CELL STRUCTURE AND FUNCTION 21: 343–349 (1996)


# A Dynein Motor Superfamily

**Kazuo Ogawa*** **and Hideo Mohri†**

**Radioisotope Facility and †Office of Director-General, National Institute for Basic Biology, Okazaki 444, Japan*

## HISTORICAL ASPECTS

**Dynein**: The name of dynein was given an ATPase that had been extracted from the cilia of *Tetrahymena* by Gibbons and Rowe (9). The impressive work of Gibbons and his colleagues (8, 51) from the late 1960s to the early 1970s led to the acceptance of dynein as a motor protein that is responsible for flagellar and ciliary movements. In the mid 1970s, studies of dynein took a new turn when it became clear that tubulin, the component protein of microtubules (32), is present at high levels not only in cilia and flagella but also in brain, and when the polymerization in vitro of tubulin from brain became possible (57). It was apparent, then, that dynein might play a much broader role in the cytoplasm, and it seemed natural to postulate that proteins of high molecular weight associated with polymerized microtubules could be forms of dynein that act as the motor for microtubule-based motility in the cytoplasm of brain cells (2, 5).

**A Brief History**: The first evidence suggesting that dynein might be located in the mitotic apparatus was presented by Mohri *et al.* (34), after the successful preparation of antibodies that were able to suppress the ATPase activity of dynein and reactivate the Triton-model in sea urchin sperm (39, 42). Figure 1A shows an electron micrograph of a sea urchin sperm axoneme after immunostaining with antibodies against dynein fragment A (formed by digestion of dynein with trypsin) and ferritin-conjugated second antibody (40). The ferritin particles are visible between the outer doublet, in the vicinity of the distal end of the outer arm. This evidence confirmed for the first time that dynein is located in the outer arm. Although the inner arm also contains dynein distinct from the outer arm dynein, the present review will focus on the outer arm dynein. On inspecting electron micrographs, we noticed that immunolabeling was quite rare on the outer arm that projected from outer doublet no.5. This feature suggested that this outer arm might be masked by a structure that projects backward from doublet no.6. This finding also indicated that the binding of antibodies to the dynein of outer arm no.5 is prevented by the presence of this projection, that the doublets no.5 and no.6 are firmly bound so that they do not move with respect to each other, and that the no.5 dynein probably does not function as an active ATPase. This observation might be related to the fact that flagellar bending occurs in the plane perpendicular to the central pairs of microtubules.

**Some Disputes**: The antibodies against dynein fragment A reacted not only with cytoplasm but also with the mitotic apparatus of the cleaving sea urchin egg (34, Fig. 1B). The weakly stained antigen in the cytoplasm might be partly due to the presence of precursor dynein stockpiled in the egg that is required for ciliogenesis. Sakai *et al.* (47) demonstrated the ATP-induced segregation of chromosomes in vitro was suppressed by the same antibodies. From these observations, we proposed that an axonemal dynein-like protein might be associated with the mitotic spindle and might be responsible for chromosome segregation. Supporting our finding, Hisanaga *et al.* (16) prepared a monoclonal antibody against 21S dynein of sea urchin sperm (corresponding to the outer arm dynein) and demonstrated that cytoplasmic dynein was located at the mitotic apparatus. Our observation was, however, called into question by a paper of Zieve and McIntosh (62) who claimed that their antibodies against axonemal dynein did not react with any cellular components. This dispute seemed fruitless since our antiserum completely inhibited the ATPase activity of dynein, while their antibodies were raised against antigen eluted from a denaturing SDS-polyacrylamide gel and did not inhibit the ATPase. Complete sequencing of both isoforms of dynein and a comparison of their primary structures should resolve this dispute. Moreover, McIntosh and colleagues (24) reported that antibodies directed against selected sequences of dynein from the cellular slime mold *Dictyostelium* reacted not only with a cytoplasmic isoform of dynein from *HeLa* cell but also with the sea urchin sperm dynein.

**Video Assay and the Discovery of Kinesin**: From the late 1970s to the early 1980s, studies aimed at the

Corresponding author: Kazuo Ogawa, Radioisotope Facility, National Institute for Basic Biology, Okazaki 444, Japan.
PHONE: +81-564-55-7662, FAX: +81-564-53-3299
e-mail: ogawaai@nibb.ac.jp

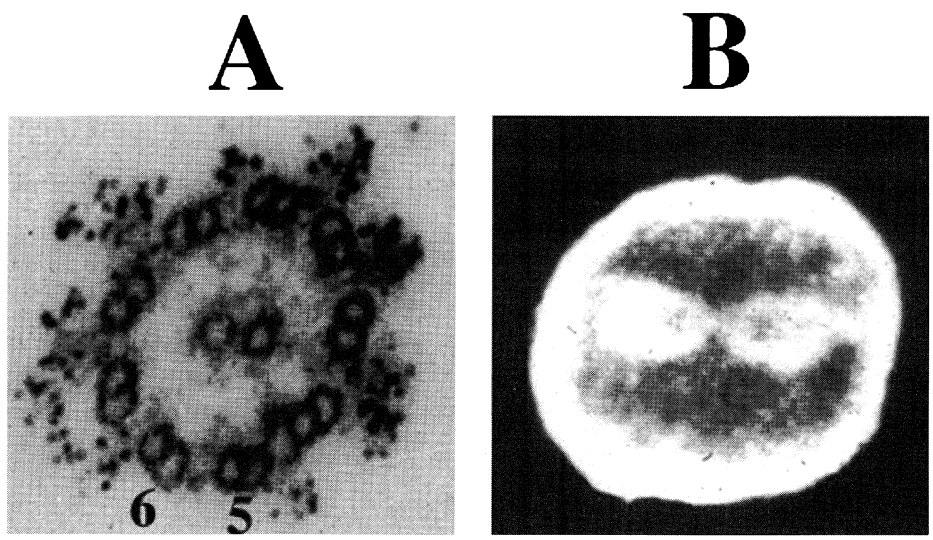


**Fig. 1.** Localization of dynein in sea urchin sperm axoneme (A) and a cleaving egg (B).

isolation of cytoplasmic dynein in a pure form were reported. Although Hisanaga and colleagues isolated "cytoplasmic" dynein from unfertilized sea urchin eggs (15), the possibility could not be ruled out that this protein might have been a precursor to dynein that had been stockpiled in eggs for subsequent ciliogenesis. Unfortunately, the presence of a cytoplasmic isoform of dynein was disputed until the successful isolation of dynein from non-ciliated tissues. During this period, a revolutionary methodology, namely, video-enhanced differential interferential contrast microscopy, which allows detection of single microtubules as little as 25 nm in diameter, was developed by Allen *et al.* (1), making it possible to monitor the motor activity of individual proteins of interest. Use of this video assay and an axoplasmic extract of squid giant axons led to the discovery of a novel, microtubule-based motor that was named "kinesin" (54). The growth of a microtubule occurs more rapidly at one end, defined as the plus-end, than at the other end, defined as the minus-end. Kinesin adsorbed to glass was able to move microtubules, which moved with the minus-end leading. Thus, kinesin was shown to be a motor protein responsible for transport towards the nerve terminal from the cell body in an axon (anterograde transport).

**Isolation of Cytoplasmic Dynein**: After the discovery of kinesin, much interest was concentrated on finding the motor protein that was responsible for the transport of vesicles towards the cell body from a nerve terminal (retrograde transport). Eventually, microtubule-associated protein 1C (MAP 1C) was isolated from rat brain and retrograde motor activity in vitro was demonstrated by the video assay (43). MAP 1C has some similarities to axonemal dynein, but there are differences as well. MAP 1C has microtubule-activated ATPase activity, as does dynein (44). Electrophoretic comparison showed that MAP 1C migrates with mobility similar to that of the outer arm dynein of *Chlamydomonas*. On the basis of these findings (43, 44), MAP 1C was defined as cytoplasmic dynein or a cytoplasmic isoform of dynein (55). Lye *et al.* (28) purified a protein of high molecular weight that combined some dynein-like properties with the in vitro motility properties of kinesin from the nematoda *C. elegans*. It is unknown whether or not this protein is a novel microtubule-translocator that is distinct from MAP 1C (cytoplasmic dynein).

## RECENT PROGRESS IN MOLECULAR CLONING

### Dynein Heavy Chains

*Introduction*: Native dyneins are very large complex proteins, which range in molecular mass up to 1 to $2 \times 10^6$ daltons. Each dynein contains two or three heavy chains (HCs) with ATPase activity, which range in molecular mass up to 500 kDa. The motor activity of dynein is associated with these chains. Some functional differences have been reported between HCs of outer arm dynein. Sea urchin outer arm dynein is a heterodimer of HCs ($\alpha$ and $\beta$) and at least the $\beta$ HC is able to induce gliding of microtubule in vitro. The $\alpha$ HC might amplify the function of $\beta$ HC and it has been reported to have no motile activity (35, 50). The outer arm dynein of *Chlamydomonas* is a heterotrimer of HCs ($\alpha$, $\beta$, and $\gamma$). Kamiya and colleagues revealed the functional differences between $\alpha$ and $\beta$ HCs using mutants that were partially defective in outer arm structure (19, 48). The outer arm of *Chlamydomonas* can be assembled without the $\alpha$ HC, and outer arms that lack the $\alpha$ HC re-

tain partial function (48). There appear to be some empirical rules with respect to the numbers of HCs in outer arm dyneins. The outer arm dyneins of unicellular organisms, such as *Tetrahymena, Chlamydomonas*, and *Paramecium*, contain three different HCs that reflect their three-headed structure, whereas those of multicellular animals, such as oyster (56), sea urchin (53), rainbow trout (6), and pig (14), contain only two HCs. This difference is due to the presence of the outermost appendage of the outer arm that is seen in unicellular organisms (Fig. 2A). Sakakibara *et al.* (48, 49) showed that this appendage is derived from the $\alpha$ HC. Examination of cross-sections of axonemes of other unicellular organisms revealed images of outer arms of the multicellular organisms listed above that are quite similar to those of a mutant (*oda11*) of *Chlamydomonas* that lacks $\alpha$ HC (33, Fig. 2B). This observation provides us with a useful tool for predicting the numbers of HCs or heads of outer arm dynein in situ. Images of outer arm dyneins of all metazoan flagella and cilia examined to date revealed the presence of two HCs. Thus, a decrease in the number of HCs in outer arm dynein seems to have occurred during evolution from protozoa to metazoa (33, 56). Scanning transmission electron micrographs of cytoplasmic dynein revealed that most particles have a pair of globular heads. This dynein can be split into two species, designated $\alpha$ and $\beta$ according to the convention used for axonemal dyneins, and it would appear, therefore, that cytoplasmic dynein consists of two species of HCs (55). However, molecular cloning of a cytoplasmic HC from a variety of organisms revealed that this cytoplasmic dynein is a homodimer (29).

*The Four P-Loop Family*: After the first cloning of $\beta$ HC from sea urchin ciliary axonemes (10, 37), the sequences of $\beta$ HC (30) and $\gamma$ HC (58) of *Chlamydomonas* outer arm dynein and of the $\beta$ HC (20) of *Paramecium* outer arm dynein were determined in their entirety. The $\alpha$ HCs of sea urchin and *Chlamydomonas* outer arm dyneins have also been almost completely sequenced. Sequence comparisons have shown that sea urchin $\alpha$ and $\beta$ HCs are much more closely related to *Chlamydomonas* $\gamma$ and $\beta$ HCs, respectively, than either sea urchin chain or *Chlamydomonas* chain is related to the other. *Chlamydomonas* $\alpha$-HC is much more closely related to $\beta$ HC than to $\gamma$ HC (7). With respect to cytoplasmic HCs, cloning was completed for *Dictyostelium* by Koonce *et al.* (24), for rat brain by Mikami *et al.* (29) and Zhang *et al.* (61), for yeast by Eshel *et al.* (3) and Li *et al.* (26), for *Drosophila* by Li *et al.* (25), for *Aspergillus* by Xiang *et al.* (60), for *Neurospora* by Plamann *et al.* (46), and for nematoda *C. elegans* by Lye *et al.* (27). Without exception, all the HCs cloned to date contain four P-loop (ATP-binding) sequences in the midregion of the molecule. Thus, they can be classified as a four P-loop family. The P-loops can be abbreviated as P1, P2, P3, and P4, from the N-terminal end. P1 and P4 are conserved in all axonemal HCs, while P1 and P3 are conserved in all cytoplasmic HCs.

*Unique Conformation*: Predictions of secondary structure suggest that all the HCs cloned to date consist of three regions with dominant $\beta$ sheet structure separated by two mainly $\alpha$ helical regions. We refer to these regions as the N, M, and C domains from the N-terminus to the C-terminus in the case of the sea urchin $\beta$ HC (37, 38, Fig. 3A), and we present a tail-head model, wherein the M and C domains form a head (motor domain) and the N domain forms an irregularly shaped tail, in Figure 3B. The leucine zipper structure found in the $\alpha$2 region promotes the association of two domains. The corresponding heptad repeats of hydrophobic amino acids are well conserved in all HCs. Thus, a similar tail-head structure can be proposed for each of them.

*Variable N Domain*: A dot-plot matrix comparison between the HCs of *Dictyostelium* and sea urchin dynein showed that sequence similarities are not uniformly distributed and are limited to the M and C domains (the motor domains). No sequence similarity was found among the 1,400 N-terminal amino acids (N domain). The sequence of the N domain is well conserved among

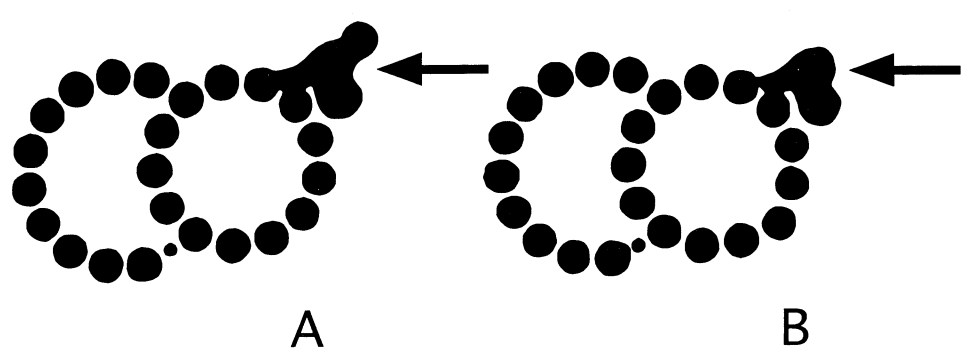


**Fig. 2.** Diagrams of outer arm dynein (arrow) projecting from an outer doublet microtubule of protozoan (a) and animal (B) axonemes (from reference 33 with copyright permission).

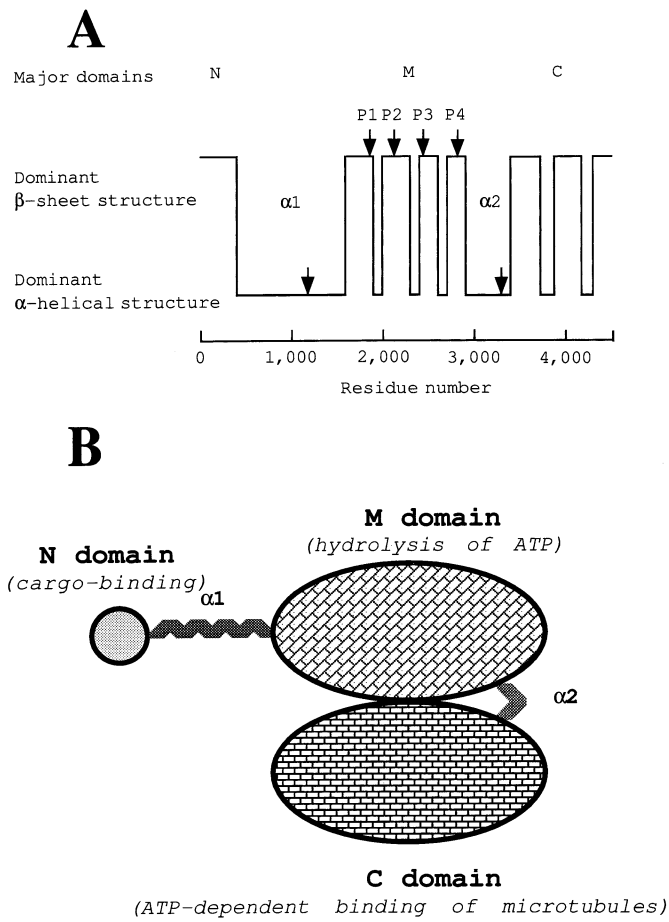


**Fig. 3.** Diagram showing that the HCs of dynein cloned to date consist of three regions with dominant $\beta$-sheet structure, separated by two regions with dominant $\alpha$-helical structure (A) and the tail-head model of these HCs (B). The two arrows on two long dominant $\alpha$-helical structures define the trypsin cleavage sites. Dynein fragment A consists of the M and C domains (motor domain).

axonemal and cytoplasmic families of HCs. Thus, the N domain could specify the function of an axonemal or cytoplasmic HC. Sakakibara *et al.* (49) showed that the N-terminal 160-kDa region in the $\beta$ HC of *Chlamydomonas* includes a so-called cargo-binding site or ATP-independent A-tubule-binding site. Because of the restricted localization of axonemal dyneins, the difference in cargoes that they bind in situ could be small. By contrast, cytoplasmic dyneins are thought to bind a variety of different cargoes that include nerve vesicles (43), spindles (3, 26), morphogens (25), and nuclei (47, 61), depending on the location of the motor. However, genomic Southern blots probed with isolated cDNA clones suggest that there is only a single copy of the gene for cytoplasmic HC in a variety of organisms. Thus, the question of whether the HC makes direct contact with each of these cargoes remains to be answered.

*Multigene Family*: Gibbons *et al.* (11) and Tanaka *et al.* (52) synthesized sets of degenerate primers for the sequences in the vicinity of the P1 site, which is the most highly conserved region, and they amplified thirteen kinds of new gene for the HCs of the sea urchin embryo and the rat brain, respectively, by involving reverse transcriptase and the polymerase chain reaction (RT-PCR) strategies. From a molecular evolutionary analysis, Tanaka *et al.* (52) concluded that there are at least two non-axonemal HCs in animals. According to Kagami and Kamiya (18), the inner arm dynein of *Chlamydomonas* probably contains as many as eight different HCs in all. Thus, a *Chlamydomonas* cell contains at least twelve different isoforms of HC, which include three outer arm HCs and one cytoplasmic HC. The multiple genes for HCs identified by the RT-PCR method might correspond to the genes for each HC in the outer and inner arm dyneins in cytoplasmic dynein (7).

## Dynein Intermediate Chains

*Introduction*: The outer arm dyneins contain two or three proteins that range in molecular mass from 70 to 120 kDa and copurify with HCs. During the preparation of cytoplasmic dynein, a set of three 74-kDa proteins was associated with the HC. They were named dynein intermediate chains (ICs). ICs of sea urchin outer arm dynein are abbreviated as IC1, IC2, and IC3. Those of *Chlamydomonas* are called IC78 and IC69, and ICs of cytoplasmic dynein are called IC74. *Chlamydomonas* IC78 has been shown to be in direct contact with the A-tubule in vivo (22), to be a microtubule-binding protein (23), and to be essential for assembly of outer arms onto axonemes (59). IC69 is also necessary for outer arm assembly (31). However, in contrast to IC78, it does not appear to bind directly to microtubules and it might function in the binding of IC78 to HCs or in the stabilization of the entire dynein complex. Since the enzymatic activities of ICs have not been determined and they are believed to be non-functional regulatory proteins, the relationship between these proteins from different species remained unknown until the successful clonings of all six proteins.

*A WD Family: Chlamydomonas* IC78 and IC69 were cloned by Wilkerson *et al.* (59) and Mitchell and Kang (31), respectively. The sequences of sea urchin IC2 and IC3 were determined by Ogawa *et al.* (41). IC1 has recently been cloned (K. Ogawa, accession number D63884). Rat brain IC74 was cloned by Paschal *et al.* (45). Thus, all the ICs found in the axonemal and cytoplasmic dyneins of the model organisms used for studies of dynein function have been completely sequenced. Comparison of amino acid sequences of IC2 and IC3 with those of IC78 and IC69 and with that of IC74 showed that, although all five ICs are homologous, IC2 is much more closely related to IC78, and IC3 is much

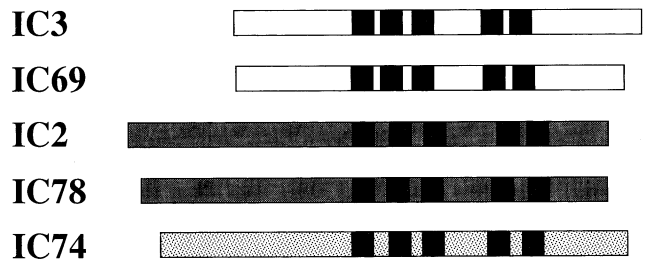


**Fig. 4.** Relative positions of WD repeats (filled boxes) in sea urchin outer arm IC2 and IC3, *Chlamydomonas* outer arm IC78 and IC69, and rat cytoplasmic IC74. Types of shading mean subclass of ICs.

more closely related to IC69, than either sea urchin chain or either *Chlamydomonas* chain is related to each other. Regions of similarity between all five ICs are limited to the C-terminal halves of the molecules. Similarities are due primarily to conservation of the WD repeats in all of these chains. The WD repeats are involved in protein-protein interactions in a large family of regulatory molecules (36). The relative positions of WD repeats in these chains are shown schematically in Figure 4. A parsimony tree for these chains shows that, although the C-terminal halves of all these chains contain WD repeats, the chains can be divided into three distinct subclasses (IC3 plus IC69, IC2 plus IC78, and IC78) on the basis of sequence similarities throughout the N-terminal halves of each homologous pair. The fact that specific structural differences between ICs have been highly conserved in different species suggests that functions specific to each of these ICs are likely to have been conserved throughout evolution. Thus, we can postulate that IC2 and IC3 might have similar functions in the outer arm dynein of sea urchin.

*Is IC1 Unique?*: Sea urchin IC1 is not a member of the WD family. Sequence analysis showed that IC1 consists of a thioredoxin-related (TRXR) sequence and three nucleoside diphosphate kinase-related (NDPKR) sequences. The relative positions of these sequences are shown schematically in Figure 5. Outer arm dyneins from rainbow trout and *Chlamydomonas* do not contain a polypeptide homologous to IC1. However, Gagnon *et al.* (4) reported that homologues of IC1 are widespread in *Oxyrrhis marina* (a primitive dinoflagellate) and human, and play a dynamic role in flagellar bending and/or wave propagation. We have recently succeeded in identifying the homologues of IC1 in the outer arm dyneins from *Halocynthia* and rainbow trout sperm axonemes.

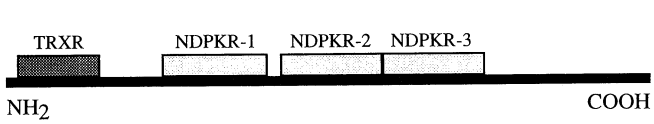


**Fig. 5.** Diagram showing the positions of TRXR and NDPKR sequences in the IC1 of sea urchin outer arm dynein.

### Dynein Light Chains

*Introduction*: An analysis by SDS-PAGE revealed several small polypeptides that ranged in molecular mass from 8 to 30 kDa and copurified with axonemal outer arm dynein, as well as proteins that ranged in molecular mass from 53 to 61 kDa and copurified with cytoplasmic dynein. These chains are known as dynein light chains (LCs). In the case of cytoplasmic dynein, they are also referred to as dynein intermediate-light chains (or ILC). Because of the great difference in molecular mass between HCs and LCs, the exact numbers of LCs reported to be associated with dynein tend to vary. Recently, interest in LCs has been generated as a consequence of the relationship between phosphorylation of LCs and a possible regulatory role in dynein function. Hamasaki *et al.* (13) observed that a 29-kDa protein (LC29) associated with the outer arm dynein of *Paramecium* is phosphorylated in a cAMP- and $Ca^{2+}$-sensitive manner. Phosphorylation of LC29 increased the velocity of translocation in vitro of microtubules by the outer arm dynein.

*Work in Progress*: King and Patel-King (21) reported the molecular cloning of LCs of 8 and 11 kDa from the outer arm of *Chlamydomonas* flagella. Both molecules contained a highly amphiphilic $\alpha$-helix, which is presumably involved in protein-protein interactions. Several LC homologues have been identified in organisms that lack cilia and flagella. Gill *et al.* (12) showed that LCs from chicken cytoplasmic dynein from two major families, a higher $M_r$ family (LC-A) and lower $M_r$ family (LC-B), and that they are tightly associated with the HC, while ICs are more readily released. All the LCs were phosphorylated. Gill *et al.* (12) also isolated a cDNA clone that encoded LC-A, which contained a consensus P-loop sequence in the N-terminal region. Hughes *et al.* (17) also isolated a cDNA clone for ILC53/55 of rat brain cytoplasmic dynein. The sequences of the proteins from chicken and rat were 64% identical and 80% similar. The enzymatic activity of these LCs/ILCs has not been examined and their functions are unknown. At present, there are no reports of proteins associated both with axonemal and cytoplasmic dyneins.

## CONCLUSIONS

Dynein was discovered 30 years ago by Professor Ian R. Gibbons, the winner of the eleventh International Prize for Biology. It then took ten years to demonstrate that dynein is the motor responsible for flagellar and ciliary movement. The study of dynein took a new turn in the next 10-year period when interest was focused on

finding a cytoplasmic homologue of dynein. Then, as a result in large part of the development of a video assay, cytoplasmic dynein was isolated from non-ciliated cells, and the idea became established that axonemal and cytoplasmic dyneins constitute a family of retrograde motors that are strikingly different from kinesins, a family of anterograde motors. In the past 5 years, axonemal and cytoplasmic dyneins were successively cloned from a variety of organisms, and primary structures not only of HCs but also of other subunits, ICs and LCs, were determined. Analysis of cloned genes revealed that HCs are members of the four P-loop family while ICs are members of the WD family, and that axonemal and cytoplasmic dyneins consist of two different families.

In human, the immotile cilia syndrome (or Kartagener's syndrome) which causes situs inversus, respiratory complaints, and male sterility is thought to the cause of a mutation occurring in the assembly of dynein onto the outer doublet. Cloning of dynein proteins such as HCs, ICs, and LCs will make it possible to isolate homologues in humans and give a new direction in the clinical investigation of this disease at the genetic level.

*Acknowledgments.* The authors' research described in the present review was supported in part by grants from the Ministry of Education, Science and Culture of Japan (K.O. and H.M.) and the Yamada Science Foundation (K.O.).

## REFERENCES

1. Allen, R.D., Metuzals, J., Tasaki, I., Brady, S.T., and Gilbert, S.P. 1982. Fast axonal transport in squid giant axon. *Science* (Wash. DC), **218**: 1127–1228.
2. Burns, R.G. and Pollard, T.D. 1974. A dynein-like protein from brain. *FEBS Lett.*, **40**: 274–280.
3. Eshel, D., Urrestarazu, L.A., Vissers, S., Jauniaux, J.-C., van Vliet-Reedijk, J.C., Planta, R.J., and Gibbons, I.R. 1993. Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Natl. Acad. Sci. USA*, **90**: 11172–11176.
4. Gagnon, C., White, A., Huitorel, P., and Cosson, J. 1994. A monoclonal antibody against the dynein IC1 peptide of sea urchin spermatozoa inhibits the motility of sea urchin, dinoflagellate, and human flagellar axonemes. *Mol. Biol. Cell*, **5**: 1051–1063.
5. Gaskin, F., Kramer, S.B., Cantor, C.B., Adelstein, R., and Shelanski, M.L. 19??. A dynein-like protein associated with neurotubules. *FEBS Lett.*, **40**: 281–286.
6. Gatti, J.-L., King, S.M., Moss, A.G., and Witman, G.B. 1989. Outer arm dynein from trout spermatozoa. Purification, polypeptide composition, and enzymatic properties. *J. Biol. Chem.*, **264**: 11450–11457.
7. Gibbons, I.R. 1995. The dynein family of motor proteins: present status and future questions. *Cell Motil. Cytoskel.*, **32**: 136–144.
8. Gibbons, B.H. and Gibbons, I.R. 1972. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. *J. Cell Biol.*, **54**: 75–97.
9. Gibbons, I.R. and Rowe, A.J. 1965. Dynein: a protein with adenosine triphosphatase activity from cilia. *Science* (Wash. DC), **149**: 424–426.
10. Gibbons, I.R., Gibbons, B.H., Mocz, G., and Asai, D.J. 1991. Multiple nucleotide-binding sites in the sequence of dynein $\beta$ heavy chain. *Nature* (Lond.), **352**: 640–643.
11. Gibbons, B.H., Asai, D.J., Tang, W.-J.Y., Hays, T.S., and Gibbons, I.R. 1994. Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins. *Mol. Biol. Cell*, **5**: 57–70.
12. Gill, S.R., Cleveland, D.W., and Schroer, T.A. 1994. Characterization of DLC-A and DLC-B, two families of cytoplasmic dynein light chain subunits. *Mol. Biol. Cell*, **5**: 645–654.
13. Hamasaki, T., Barkalow, K., Richmond, J., and Satir, P. 1991. cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with 22S dynein arm regulates microtubule translocation velocity and swimming speed in *Paramecium*. *Proc. Natl. Acad. Sci. USA*, **88**: 7918–7922.
14. Hastie, A.T., Marchese-Ragona, S.P., Johnson, K.A., and Wall, J.S. 1988. Structure and mass of mammalian respiratory ciliary outer arm 19S dynein. *Cell Motil. Cytoskel.*, **11**: 157–166.
15. Hisanaga, S. and Sakai, H. 1983. Cytoplasmic dynein of the sea urchin egg. II. Purification, characterization, and interactions with microtubules and Ca-calmodulin. *J. Biochem.*, **93**: 87–98.
16. Hisanaga, S., Tanaka, T., Masaki, T., Sakai, H., Mabuchi, I., and Hiramoto, Y. 1987. Localization of sea urchin egg cytoplasmic dynein in mitotic apparatus studied by using a monoclonal antibody against sea urchin sperm flagellar 21S dynein. *Cell Motil. Cytoskel.*, **7**: 97–109.
17. Hughes, S.M., Vaughan, K.Y., Herskovits, J.S., and Vallee, R.B. 1995. Molecular analysis of a cytoplasmic dynein light intermediate chain reveals homology to a family of ATPases. *J. Cell Sci.*, **108**: 17–24.
18. Kagami, O. and Kamiya, R. 1992. Translocation and rotation of microtubules caused by multiple species of *Chlamydomonas* inner-arm dynein. *J. Cell Sci.*, **103**: 653–664.
19. Kamiya, R. 1988. Mutations at twelve independent loci result in absence of outer dynein arms in *Chlamydomonas reinhardtii*. *J. Cell Biol.*, **107**: 2253–2258.
20. Kandl, K.A., Forney, J.D., and Asai, D.J. 1995. The dynein genes of *Paramecium tetraurel*: the structure and expression of the ciliary and cytoplasmic heavy chains. *Mol. Biol. Cell*, **6**: 1549–1562.
21. King, S.M. and Patel-King, R.S. 1995. The $M_r$=8,000 and 11,000 outer arm dynein light chains from *Chlamydomonas* flagella have cytoplasmic homologues. *J. Biol. Chem.*, **270**: 11445–11252.
22. King, S.M., Wilkerson, C.G., and Witman, G.B. 1991. The $M_r$ 78,000 intermediate chain of *Chlamydomonas* outer arm dynein interacts with $\alpha$-tubulin in situ. *J. Biol. Chem.*, **266**: 8401–8407.
23. King, S.M., Patel-King, R.S., Wilkerson, C.G., and Witman, G.B. 1995. The 78,000-$M_r$ intermediate chain of *Chlamydomonas* outer arm dynein is a microtubule-binding protein. *J. Cell Biol.*, **131**: 399–409.
24. Koonce, M.P., Grissom, P.M., and McIntosh, J.R. 1992. Dynein from *Dictyostelium*: primary structure comparisons between a cytoplasmic motor enzyme and flagellar dynein. *J. Cell Biol.*, **119**: 1597–1604.
25. Li, M.-G., McGrail, M., Serr, M., and Hays, T.S. 1994. *Drosophila* cytoplasmic dynein, a microtubule motor that is

asymmetrically localized in the oocyte. *J. Cell Biol.*, **126**: 1475–1494.

26. Li, Y.-Y., Yeh, E., Hays, T., and Bloom, K. 1993. Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Natl. Acad. Sci. USA*, **90**: 10096–10100.
27. Lye, R.J., Wilson, R.K., and Waterston, R.H. 1995. Genomic structure of a cytoplasmic dynein heavy chain gene from the nematode *Caenorhabditis elegans*. *Cell Motil. Cytoskel.*, **32**: 26–36.
28. Lye, R.J., Porter, M.E., Scholey, J.M., and McIntosh, J.R. 1987. Identification of a microtubule-based cytoplasmic motor in the nematoda C. elegans. *Cell*, **51**: 309–318.
29. Mikami, A., Paschal, B.M., Mazumdar, M., and Vallee, R.B. 1993. Molecular cloning of the retrograde transport motor cytoplasmic dynein (MAP 1C). *Neuron*, **10**: 787–796.
30. Mitchell, D.R. and Brown, K.S. 1994. Sequence analysis of the *Chlamydomonas* alpha and beta dynein heavy chain genes. *J. Cell Sci.*, **107**: 635–644.
31. Mitchell, D.R. and Kang, Y. 1991. Identification of *oda6* as a *Chlamydomonas* dynein mutant by rescue with the wild-type gene. *J. Cell Biol.*, **113**: 835–842.
32. Mohri, H. 1968. Amino-acid composition of "tubulin" constituting microtubules of sperm flagella. *Nature* (Lond.), **217**: 1053–1054.
33. Mohri, H., Kubo-Irie, M., and Irie, M. 1995. Outer arm dynein of sperm flagella and cilia in the animal kingdom. *Mém. Mus. Natn. His. Nat.*, **166**: 15–32.
34. Mohri, H., Mohri, T., Mabuchi, I., Yazaki, I., Sakai, H., and Ogawa, K. 1976. Localization of dynein in sea urchin eggs during cleavage. *Develop. Growth Differ.*, **18**: 391–398.
35. Moss, A.G., Sale, W.S., Fox, L.A., and Witman, G.B. 1992. The $\alpha$ subunit of sea urchin sperm outer arm dynein mediates structural and rigor binding to microtubules. *J. Biol. Chem.*, **118**: 1189–1200.
36. Neer, E.J., Schmidt, C.J., Nambudripad, R., and Smith, T.F. 1994. The ancient regulatory-protein family of WD-repeat proteins. *Nature* (Lond.), **371**: 297–300.
37. Ogawa, K. 1991. Four ATP-binding sites in the midregion of the $\beta$ heavy chain of dynein. *Nature* (Lond.), **352**: 643–534.
38. Ogawa, K. 1992. Primary structure and function of a dynein motor molecule. *Zool Sci.*, **9**: 265–274.
39. Ogawa, K. and Mohri, H. 1975. Preparation of antiserum against a tryptic fragment (fragment A) of dynein and an immunological approach to the subunit composition of dynein. *J. Biol. Chem.*, **250**: 6476–6483.
40. Ogawa, K., Mohri, T., and Mohri, H. 1977. Identification of dynein as the outer arms of sea urchin sperm axonemes. *Proc. Natl. Acad. Sci. USA*, **74**: 5006–5010.
41. Ogawa, K., Kamiya, R., Wilkerson, C.G., and Witman, G.B. 1995. Interspecies conservation of outer arm dynein intermediate chain sequences defines two intermediate chain subclasses. *Mol. Biol. Cell*, **6**: 685–696.
42. Okuno, M., Ogawa, K., and Mohri, H. 1976. Inhibition of movement and ATPase activity of demembranated sea urchin spermatozoa by anti-dynein antiserum. *Biochem. Biophys. Res. Commun.*, **68**: 901–906
43. Paschal, B.M. and Vallee, R.B. 1987. Retrograde transport by the microtubule-associated protein MAP 1C. *Nature* (Lond.), **330**: 181–183.
44. Paschal, B.M., Shpetner, H.S., and Vallee, R.B. 1987. MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties. *J. Cell Biol.*, **105**: 1273–1282.
45. Paschal, B.M., Mikami, A., Pfister, K.K., and Vallee, R.B. 1992. Homology of the 74-kD cytoplasmic dynein subunit with a flagellar dynein polypeptide suggests an intracellular targeting function. *J. Cell Biol.*, **118**: 1133–1143.
46. Plamann, M.P., Minke, P.F., Tinsley, J.H., and Bruno, K.S. 1994. Cytoplasmic dynein and actin-related protein *Arp1* are required for normal nuclear distribution in filamentous fungi. *J. Cell Biol.*, **127**: 139–149.
47. Sakai, H., Mabuchi, I., Shimoda, S., Kuriyama, R., Ogawa, K., and Mohri, H. 1976. Induction of chromosome motion in the glycerol-isolated mitotic apparatus: Nucleotide specificity and effects of anti-dynein and myosin sera on the motion. *Develop. Growth Differ.*, **18**: 211–219.
48. Sakakibara, H., Mitchell, D.R., and Kamiya, R. 1991. A *Chlamydomonas* outer arm dynein mutant missing the $\alpha$ heavy chain. *J. Cell Biol.*, **113**: 615–622.
49. Sakakibara, H., Takada, S., King, S.M., Witman, G.M., and Kamiya, R. 1993. A *Chlamydomonas* outer arm dynein mutant with a truncated $\beta$ heavy chain. *J. Cell Biol.*, **122**: 653–661.
50. Sale, W.S. and Fox, L.A. 1988. Isolated $\beta$-heavy chain subunit of dynein translocates microtubules in vitro. *J. Cell Biol.*, **107**: 1793–1797.
51. Summers, K.E. and Gibbons, I.R. 1971. Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. *Proc. Natl. Acad. Sci. USA*, **68**: 3092–3096.
52. Tanaka, T., Zhang, Z., and Hirokawa, N. 1995. Identification and molecular evolution of new dynein-like protein sequences in rat brain. *J. Cell Sci.*, **108**: 1883–1893.
53. Tang, W.-J.Y., Bell, C.W., Sale, W.S., and Gibbons, I.R. 1982. Structure of the dynein-1 outer arm in sea urchin sperm flagella. I. Analysis by separation of subunits. *J. Biol. Chem.*, **257**: 508–515.
54. Vale, R.D., Reese, T.S. and Sheetz, M.P. 1985. Identification of a novel force generating protein, kinesin, involved in microtubule-based motility. *Cell*, **42**: 39–50.
55. Vallee, R.B., Wall, J.S., Paschal, B.M., and Shpetner, H.S. 1988. Microtubule-associated protein 1C from brain is a two-headed cytosolic dynein. *Nature* (Lond.), **332**: 561–563.
56. Wada, S., Okuno, M., Nakamura, K.-I., and Mohri, H. 1992. Dynein of sperm flagella of oyster belonging to Protostomia also has a two-headed structure. *Biol. Cell*, **76**: 311–317.
57. Weisenberg, R.C. 1972. Microtubule formation in vitro in solutions containing low calcium concentrations. *Science* (Wash. DC), **177**: 1104–1105.
58. Wilkerson, C.G., King, S.M., and Witman, G.B. 1994. Molecular analysis of the $\gamma$ heavy chain of *Chlamydomonas* flagellar outer-arm dynein. *J. Cell Sci.*, **107**: 497–506.
59. Wilkerson, C.G., King, S.M., Koutoulis, A., Pazour, G.J., and Witman, G.B. 1995. The $M_r$ 78,000 intermediate chain of *Chlamydomonas* outer arm dynein is a WD-repeat protein required for arm assembly. *J. Cell Biol.*, **129**: 169–178.
60. Xiang, X., Beckwith, S.M., and Morris, N.R. 1994. Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA*, **91**: 2100–2104.
61. Zhang, Z., Tanaka, Y., Nonaka, S., Aizawa, H., Kawasaki, H., Nakata, T., and Hirokawa, N. 1993. The primary structure of rat brain (cytoplasmic) dynein heavy chain, a cytoplasmic motor enzyme. *Proc. Natl. Acad. Sci. USA*, **90**: 7928–7932.
62. Zieve, G.W. and McIntosh, J.R. 1981. A probe for flagellar dynein in the mammalian mitotic apparatus. *J. Cell Sci.*, **48**: 241–257.