The molecular anatomy of dynein

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Introduction

Dyneins act as molecular motors using energy derived from the hydrolysis of ATP to translocate towards the minus-end of microtubules. From the initial identification of dynein as an ATPase in *Tetrahymena* cilia and sea urchin sperm flagella, these enzymes and their components are now known or suggested to be involved in such varied processes as mitosis, left–right asymmetry during mammalian development, sperm development, the movement of membranous organelles, rhodopsin trafficking and intraflagellar transport. Classically, dyneins can be divided into three major classes dependent primarily on subcellular localization: these are the dyneins of the inner and outer arms of flagella, and cytoplasmic dynein. All of these enzymes are involved in the generation of force relative to a microtubule (Figure 1). Inner- and outer-arm dyneins generate complex flagellar waveforms by producing differential lateral forces between adjacent microtubules. The outer arms transduce a substantial proportion of the total force, while the inner arms are responsible for the fine control of the waveform. Conversely, cytoplasmic dynein has a much more varied role. It is involved in the movement of organelles and polarity determinants as well as retrograde transport in axons and maintenance of the Golgi apparatus. Cytoplasmic dynein also cross-links

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and positions microtubules of the mitotic spindle. Furthermore, recent studies support a role for cytoplasmic dynein in sperm morphogenesis and intraflagellar transport.

Dyneins are massive multimeric enzymes with molecular masses between 1 and 2 MDa. The multiplicity of known dynein functions is mirrored by a corresponding structural complexity. They are composed of a number of heavy, intermediate and light polypeptide chains, the arrangement of which determines the identity and functioning of the dynein in question. Therefore, to understand how a particular dynein enzyme acts to transport the correct
cargo to the appropriate location, it is essential to understand the underlying structural organization of the motor complex.

**Force generation and microtubule movement**

Dynein-mediated motility is powered by the hydrolysis of ATP. During the mechanochemical cycle, the enzyme binds ATP, hydrolyses it to ADP and phosphate, and subsequently releases the products. Conformational changes in protein structure are intimately associated with each step of the ATPase cycle and allow the motor enzyme to interact transiently with the microtubule to generate force. Following the force-generating step, the dynein must release from the microtubule in order to return to the original conformation and thus allow for the next cycle. In this manner the dynein motor is able to translocate along microtubules at rates of 1–6 μm·s⁻¹ (the velocity depends on the source of dynein, buffer conditions etc.).

**The structure of dynein**

The basic structure of a generic dynein is illustrated in Figure 2. However, it is important to keep in mind that some dyneins diverge significantly from this overall plan. These enzymes are constructed around the massive (≈500 kDa) dynein heavy chains, which form the globular motor domains, and the stems. The number of heavy chains can be from one to three depending on the dynein in question (see below). It is these components that hydrolyse ATP and interact with microtubules to generate force. At the base of the stems are located several intermediate chains that are involved in the attachment of the dynein to its particular cargo. Additional components are also present, including light chains associated with the stems (and in one case the head) of the heavy chains. Two classes of light chain (LC8- and Tctex1-family proteins) are found at the base as part of an intermediate-chain–light-chain complex.

**Specific features of different dynein classes**

**Inner-arm dynein**

In the alga *Chlamydomonas*, the inner flagellar arms (I1, I2 and I3) are arranged into groups of three that repeat every 96 nm. The inner arms are responsible for the initiation and propagation of flagellar bends. Inner arm I1 is located proximal to the first spoke of the radial spoke pair. It contains two heavy chains, 1α and 1β, at least two intermediate chains as well as Tctex1 and LC8. The groups of inner arms termed I2 and I3 are distinct from I1 and consist of single heavy chains associated with several other components, including p28, actin and centrin (there are multiple different members of these inner-arm groups). Moreover, these dyneins have a complex localization, with some being present in the region of the flagellum proximal to the cell body while others are assembled more distally.
Unlike the inner arms, there is only a single type of outer arm within a flagellum. The outer arms contain either two or three different heavy chains depending on source. These each have light chains bound and also interact with an intermediate-chain–light-chain complex at the base. Mutational analysis has revealed that the role of the outer arms is to provide the power for flagellar beating. Lack of these components reduces beat frequency by \( \approx 50\% \) but has little effect on the waveform itself. The outer arm interacts in situ with an additional trimeric structure known as the docking complex. This complex is essential for binding of the outer arm to the axoneme and appears to determine the attachment site.

### Cytoplasmic dynein

Unlike flagellar dyneins, the major cytoplasmic isozyme (known as 1a) contains two apparently identical heavy chains, as well as an intermediate-chain–light-chain complex analogous to that found in the outer arm. This basal structure is involved in attachment to dynactin, which is essentially an adaptor to mediate dynein–vesicle interactions. In addition, this complex also includes four light intermediate chains of \( \approx 53–59 \text{ kDa} \), but it is not yet clear where they are located. Recently, a second class of cytoplasmic dynein (termed 1b) has
been recognized. This enzyme appears to consist of a single heavy chain; no accessory proteins have yet been described.

The heavy chains

Dyneins are constructed around large (>500 kDa) heavy chains that contain the ATPase motor domain of the enzyme. Many organisms express a number of different heavy chains: for example, *Chlamydomonas* has 16 such chains, two cytoplasmic and 14 flagellar-specific (M.E. Porter, personal communication). All of these proteins have a number of common features (Figure 3), including four centrally located nucleotide-binding consensus sequences (P-loops) that are separated by ≈350 amino acid residues [1]. P1, the P-loop (or phosphate-binding loop) nearest to the N-terminus is absolutely conserved in heavy chains from both cytoplasmic and axonemal dynein [1,2], whereas the other three loops are more diverse. Interestingly, the P3 and P4 loops are more highly conserved among cytoplasmic and axonemal dyneins, respectively, although the functional significance of this is unclear. The absolute conservation of the P1 loop between all heavy chains suggests functional importance. Indeed, this loop forms part of the ATP-hydrolysing site of the dynein heavy chain; disrupting the P1 loop leads to a complete loss of ATPase activity. The other P-loops still lack a clearly assigned function, although several lines of evidence suggest that they bind nucleotides and may therefore be involved in dynein regulation [3].

Co-operative interactions between heavy chains also occur. For example, in *Chlamydomonas* outer-arm dynein, the ATPase activity of the β heavy chain is down-regulated by association with the α heavy chain. Furthermore, the enzymic properties of the resulting αβ dimer are modified further by interaction with the γ heavy chain. This suggests that the overall activity of the outer arm, under different conditions, is determined by intercommunication between the heavy chains. Genetic and structural analyses have revealed that the motor domains of the three outer-arm heavy chains are arranged with the γ chain innermost and the α chain to the outside of the axoneme (Figure 4) [4]. The apparent direct contact between heavy chains could allow conformational changes in adjacent subunits to co-ordinate the control of force production. Indeed, analysis of mutations in the β and γ chains, which suppress the paralysis caused by defects in the radial-spoke and central-pair complexes, supports this interpretation. Interestingly, the αβ dimer and the single β and γ heavy chains have different microtubule-binding and -translocation properties [5]. This raises the important question of how these different motor properties can be co-ordinated into a co-operative action leading to a functional power stroke. Other studies have found that the α heavy chain is the only phosphorylated component within the *Chlamydomonas* outer dynein arm. This heavy chain contains at least six phosphorylated sites, most of which are in the head domain. Perhaps importantly, one of these residues is adjacent to the ATP
hydrolysis site while an additional phosphorylated region was identified near to the coiled-coil domain towards the C-terminus (Figure 3). These locations, in concert with the high rates of turnover, suggest that reversible phosphorylation/dephosphorylation has an important regulatory role in dynein function. This hypothesis is supported further by the observation that the rate at which Paramecium dynein translocates microtubules in an assay in vitro is controlled by the cAMP-dependent phosphorylation of a 29 kDa light-chain component [6].

Recent studies have made progress towards the identification of the microtubule-binding site within the heavy chain. Deletion of a well-conserved region C-terminal to the P4 loop abolished microtubule-binding activity and confirmed the importance of this heavy-chain segment in dynein activity [7,8]. High-resolution structural studies of the head domain of Dictyostelium cytoplasmic dynein have visualized seven to eight globular lobes that surround a large central cavity. There is also a stalk domain protruding from the globular domain that is thought to connect the dynein head to its adjacent microtubule in an ATP-sensitive manner and impart lateral force [9,10]. The microtubule-binding C-terminal region downstream of the P4 loop contains conserved sequences predicted to form α-helical coiled-coils. These coil structures may interact in an anti-parallel manner to form the stalk with a small globular (presumably microtubule-binding) unit at the tip. When this region was expressed in vitro it was found to co-sediment with microtubules [8]. The N-terminal region (∼160 kDa) of the heavy chains is much more divergent and may impart additional individuality to the heavy chains by determining which accessory components are able to bind and thus modulate cargo attachment and other regulatory activities.

The intermediate chains

Two specific classes of intermediate chain (∼70–120 kDa) are known at present. Outer-arm dynein from both sea urchin and Chlamydomonas, as well as mammalian cytoplasmic dynein, contain two copies of related intermediate-chain proteins. These polypeptides have five or six repeated segments in the C-
terminal region and are members of the WD-repeat protein family (Figure 5).

The WD-repeat motif is ~40 residues long and the three-dimensional structure of one protein containing these repeats (the G\textsubscript{\beta} subunit of heterotrimeric G-proteins) has been solved [11]. Each repeat provides four \( \beta \) strands that come together to form the blades of a toroidal structure. The N-termini of each intermediate chain are apparently unrelated and thus far each appears to exhibit a distinct function. For example, IC1 (formerly IC78 or IC80) from the \textit{Chlamydomonas} outer arm interacts directly with tubulin \textit{in situ} [12]. Likewise, IC74 of cytoplasmic dynein binds directly to the p150\textit{Glued} component of dynactin (an activator of dynein-mediated vesicular transport) [13]. Electron-microscopic studies of sea urchin and \textit{Chlamydomonas} dyneins have revealed that the intermediate chains are located at the base of the soluble dynein particle. Furthermore, \textit{Chlamydomonas} null

**Figure 4. Structure of the outer dynein arm in situ**

Averaged high-resolution electron micrograph of 16 outer doublet microtubules from \textit{Chlamydomonas} flagellar axonemes. The location of the three outer-arm motor domains (\( \alpha \), \( \beta \) and \( \gamma \)) and the microtubule-binding basal complex are indicated. The inner row of dynein arms (IA) and the radial spokes (RS) are also indicated. Figure provided kindly by Dr. Ritsu Kamiya (University of Tokyo, Tokyo, Japan).
mutants defective for either IC1 or IC2 (formerly IC69 or IC70) fail to incorporate outer dynein arms into the axonemal superstructure, indicating that these components are essential for assembly. Together, these observations have led to the hypothesis that the intermediate chains are involved in the attachment of the dynein motor to its specific cargo.

The flagellar dynein intermediate chains are not post-translationally modified. However, IC74 of rat brain cytoplasmic dynein is encoded by two separate genes and is subject to both alternative splicing and differential phosphorylation. Together, these modifications generate multiple isoforms that are developmentally regulated. This suggests that different intermediate-chain isoforms are required at different times during brain development, possibly in concert with changes in cargoes for retrograde axonal transport and/or other dynein-mediated activities [14].

The light chains

The light chains (<30 kDa) are by far the most structurally and functionally diverse dynein components. There are essentially two classes of light chain: those associated with the intermediate chains at the base of the particle (see below) and those that are bound tightly to individual heavy chains. In Chlamydomonas, this latter group includes two Ca\(^{2+}\)-binding proteins, centrin (associated with inner arms I2 and I3) and LC4 (bound to the outer-arm \(\gamma\) heavy chain). As both phototactic steering (differential regulation of flagellar beat frequency) and the photoshock response (which involves a reversal in swimming direction) are Ca\(^{2+}\)-mediated, it is likely that these light chains are involved in the control of specific motor functions. In the Chlamydomonas outer arm, both \(\alpha\) and \(\beta\) heavy chains interact with members of the thioredoxin superfamily. These polypeptides are redox-active and indeed appear to be functional sulphhydryl oxidoreductases. As mentioned above, one Paramecium light chain is phosphorylated in a cAMP-dependent manner and appears also to control dynein activity in that organism.
The remaining light chains are located at the base of the dynein particle and are more generic in nature as both cytoplasmic and flagellar dyneins contain similar (and in some cases identical) chains. This group includes the highly conserved LC8 protein and several members of the Tctex1 protein family (these polypeptides are discussed in more detail below). Disruption of the genes encoding some of these proteins leads to the failure of the dynein particle to assemble, emphasizing the importance of these components in dynein structure.

**Recent advances**

**LC8 and retrograde intraflagellar transport**

Probably the most fascinating of the dynein light chains is LC8, which was initially identified in *Chlamydomonas* outer-arm dynein [15]. It was subsequently found to be an integral component of mammalian cytoplasmic dynein, inner arm I1 and the unconventional actin motor, myosin V. LC8 has also been identified in yeast two-hybrid screens as physically interacting with a number of additional proteins, including neuronal nitric oxide synthase and IκBα (inhibitor of nuclear factor κB). Indeed, LC8 has been reported to inhibit nitric oxide synthase activity. Intriguingly, LC8 expression is up-regulated in neuronal cells that are at risk from damage by nitric oxide subsequent to cerebral ischaemia. In *Drosophila*, partial loss-of-function mutants in LC8 result in morphogenetic defects and female sterility, whereas total loss-of-function is embryonic lethal. Together, these observations have led to the suggestion that LC8 plays an important regulatory function in a large number of different systems in a manner analogous to calmodulin.

Intraflagellar transport was originally identified in *Chlamydomonas* as the bidirectional movement of raft-like particles along the flagellar microtubules, in a process independent of flagellar beating [16]. The rafts are located just underneath the flagellar membrane and appear to contain materials involved in the maintenance of flagellar integrity. Movement of rafts away from the cell body is powered by a kinesin-like protein that is encoded at the *fla10* locus. Transport of the rafts back to the cell body is predicted to require a minus-end-directed microtubule motor and it has recently been found that the mutant *fla14* lacks this activity [17]. Flagella from the *fla14* mutant are very short, immotile and have many morphological defects. Most importantly they also have large accumulations of the raft-like particles at the tips. *fla14* actually encodes the *Chlamydomonas* LC8 protein, suggesting that retrograde intraflagellar transport is mediated by a cytoplasmic dynein which is disrupted in the mutant.

**Docking complex**

Reconstitution experiments have revealed that for the successful assembly of the outer arm on to dynein-depleted axonemes, an additional particle that
sediments at 7 S is required [18]. This particle was subsequently termed the outer-arm dynein-docking complex and is composed of three polypeptides of $\approx$83, 62.5 and 25 kDa. Axonemes from outer-dynein armless mutants that retain the docking complex have small projections at the correct location on the outer doublets, which therefore may represent the docking particle. Structural analysis of docking-complex components identified extensive coiled-coil regions in both the $\approx$83 and 62.5 kDa polypeptides. The larger protein also contains stretches of highly basic and highly acidic residues that may provide sites for mediating interactions between tubulin of the outer doublets and the basal domain of the outer dynein arm. This flagellar structure appears to be analogous in function to dynactin, which promotes the interaction between cytoplasmic dynein and its vesicular cargo.

**Motility in vitro and control of dynein function**

It has proved possible to assess dynein motor function in two relatively simple assays in vitro. One entails coating microscope coverslips with purified dynein, adding ATP and microtubules, and then directly visualizing microtubule translocation across the coverslip [19]. The second method examines dynein function in situ by performing controlled proteolysis of flagellar axonemes in the presence of ATP. Proteolysis breaks the linkage holding the axonemal doublet microtubules together and allows them to be moved with respect to each other, thus leading to the sliding disintegration of the entire structure.

The various inner and outer dynein arm species cause microtubules to glide with a large range of velocities [20]. This suggests that different dynein components have different functions in flagellar beating; for example, the inner arms exhibiting the faster gliding velocities may be involved in bend initiation. Also, a subset of the inner arms has been observed to generate torque and thus rotate the gliding microtubules. The physiological significance of this activity is unknown, although it may be involved in the formation of complex flagellar waveforms.

The sliding disintegration assay has yielded important clues as to how dynein arms are regulated in vivo by measuring differences in sliding disintegration velocity of axonemes from numerous mutant strains. Dynein inner arms derived from a strain lacking radial spokes translocate microtubules in the sliding disintegration assay at a slower velocity than inner arms from a radial-spoke-containing strain. This indicates that the radial spokes activate the inner arms and that this activated state is maintained even after reconstitution of these active dynein particles into radial-spoke-less axonemes [21].

**Dynein and meiotic drive of the murine t complex**

Recent observations indicate that several dynein light chains are proteins previously cloned as candidates for involvement in meiotic drive exhibited by the murine t haplotypes, which is thought to be caused by defects in
spermiogenesis [22,23]. The t complex encompasses the proximal 30–40 Mb of mouse chromosome 17 (Figure 6). In wild mouse populations variant forms of this region exist (known as t haplotypes), characterized by four large inversions that prevent the haplotype from undergoing recombination with the wild-type t complex. A number of genes and other DNA markers have been located in this region, including the dynein light chains Tctex1 and Tctex2, the hybrid-sterility locus (Hst6) and a presumptive axonemal dynein heavy chain (Dnahc8). On the t haplotype map are the locations of the distorter (Tcd1−4) and responder (Tcr) loci that are mutated in the t haplotype and are responsible for the non-Mendelian transmission of the t haplotype-containing copy of chromosome 17. Modified from Pilder et al. [24] with permission. ©1993, Academic Press.

**Figure 6. The t complex region of mouse chromosome 17**
Genomic organization of the two variant forms of the proximal region of chromosome 17: the upper map shows the wild-type t complex, while the lower map illustrates the rearrangements present in the variant t haplotypes. The large inversions in the t haplotype serve to suppress recombination with the wild-type t complex. A number of genes and other DNA markers have been located in this region, including the dynein light chains Tctex1 and Tctex2, the hybrid-sterility locus (Hst6) and a presumptive axonemal dynein heavy chain (Dnahc8). On the t haplotype map are the locations of the distorter (Tcd1−4) and responder (Tcr) loci that are mutated in the t haplotype and are responsible for the non-Mendelian transmission of the t haplotype-containing copy of chromosome 17. Modified from Pilder et al. [24] with permission. ©1993, Academic Press.

The fascinating feature of this system is that heterozygous (+/t) males pass the t haplotype form of chromosome 17 to essentially all of their progeny in a non-Mendelian process known as transmission ratio distortion or meiotic drive. Genetic analysis has implicated mutations in a series of interacting distorter and responder proteins that lead to defective sperm bearing specifically the wild-type genotype. Therefore, t haplotype-containing sperm have a major competitive advantage during fertilization. Two distorter candidates have now been identified as dynein light chains. Tctex1 is a component of inner arm I1 from *Chlamydomonas* flagella, outer-arm dynein from sea urchin sperm and rat brain cytoplasmic dynein. The *Chlamydomonas* outer arm also contains a light chain homologous to a second candidate distorter Tctex2. Importantly, cytoplasmic dynein containing Tctex1 is present in many tissues but the phenotype due to the t haplotype-encoded mutations is observed only in the male germ cells. This suggests that these mutations specifically affect the activity of Tctex1 and Tctex2 in axonemal dyneins, which could lead directly to flagellar dysfunction. This has been the first indication that alterations in flagellar dynein light chains can result in aberrant sperm motility and consequently profoundly affect male fertility. Clearly much remains to be learnt about this intriguing phenomenon, including the molecular identity of the responder, which is thought to be responsible for differential distribution of wild-type and t mutant dyneins during spermiogenesis.
Summary

• Recent molecular, genetic and functional studies have led to an unparalleled growth in our understanding of dynein and the roles played by the various polypeptides of these massive macromolecular assemblies.

• Dyneins are highly complex 1–2MDa complexes that function as molecular motors and move the cargo to which they are attached towards the minus-end of a microtubule.

• Dynein motor function is a property of the heavy chains, whereas the intermediate chains are involved in attachment to the appropriate cargo.

• In order for useful work to be obtained, motor and cargo-binding activities must be tightly controlled. Current data suggest that this is the role played by certain accessory light-chain proteins.

• The LC8 is highly conserved and found in many enzyme systems. This protein is essential in multicellular organisms.

• The dynein light chains Tctex1 and Tctex2 have been implicated in the non-Mendelian transmission of variant forms of mouse chromosome 17.

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