The dynein family at a glance

Peter Höök* and Richard B. Vallee

Department of Pathology and Cell Biology, Columbia University, New York, NY 10032, USA.
*Author for correspondence (e-mail: ph2041@columbia.edu)

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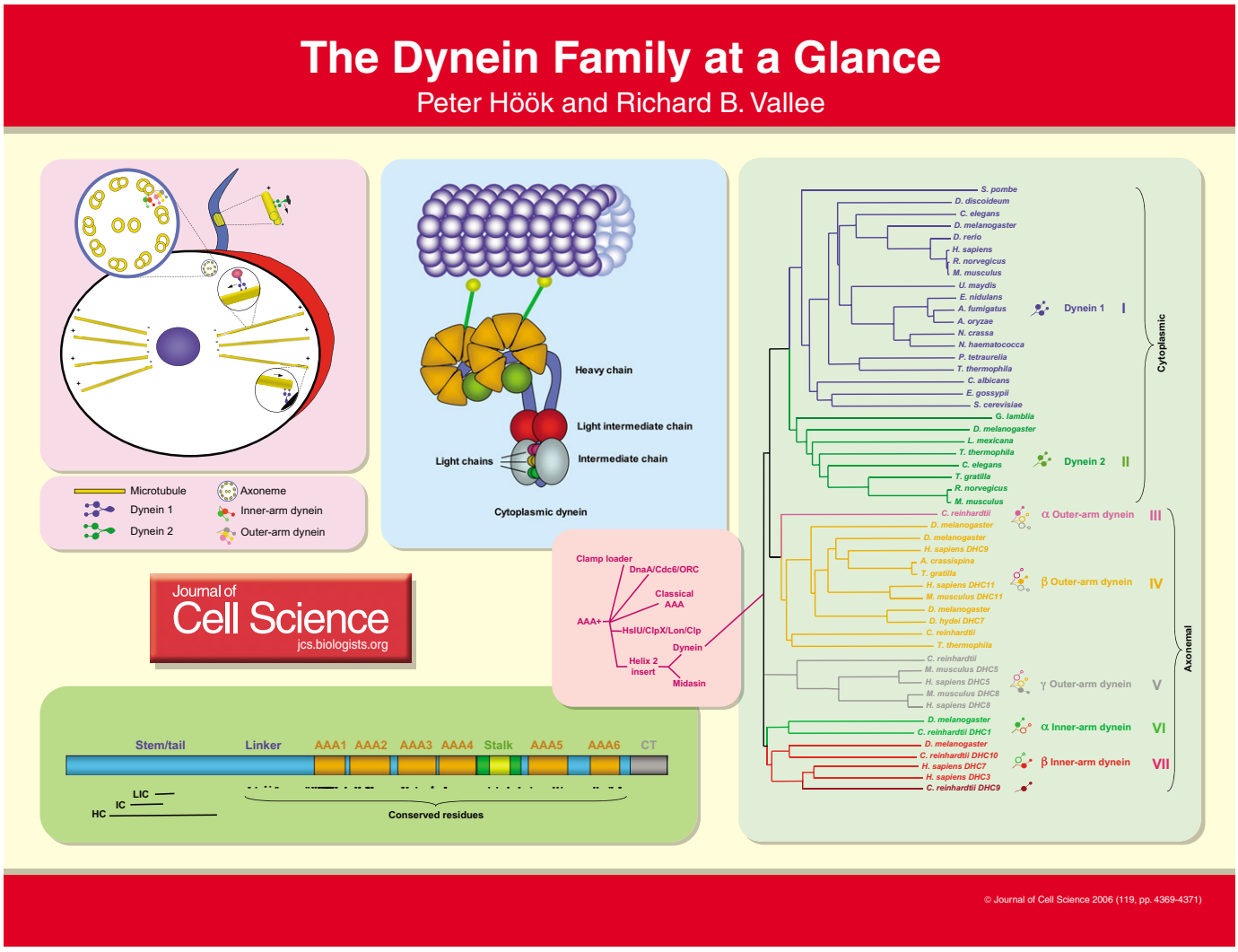
Three families of cytoskeletal motor protein – the myosins, kinesins and dyneins – have evolved to mediate transport of cells and of structures and materials within cells in eukaryotes. Whereas myosin uses actin polymers to carry out its tasks, kinesin and dynein are microtubule-associated motors. Dyneins use energy from ATP hydrolysis to power a wide variety of cellular functions. Although at least 14 classes of kinesin and 17 classes of myosin have been identified, the dyneins fall into only two major classes, axonemal and cytoplasmic dyneins, based on both functional and structural criteria. Axonemal dyneins are responsible for ciliary and flagellar beating; cytoplasmic dyneins are involved in intracellular transport, mitosis, cell polarization and directed cell movement

All dynein forms that have been identified biochemically are multisubunit proteins. Each has one to three heavy chains (HCs) of >500 kDa; these correspond to the number of morphologically identifiable heads and contain the motor domains of the molecule. The dynein HC forms two prominent structures: a ~160 kDa N-terminal domain that forms the base of the molecule, to which most of the accessory subunits bind; and a ~380 kDa motor domain. The motor domain contains six discernible AAA ATPase units, identifying the dynein HC as a divergent member of the AAA+ family of ATPases (Neuwald et al., 1999). Members of the AAA+ family are involved in a very wide range of functions but have a common feature: the formation of ring-shaped oligomeric complexes of the AAA ATPase module. Within the AAA+ proteins, dynein occupies a divergent branch along with midasin (Iyer et al., 2004). This branch is characterized by the incorporation of all six AAA modules within a single giant polypeptide. The AAA family has members in prokaryotes and it seems likely, therefore, that the dyneins had their origin very early in evolution. In dynein, energy from nucleotide hydrolysis at the AAA units is conveyed to the base of the molecule and to the



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microtubule-binding stalk for force production (Burgess et al., 2003). The stalk is predicted to consist of a 15-nm-long antiparallel coiled-coil α-helix crowned with a globular structure for microtubule association (Gee et al., 1997).

A diversity of accessory subunits, referred to as intermediate, light intermediate and light chains are also found associated with the dyneins. Most, but not all, of these subunits are associated with the cargo-binding base of the dynein molecule. Dynein-isoform-specific intermediate chains are found in some axonemal dyneins as well as in cytoplasmic dynein 1. Some light chains are shared between axonemal dyneins, cytoplasmic dynein 1 and other proteins. Isoform-specific light intermediate chains are associated with both cytoplasmic dyneins but not with axonemal dyneins.

Axonemal dyneins

The axoneme is a highly specialized and highly conserved, array of microtubules. Motile axonemes in flagella and many forms of cilia almost universally consist of 20 microtubules, of which two are centrally located and nine are fused pairs (the outer doublet) forming a surrounding cylinder. Dyneins connect the outer doublets and force them to slide against each other (Summers and Gibbons, 1971). Most ultrastructural and genetic evidence for subspecialization of the axonemal dyneins has been obtained in studies of the biflagellate green alga *Chlamydomonas reinhardtii* and the ciliate protozoan *Tetrahymena pyriformis*. Sea urchin sperm flagella have also been used for biochemical analysis of axonemal dyneins, but limited work has been performed in vertebrates. At least five biochemically defined forms of dynein (and even more gene products) can be found in a given type of cilium or flagellum, but correlating dynein genes with specific dynein structures in the axoneme has been a long and challenging process. All axonemal dyneins are stably associated with the complete microtubule (the A microtubule) of the outer doublet and aligned as rows along the outer or inner side of the A microtubule. The outer-arm dyneins have three motor domains (heads) in protozoans, but only two are retained in metazoans. The inner-arm dyneins are two- and one-headed and arrayed in a repeating pattern along the A-microtubule. Each of the flagellar dyneins interacts with the B microtubule of the adjacent outer doublet. Seven subtypes of *Chlamydomonas* axonemal inner-arm dynein have been identified (Kagami and Kamiya, 1992); one forms a HC heterodimer; the rest are monomeric, one-headed structures.

Cytoplasmic dyneins

Two forms of cytoplasmic dynein have been identified: cytoplasmic dynein 1 and cytoplasmic dynein 2. Both assemble as homodimers, but their distributions and functions within the cell differ markedly. Cytoplasmic dynein 1 is far more abundant and is found in all microtubule-containing cells. It associates with and transports elements of the Golgi apparatus, lysosomes, and late and recycling endosomes, and is responsible for retrograde axonal transport. It also associates with diverse protein and RNA-containing complexes, which it transports or maintains at microtubule minus ends. Cytoplasmic dynein 1 also associates with kinetochores, where it has been implicated in microtubule capture and the removal of checkpoint proteins to permit entry into anaphase. This form of cytoplasmic dynein also associates with the cell cortex during division and during directed migration, as well as residing at other sites. These latter roles have suggested an additional feature of dynein function: it may serve not only in transport motor, but also in producing tension along or at the ends of microtubules from fixed cellular sites.

By contrast, cytoplasmic dynein 2 is found almost exclusively within and around the base of cilia and flagella, where it is engaged in retrograde intraflagellar transport (Porter et al., 1999; Pazour et al., 1999; Mikami et al., 2002). This form of motility is required for axonemal maintenance. In addition, cytoplasmic dynein 2 is responsible for transport through modified ciliary structures, such as the connecting cilium of photoreceptors. The transported material is in the form of proteinaceous rafts, which travel along the outer surface of flagellar microtubules. Thus, although this form of dynein is cytoplasmic, as judged by a number of criteria (see below), it coexists with axonemal dyneins in cilia and flagella.

Evolutionary relationship among dyneins

The diverse forms of kinesin and myosin have been judged to comprise clear subfamilies. The definition of the subcategories has been made on the basis of phylogenetic analysis of the motor subunits (HCs), but also on the composition and organization of the nonmotor domains, which are highly distinctive between subfamilies. Differences in physiological and mechanochemical properties have correlated well with family boundaries. By contrast, the organization of both the motor and nonmotor portion of the dynein HCs is remarkably well conserved. Differences in physiological function and subcellular localization have provided the clearest indication of boundaries. Some differences in domain organization have been discerned between dyneins, but these are very limited in scope. For example, the C-terminal (CT) region of the motor domain is found in some, but not all, dynein HCs, although the functional significance of this region is incompletely understood (Vallee and Höök, 2006). Isoforms of the intermediate chain (IC) family bind to some axonemal dyneins and cytoplasmic dynein 1 but not cytoplasmic dynein 2. The full range of IC-binding axonemal dynein forms remains to be explored. The light chains (LCs), which bind to dynein almost exclusively through the ICs as scaffolds, are found in cytoplasmic dynein 1 and some, but not all, axonemal dyneins, as well as non-dynein proteins. The LCs, therefore, like the ICs, have not been of significant value in distinguishing dynein subfamilies. The LICs, however, are associated with cytoplasmic dynein 1 and dynein 2, but, so far, with no known axonemal dyneins. These subunits, therefore, serve as a further means to distinguish the two major dynein subfamilies. Their binding site within their respective dynein HCs is also conserved (Habura et al., 1999; Mikami et al., 2002).

We have carried out a phylogenetic analysis of 51 full-length dynein HCs from 24 diverse organisms by multiple

sequence alignment (using the Clustal W program with default parameters) and constructed a phylogenetic tree (using the Neighbor Joining method). Based on this and existing functional analysis we have labelled subclasses of dynein from I-VII, which is consistent with current kinesin and myosin family nomenclature. The axonemal dyneins appear to have diverged into five subclasses, each of which is represented by a single gene in *Chlamydomonas*: the α inner-arm dynein; the β inner-arm dynein; the γ outer-arm dynein, which is present only in protozoans; the α and β outer-arm dyneins, which branched from a common trunk to form separate α and β dyneins. The phylogenetic origin of one-headed inner-arm dyneins is poorly understood. We included the sequence of *Chlamydomonas* DHC9, or subspecies c, the only one-headed full-length dynein sequence presently available. The alignment assigned subspecies c to the β inner-arm dyneins, suggesting that human DHC7 and DHC3 represent single-headed inner-arm dyneins. Five additional one-headed dyneins have been identified biochemically (Kagami and Kamiya, 1992). Which subclass of inner-arm dynein these represent should be determined as their sequences become available.

Sequence conservation within dynein HC functional domains

Whereas the motor domain displays a high level of sequence conservation, especially among motifs implicated in nucleotide binding and hydrolysis, the sequence within the nonmotor region (traditionally referred to as the 'stem' and more recently as the 'tail') is more divergent, and only a few residues are entirely conserved. As noted above, the LIC binding region within cytoplasmic dynein 1 and cytoplasmic dynein 2 shows evidence of conservation, but can be identified in intraclass comparisons (Mikami et al., 2002). Conserved residues are more readily detected along the ~600-residue portion of the stem proximal to AAA1. This region, referred to as the 'linker', has been recently implicated in force transduction and has been proposed to generate force through its interaction with the AAA ring (Burgess et al., 2003). AAA1 has been deduced to serve as the principal site for ATP hydrolysis, based on the effects of vanadate-mediated UV photocleavage at this site (Gibbons et al., 1987; Gee et al., 1997). More recent studies on the dynein motor domain suggest that hydrolytic activity in AAA1 also requires the structural involvement of AAA2 (Takahashi et al., 2004; Höök et al., 2005). Consequently, more than half of the conserved residues are located within the boundaries of the linker and the first two AAA domains, stressing the region's significance as the source of force production within the dynein motor. Additional clusters of conserved residues are found within AAA3, in particular among motifs that are involved in nucleotide binding and hydrolysis, which is consistent with the role of this ATPase in dynein function (Silvanovich et al., 2003; Kon et al., 2004; Reck-Peterson, 2004).

Sporadic residues along the dynein polypeptide sequence, including the microtubule-binding stalk, have been kept remarkably unchanged throughout dynein evolution. Three of the four entirely conserved residues within the stalk are prolines, which have the unique ability to act as coiled-coil breakers and may in that role function as the boundary between the coiled coil and microtubule-binding elements

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