGAGTACATTAAGGACCTGGAGCGGCAGCGGATAGATGAGGCGATGAAGTCCAAAGGCGGCAAGAAGG
      P  P  P  T  A  T  V  S  G  A  C  T  Q  W  E  I  Y  D  E  Y  I  K  D  L  E  R  Q  R  I  D  E  A  M  K  S  K  G  G  K  K  229
841   CGGCTGCGGCGGCACGAGCGGCGGGCGCGGCGCACCGGCAAAGGAACGAGCACGTGCCCACGCTGCAAAGCCCGACGCTGATGCACAGCCTGGGGACACTAGACCGCATGGTGAACCAGA
      A  A  A  A  R  A  A  G  A  H  R  Q  R  E  H  V  P  T  L  Q  S  P  T  L  M  H  S  L  G  T  L  D  R  M  V  N  Q  269
961   ACATGTACGAGGAGGTGGCTATGGACTTCAAGTACTGGGACGACGCGGTCGGACGCCTTCCGGCCCGGGGAAGGCTCGCTGCTGCTGCACTCTGGCGCTTTGTGTCGGACAAGAGCAAGCGGC
      N  M  Y  E  E  V  A  M  D  F  K  Y  W  D  D  A  S  D  A  F  R  P  G  E  G  S  L  L  P  L  W  R  F  V  S  D  K  S  K  R  309
1081  GGCAGGTGACGAGCGTTTGCTGGAACCCGCTGTACGACGACATGTTCGCGGTGGGCTACGGCAGCTACGAGTTCCTGAAGCAGGCGTCCGGCCTCATCAACATCTACTCGCTGAAGAACC
      R  Q  V  T  S  V  C  W  N  P  L  Y  D  D  M  F  A  V  G  Y  G  S  Y  E  F  L  K  Q  A  S  G  L  I  N  I  Y  S  L  K  N  349
1201  CCTCGCACCCCGAGTACACCTTCCACACCGAGTCGGGCGTCATGTGCGTGCACTTCCACCCCGAGTTCGCTAACCTGCTGGCGGTGGGCTGCTACGACGGCTCCGTGCTTGTGTACGACG
      P  S  H  P  E  Y  T  F  H  T  E  S  G  V  M  C  V  H  F  H  P  E  F  A  N  L  L  A  V  G  C  Y  D  G  S  V  L  V  Y  D  389
1321  TGCGGTTGAAGAAGGACGAGCCCATCTACCAGGCCTCGGTGCGCACCGGCAAGCTCAACGACCCAGTGTGGCAGATCTACTGGCAGCCCGACGACGCGCAGAAGAGCCTGCAGTTCGTGT
      V  R  L  K  K  D  E  P  I  Y  Q  A  S  V  R  T  G  K  L  N  D  P  V  W  Q  I  Y  W  Q  P  D  D  A  Q  K  S  L  Q  F  V  429
1441  CGATCTCGTCGGACGGCGCGGTGAACCTGTGGACGCTGACAAAGAGCGAGCTCATACCCGAGTGCCTCATGAAGCTGCGTGTGGTGCGCGCCGGCGAGACGAGGGAGGAGGAGGATCCCA
      S  I  S  S  D  G  A  V  N  L  W  T  L  T  K  S  E  L  I  P  E  C  L  M  K  L  R  V  V  R  A  G  E  T  R  E  E  E  D  P  469
1561  ACGCCTCCGGCGCGGCGGGCGGCTGCTGCATGGACTTCTGCAAGATGCCGGGCCAGGAGTCCATCTACCTGGTGGGCACGGAGGAGGGCGCCATCCACCGCTGCTCCAAGGCCTACAGCT
      N  A  S  G  A  A  G  G  C  C  M  D  F  C  K  M  P  G  Q  E  S  I  Y  L  V  G  T  E  E  G  A  I  H  R  C  S  K  A  Y  S  509
1681  CGCAGTACCTGTCCACCTACGTCTCGCACCACCTGGCCGTGTACGCGGTGCACTGGAACAACATCCACCCCTCCATGTTCCTGTCCGCCTCCTGCCGACTGGACCATCAAGCTGTGGGAC
      S  Q  Y  L  S  T  Y  V  S  H  H  L  A  V  Y  A  V  H  W  N  N  I  H  P  S  M  F  L  S  A  S  C  R  L  D  H  Q  A  V  G  549
1801  TGTGTCACGACCCCAAGCGCGCGGTCATGAACTTTGACCTGAACGACTCCATCGGCGATGTGTCCTGGGCTGCCCTACACGCCGACCGTGTTCGCTGCCGTCACCGACGACGGCCGCGTGC
      L  C  H  D  P  K  R  A  V  M  N  F  D  L  N  D  S  I  G  D  V  S  W  A  A  L  Q  P  T  V  F  A  A  V  T  D  D  G  R  V  589
1921  ACGTGTTCGACCTGGCGCAGAACAAGCTGCTGCCGCTGTGCAGCCAGAAGGTGGTGAAGAAGGCCAAGCTGACCAAGCTGGTGTTCAACCCCAAGCACCCCATCGTGCTGGTGGGCGACG
      H  V  F  D  L  A  Q  N  K  L  L  P  L  C  S  Q  K  V  V  K  K  A  K  L  T  K  L  V  F  N  P  K  H  P  I  V  L  V  G  D  629
2041  ACAAGGGCTGCGTCACCTCGCTCAAGCTGAGCCCCAACCTGCGCATCACGTCCAAGCCGGAGAAGGGTCAGAAGTTTGAGGACCTTGAGGTCGCCAAGTTGGACGGCGTGGTGGAGATTG
      D  K  G  C  V  T  S  L  K  L  S  P  N  L  R  I  T  S  K  P  E  K  G  Q  K  F  E  D  L  E  V  A  K  L  D  G  V  V  E  I  669
2161  CGCGCAAGAGCGACGCCGACCTCGCCAAGAACGCGGGCGCACTGATGAGGGGCTTTCGGGTGGTTGTGCAATAGCCGTGTGTGTTGTGGCGGTGGAGCGGATGGTAATGGGAGGGGGTTTG
      A  R  K  S  D  A  D  L  A  K  N  A  A  H  *                                                                            683
2281  GAGGGTGCGTGCAGTGGATACGGTACAGATGACAGGGTCAAGACATTGTGCGATGGTACAGGGCGAGGAGGATTGATGGCAAAGCAAGGTGTAGGAGTGGGGTTGCTGGGGCTGACAGGA
2401  CAGGCAGCGGCGCAGACCACACTGCTCAGGTATGGTATCGGTTGAATGTTTGCGTGCTGCTGCCCATCGCGCGTCCAGGGTACTGCCATTAGGGCGGTTTGTGCAGCTCGCCGGACATGA
2521  CCGCAGACTGGTGCATGCGTCTGGGGCTTTGTGACTGACACATGAATGCGTGGGATTCATGGTTGCATTTGGCTGAATATGTTACGGTGTAAAGCGAACGCGATGAAAAAAAAAAAAAAA
2641  AAAA
```

Figure 3. Nucleic acid and deduced amino acid sequence of IC78. The numbers on the right refer to amino acid residues whereas those on the left refer to nucleotides. The two single underlines indicate amino acids matching two sequences obtained by direct protein sequencing. The double underline marks bases matching the poly A addition signal sequence of *Chlamydomonas*. These sequence are available from EMBL/GenBank/DDBJ under accession number U19120.

A

```
          **   *  *      *   *  *    ******* *  * ***
IC78 A)   SKRRQVTSVC.....W..NPLYDD...MFAVGYGSYEFLKQAS (307-339)
     B)   HTESGVMCVH.....F..HPEFAN...LLAVGCYDGSVLVYDV (358-390)
     C)   KLNDPVWQIY.....W..QPDDAQKSLQFVSISSDGAVNLWTL (407-442)
     D)   SHHLAVYAVH.....W..NNIHPS...MFLSASCRLDHQAVGL (518-550)
     E)   DLNDSIGDVS.....W..AALQPT...VFAAVTDDGRVHVFDL (567-594)
     F)   SPNLRITSKPEKGQKF..ED..LE...VAKLDGVVEIARKSDA (640-675)
IC69 A)   HPDGSVPKVVVA...Y...SILQF...QQQPAGMPLSSYIWDV (170-203)
     B)   VPTSQICCAK.....FNLKD..NN...LVGAGQYNGQLAYFDV (214-246)
     C)   SHRDPIYDFA.....W.LQSKTGT...ECMTVSTDGNVLWWDL (261-294)
     D)   GHHGPIYGLRRNP..F...NS......KYFLSIGDWTARVWVE (360-391)
     E)   KYHPTYLTGGT....W..SPSRPG...VFFTIKMDGAMDVWDL (403-436)
IC74 A)   SKHRVVTCMDWSLQ.Y...PELMV...ASYSNNXDGVALVWNM (283-324)
     B)   HCQSSVMSVCFAR..F..HP..N....LVVGGTYSGQIVLWDN (336-368)
     C)   AHTHPVYCVNV....V...GTQNAH..NLITVSTDGKMCSWSL (385-418)
     D)   GHQGPVTGINC....HMAVG..P....IDFSHLFVTSSFDWTV (480-512)
     E)   DNADYVYDVM.....W..SPVHPA...LFACVDGMGRLDLWNL (528-557)
```

B

Figure 4. (*A*) Alignment of repetitive elements present in IC78, IC69, and IC74 which conform to the WD repeat consensus of Neer et al. (1994). Examination of the IC78 sequence with the program REPEAT (window size 40, stringency 20, and a range of 300) found five repetitive elements (*A-C*, *E*, and *F*) in IC78. We recognized these as conforming to the regular expression that describes the WD repeat. Visual inspection of the IC78 sequence revealed a sixth repeat (*D*) which matched the WD repeat consensus. Further analysis indicated that similar repeats also are present in IC69 and IC74. The alignment in A was accomplished by hand using only the spacing (dots) allowed by the consensus of Neer et al. (1994). The only exception is in the A repeat from IC74, where the symbol X represents the amino acid sequence EDAPHEP. The asterisks at the top of the aligned sequences indicate constrained amino acids in the WD repeat consensus sequence. Amino acids shown in bold text are those which match the consensus. Only two of three amino acids need to match under the asterisks marked by the overline. (*B*) Diagrammatic representation of the location of the six repetitive elements in the IC78 sequence. *N* and *C* indicate the NH_2 and COOH termini.

chain is a poorer match to the consensus sequence (not shown). The WD repeat elements B, C, and E in IC78 (corresponding to positions 379-411, 428-463, and 597-630, respectively, in Fig. 6) are among the regions most highly conserved between these three ICs.

The third sequence that is homologous to IC78 is a human cDNA encoding a 56-kD protein (L07758) of unknown function (the probability that this match occurred by chance was 1.9×10^{-4}). Fig. 7 *a* shows a dot plot comparison between L07758 and IC78. Four regions of homology are revealed by the dot plot. One region (residues 302-376) in the clone L07758 matches two sequences separated by 162 amino acids in the IC78 sequence (Fig. 7 *b*). These two regions in IC78 correspond to the WD repeats B and E. As indicated by the offsets in Fig. 7 *a*, L07758 contains a second region (residues 270-285) that also is homologous to the repeats B and E in IC78.

IC78 Is Required for Outer Arm Assembly

If IC78 is involved in attaching the outer arm to the outer doublet microtubule, then a defect in IC78 might result in

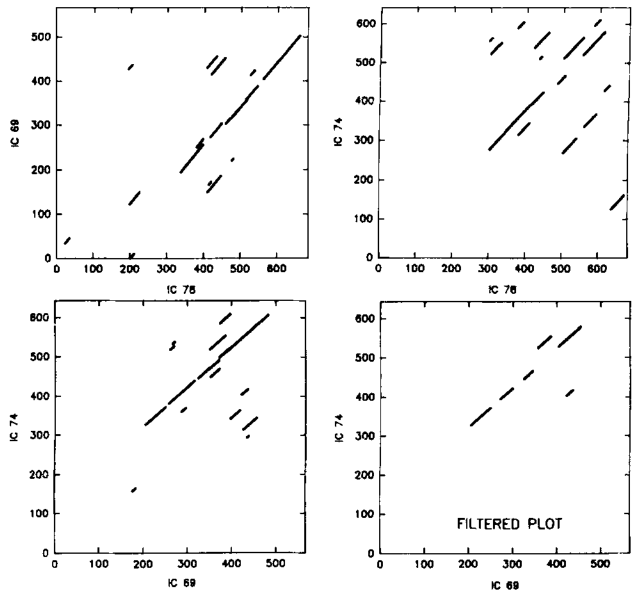

Figure 5. Dot plot comparisons of IC78, IC69 (Mitchell and Kang, 1991), and IC74 (Paschal et al., 1992). Each IC was compared to each of the other ICs using the program ALIMAT. The filtered plot shows regions of similarity shared by all three ICs. This plot, generated using the program FILTER, arbitrarily uses the sequences of IC74 and IC69 for the axes, even though all three chains are compared. Points in regions having a match four standard deviations above a random match were plotted.

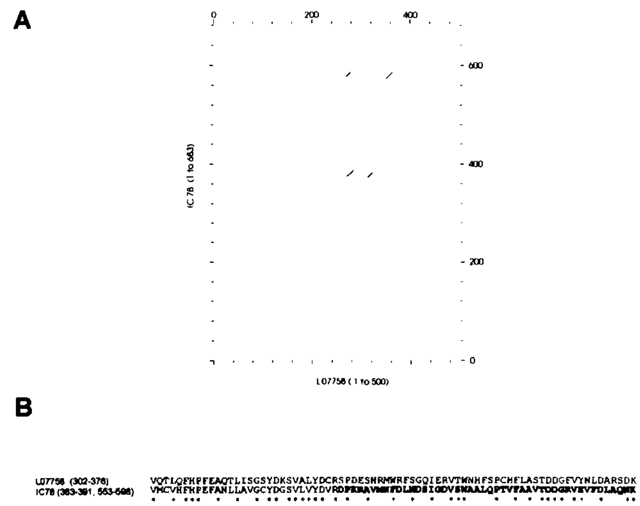

Figure 7. (*A*) Dot plot comparison between IC78 and the predicted amino acid sequence of the protein encoded by the human cDNA L07758. The dot plot was generated by the program COMPARE using a window size of 30 and a stringency of 19. (*B*) Alignment of two regions of IC78 with a single region of L07758. The program BLAST matched the regions of IC78 from residues 363-391 and 553-598 (indicated by bold text) with residues 302-376 of L07758. The program marks amino acid identities with asterisks and conservative replacements with pluses.

loss of the outer arm. The availability of a cDNA clone for IC78 made it possible to identify mutations with disruptions or deletions in the IC78 gene and thus test this prediction. To do this, we took advantage of the fact that when *Chlamydomonas* is transformed, the inserting DNA usually inserts at random into the genome, disrupting the gene at the site of entry or, more commonly, causing large deletions of the flanking DNA (Tam and Lefebvre, 1993; Gumpel and Purton, 1994; and see below).

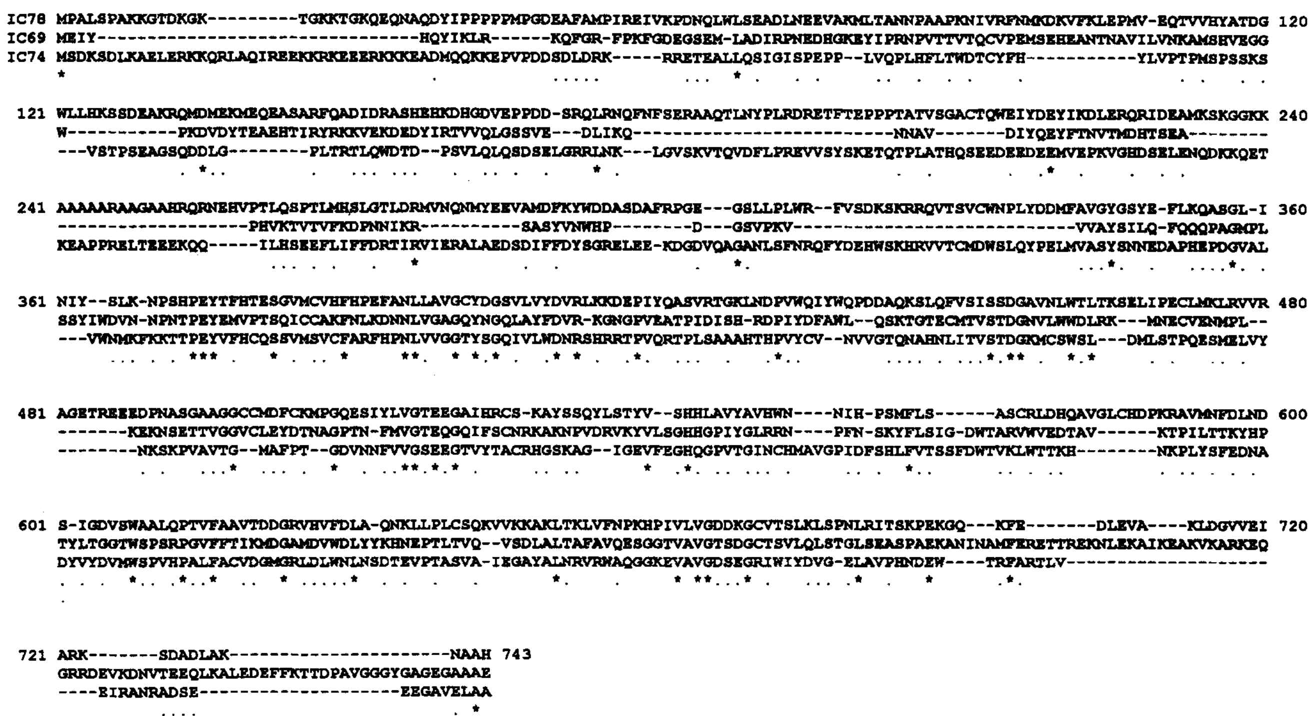

Figure 6. Three-way alignment of IC78, IC69, and IC74. The numbers are arbitrary and include gaps introduced by the alignment program CLUSTAL. Asterisks indicate amino acids conserved between all three sequences whereas dots indicate conservative replacements.

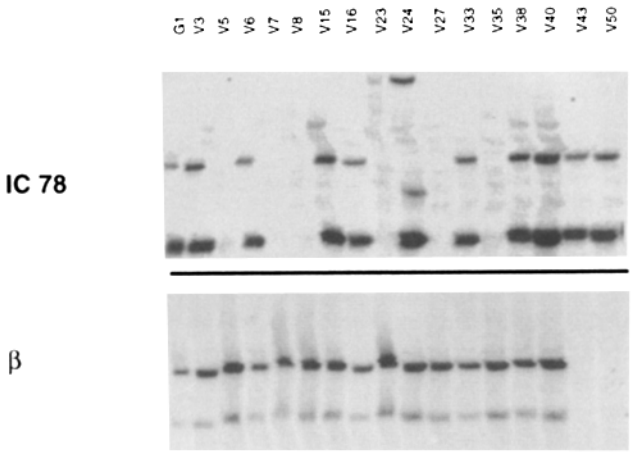

Figure 8. Southern blot analysis of 16 slow-swimming strains isolated following insertional mutagenesis. DNA isolated from the wild-type strain g1 (*G1*) or slow-swimming strains (*V3–V50*) was digested with PstI and used to prepare the blot. (*Top*) The blot was probed first with the IC78 clone pc78k3. In the wild-type g1 strain, two hybridizing bands were observed with the IC78 probe. In strains V5, V7, V8, V23, V27, and V35, no bands were observed, indicating that the IC78 sequences are completely deleted. In strain V24, the upper band present in g1 has split into two bands, indicating that an insertion is present in the IC78 gene. (*Bottom*) To confirm that DNA was present in each of the lanes, the blot was then stripped and reprobed with the clone pEB1.2, which encodes the NH_2-terminal portion of the β heavy chain of outer arm dynein (Mitchell, 1989). The pEB1.2 sequences are normal in the first 14 strains, but have been deleted in strains V43 and V50.

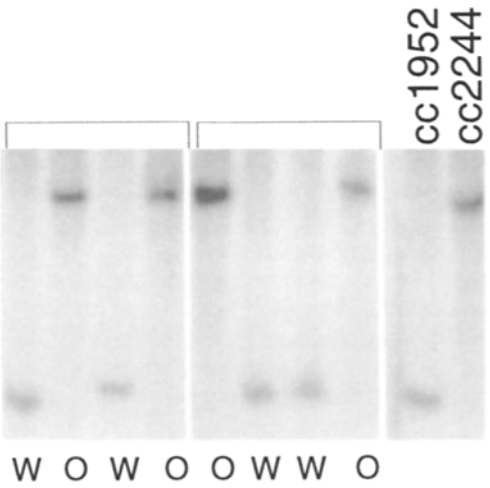

Figure 10. Tetrad analysis of the segregation of IC78 sequences and the *ODA9* locus. 15 tetrads were dissected from a cross between CC-1952 and CC-2244 (*oda9*). DNA isolated from each of the progeny was digested with BamHI and used to prepare Southern blots. The blots were then probed with pc78k3, which hybridizes with restriction fragments of 9 kb in the *oda9* mutant and 4 kb in CC-1952. The figure shows two tetrads (*bracketed*) from the cross. The swimming phenotype is indicated by an O for the slow jerky swimming characteristic of *oda* mutants and a W for wild-type swimming. The 9-kb band segregated with the *oda9* phenotype in all 15 tetrads.

Chlamydomonas strain g1, carrying a mutant allele (*nit1*) of the nitrate reductase gene, was transformed with DNA carrying the wild-type allele. Transformed cells were selected on the basis of their ability to grow on medium containing nitrate as the sole nitrogen source. Transformants were then screened for a slow swimming phenotype, which is characteristic of mutants with defects in the dynein arms. Of 2978 independently isolated colonies that were screened, 24 had reduced swimming speed. The DNA from these cells was isolated and analysed in Southern blots probed with the IC78 cDNA or with cloned DNA encoding portions of the α, β, or γ DHCs or IC69. Of the 24 mutants, seven had a complete deletion of the IC78 gene, and one had a large insertion in the gene (Fig. 8). Two other mutants had deletions of all or part of the β DHC gene. No alterations were detected with the probes for the other dynein genes.

Two of the IC78 deletion mutants (V5 and V27) and the IC78 disruption mutant (V24) were selected for further study. All three of the mutants had normal length flagella but swam with a slow jerky movement, which is indicative of a defect in the outer dynein arm (Kamiya, 1991). Electron microscopic analysis revealed that the outer arm was completely missing in each of these strains (Fig. 9), although the inner arms, the radial spokes, and the central microtubules and their projections appeared normal. Therefore, deletion or disruption of IC78 specifically prevents assembly of the outer arm.

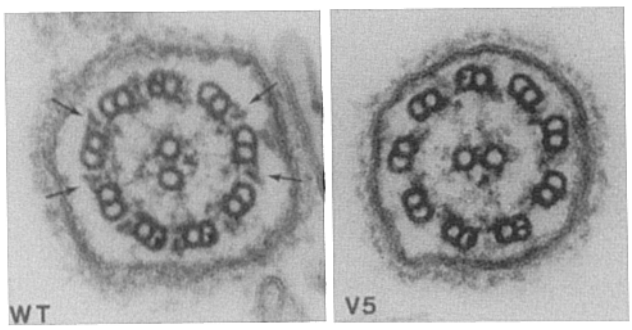

Figure 9. Representative electron micrographs of cross-sections of flagella of strains g1 (*WT*) and V5. The outer arms (four of which are marked by *arrows* in *WT*) are specifically absent in V5, in which the IC78 gene has been deleted.

Mapping the IC78 Gene

To determine the location of the IC78 gene on the *C. reinhardtii* genetic map, we collaborated with Drs. P. Lefebvre and C. Silflow (University of Minnesota), who have been using RFLP analysis to map cloned nuclear sequences throughout the *C. reinhardtii* genome (Ranum et al., 1988). This mapping effort relies on RFLPs between *C. reinhardtii* and the closely related interfertile strain *C. smithii*. No RFLPs were observed between these two strains using the IC78 cDNA clone as a probe. However, using a 9-kb genomic clone encoding IC78, bands of 9 and 12 kb were observed in Southern blots of BamHI digests of *C. reinhardtii* and *C. smithii* DNA, respectively. The pattern of segregation of the RFLP in random tetrad products from a *C. reinhardtii* × *C. smithii* cross-indicated that the gene encoding IC78 was on the left arm of chromosome XII/XIII, approximately 13 map units from a β-2 tubulin RFLP marker.

IC78 Probably Is Encoded by the ODA9 Locus

The region of chromosome XII/XIII which contains the IC78 gene also contains the *ODA9* locus, which is necessary for outer arm assembly. To determine how closely linked the IC78 and *ODA9* loci are, we crossed strain CC-2244 containing the *oda9* mutation, which causes loss of the outer arm and slow swimming, to the wild-type *C. reinhardtii* strain CC-1952, which has abundant RFLPs with respect to the standard laboratory strains (Gross et al., 1988). The CC-2244 and CC-1952 strains exhibit restriction fragments of 9 and 4 kb, respectively, when Southern blots of BamHI digests of their DNAs are probed with the IC78 cDNA clone pc78k3. Tetrad progeny were scored for the IC78 RFLP marker and the slow swimming phenotype indicative of the *oda* mutation (Fig. 10). Only parental ditypes were found in 15 tetrads, indicating that the two markers are less than 3 map units apart.

To determine if mutations at the *ODA9* and IC78 loci could complement one another, we constructed stable diploids between the IC78-defective strains V24 or V27 and strains containing either the mutations *oda9* or *oda1*, which also causes loss of the outer arm and slow swimming. Diploids containing V24 or V27 and *oda1* swam at wild-type speeds, indicating that there was complementation between these loci. In contrast, there was no complementation between V24 or V27 and *oda9*. These results strongly suggest that the *ODA9* locus encodes IC78.

Discussion

Previous studies have shown that IC78 is located in an IC/LC complex at the base of the isolated $\alpha\beta$ heterodimer of the outer arm dynein (King and Witman, 1990), and is in direct contact with α tubulin in the axoneme of the *Chlamydomonas* flagellum (King et al., 1991). These findings suggested that IC78 might be involved in binding the outer arm to the outer doublet microtubule. To learn more about the structure of IC78, and thus ultimately to better understand its function and its interaction with tubulin and the other proteins with which it associates, we have cloned and sequenced a full-length cDNA encoding IC78.

The sequence reveals that IC78 shares significant homology with IC69 (the other IC of *Chlamydomonas* outer arm dynein) and with IC74 (the IC of cytoplasmic dynein). A previous study showed that IC69 and IC74 were homologous (Paschal et al., 1992). The finding that IC78 also is related is of interest for several reasons. First, this is the first demonstration that different ICs within a single dynein are homologous. Thus, *Chlamydomonas* IC78 and IC69 are members of the same protein family, despite differences in their size, antigenicity (King et al., 1985, 1986) and apparent function (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication; and see below). Second, whereas IC69 may have a role in the regulation of dynein activity (Mitchell and Kang, 1993), IC78 probably is involved in binding dynein to the outer doublet microtubule (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication, and see below). This raises the possibility that the ICs of other dynein isoforms may be similarly involved in structural attachment to the cell organelles to which those dyneins bind. Third, the fact that IC78 is related to the other two ICs has permitted a more detailed analysis of the domains and specific residues conserved between the ICs (see below). The finding that residues which are conserved between any two of the ICs may be different in the third IC suggests that, during evolution, the outer arm ICs diverged from each other soon after the separation of axonemal and cytoplasmic dyneins.

The IC78 sequence can be divided into two regions based on its similarity to IC69 and IC74. One region, representing the COOH-terminal one-half of the protein, is fairly conserved among the three ICs, whereas the NH_2-terminal third is very divergent. The conserved region contains six repetitive elements that are related to the WD repeats first identified in β-transducin (Fong et al., 1986). These repeats, each of which is about 40–residues long and contains a variable and a conserved region, are present in nonenzymatic, regulatory proteins with widely different functions and cellular locations (van der Voorn and Ploegh, 1992; Neer et al., 1994). It has been proposed that they play a role in the assembly of multiprotein complexes (Neer et al., 1994). Neer et al. (1994) have speculated that the conserved core of the WD repeat provides a "scaffold" to present the variable region, which may dictate the specificity of binding, on the surface of the protein. They further suggest that the differentiated repeats in a single protein may allow interactions with different subunits. The presence of WD repeats in all three dynein ICs would be consistent with a role for the repeats in subunit–subunit interactions within the dyneins, as such interactions are common to these dynein ICs. Repeats A and B of IC78 correspond precisely with the region of that polypeptide which appears to bind IC69 (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication), suggesting that these particular repeats are involved in IC78/IC69 interaction, and supporting the general hypothesis that WD repeats are involved in subunit–subunit binding.

Some of the proteins containing WD repeats have been shown by genetic means to be associated with proteins containing tetratricopeptide (TRP) repeats (Goebl and Yanagida, 1991). This has led to the suggestion that proteins containing WD and TRP elements interact with each other via their respective repeats (van der Voorn and Ploegh, 1992). We have not found evidence of TRP repeats in any of the ICs, and have found only weak matches in the DHCs. Nevertheless, there is a precedent for TRP repeats in the nonenzymatic subunits of molecular motors, as the kinesin light chain contains a large number of repeats (Fan and Amos, 1994) which are related to the TRP repeats in the yeast glucose repression mediator protein (P14922) (Wilkerson and Witman, unpublished results).

The conserved half of IC78 also contains one of the chain's microtubule-binding domains (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication). Inasmuch as IC78 is the only one of the ICs to bind to microtubules, the critical determinants for this microtubule-binding site must not have been retained, or did not evolve, in the other chains. The other microtubule-binding sites, which appear to be responsible for the cross-linking between IC78 and α-tubulin, occur in the non-conserved NH_2-terminal half of IC78.

Although the NH_2-terminal one-third is poorly conserved among members of the IC family, the extreme NH_2 termi-

nus of both IC78 and IC74 is very highly charged. In IC78, this region is separated from the rest of the protein by a short segment enriched in the amino acids glutamine and proline. Similar segments have been shown to form flexible structures that link functional elements of proteins and are known as polyproline II helices (Adzhubei and Sternberg, 1994) or Q-linkers (Wootton and Drummond, 1989). It is therefore of interest that in IC78, the presence or absence of the NH_2 terminus affects the protein's ability to bind to microtubules (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication). IC69, which does not bind microtubules, lacks both the highly charged NH_2-terminal region and the flexible linker. It is tempting to speculate that the highly charged NH_2 terminus of IC74 may have a role comparable to that of the NH_2 terminus of IC78.

A search of the Genbank database revealed that IC78 is similar to a human cDNA sequence encoding a 56-kD protein. The BLAST program listed this sequence as having a smallest Poisson probability (which is a measure of the likelihood that the match occurred by chance) of 1.9×10^{-4}, which was even smaller than the value of 2.6×10^{-4} obtained for IC69. The 56-kD protein was not found when the database was searched with either the IC69 or IC74 sequences, indicating that this match was specific for IC78. A dot plot comparison revealed that two contiguous amino acid stretches in the 56-kD protein match two of the WD repeats (B and E) that are separated by 162 amino acids in the IC78 sequence. In addition, a third sequence between amino acids 270 and 285 in the 56-kD protein matches both the B and E WD repeats in IC78. Nothing is known about the function of either the 56-kD protein or its closest match in the database, the yeast PWP1 protein. However, the similarity between the WD repeats in IC78 and the 56-kD protein suggests that they interact with a similar protein.

Using the IC78 cDNA as a probe, we have identified several mutants in which the IC78 gene was either disrupted or completely deleted. These mutants initially were selected on the basis of a slow-swimming phenotype, which previously has been used to isolate outer dynein arm-less (*oda*) mutants (Kamiya, 1988). Although EM analysis revealed that not all of the slow-swimming mutants were lacking the outer arm (Koutoulis, A., G. J. Pazour, and G. B. Witman, unpublished results), the outer arm was missing in all of the IC78 deletion or disruption mutants that were examined. This is the first direct evidence that IC78 is essential for the assembly of the outer arm or for its attachment to the outer doublet microtubule. In light of the evidence that IC78 is a microtubule-binding protein (King, S. M., C. G. Wilkerson, and G. B. Witman, manuscript submitted for publication), it is reasonable to assume that loss of IC78 greatly decreases the affinity of the outer arm for the outer doublet.

IC78 appears to be encoded by the *ODA9* locus. First, mutations in *ODA9* and the IC78 gene both lead to loss of the outer arm. Second, we have mapped the IC78 gene to within 3 map units of *ODA9*. Third, insertional mutants in which the IC78 gene is disrupted or deleted do not complement a mutant allele of *ODA9* in diploids, although they do complement a mutant allele of *ODA1*, which also causes loss of the outer arm. Absolute proof that the *ODA9* locus encodes IC78 would require rescue of an *ODA9* mutant by transformation with a genomic clone or minigene encoding the wild-type IC78. In the absence of such definitive evidence, we tentatively assign IC78 to the *ODA9* locus.

Although IC78 appears to be a microtubule-binding protein necessary for assembly of an outer arm onto an outer doublet microtubule, its affinity for microtubules is not adequate to explain the specificity of outer arm binding. Previous studies have shown that *Chlamydomonas* outer arm dynein binds to and saturates the entire surface of microtubules prepared from purified bovine brain tubulin (Haimo and Fenton, 1984, 1988). However, the outer arm binds to a specific site on the outer doublet microtubule in vivo. Moreover, when outer arms are added back to *Chlamydomonas* axonemes lacking the arms, they reattach to the correct site (Takada et al., 1992). Therefore, there must be some axonemal component in addition to tubulin which directs the outer arm to its proper location. Recently, it has been shown that a 7S factor is necessary for efficient assembly of isolated outer arms onto axonemes from the mutants *oda1* and *oda3*, but not onto axonemes from the mutants *oda2*, *oda4*, *oda5*, or *oda6* (Takada and Kamiya, 1994). The 7S factor is correlated with a 70-kD protein present in KCl extracts of *oda2*, *oda4*, *oda5*, and *oda6* axonemes but absent in extracts of *oda1* and *oda3* axonemes; the absence of this factor also is correlated with the absence in *oda1* and *oda3* of a small structure which in the other *oda* mutants projects from the outer doublet at the site where the outer arm normally would attach. Therefore, this structure probably has an important role in forming the outer arm binding site on the outer doublet. It will be very interesting to determine if this component is structurally related to the dynein ICs, and if it interacts with IC78 or with some other outer arm polypeptide. In any case, it is likely that the interaction of IC78 with tubulin provides much of the adhesive force for binding the outer arm to the outer doublet.

Mutations in at least 10 different genes spread throughout the *Chlamydomonas* genome can cause loss of the outer arm and slow swimming (Kamiya, 1988). Consequently, if nonhomologous insertional mutagenesis were random, and considerations of target size notwithstanding, one would expect that insertional mutants involving the IC78 gene would be relatively infrequent among outer arm-less mutants. It was surprising to find that the IC78 gene was specifically affected in 8 out of 24 slow-swimming mutants generated by insertional mutagenesis. Since not all of the latter are outer arm-less, the percent of outer arm-less mutants having a defect in IC78 was even higher. These results suggest that insertional events occur more frequently in some parts of the *Chlamydomonas* genome than in others. Thus, it may be difficult or impossible to saturate a phenotype by insertional mutagenesis alone. Nevertheless, it is clear that insertional mutagenesis is an extremely valuable tool for obtaining the null phenotype for many genes. The availability of null mutants for the IC78 gene will greatly facilitate future studies of IC78 using site-directed mutagenesis and transformation of cells with the altered gene, because the effects of the alteration can be analyzed in the null mutant without complications caused by the presence of the protein product of the wild-type or a different mutant allele.

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