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Interspecies Conservation of Outer Arm Dynein Intermediate Chain Sequences Defines Two Intermediate Chain Subclasses

Kazuo Ogawa,*† Ritsu Kamiya,‡ Curtis G. Wilkerson,§ and George B. Witman§

*Department of Cell Biology, National Institute for Basic Biology, Okazaki 444, Japan; ‡Zoological Institute, The University of Tokyo, Tokyo 113, Japan; and §Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

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Immunological analysis showed that antibodies against the intermediate chains (ICs) IC2 and IC3 of sea urchin outer arm dynein specifically cross-reacted with intermediate chains IC78 and IC69, respectively, of *Chlamydomonas* outer arm dynein. In contrast, no specific cross-reactivity with any *Chlamydomonas* outer arm polypeptide was observed using antibody against IC1 of sea urchin outer arm dynein. To learn more about the relationships between the different ICs, overlapping cDNAs encoding all of IC2 and IC3 of sea urchin were isolated and sequenced. Comparison of these sequences with those previously obtained for the *Chlamydomonas* ICs revealed that, although all four chains are homologous, sea urchin IC2 is much more closely related to *Chlamydomonas* IC78 (45.8% identity), and sea urchin IC3 is much more closely related to *Chlamydomonas* IC69 (48.5% identity), than either sea urchin chain is related to the other (23.5% identity). For homologous pairs, the similarities extend throughout the full lengths of the chains. Regions of similarity between all four ICs and the IC (IC74) of cytoplasmic dynein, located in the C-terminal halves of the chains, are due primarily to conservation of the WD repeats present in all of these ICs. This is the first demonstration that structural differences between individual ICs within an outer arm dynein have been highly conserved in the dyneins of distantly related species. The results provide a basis for the subclassification of these chains.

INTRODUCTION

Dyneins are molecular motors responsible for many different types of microtubule-based motility. Within cilia and flagella, dyneins are contained in the inner and outer arms and generate the force required for ciliary and flagellar beating. These arms are structurally bound to the A-tubules of the outer doublet microtubules and extend toward the B-tubules of the adjacent outer doublets, to which they transiently bind during force generation. The outer arm dynein is probably the most extensively studied of all dynein isoforms (reviewed by Witman *et al.*, 1994); detailed structural and biochemical studies have been carried out on the outer arm dynein from sea urchin sperm (Tang *et al.*, 1982; Moss *et al.*, 1992), *Chlamydomonas* flagella (reviewed by King and Witman, 1989), rainbow trout sperm (Gatti *et al.*, 1989; King *et al.*, 1990), and *Tetrahymena* cilia (Porter and Johnson, 1983). These studies revealed that outer arm dynein is a complex macromolecular assembly containing a large number of polypeptide chains that have been classified according to their sizes as heavy, intermediate, and light chains.

The dynein heavy chains (DHCs)[1] are larger than 500 kDa and contain the ATPase activity that forms the basis for dynein's mechanochemical properties.

† Corresponding author.

[1] Abbreviations used: DHC, dynein heavy chain; IC, dynein intermediate chain; LC, dynein light chain.

The outer arm dyneins of protistans such as *Chlamydomonas* and *Tetrahymena* contain three different DHCs (termed α, β, and γ DHCs), whereas those of multicellular organisms such as sea urchin and trout contain only two different DHCs (termed α and β DHCs).

The outer arm intermediate chains (ICs) range in size from approximate M_r 55,000–120,000 (reviewed in Witman *et al.*, 1994). They have no known enzymatic activity, but at least one of the chains appears to be essential for binding the arm to the A-tubule (Wilkerson *et al.*, 1995; King, Patel-King, Wilkerson, and Witman, unpublished data), and others may have roles in the regulation of dynein activity (Mitchell and Kang, 1993; Gagnon *et al.*, 1994). The number of ICs varies widely from organism to organism. In *Chlamydomonas*, there are two ICs (IC78 and IC69[2]; M_r 78,000 and 69,000, respectively). Sea urchin sperm outer arm dynein contains three ICs (IC1, IC2, and IC3; M_r 112,000–122,000, 79,000–98,000, and 70,000–76,000, respectively, depending on species) (Bell *et al.*, 1979; Ogawa *et al.*, 1990; Moss *et al.*, 1992). The outer arm dynein of trout sperm contains five ICs (ICs 1–5; M_r 85,000, 73,000, 65,000, 63,000, and 57,000, respectively) (Gatti *et al.*, 1989). The ICs interact with each other and/or with the DHCs and light chains (LCs). Although their subunit affinities suggest that there are structural and functional similarities between the specific ICs of different species (Witman *et al.*, 1992), the variability in the number and size of the chains has precluded subclassification of the chains, as well as any firm conclusions regarding their relationships across species lines.

Until now, the only outer arm IC sequence that has been reported is that of *Chlamydomonas* IC69 (Mitchell and Kang, 1991); this chain is homologous to the 74,000 M_r IC (IC74) of cytoplasmic dynein (Paschal *et al.*, 1992). Recently, we have cloned a cDNA encoding *Chlamydomonas* IC78 (Wilkerson *et al.*, 1995); IC69 and IC78 are homologous to each other. To determine the extent to which individual ICs are related in separate species, we have now obtained cDNAs encoding sea urchin IC2 and IC3. Sequence comparisons indicate that IC2 of sea urchin is more closely related to IC78 of *Chlamydomonas* than to any of the other cloned ICs, whereas sea urchin IC3 is more similar to *Chlamydomonas* IC69 than to the other ICs. Based on these results, we propose that a pair of ICs equivalent to sea urchin IC2/IC3 and *Chlamydomonas* IC78/IC69 is a general and highly conserved structural feature of outer arm dyneins, and that the chains that form homologous pairs between each species (e.g., sea urchin IC2 and *Chlamydomonas* IC78) are likely to have similar roles within their respective dyneins. Comparison between the members of such homologous pairs reveal extended regions of sequence that are very highly conserved between species; these conserved regions are apt to be especially important in the structure or function of a particular subclass of IC.

[2] This chain also is referred to as IC70 (Mitchell and Kang, 1991).

MATERIALS AND METHODS

Sea Urchin Egg cDNA Libraries

Total RNA was extracted from the unfertilized eggs of the sea urchin *Anthocidaris crassispina*. cDNA syntheses were performed by priming poly(A)$^+$ RNA with oligo-d(T) and specific oligonucleotides complementary to the nucleotide sequence of cDNAs of interest using the Time-Saver cDNA Synthesis kit (Pharmacia, Uppsala, Sweden). Double-stranded cDNAs were ligated to λgt11 at the *Eco*RI site. Phage DNA isolation and the subcloning of cDNA inserts into plasmids were performed according to basic procedures (Sambrook *et al.*, 1989).

Sequencing

The cDNA inserts in the λ vector were subcloned into pBluescript IIKS+ (Stratagene, La Jolla, CA) or pTZ19R. Plasmids with the cDNA in opposite orientations were obtained and a number of nested deletions were generated from the DNAs using the Nested Deletion kit (Pharmacia). DNA sequences were determined by Sanger's chain-termination method using Sequenase Ver. 2 (United States Biochemical, Cleveland, OH) and double-stranded DNA as template.

Sequence Analysis

The GCG programs COMPARE and DOTPLOT (Devereux *et al.*, 1984) were used to generate dot plot comparisons. The GCG program PILEUP was used to generate relationship trees. Pairwise and multiple sequence alignments were obtained using the program CLUSTAL (Higgins, 1994). The program RDF2 was used to calculate *z* values (Lipman and Pearson, 1985).

Cilia and Flagella Isolation

Embryonic cilia and sperm flagella from *A. crassispina* were prepared as reported previously (Ogawa *et al.*, 1990). Flagella from wild-type and *oda6* (Kamiya, 1988) strains of *Chlamydomonas* were isolated and demembranated as previously described (Witman *et al.*, 1978).

Antibodies and Immunoblotting

All antibodies, including affinity-purified and monoclonal antibodies directed against IC2 and IC3 of sea urchin outer arm dynein, were the same as those reported previously (Ogawa *et al.*, 1990). These antibodies do not react with polypeptides of cytoplasmic dynein isolated from sea urchin eggs (Ogawa *et al.*, 1990). They also are unlikely to react with sea urchin inner arm dynein ICs, as these would be expected (based on studies of *Chlamydomonas* [reviewed in Witman *et al.*, 1994]) to migrate very differently from the outer arm ICs in sodium dodecyl sulfate (SDS)-polyacrylamide gels.

For immunoblots, ciliary and flagellar proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose filters as described previously (Ogawa *et al.*, 1990). Alkaline-phosphatase–conjugated antibodies were used as secondary antibodies and immuno-complexes were detected with 4-nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Mierendorf *et al.*, 1987).

RESULTS

Antibodies to Specific Sea Urchin ICs Cross-react with Specific Chlamydomonas ICs

We previously reported the production of both affinity-purified antibodies and monoclonal antibodies that were specific for IC1, IC2, and IC3 of sea urchin sperm flagellar and blastula ciliary outer arm dynein (Ogawa *et al.*, 1990). To determine if these antibodies would cross-react with *Chlamydomonas* outer arm dynein ICs, flagellar proteins from wild-type and *oda*6 (an outer dynein arm-less mutant) *Chlamydomonas* cells were electrophoresed and subjected to Western blotting (Figure 1). As expected, affinity-purified anti-IC3 antibody reacted specifically with IC3 in sea urchin embryonic cilia (Figure 1, panel B). This antibody also reacted strongly with a polypeptide of approximately 69,000 M_r in flagella of wild-type *Chlamydomonas* cells but did not react with any similar protein in flagella of the mutant *oda*6. Inasmuch as *oda*6 specifically lacks the outer arm, we can conclude that this immunoreactive band represents IC69 of the *Chlamydomonas* outer arm dynein. When a similar blot was probed with affinity-purified anti-IC2 antibody, the antibody reacted specifically with IC2 in the sea urchin embryonic cilia and with a single protein of approximately 78,000 M_r in flagella of wild-type *Chlamydomonas*, but did not react with any protein in flagella of *oda*6 (Figure 1C). We conclude that this antibody cross-reacts specifically with *Chlamydomonas* IC78. Therefore, sea urchin IC2 and *Chlamydomonas* IC78 are immunologically related, but distinct from sea urchin IC3 and *Chlamydomonas* IC69, which also are immunologically related.

When similar blots were probed with affinity-purified anti-IC1 antibody specific for sea urchin IC1, multiple faint bands were observed in lanes of flagella of wild-type and *oda*6 *Chlamydomonas* (Ogawa, unpublished results). No bands specific to wild-type flagella were observed.

cDNA Sequence of IC3 from Sea Urchin Outer Arm Dynein

To more definitively characterize the relationship between the sea urchin outer arm dynein ICs and those of other organisms, we isolated and sequenced overlapping cDNA clones covering the entire coding region of these two ICs. The cDNA sequence of IC3 (Figure 2) was determined from three overlapping clones. A λgt11 cDNA library prepared from *A. crassispina* total RNA was screened with an affinity-purified polyclonal antibody against IC3. Five positive clones were isolated from 2×10^5 independent clones. These were further screened with monoclonal antibodies D6 and D16 directed against IC3. Two clones were identified as positive. The longer clone (λD154) was subcloned into the *Eco*RI site of pTZ19R to yield pD154. This clone corresponded to bases 56–3723 in the final sequence (Figure 2). A *Kpn*I fragment (base position 56–582) of this clone was then used to screen the original library to obtain an overlapping cDNA clone (pJ221), which corresponded to bases 74–3731 in the final sequence and contained the 3′-end of the cDNA. To isolate a clone containing the 5′-end of the cDNA, a mini-library was prepared by priming poly(A)$^+$ RNA with the specific oligonucleotide (5′-CTC CTG TGG GTT GAT GTC CTT TGG-3′), which is complementary to a portion of the nucleotide sequence (bases 316–339) of pD154. This library was then probed with the above *Kpn*I fragment to yield a clone (pSP192) containing bases 1–332. Assuming

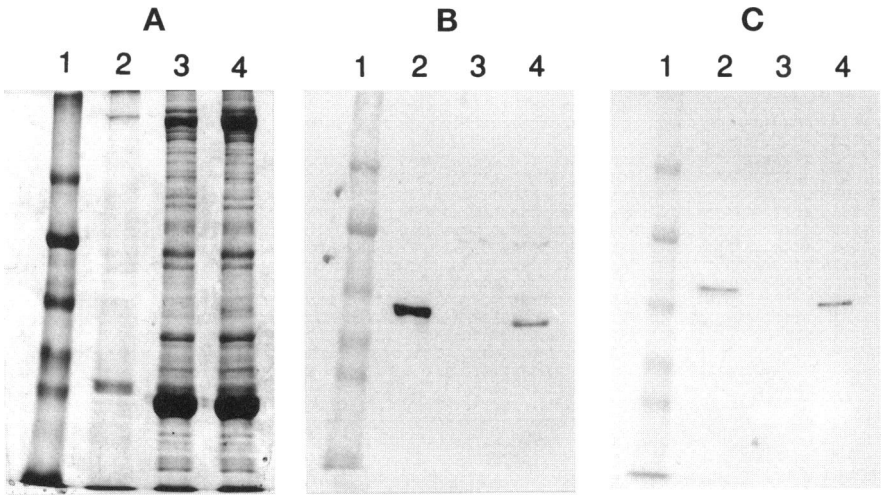

Figure 1. Western blot analysis of sea urchin ciliary and *Chlamydomonas* flagellar proteins. Panel A is a gel stained with Coomassie blue to show total proteins. Lane 1, prestained markers (top to bottom: 180, 116, 84, 58, and 48.5 kDa; SDS-7B kit, Sigma Chemical, St. Louis, MO); lane 2, sea urchin embryonic cilia; lane 3, flagella of *Chlamydomonas oda*6 cells; lane 4, flagella of *Chlamydomonas* wild-type cells. Three identical gels were blotted to nitrocellulose membrane. One was incubated with affinity-purified antibody against IC3 of sea urchin outer arm dynein and stained to reveal antibody-protein interaction (panel B). The faint band at about 110 kDa in lanes 3 and 4 of Panel B is not an outer arm polypeptide as it is present in *oda*6 flagella; it may be due to a nonspecific reaction, or to cross-reactivity with an inner arm dynein IC of about the same relative mobility (Smith and Sale, 1991; Porter *et al.*, 1992). Another blot was probed with affinity-purified antibody against IC2 (panel C). The third was probed with affinity-purified antibody against IC1 (blot not shown; see text).

that translation initiation occurs at the first in-frame AUG triplet (position 73), the open reading frame encodes a polypeptide of 597 amino acids with a predicted isoelectric point of 4.85 and a predicted unmodified molecular mass of 68,221 Da. The open reading frame terminates with a stop codon (UGA, indicated by an asterisk in Figure 2) and is followed by a long 3′-untranslated sequence that includes three polyadenylation signals (AAUAAA, underlined in Figure 2). The presence of the unusually long, 3′-untranslated sequence in this cDNA is not an artifact of the cDNA synthesis, because the sequence was present in the two overlapping clones (pD154 and pJ221). The predicted mass of the chain is consistent with the apparent molecular weight of *A. crassispina* IC3 (74,000) as estimated by SDS-PAGE (Ogawa *et al.*, 1990).

cDNA Sequence of IC2 from Sea Urchin Outer Arm Dynein

The cDNA sequence of IC2 (Figure 3) also was deduced from three overlapping clones. The λgt11 cDNA library was screened with an affinity-purified polyclonal antibody specific for IC2. Three positive clones were isolated from 2×10^5 independent clones.

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   1 CAACAACTAGCTTACTAGAAGTGATGCTGAGAAGTTGTATTATATCAGGAGTAGAATAGATTGTTAATCAAAATGGAGATCGTG   84
   1                                                                   M  E  I  V     4
  85 TACGTGTACACGAAGAAGAGGAGTGAGTTTGGGCGGCAGTGCAACTTCTCTGATCGTCCTGCAGAGCTCCACGTTGATATCCTT  168
   5  Y  V  Y  T  K  K  R  S  E  F  G  R  Q  C  N  F  S  D  R  P  A  E  L  H  V  D  I  L    32
 169 CCAGATGAGAGCCTTCTGTCAAATTTCATCGAGAAGAACCCGGTAGACAGAGGGATACAATGTGTGCAAGAGATGTCGGAACAT  252
  33  P  D  E  S  L  L  S  N  F  I  E  K  N  P  V  D  R  G  I  Q  C  V  Q  E  M  S  E  H    60
 253 GAGATCAACACGGAGCGGTTTGAGACAGACAGCAGAGGTATCAACCATATGGAAGGAGGCTGGCCAAAGGACATCAACCCACAG  336
  61  E  I  N  T  E  R  F  E  T  D  S  R  G  I  N  H  M  E  G  G  W  P  K  D  I  N  P  Q    88
 337 GAGGTGGAGCAGGTCCTTAGGTTCAGGAAAAAAGTGGAGAAAGACGAATCGTATGTCACAACCATCCAGAACCTGGCCAGTTCG  420
  89  E  V  E  Q  V  L  R  F  R  K  K  V  E  K  D  E  S  Y  V  T  T  I  Q  N  L  A  S  S   116
 421 ATGGAACACACAATCAAACAAAACAATGCAATAGACATCTATGAAGATTACTTTGCAGACCTAGAGATAGAGGAGGTAGAAGAA  504
 117  M  E  H  T  I  K  Q  N  N  A  I  D  I  Y  E  D  Y  F  A  D  L  E  I  E  E  V  E  E   144
 505 ACACCATCAGCTAAAACCATCAATGTCTTCAGAGATCCTAACGAGATCAAGAGACCAGCCACTCACTTGTCATGGTACCCTGAT  588
 145  T  P  S  A  K  T  I  N  V  F  R  D  P  N  E  I  K  R  P  A  T  H  L  S  W  Y  P  D   172
 589 GGGCCCAAGAAGCTTGCTGTAGCCTACTCAAGTTTGGAGTTCCAGAAGACATCAGCAGAAACCAGCATGGATTCCTATATCTGG  672
 173  G  P  K  K  L  A  V  A  Y  S  S  L  E  F  Q  K  T  S  A  E  T  S  M  D  S  Y  I  W   200
 673 GACATAGAAAACCCTAACAAGCCAGAGTTTGTTTTGAAGCCAGTATCACCCCTGGTGTGCCTAGAATACAACCCTAAAGATGTC  756
 201  D  I  E  N  P  N  K  P  E  F  V  L  K  P  V  S  P  L  V  C  L  E  Y  N  P  K  D  V   228
 757 CATGTCCTCATAGGTGGCTGCTACAATGGACAAGTTGCATTCTGGGATACAAGAAAAGGTTCACAAGCAGTTGAGATGTCACCC  840
 229  H  V  L  I  G  G  C  Y  N  G  Q  V  A  F  W  D  T  R  K  G  S  Q  A  V  E  M  S  P   256
 841 GTAGAACACAGCCATCATGATCCAGTCTATAAGACGATCTGGCTCCAATCAAAGACAGGAACAGAGTGCTTCTCAGCATCAACA  924
 257  V  E  H  S  H  H  D  P  V  Y  K  T  I  W  L  Q  S  K  T  G  T  E  C  F  S  A  S  T   284
 925 GATGGACAAGTGCTGTGGTGGGATATGAGGAAGCTTGGAGAACCGACAGAAAAACTCATCATGGACCCCTCGAAGAAGGGCAAG 1008
 285  D  G  Q  V  L  W  W  D  M  R  K  L  G  E  P  T  E  K  L  I  M  D  P  S  K  K  G  K   312
1009 ATGGAAAACGCACAAGGAGTCATCTCATTAGAATATGAACCAACCATTCCAACTAAATTCATGGTAGGTACGGAGCAGGGTACG 1092
 313  M  E  N  A  Q  G  V  I  S  L  E  Y  E  P  T  I  P  T  K  F  M  V  G  T  E  Q  G  T   340
1093 ATAATCTCTTGCAACAGGAAAGCCAAGACACCTCCAGAGAAGATTGTAGCCATATACAAGGAGCACATCGGTCCTGTCTACTCA 1176
 341  I  I  S  C  N  R  K  A  K  T  P  P  E  K  I  V  A  I  Y  K  E  H  I  G  P  V  Y  S   368
1177 CTCCAGAGGAATCCATTCTTCCCCAAGAACTTCTTGACGGTTGGTGACTGGACTGCTAGGATCTGGTCAGAAGATATCAGGGAC 1260
 369  L  Q  R  N  P  F  F  P  K  N  F  L  T  V  G  D  W  T  A  R  I  W  S  E  D  I  R  D   396
1261 TCGTCAATTATGTGGACCAAGTATCACATGTCCTACCTAACCGATGGTTGTTGGGAGTCCTGTCAGACCAGCTGTGTTCTTCACA 1344
 397  S  S  I  M  W  T  K  Y  H  M  S  Y  L  T  D  G  C  W  S  P  V  R  P  A  V  F  F  T   424
1345 ACTAAGATGGACGGCTCTCTTGATGTGTGGGACTATCTCTTTAAGCAGAAAGATCCAACTCTTAGCCTACAGGTCAGTGATGAT 1428
 425  T  K  M  D  G  S  L  D  V  W  D  Y  L  F  K  Q  K  D  P  T  L  S  L  Q  V  S  D  D   452
1429 GCCCTACACAGCCTCCGCGTTCAGGATCAGGGTCGACTCATTGCCACCGGCTCGAACAGCGGAACCACCACGCTACTGGAGCTC 1512
 453  A  L  H  S  L  R  V  Q  D  Q  G  R  L  I  A  T  G  S  N  S  G  T  T  T  L  L  E  L   480
1513 TCCAGTGGTCTCTGTACCATGCAGCGTAATGAGAAGGCCCTCGTCACCGCCATGTTTGAGAGGGAGACCAAGAGGGAGAAGATA 1596
 481  S  S  G  L  C  T  M  Q  R  N  E  K  A  L  V  T  A  M  F  E  R  E  T  K  R  E  K  I   508
1597 CTGGAGTCCAGGCAGAGGGAGCTGAGGCTCAAGAGACAAGGAGCCAGTGCTCAAGGCCAAGATGATGACGAAGAAGGAGGACCA 1680
 509  L  E  S  R  Q  R  E  L  R  L  K  R  Q  G  A  S  A  Q  G  Q  D  D  D  E  E  G  G  P   536
1681 GATGAAGAGGAAGACCTGGTGGCAGCAGCAGAGAAGGAGTTCTTCCAGATCGTACAGGCAGATAAGAAGAAGTTTGCTGCTCAA 1764
 537  D  E  E  E  D  L  V  A  A  A  E  K  E  F  F  Q  I  V  Q  A  D  K  K  K  F  A  A  Q   564
1765 CAGGCCAAGCTGTCAGAACAGGATAATAAGATCATTGAAGAGGCAGAGGAAAATAATGGAAGCGAAAAGAAGGACACTGAAAAT 1848
 565  Q  A  K  L  S  E  Q  D  N  K  I  I  E  E  A  E  E  N  N  G  S  E  K  K  D  T  E  N   592
1849 GGAGAGAAAGAAGGATAGGTGAAAAGCTGAGATTCATTCCTTGGTCAATGTGTGACATCAAAGACTACCGCCATCGTTTCTGTC 1932
 593  G  E  K  E  G  *                                                                      597
1933 TTGTTTATATCTCGACCTACATCTGGCATTGCCATAATTTACAATGGTTCCTGCTTCATTCCATCAATCAACACTTAGTGTTTT 2016
2017 GCTGTCAGAAGCCAGAACTCACTCTAGGATGCAATTGTATTGGAAAACATGATCTTAAGTTTGTATAATCTTGTAGCCAGCATA 2100
2101 GAGAGAAAGAATTTTTAAAGTTTTCTGCAACATTTGGAAAGATAGTAGGTTACGATAGATTTACGGGAATGCATGGTTGCATCA 2184
2185 TTTTTGCCATGTAATAACATATTACAATCGAGAAATTGTAAATTGTGTTATATCTGATTCTATTACAATGTACCTCCTTTTTGT 2268
2269 GATCTGGTTTCTGCAATCAAGATATTACTGTGCAGCTGTTACAAATCTCACTCCTTAATATTTCCTTTTCTTTTTACATTATAG 2352
2353 TGAACCAAAATTCTCATTGTTCTGGCATCTATTTGAAACGAGGTAAATAATGCTGTTGGGCAAACAATTTTATCTCCTCTCATC 2436
2437 TCTGTATGGGTAGAAGTCAATTGTTCCCCATCATAGGAGTTACATTGATATCTCACTCCCTGATATTTCCTTTTCTTTTTCACA 2520
2521 TTATATTGAGCAAAATTATCATTGCGCTGGAATCTAATTGGAATGAGGTCGATCATGCTGATGGGCAAGTGAGTTTGTCTCCTT 2604
2605 TCTTCCCAGTATTGGTAGAAGTCAACTTTTATCCATCATAGGAGTTACATCATTATCATACATGTTCATTTTCGTGTGAGGTTG 2688
2689 TTATTAAGAGGAGCAGTGTTTTCATTGAGAACAGTAAATTTGCTCTTGAAGCTCATTTGCAACTGAAGTTAATAAGGGGAATGC 2772
2773 AAATTTTTACTCTTGGTTTATCTCATAAGTTGCATCAAAAGGATTTCTCTTCTGTTACGTGAAAACTTTAAGAAGAGGAGGGTC 2856
2857 TACACTTTTATCTGCTAGAAGTGAAGTTAAAGCATCTGTTGTCATCACTATGAATCACATATTGTAAGCAAGTAGCCAGGGCCT 2940
2941 TGTGAATGAAATGCATTAGGTCATACAGGATTTGTCCAATCAGTGTTGAAATAGATCCAAGATTTTAACTTATTGTCAGCGATT 3024
3025 TATGGATTGTGAACCATATTCATTGTTTTATTCAAAAGTACATGTAGTGCCAGGTCAACACAGATGGAAAGACTGTATTTCAGG 3108
3109 GGGAATGTGTCAAATTCTTGGGGAAATGTTTTTTATTTTTCTTTTCTTCTTTTGTTTTTTTAAATAAAGTATCCTTAACATATT 3192
3193 TAAGACTGCAAAAATATCATGCAGATGATAACCTCCCTCCCCCCAACTATGTTTATCTTTTCAACAGTTATATGTGGATATTTG 3276
3277 GCAGTACAAAAAGACAAATCTTTGTGATTTCCAGTATGGTTTGAAGATTCATGTCAATAATCTTTAATATCTCCAAAAAGAAAA 3360
3361 GGGAAATTCAATAAACAGTCAGTTTGGTTGTTTGCAAAATGACAATAGAACAGGTGAGGCTTCGCTATCTACGTTACCGAATGC 3444
3445 CTTTACGATAAAAATAATATCGACCTTAAGGTAATAGCAAGAAGAATGAGATGTATTGGGGGAAAAAAAAGAAAAACAGATATG 3528
3529 CAGTGACTTGATATTTTTTCCATTGTAAATAATAATCCATAATGAATATTTGGGAGTTCTATATAGAGAGCTGTGCCTTTGTCA 3612
3613 TTGTGCAGCCTTATCTATGTATCTGTGTACAGAGTGAAAATGTATCACACTATATTGTACCCAATTTAAAATGTACATGTGTAT 3696
3697 ATTTGTTCTAAATAAATGTGTTTTATAACTTAAAA                                                   3731
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Figure 2. Nucleotide and predicted amino-acid sequences of the full-length cDNA that encodes the sea urchin ciliary outer arm dynein IC3. The sequence reported here will appear in the GSDB, DDBJ, EMBL, and NCBI Nucleotide Sequence Databases under the accession number D28863.

These were screened further with monoclonal antibodies D9, D58, and D452 directed against IC2. One clone (λA263) was identified as positive. This was subcloned into the *Not*I site of pBluescript IIKS+ to yield pA263. This clone contains bases 5–3058 in the final sequence and contains a stop codon (UGA) at base position 16 of the 5′-upstream sequence as well as a stop codon in the 3′-downstream sequence. The original library was rescreened using an *Eco*RI fragment (consisting of bases 5–301) of this clone to obtain an overlapping cDNA clone (pJ126), which contained bases 3–3039. To obtain the extreme 5′-end of the transcript, a mini-library was prepared by priming poly(A)$^+$ RNA with a specific oligonucleotide (5′-TTT CTG GTC TGA CTT GGC CTC GGC-3′) complementary to a portion (bases 616–639) of pA263. This library was then probed with the above *Eco*RI fragment to yield clone λSP151, which contains bases 1–627. The open reading frame terminates with the stop codon UAA (indicated by an asterisk in Figure 3) and is followed by an untranslated sequence that includes one polyadenylation signal (AAUAAA; underlined). The open reading frame predicts a polypeptide of 702 amino acids with an isoelectric point of 5.64 and an unmodified molecular mass of 79,134 Da. The extreme N-terminal portion of the predicted sequence contains a high proportion of positively charged amino acids (Lys).

Sea Urchin IC2 and IC3 Correspond to Chlamydomonas IC78 and IC69, Respectively

A search of the databases using the BLAST program revealed that the predicted amino-acid sequences of

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1    CTGCATGCAGTGTGTTGATGTTTGCCTTTTTATCGAATATTTAGCCATCTTTTACTATCATTTTTCAGAGGAATGCCGGTGAAA   84
1                                                                    M  P  V  K            4
85   TCAACCAAGACCAAAGGGGGGTCCACCCAAGGCGGTAGCCAGGCAGGTGGACCTAGTACCCTGAAGGTCAACAAAGCCAGAGTA  168
5     S  T  K  T  K  G  G  S  T  Q  G  G  S  Q  A  G  G  P  S  T  L  K  V  N  K  A  R  V   32
169  CCGGCTAAAGGAAAGAAGGATGATGATGATGCCACAGAGGCTGGTGAACATGGAGGTGAAGAGTGGATGCAGACAAAGTCATTG  252
33    P  A  K  G  K  K  D  D  D  D  A  T  E  A  G  E  H  G  G  E  E  W  M  Q  T  K  S  L   60
253  ATAAAGCCTGATGACCAGCTGGAGTTAAATGATCAGGAGTTGAAGGAAGAATTCACCAGGATTCTAACAGCCAACAATCCCCAT  336
61    I  K  P  D  D  Q  L  E  L  N  D  Q  E  L  K  E  E  F  T  R  I  L  T  A  N  N  P  H   88
337  GCACCACAGAACATTGTCAGATACAGCTTCAAGGATTGTTCCTTTAAGCAGACGTCCCATGTAGACCAGCTAGCCATCCATTTC  420
89    A  P  Q  N  I  V  R  Y  S  F  K  D  C  S  F  K  Q  T  S  H  V  D  Q  L  A  I  H  F  116
421  TCCCTGGATGGGAACATGATCCACAAGGACTCTGATGAGGCTAGGAGACAGCAACAGAGACATGGCGCTTCAGAAGCTATCAGT  504
117   S  L  D  G  N  M  I  H  K  D  S  D  E  A  R  R  Q  Q  Q  R  H  G  A  S  E  A  I  S  144
505  GAACAGGCCATAAGCGAAGCTGGAGAGGAGAAGAAGGAAGAAGATGGAGAACAAAAAACAGAAGAGCCCAAGGAAGGTGAGAAG  588
145   E  Q  A  I  S  E  A  G  E  E  K  K  E  E  D  G  E  Q  K  T  E  E  P  K  E  G  E  K  172
589  AGGGACGAGGAGTCTACCCCTGCCCCTGCCGAGGCCAAGTCAGACCAGAAACTGACCAATCAGTTTAACTTCAGCGAGAGGGCC  672
173   R  D  E  E  S  T  P  A  P  A  E  A  K  S  D  Q  K  L  T  N  Q  F  N  F  S  E  R  A  200
673  TCGCAAACATACAACAACCCATACAGGGAAAGAGGAACACAGACTGAGCCGCCACCTCGTGCTAACTTCTCATCCACTGCCAAC  756
201   S  Q  T  Y  N  N  P  Y  Y  R  E  R  G  T  Q  T  E  P  P  P  P  R  A  N  F  S  S  T  A  N  228
757  CAATGGGAGATATATGATGCATACATGGAGGATCTTGAGAAACAGGAGAAAGCTAAGGAGAAGAAGGCAGCACCCTCCAAGAAG  840
229   Q  W  E  I  Y  D  A  Y  M  E  D  L  E  K  Q  E  K  A  K  E  K  K  A  A  P  S  K  K  256
841  GATGATGATAAGAGTAAGAAGAAGCTGACAGCGTTGGAAACCCAGTCAGATGACATGAGCCGCATCAGCCATGCTGCCAAGATC  924
257   D  D  D  K  S  K  K  K  L  T  A  L  E  T  Q  S  D  D  M  S  R  I  S  H  A  A  K  I  284
925  ATTGAGAGGATGGTCAACCAGAACACTTTTGATGATGTCTCACAAGATTTCAAGTACTACGAAGATATGTCAGATGAGTTTCGC 1008
285   I  E  R  M  V  N  Q  N  T  F  D  D  V  S  Q  D  F  K  Y  Y  E  D  M  S  D  E  F  R  312
1009 GACCAAGAAGGAACCCTCTTGCCTCTCTGGAAGTTCTCCTACGACAAGTCCAAGCGGTTGGCTGTCACATCAGTTTGCTGGAAT 1092
313   D  Q  E  G  T  L  L  P  L  W  K  F  S  Y  D  K  S  K  R  L  A  V  T  S  V  C  W  N  340
1093 CCCAAGTATAAGGATCTCTTTGCCGTCGCTCATGGCTCTTATGATTTCATGAAGCAATCTCGTGGCATGATCCTCTTCTACACA 1176
341   P  K  Y  K  D  L  F  A  V  A  H  G  S  Y  D  F  M  K  Q  S  R  G  M  I  L  F  Y  T  368
1177 CTCAAGAACCCATCATTCCCGGAGTTCGTCTACCCTACCGACAGCGGCGTCATGTGCATCGACATCCGCCCTGAGCACCCGTAC 1260
369   L  K  N  P  S  F  P  E  F  V  Y  P  T  D  S  G  V  M  C  I  D  I  R  P  E  H  P  Y  396
1261 CTGATCTGCGTGGGCTACTGTGATGGTTCCGTTGGGGTCTTTAACGTGACTTCCACCGATGCCAACCCTGTCTTTCAGAGCACA 1344
397   L  I  C  V  G  Y  C  D  G  S  V  G  V  F  N  V  T  S  T  D  A  N  P  V  F  Q  S  T  424
1345 GCCAAGACTGGCAAGCACACAGACCCGGTCTGGCAGGTGGCCTGGCAAAAGGATGATCTGGACAACAACCTCAACTTCTTTTCT 1428
425   A  K  T  G  K  H  T  D  P  V  W  Q  V  A  W  Q  K  D  D  L  D  N  N  L  N  F  F  S  452
1429 GTATCGTCAGATGGGCGAGTGGTTGCATGGACATTGGTCAAGAATGAATTGACGTACACTGACGTGATTCAGCTTCAGCTTGAT 1512
453   V  S  S  D  G  R  V  V  A  W  T  L  V  K  N  E  L  T  Y  T  D  V  I  Q  L  Q  L  D  480
1513 TCAGCTCCTCAGGATGGCCCCGAAGGAACCCAACTAACACCTCTAGGCTGTGGTACATGCTTTGACTTCCACAAGCAGACAGAT 1596
481   S  A  P  Q  D  G  P  E  G  T  Q  L  T  P  L  G  C  G  T  C  F  D  F  H  K  Q  T  D  508
1597 TACCTGTTCCTTGTTGGCACAGAGGAGGGAAAGATCCACAAGTGTTCCAAGGCCTACTCCAGCCAGTTCCTGGACACGTTTGAG 1680
509   Y  L  F  L  V  G  T  E  E  G  K  I  H  K  C  S  K  A  Y  S  S  Q  F  L  D  T  F  E  536
1681 GCGCACCACATGGCTGTCTACAAGGTGATGTGGAACCACTTCCATCCCAAGATCTTTATCTCCTGCAGTGCTGATTGGAGTGTC 1764
537   A  H  H  M  A  V  Y  K  V  M  W  N  H  F  H  P  K  I  F  I  S  C  S  A  D  W  S  V  564
1765 AAGATCTGGGACCACACCTACAGGAATGGCCCGATGTTCACCTTTGACCTTGGAAGTGCGGTAGGTGATGTAGCATGGGCTCCC 1848
565   K  I  W  D  H  T  Y  R  N  G  P  M  F  T  F  D  L  G  S  A  V  G  D  V  A  W  A  P  592
1849 TACTCCTCGACAGTCTTTGCAGCAGTCACAGCAGATGGCAAGGTGCATGTGTTTGACCTCAATCTCAACAAGTACGAGCCGATC 1932
593   Y  S  S  T  V  F  A  A  V  T  A  D  G  K  V  H  V  F  D  L  N  L  N  K  Y  E  P  I  620
1933 TGCGAGCAGGCTGTCGTGCAGAAGAAAAAGACCAAGCTGACCCACATCACTTTCAACCCGAATTTCCCCATCGTGCTGGTGGGA 2016
621   C  E  Q  A  V  V  Q  K  K  K  T  K  L  T  H  I  T  F  N  P  N  F  P  I  V  L  V  G  648
2017 GACGACCGGGGCTACGTCTCCAGCCTCAAGCTCTCGCCCAACCTGCGGAAGGTGCCCAAGGACAAAAAGGGGGCTGCCCTCAAC 2100
649   D  D  R  G  Y  V  S  S  L  K  L  S  P  N  L  R  K  V  P  K  D  K  K  G  A  A  L  N  676
2101 CACGGACCCGAGGCTGAGATCGCCAAGATGGACAAGCTGCTAGCCCTAGTGAGGGAGCCGCCCAAGGATAACAAGAGCTAAAGA 2184
677   H  G  P  E  A  E  I  A  K  M  D  K  L  L  A  L  V  R  E  P  P  K  D  N  K  S  *       702
2185 TGTCACCTTCAACAAGTTCTAGCAAACGATGGTTTTTTGCCAAGTGCACAGACTTGATGAGATTTTTCCCCATACATTTGTATA 2268
2269 AGGAATTTTCCCTGAATATGATCTTGGAACAATCTGAAGTTTACAGTCACTCCATGAAGTTGGTCCATAGGGAAGGGAAGAAAG 2352
2353 TAATAACCATGGCTATCATTCTAGATACAGAACATTAAGAATATCCAGTTATTCCCTTGTTTTTACATCAAGCTGCTGTTTTGT 2436
2437 CAATCATATTGACATGCATATTTCCTCAAACATTTGACGCAATTTTATTCTTCTGAAAACAGGTCGAACAACACACCTACCCCA 2520
2521 AAAAACAAAATCAGTGAAGGCATTACATCTTTCTCACTTTCAACTGTCATCCACATGAATATGTTGCCTTTTTGCTAAATTTCC 2604
2605 TTTTGGACCATTACTTGTACATATGTATGATATTAGAGTGTGTATAAATTTGAAGAAAAGAAAGATAGAAAAGATGTATTCGCC 2688
2689 TGTCATTGAGACAGAAGATGTGTTCCTTTTAAATTATTATTTTCATTTTCCAGAATTATTTTTTTTCAAAAAGGAAATCCATGC 2772
2773 CCGTTACATTTGTTTTACTGATTCTGATGCAAAAACAGACAGTTGAGTCCTCTGTGATCTCAAAATGAAACATAATGTGCTTAT 2856
2857 TAATTATTTGATAGTTTTACAAACACATAATAGTTCACATTGTGAATGCCGTCCTATTAGTGTAGTACTTTTTCACAATTTTTT 2940
2941 ATAAATATCTGCAGGAATATATTTAAGTGTATTTGAAAATGTATTCACGATACTCATTTTATGTCATATTGAATTCAAAAATAA 3024
3025 ACGCTGACTTAACAACAAAAATTTTAAAAAAAAA                                                    3058
```

Figure 3. Nucleotide and predicted amino-acid sequences of the full-length cDNA that encodes the sea urchin ciliary outer-arm dynein IC2. This sequence will be available from the GSDB, DDBJ, EMBL, and NCBI Nucleotide Sequence Databases under the accession number D38538.

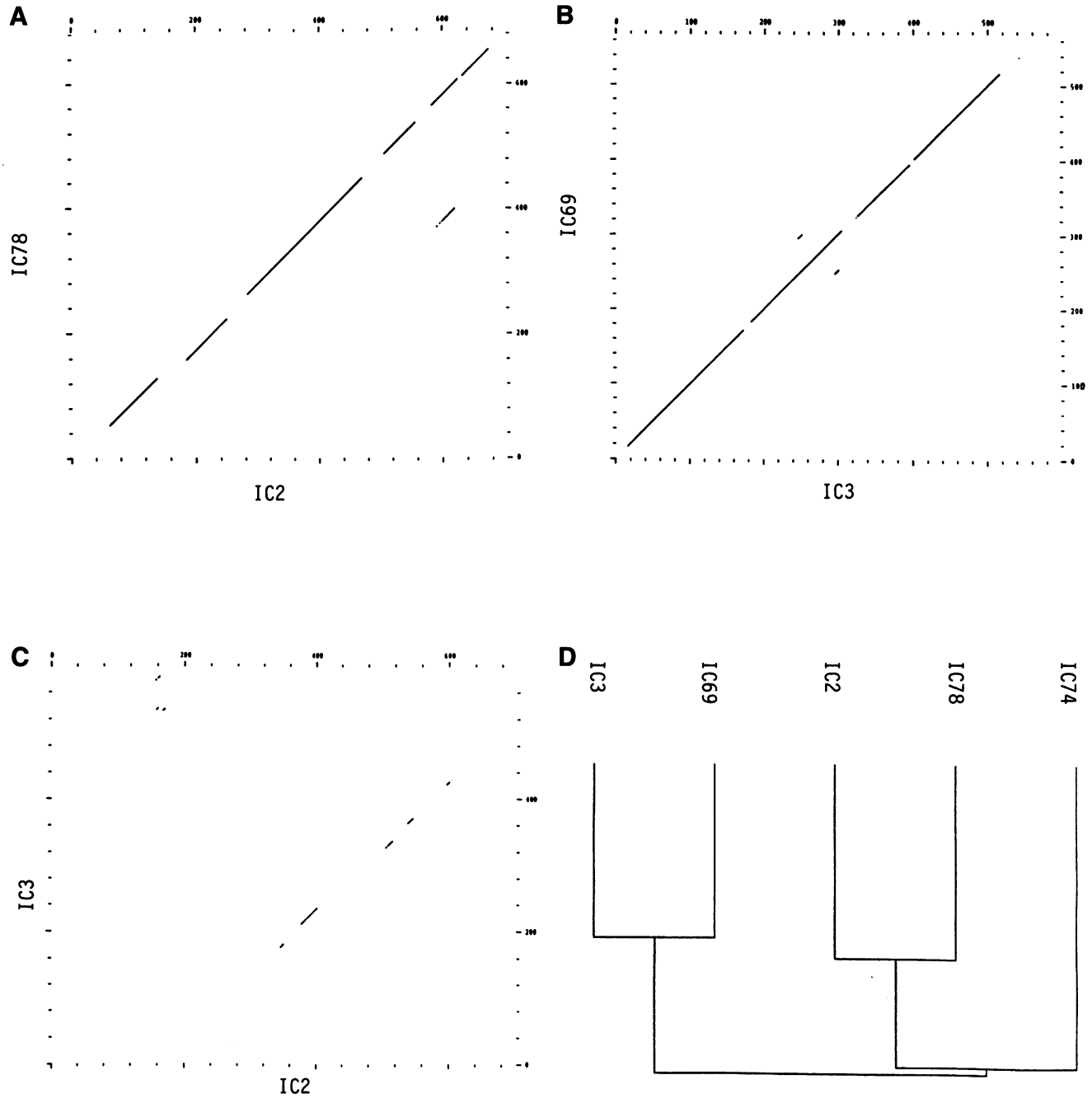

Figure 4. Dot plot comparisons of sea urchin IC2 vs. *Chlamydomonas* IC78 (Wilkerson *et al.*, 1995) (panel A), sea urchin IC3 vs. *Chlamydomonas* IC69 (Mitchell and Kang, 1991) (panel B), and sea urchin IC2 vs. sea urchin IC3 (panel C). The dot plots were generated by the program COMPARE using a window size of 30 and a stringency of 19. Panel D shows a parsimony tree for sea urchin IC2 and IC3, *Chlamydomonas* IC78 and IC69, and IC74 of rat cytoplasmic dynein (Paschal *et al.*, 1992). This comparison was carried out using the program PILEUP.

IC2 and IC3 are closely related to the sequences of IC69 from *Chlamydomonas* (Mitchell and Kang, 1991) and of the 74-kDa intermediate chain (IC74) of cytoplasmic dynein (Paschal *et al.*, 1992). The sequences of IC2 and IC3 also are very similar to that of IC78 from *Chlamydomonas*, which was recently reported (Wilkerson *et al.*, 1995).

Figure 4, A, B, and C show dot plot comparisons of IC2 versus IC78, IC3 versus IC69, and IC2 versus IC3, respectively. IC2 and IC78 are related throughout their

Table 1. Calculated z values for each pairwise comparison between outer arm dynein intermediate chains

	IC3	IC78	IC69
IC2	39	186.8	37.2
IC3	—	29	148.7
IC78	—	—	26.3

The shuffled sequence (see text) in each pairwise comparison was that listed across the top of the table. The z values were calculated for optimized scores (Lipman and Pearson, 1985).

entire lengths; likewise, IC3 and IC69 are similar throughout their entire lengths. In contrast, the two sea urchin sequences IC2 and IC3 exhibited much less similarity to one another; this was confined primarily to their C-terminal halves. This relationship between the chains was confirmed by parsimony analysis (Figure 4D): IC2 and IC78 were grouped with each other but well separated from IC3 and IC69, which were grouped together. In the same parsimony tree, IC74 of cytoplasmic dynein was placed closer to IC78 and IC2.

The significance of the sequence similarities between these ICs was further evaluated quantitatively using the program RDF2 (Lipman and Pearson, 1985). For each IC, a similarity score was obtained for pairwise comparisons between the query chain and one of the other ICs, and between the query chain and a collection of 100 sequences generated by a random shuffle of the IC sequence to which the query chain was being compared. The shuffled sequences therefore have the same length and amino acid composition as the IC used to generate them. A z value, which is defined as (similarity score of unshuffled sequence − mean of scores of shuffled sequences)/standard deviation of scores of shuffled sequences, was then calculated. Comparisons that generate z values > 10 are generally considered significant. All two-way comparisons between these four ICs yielded z values >10 (Table 1). The z values for IC2 versus IC78 and for IC3 versus IC69 are between four and ten times greater than those for the other pairwise combinations. These results provide further evidence that IC2 is much more closely related to IC78, and IC3 to IC69, than either is to any of the other ICs.

A detailed comparison of the IC2 and IC78 sequences (Figure 5) revealed that there is extensive amino acid identity (45.8%) spread throughout the chains; there are numerous short segments (up to nine residues each) that are absolutely conserved. The N-terminal one-half of IC2 contains two small insertions relative to IC78 that account for its slightly larger mass. There is somewhat less amino acid identity in the extreme N-terminal regions of the sequences, although in both chains the extreme N-terminus contains a high proportion of positively charged residues

Figure 5. Sequence comparison of sea urchin IC2 and *Chlamydomonas* IC78. The sequences were aligned using the program CLUSTAL. Gaps introduced by the program to maximize alignment are indicated by dashes. Asterisks indicate amino acid identities whereas dots indicate conservative replacements.

(Lys) followed by a region containing a high proportion of negatively charged residues.

The IC3 and IC69 sequences also exhibit extensive amino acid identity (48.5%), which is spread throughout their lengths (Figure 6). Again, there are several short segments (up to 10 residues long) that are absolutely conserved; one of these regions (residues 97–104 in both chains) is especially notable in that all but one of its residues is charged. IC3 appears to contain an insert of about 26 residues near its C-terminus that accounts for its greater mass relative to IC69.

Although sea urchin IC2 and IC3 are clearly homologous proteins, they exhibit considerably less conservation of sequence (23.5% identity) between each other than either does relative to its homologue from *Chlamydomonas*.

An objective four-way comparison of IC2, IC3, IC78, and IC69 (Figure 7) suggests that most of the difference in size between the IC2/IC78 and IC3/IC69 pairs is due to one or two large inserts in the centers of IC2 and IC78, and to large inserts at both the extreme N-terminus and extreme C-terminus of these two proteins. The comparison also reveals domains that ap-

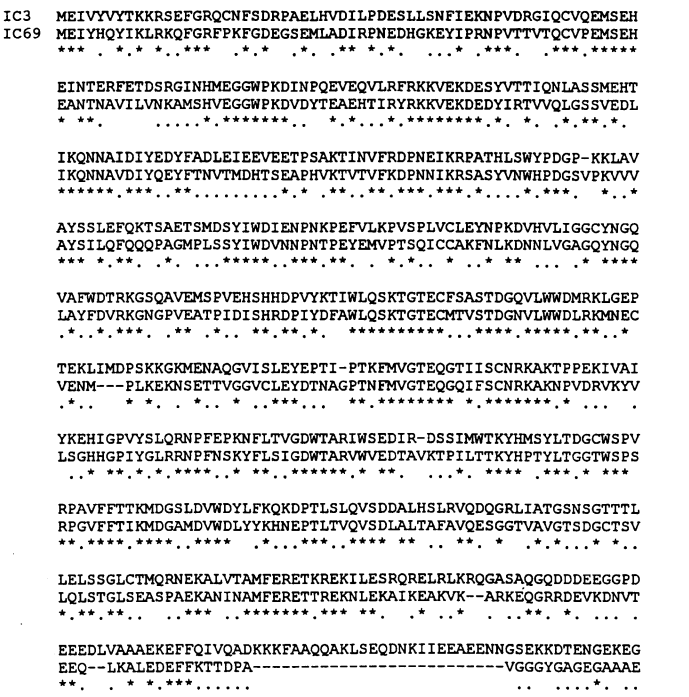

Figure 6. Sequence comparison of sea urchin IC3 and *Chlamydomonas* IC69 aligned as in Figure 5.

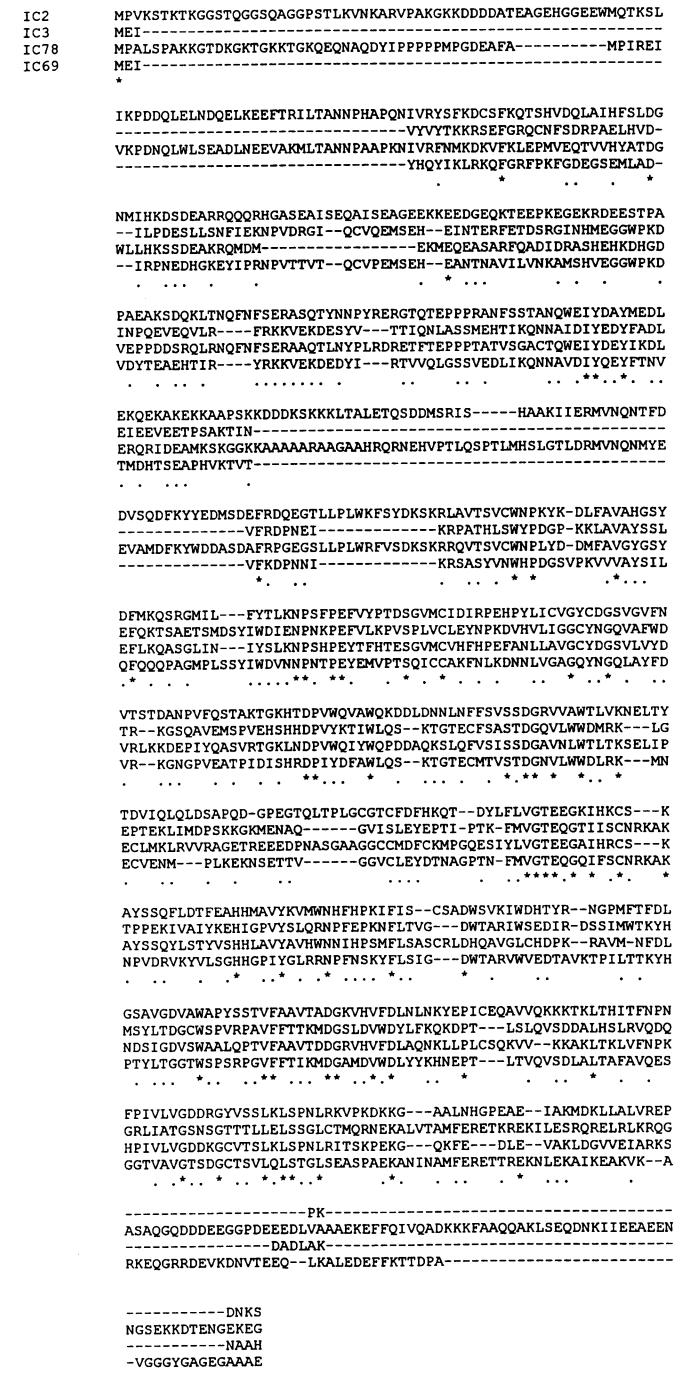

Figure 7. Four-way comparison of IC3, IC2, IC78, and IC69 sequences aligned using the program CLUSTAL. Dashes indicate gaps introduced to obtain optimum alignment. Asterisks indicate amino acids identical in all four sequences, whereas dots indicate conservative replacements.

pear to be conserved between all four ICs. Toward the N-terminus of the proteins, there is a region of conservative substitution between the IC2/IC78 and IC3/IC69 pairs. This region corresponds to residues 97–104 in IC3/IC69, which, as noted above, is absolutely conserved and very highly charged in these two proteins; this region is aligned with most of a region (residues 192–200 in IC2) that is absolutely conserved in the IC2/IC78 pair. Numerous residues are absolutely conserved between all four chains; these are located predominantly in the C-terminal halves of the chains and in many cases correspond to consensus residues of the WD repeats (see below).

The Sea Urchin ICs Contain Repeated Elements that Conform to the WD-Repeat Consensus Sequence

Sea urchin IC2 and IC3 each contain five WD repeats (van der Voorn and Ploegh, 1992; Neer *et al.*, 1994), which account for much of the similarity between these two chains. Similar repeats previously were shown to be present in the *Chlamydomonas* ICs and in IC74 of cytoplasmic dynein (Wilkerson *et al.*, 1995). Figure 8 shows an alignment of the WD repeats found in the four axonemal dynein ICs; Figure 9 shows the relative positions of the WD repeats in all five ICs. WD repeats may vary considerably from the consensus expression; Neer *et al.* (1994) define "WD-repeat proteins as having at least one unit that matches the expression with zero or one mismatch, and at least one other unit that has three or fewer mismatches." Repeat D of IC2 matches the consensus sequence with only

```
                **  *  *      *  *  *   ******* *  * ***
IC2      A)   SKRLAVTSVC.....W..NPKYKD...LFAVAHGSYDFMKQSR(329-361)
IC78     A)   SKRRQVTSVC.....W..NPLYDD...MFAVGYGSYEFLKQAS(307-339)
IC3      A)   YPDGPKKLAVA....Y..SSLEF....QKTSAETSMDSYIWDI(170-202)
IC69     A)   HPDGSVPKVVVA...Y..SILQF....QQQPAGMPLSSYIWDV(170-203)

         B)   PTDSGVMCID.....I..RPEHPY...LICVGYCDGSVGVFNV(380-412)
         B)   HTESGVMCVH.....F..HPEFAN...LLAVGCYDGSVLVYDV(358-390)
         B)   KPVSPLVCLE.....Y..NPK.DVH..VLIGGCYNGQVAFWDT(213-245)
         B)   VPTSQICCAK.....FNLKD..NN...LVGAGQYNGQLAYFDV(214-246)

         C)   KHTDPVWQVA.....W.QKDDLDN.NLNFFSVSSDGRVVAWTL(429-464)
         C)   KLNDPVWQIY.....W..QPDDAQKSLQFVSISSDGAVNLWTL(407-442)
         C)   SHHDPVYKTI.....W.LQSKTG...TECFSASTDGQVLWWDM(260-293)
         C)   SHRDPIYDFA.....W.LQSKTGT...ECMTVSTDGNVLWWDL(261-294)

         D)   AHHMAVYKVM.....W..NHFHPK...IFISCSADWSVKIWDH(537-569)
         D)   SHHLAVYAVH.....W..NNIHPS...MFLSASCRLDHQAVGL(518-550)
         D)   EHIGPVYSLQ.....R..NPFEP....KNFLTVGDWTARIWSE(361-392)
         D)   GHHGPIYGLRRNP..F...NS......KYFLSIGDWTARVWVE(360-391)

         E)   DLGSAVGDVA.....W..APYSST...VFAAVTADGKVHVFDL(580-612)
         E)   DLNDSIGDVS.....W..AALQPT...VFAAVTDDGRVHVFDL(567-594)
         E)   YHMSYLTDGC.....W..SPVRPA...VFFTTKMDGSLDVWDY(404-436)
         E)   YHPTYLTGGT.....W..SPSRPG...VFFTIKMDGAMDVWDL(404-436)
```

Figure 8. Alignment of WD repeats A, B, C, D, and E from (top to bottom) IC2, IC78, IC3, and IC69. Amino acids shown in bold text are those that match the consensus sequence of Neer *et al.* (1994). Only two of three amino acids need to match under the asterisks marked by the overline. Gaps (indicated by dots) are allowed by the consensus sequence.

one mismatch, and repeat A of IC2 has only three mismatches. Thus, IC2 meets the stringent requirements of Neer *et al.* for a WD-repeat protein, and establishes that the outer arm dynein ICs represent a subfamily of WD-repeat proteins. Although the match to the expression of Neer *et al.* is not perfect for the repeats in the other ICs, the WD elements in these chains are readily recognized by their close similarity to the consensus sequence and by their repeating nature.

Van der Voorn and Ploegh (1992) divided the WD repeat into parts A and B. Part A corresponds to the first one-half of the repeat and includes the GH (or their unambiguous equivalents) at the beginning of the expression of Neer *et al.*, whereas part B corresponds to the second one-half of the repeat and ends with WD (or their equivalents). Van der Voorn and Ploegh noted that in many WD repeats, only part B can be identified, suggesting that part A may be dispensible. This suggestion is supported by the pattern of WD motifs in the outer arm dynein ICs; in most of these repeats, part A exhibits a poor match to the consensus sequence, whereas part B is a much better match.

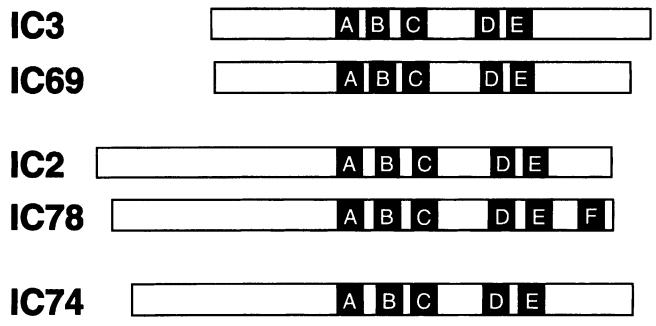

Figure 9. Relative positions of WD repeats (filled boxes) in sea urchin outer arm dynein IC2 and IC3, *Chlamydomonas* outer arm dynein IC69 and IC78, and rat cytoplasmic dynein IC74. WD repeat F in IC78 is a weak match to the consensus sequence; the equivalent region in IC2 is even more degenerate and so is not indicated here. Although the C-terminal halves of all of these chains contain WD repeats, the chains may be placed into three distinct subclasses (IC3/IC69, IC2/IC78, and IC74) on the basis of sequence similarities throughout the N-terminal halves of each homologous pair (see Figure 4).

DISCUSSION

In the work reported here, we have isolated and sequenced cDNA clones covering the complete coding regions of the intermediate chains IC2 and IC3 of sea urchin outer arm dynein. The results indicate that sea urchin IC2 is more closely related to IC78 of *Chlamydomonas* outer arm dynein, and sea urchin IC3 is more closely related to IC69 of *Chlamydomonas* outer arm dynein, than either sea urchin chain is related to the other. These findings provide the first unequivocal evidence that structural differences between individual ICs within an outer arm dynein are highly conserved in outer arm dyneins of distantly related species, and indicate that the ICs may be subclassified on the basis of these differences, particularly those in the N-terminal halves of the chains.

The fact that specific structural differences between the ICs have been highly conserved in different species also suggests that functions specific to each of these ICs are likely to have been conserved throughout evolution. There is very little information available on the functions of IC2 and IC3 in sea urchin outer arm dynein. In *Chlamydomonas*, IC78 has been shown to be in direct contact with the A-tubule in vivo (King *et al.*, 1991), to be a microtubule-binding protein (King, Patel-King, Wilkerson, and Witman, unpublished data), and to be essential for outer arm assembly onto the axoneme (Wilkerson *et al.*, 1995). Taken together, these results strongly suggest that IC78 has a role in binding the outer arm to the A-tubule of the outer doublet. It thus seems likely that IC2 has a similar function in sea urchin outer arm dynein. IC69 also is necessary for outer arm assembly in *Chlamydomonas* (Mitchell and Kang, 1991). However, in contrast to IC78, it does not appear to bind directly to microtubules (King *et al.*, 1991; King, Patel-King, Wilkerson, and Witman, unpublished data), although it has been shown to bind to the β DHC. Therefore, it may function in holding IC78 to the DHCs, or in stabilizing the entire dynein complex. It also may have a role in the regulation of DHC activity (Mitchell and Kang, 1993). Based on the results presented here, it may be presumed that IC3 of sea urchin has comparable functions.

Previous studies of dynein subfractions and subunit-subunit interactions revealed similarities in IC/LC affinities, which suggested that specific IC pairs were structurally equivalent in evolutionarily distant species. In *Chlamydomonas*, IC78 and IC69 are tightly associated with each other and with several LCs (Mitchell and Rosenbaum, 1986; King *et al.*, 1991) to form a discrete IC/LC complex that is located at the base of the fibrous stem of the soluble outer arm (King and Witman, 1990). In sea urchin, IC2 and IC3 together with at least five light chains remain associated with one another as a discrete particle when the isolated dynein is disrupted by low ionic strength dialysis; in contrast, IC1 remains bound to the β DHC (Tang *et al.*, 1982; Sale and Fox, 1988; Moss *et al.*, 1992). A cluster of small bead-like subunits, which probably corresponds to the IC/LC complex, was observed when isolated sea urchin outer arm dynein was dialyzed and then examined by electron microscopy using the quick-freeze, deep-etch technique (Sale *et al.*, 1985). In trout, ICs 1 and 2 together with four LCs dissociate as a separate IC/LC complex when the isolated outer arm is subjected to low ionic strength dialysis, whereas ICs 3–5 tend to remain bound to the β DHC (King *et al.*, 1990). Based on these subunit affinities, Witman *et al.* (1992) proposed that a discrete IC/LC complex consisting of two ICs (IC69 and IC78 of *Chlamydomonas*, ICs 2 and 3 of sea urchin, and ICs 1 and 2 of trout) and several LCs was a common structural feature of outer arm dyneins. The present work confirms and further defines the structural homology between these sea urchin ICs and the *Chlamydomonas* ICs. We propose that IC1 of trout is homologous to IC2 of sea urchin and to IC78 of *Chlamydomonas*, and that trout IC2 is similarly homologous to IC3 and IC69 of sea urchin and *Chlamydomonas*, respectively.

Comparison of the homologous ICs from sea urchin and *Chlamydomonas* revealed numerous short sequence segments of up to 10 residues that are absolutely conserved between these two species. These results should now permit use of the polymerase chain reaction and degenerate oligonucleotide primers to obtain polymerase chain reaction clones that encode portions of homologous ICs from other species. Similarly, it will now be possible to use oligonucleotide probes based on the conserved segments to screen cDNA or genomic libraries of other species for clones that encode the homologous ICs.

Chlamydomonas IC69 and IC78 and rat brain IC74 are similar primarily in their C-terminal halves (Paschal *et al.*, 1992; Wilkerson *et al.*, 1995); Wilkerson *et al.* (1995) identified the regions of similarity as members of a repetitive sequence element termed the WD repeat (Van der Voorn and Ploegh, 1992; Neer *et al.*, 1994). The current work demonstrates that these elements also are present in sea urchin IC2 and IC3, and firmly establishes that the outer arm dynein ICs represent a true subfamily of WD-repeat proteins. Neer *et al.* (1994) proposed that these repeats are involved in subunit-subunit interactions within multicomponent complexes, and suggested that the wide variability in the repeats within a single protein may allow the protein to associate with several different partners. Such a hypothesis is particularly attractive in the case of the outer arm dynein ICs, which are known to interact directly with each other and with both DHCs and LCs as well as tubulin (see above). The region of IC78 that is believed to be involved in binding IC69 (King, Patel-King, Wilkerson, and Witman, unpublished data) corresponds closely to the region containing repeats A and B (Wilkerson *et al.*, 1995); it is therefore of interest that WD repeat A (along with E) is most highly conserved (66% amino acid identity) between IC2 and IC78. In contrast, between IC3 and IC69, repeat A is much less conserved (52% identity) whereas repeats C and E are very highly conserved (70 and 71% amino acid identity) and repeat D is nearly as well conserved (64% amino acid identity). Thus, these latter repeats are likely to be very important in subunit-subunit interactions involving IC3 or IC69. Finally, it is of interest that all WD-repeat proteins identified to date appear to be regulatory proteins (Neer *et al.*, 1994). As noted above, there is evidence that IC69 may have a role in regulating dynein arm activity (Mitchell and Kang, 1993). This raises the possibility that one or more of the WD repeats in the ICs may serve as conformationally sensitive mechanical switches that transmit information between dynein subunits.

Our observation that antibodies against sea urchin IC2 and IC3 cross-reacted with *Chlamydomonas* IC78 and IC69, respectively, provided the first indication in the current work that these ICs were homologous to each other and that unique structural features of specific ICs were conserved across distant species lines. Previous studies of immunological cross-reactivity also had suggested that the outer arm dynein intermediate chains of distantly related species were structurally related. King *et al.* (1985) observed that a monoclonal antibody against *Chlamydomonas* IC69 exhibited strong cross-reactivity with IC3 of sea urchin. The same monoclonal antibody against *Chlamydomonas* IC69 also reacted with IC2 of trout outer arm dynein (King *et al.*, 1990). Interestingly, the trout IC2 also was recognized by a monoclonal antibody against *Chlamydomonas* IC78, indicating that a single trout IC contained epitopes occurring separately on two different *Chlamydomonas* ICs. Our finding that the WD repeats are present and relatively well conserved in ICs both within and between species offers a possible explanation for this latter observation.

Our studies of antibody cross-reactivity failed to detect a specific outer arm polypeptide in *Chlamydo-*

monas that was recognized by an antibody against IC1 of sea urchin. Therefore, it remains undetermined if the *Chlamydomonas* outer arm contains a polypeptide homologous to IC1. The presence of an outer arm IC of such large size (M_r 112,000–120,000) has not been confirmed in any other organism. Porter and Johnson (1983) reported that *Tetrahymena* outer arm dynein contained ICs of M_r 70,000, 85,000, and ~100,000, but more recently the larger polypeptide was reported to be a proteolytic fragment of the γ DHC (Marchese-Ragona and Johnson, 1989). On the other hand, it was recently reported that a different monoclonal antibody that recognizes an epitope on sea urchin IC1 inhibited the motility of sperm of other species (*Oxyrrhis marina*, a primitive dinoflagellate, and human), suggesting that IC1 homologues are widespread and play a dynamic role in flagellar bending and/or wave propagation (Gagnon *et al.*, 1994). One researcher in our group (K.O.) is currently in the process of cloning and sequencing cDNAs encoding sea urchin IC1; the sequence obtained so far covers approximately two-thirds of the molecule and is unrelated to that of IC2 or IC3. Determination of the complete sequence of this enigmatic protein should shed light on its role in dynein structure and function.

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