Published in final edited form as:

J Struct Biol. 2012 August ; 179(2): 222–228. doi:10.1016/j.jsb.2012.02.013.

Integrated Control of Axonemal Dynein AAA+ Motors

Stephen M. King

Department of Molecular, Microbial and Structural Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, Connecticut 06030-3305 USA, Tel: (860) 679 3347, Fax: (860) 679 3408

Stephen M. King: king@neuron.uchc.edu

Abstract

Axonemal dyneins are AAA⁺ enzymes that convert ATP hydrolysis to mechanical work. This leads to the sliding of doublet microtubules with respect to each other and ultimately the generation of ciliary/flagellar beating. However, in order for useful work to be generated, the action of individual dynein motors must be precisely controlled. In addition, cells modulate the motility of these organelles through a variety of second messenger systems and these signals too must be integrated by the dynein motors to yield an appropriate output. This review describes the current status of efforts to understand dynein control mechanisms and their connectivity focusing mainly on studies of the outer dynein arm from axonemes of the unicellular biflagellate green alga *Chlamydomonas*.

Keywords

AAA⁺ domain; Cilia; Dynein; Flagella; Microtubule

Introduction

The ciliary axoneme contains a complex array of AAA⁺ motors that form the inner and outer rows of dyneins arms within these motile organelles. The outer arm dyneins contain either two or three (depending on organism of origin) distinct heavy chain motors that are associated with a large array of additional components that serve to stabilize the enzyme, act as adaptors to attach it to the correct axonemal location and transmit regulatory signals in response to alterations in environmental conditions. Inner arm dyneins fall into two distinct classes: a HC dimer that is required to define waveform and a series of monomeric motors that play a variety of roles including a response to increased viscous load [see (King and Kamiya, 2009) and various chapters in (King, 2012)].

In order to generate and propagate a ciliary waveform, dynein activity must be carefully coordinated such that a wave of activity is passed along the structure. In addition, to form a bend, dyneins on certain doublet microtubules must be active whereas those on other doublets are inactive; the doublets undergoing active sliding must also change in order to generate forward and reverse bends. This complex switching pattern must be controlled at the same rate as ciliary beat frequency which can exceed 60 Hz, and also, on a slower time

© 2012 Elsevier Inc. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

scale, respond to second messenger signaling (through increased Ca^{2+} levels for example) to alter the generated waveform. Reactivation of isolated axonemes and cell models has revealed that all the components necessary for generating and propagating a beat (Gibbons and Gibbons, 1972) and for altering the waveform in response to Ca^{2+} are integrated within the axonemal superstructure (Hyams and Borisy, 1978; Bessen *et al.*, 1980). This in turn lead to the hypothesis that coordination of dynein activity within the cilium must include a mechanical feedback system that in essence monitors the bending state along the axonemal long axis and allows dynein motors to fire at appropriate time points. In the green alga *Chlamydomonas*, direct experimental evidence in support of this concept came from experiments where immotile mutant flagella were induced to beat for one or more cycles by direct mechanical activation with a microneedle or by holding the cell in a micropipette and moving it rapidly backwards (Hayashibe *et al.*, 1997). Analysis of various mutants lacking combinations of inner and outer arms, radial spokes and/or the central pair microtubule complex suggested that axonemes contain two mechano-sensitive systems that are distinct at the molecular level. One system integrates signals from the central pair microtubule complex and passes them through the radial spokes, and perhaps the dynein regulatory complex, to the inner arm system. The second mechanosensory system appears to independently control outer arm function. It is important to note though that although these systems appear quite distinct their action must be coordinated either through direct interactions or *via* mechanical coupling such that a functional waveform is generated.

In addition, to changes in intraciliary Ca^{2+} levels, axonemal dyneins also respond to other regulatory pathways including direct phosphorylation/dephosphorylation by kinases/phosphatases that are integral components of the axoneme (Barkalow *et al.*, 1994; Howard *et al.*, 1994; Habermacher and Sale, 1996) and to changes in redox state (Wakabayashi and King, 2006) that appear to be detected by specialized thioredoxins associated with the outer dynein arm (Patel-King *et al.*, 1996; Harrison *et al.*, 2002). In some organisms, such as *Chlamydomonas*, these thioredoxins bind directly to individual HCs whereas in metazoans (*e.g.* sea urchins and mammals) the thioredoxin is included as part of a modular protein that also contains two or more nucleoside diphosphate kinase catalytic domains (Ogawa *et al.*, 1996; Padma *et al.*, 2001). This latter feature of course raises the possibility that certain dyneins might respond to nucleotides other than ATP although there is as yet no direct *in vivo* evidence for this hypothesis.

Although it is now clear that axonemal dyneins monitor multiple external cues, how these various signals are integrated at the level of individual dynein AAA⁺ motors to yield a coordinated response which results in defined changes in beat frequency and waveform is much less clear. In this review, I describe some recent advances that provide some structural insight into how multiple signals might impinge on single dynein motor units to yield a graduated response in motor output.

Axonemal Organization of Dynein Motors

Axonemes are built from a repeated 96 nm arrangement of components attached to the outer doublet microtubules (Goodenough and Heuser, 1985; Mastronarde *et al.*, 1992; Nicastro *et al.*, 2006; Ishikawa *et al.*, 2007). This repeat unit includes the radial spokes that transmit information from the central pair microtubule complex, the inner and outer rows of dynein arms and associated dynein regulatory complex. Each repeat unit consists of four outer arm dyneins that appear identical in composition although one actually is physically attached to the dimeric inner arm I1/f and another to the dynein regulatory complex. Thus, at least two of the outer arms must be modified in some manner. Tomographic reconstructions of axonemes from electron micrographs of frozen hydrated samples have clearly demonstrated that the inner arm repeat system consists of one dimeric I1/f dynein followed by six

monomeric HC motors (Nicastro *et al.*, 2006; Ishikawa *et al.*, 2007; Bui *et al.*, 2008). Both outer arm and inner arm I1/f HCs have the plane of their AAA⁺ ring domains arranged orthogonally with respect to the axonemal long axis. However, in some of the monomeric inner arms these HCs domains are arrayed at varying angles suggesting that their powerstrokes may generate an off-axis force component; this may be responsible for providing the torque necessary to yield the rotation of microtubules observed in *in vitro* gliding assays (Kagami and Kamiya, 1992).

Compositional Complexity of Axonemal Dyneins

In *Chlamydomonas*, the simplest axonemal dyneins are the monomeric species (termed a, b, c, d, e, and g) within the inner arm system (Kagami and Kamiya, 1992). In addition to a HC motor, these dyneins all contain a single molecule of actin and either the Ca²⁺-binding protein centrin or a dimer of the p28 light chain (LC) (Piperno and Luck, 1981); dynein d contains two additional components that are apparently unique to this motor (Yamamoto *et al.*, 2006; Yamamoto *et al.*, 2008).

Inner arm dynein I1/f and the outer arm consist of two or three (for the outer arms of certain bikonts such as *Chlamydomonas* and *Tetrahymena*) distinct HCs that are associated with a complex array of additional components (Bell *et al.*, 1979; Piperno and Luck, 1979; Pfister *et al.*, 1982). These include at least two WD-repeat intermediate chains (ICs), dimers of several LCs of the LC8/DYNLL, Tctex1/DYNLT and LC7/DYNLRB families, and an apparently monomeric Tctex2-related protein. Beyond this core, additional components specific for individual dyneins provide for further functional diversity including the response to various signaling ligands (see (King and Kamiya, 2009) for review). The composition and organization of *Chlamydomonas* outer arm dynein is illustrated in Figure 1.

There are also several axonemal dynein species present in very low amount in the axoneme whose composition has yet to be completely defined (Yagi *et al.*, 2009). These dyneins localize to the region of the axoneme proximal to the cell body and it is possible that these motors are specifically involved in the initiation of ciliary bending.

Subdomain Structure of Dynein Heavy Chains

Dynein HC motors are very large proteins (~530 kDa) consisting of multiple subdomains including AAA⁺ domains that form the core of the motor. Current phylogenetic analyses propose that the dynein motor and the ribosomal chaperone midasin, the only other known member of the AAA⁺ superfamily that consists of six tandemly arranged dissimilar AAA⁺ modules, derived from a common ancestor at a very early stage in eukaryotic evolution (Garbarino and Gibbons, 2002). Indeed, it now seems clear that the entire axonemal and cytoplasmic dynein repertoire was already in place prior to divergence from the last common eukaryotic ancestor and that the observed variation in the HC content of extant organisms is due to secondary loss and/or duplication (see (Wickstead and Gull, 2012) for a detailed discussion of dynein evolution).

Our current understanding of dynein HC structure has come from detailed electron microscopic analysis of several different dyneins (Samsó and Koonce, 2004; Nicastro *et al.*, 2006; Ishikawa *et al.*, 2007; Roberts *et al.*, 2009) and more recently from crystal structures of the yeast cytoplasmic dynein motor domain (Carter *et al.*, 2008; Carter *et al.*, 2011; Kon *et al.*, 2011). In general, dynein HCs consist of an N-terminal subdomain (~1800 residues) that provides numerous protein-protein interaction interfaces which mediate association with multiple components of each dynein complex. In addition, this subdomain contains the segment (now termed “the linker”) proposed to undergo a large conformational change in response to ATP hydrolysis that appears to be a key feature of the mechanochemical

mechanism. The N-terminal domain terminates at the first of six tandemly repeated AAA⁺ domains that comprise most of the motor unit. Unlike most other AAA⁺ proteins, these modules are each distinct at the sequence level and exhibit different nucleotide binding and hydrolytic properties. Furthermore, there are some highly conserved sequence differences between canonical cytoplasmic dynein HCs and their axonemal counterparts although the functional significance of these alterations has not yet been completely defined. An anti-parallel coiled coil region ~10 nm in length with a terminal globular domain that binds microtubules in an ATP-dependent manner emanates from the AAA4 domain, and a second coiled coil protrudes from AAA5 to interact with it. In nearly all HCs there is also an additional subdomain C-terminal of AAA6 that traverses the plane of the AAA ring and may impart autoregulatory activity (Hook *et al.*, 2005).

Key Dynein Regulatory Systems

A common theme in the response of axonemal dyneins to alterations in signaling ligands (i.e. Ca²⁺ and redox substrates) is that the signals themselves are either detected by HC-associated components that interact directly with the N-terminal region of the HC. Other signals may be detected by the motor domains or by components of the IC/LC complex. In this latter case, the signals are presumably transmitted through the HC N-terminal regions to the motor.

Ca²⁺

In *Chlamydomonas* dyneins, Ca²⁺ signals are detected by centrin (in monomeric inner arms b, e and g) and LC4 and DC3 (within outer arms). Although there is currently no information on the consequences of ligand binding by centrin, it exhibits a high affinity for Ca²⁺ and might be involved in the differential modulation of beat frequency of the two flagella in response to submicromolar changes in Ca²⁺ levels. In contrast, it has been demonstrated that the outer arm γ HC N-terminal domain undergoes a profound structural reorganization when LC4 (a calmodulin homologue) binds its ligand (Sakato *et al.*, 2007). Specifically, this LC releases one of two HC-binding sites and instead now interacts with IC1 at the base of the particle. This transition will likely result in a change in motor output, by limiting this dynein HC to an ATP-dependent interaction with the adjacent microtubule without a concomitant translocation of the microtubule; *i.e.* the motor is predicted to act as an ATP-dependent brake to limit sliding generated by other motors and lead to conversion of the waveform from an asymmetric to a symmetric pattern (King, 2010). This hypothesis is consistent with the observation that mutants lacking outer arms undergo flagellar quiescence as Ca²⁺ increases but not waveform conversion (Kamiya and Okamoto, 1985). Although the outer arm docking complex protein DC3 binds Ca²⁺, disrupting this interaction by mutating the EF hand did not yield any obvious phenotype (Casey *et al.*, 2003a; Casey *et al.*, 2003b). Intriguingly, unlike LC4 (King and Patel-King, 1995), DC3 also binds Mg²⁺ with low affinity, and this may explain the requirement for Mg²⁺ to keep the outer dynein arm intact during biochemical purifications.

Redox State

Axonemal dynein activity is also controlled by the redox state of the cell and, in *Chlamydomonas*, this leads to the modulation of beat frequency, altered duration of the Ca²⁺-signaled photophobic response which involves the transition from an asymmetric to a symmetric waveform and a change in the sign of the phototactic response (Wakabayashi and King, 2006; Wakabayashi *et al.*, 2011). In outer arm dynein this parameter is sensed by two redox-active thioredoxins associated with the N-terminal regions of the α (LC5) and β/γ (LC3) HCs (Patel-King *et al.*, 1996; Harrison *et al.*, 2002). As one EF hand within DC3 is also redox-sensitive, this protein might act to integrate both redox and Ca²⁺ signals (Casey

et al., 2003b). These proteins all transiently bind a series of flagellar matrix components in a redox-sensitive manner (Wakabayashi and King, 2006).

Although the precise mechanism for transduction of redox signals remains uncertain, it is clear that these LCs form transient mixed disulfides with other flagellar components and that the binding partner(s) change depending on the redox state. When assessed *in vitro* by reactivating cell models in various GSH/GSSG glutathione buffers, lack of the α HC/LC5 thioredoxin unit was found to decrease beat frequency under reducing conditions but to allow for greater than wildtype beat frequency in an oxidizing environment (Wakabayashi and King, 2006). As these cell models lack the soluble redox-sensitive binding partners from the flagellar matrix, this result demonstrates that the thioredoxins themselves (and presumably the state of their vicinal dithiols) have a direct effect on the power output of dynein motors. Furthermore, analysis of the ATPase activity of isolated axonemes and purified dyneins from a variety of mutants and model systems has revealed a two phase response to sulfhydryl oxidizing reagents such as dithionitrobenzoic acid (DTNB), N-ethylmaleimide and p-mecuribenzoate (Shimizu and Kimura, 1974; Gibbons and Fronk, 1979; Harrison *et al.*, 2002). At low oxidant concentrations, there was an enhancement of ATPase that was due to activation of the outer dynein arm ATPase whereas at higher concentrations both outer and inner ATPase activities were completely inhibited. Thus, the negative effect at high oxidant concentration appears to be a generic response of all dynein HCs, whereas the ATPase enhancement at low oxidant levels was specific for the outer arm. Genetic/biochemical dissection of the redox activation effect, using *Chlamydomonas* mutants lacking outer arms and purified dynein HC subunits, revealed that it was a direct result of activating the ATPase of only the γ HC (Harrison *et al.*, 2002).

As mentioned above, the thioredoxin modules in metazoan dyneins are not single LCs but rather part of a modular protein that also contains several nucleoside diphosphate kinase catalytic units. This raises the possibility that there is an intimate connection between dynein's response to changes in redox state and the generation/utilization of nucleotides other than ATP. Although some dyneins have been demonstrated to hydrolyze other nucleotides [*e.g.* CTP by mammalian cytoplasmic dynein (Shpetner *et al.*, 1988) and GTP/ITP by axonemal dyneins (Watanabe and Flavin, 1976; Piperno and Luck, 1979)], the current evidence suggests that this cannot be mechanochemically coupled to power motility; however, as dynein contains four functional dissimilar nucleotide binding sites (Mocz and Gibbons, 1996) one caveat here is that the effects of various ATP/NTP combinations have yet to be reported. An extra complexity arises in *Chlamydomonas*, where in addition to the NDK found in all motile axonemes, there is a second NDK module within the radial spokes that is controlled in a Ca^{2+} -dependent manner through Ca^{2+} /calmodulin binding to a series of C-terminal IQ motifs (Patel-King *et al.*, 2004). This leaves the fascinating question of whether nucleotides generated by NDKase activity help propagate redox signals in metazoans but have been co-opted into the Ca^{2+} control mechanism in *Chlamydomonas*.

Phosphorylation

Multiple axonemal dynein HCs in the inner and outer arms are phosphorylated and this presumably exerts control in some manner (Piperno and Luck, 1981; King and Witman, 1994). However, there remains no clear experimental test of this and the enzymes responsible for these modifications have not yet been identified. In contrast, phosphorylation of the *Chlamydomonas* IC138 intermediate chain within inner arm II/f has been clearly demonstrated to occur in response to signals from the radial spokes and to yield an alteration in motor output that is important for the phototactic response where the two flagella beat with different frequencies (Habermacher and Sale, 1997; King and Dutcher, 1997). Both the kinases and phosphatases responsible for this reversible modification (Howard *et al.*, 1994; Yang *et al.*, 2000; Yang and Sale, 2000) are integrated within the axonemal superstructure

through specific anchoring proteins (Gaillard *et al.*, 2001). These modifying enzymes (casein kinase and 1 and protein phosphatases 2A) directly control the rate of microtubule sliding powered by axonemal dyneins (Gokhale *et al.*, 2009 ; Elam *et al.*, 2011). Similarly, there is evidence that cAMP- and/or Ca²⁺-dependent phosphorylation of the p29 light chain within *Paramecium* outer arm dynein is directly linked to the control of motor output (Hamasaki *et al.*, 1991; Barkalow *et al.*, 1994).

Mechano-sensors

There are also two outer arm dynein components (LC1/DNAL1 and Lis1) that associate with the motor domains of the HCs rather than the N-terminal regions (Benashski *et al.*, 1999; Pedersen *et al.*, 2007; McKenney *et al.*, 2010). LC1 contains six leucine-rich repeats that form an elongated barrel and a C-terminal helical domain (Wu *et al.*, 2000). This protein interacts with the innermost HC within the outer arm (γ in *Chlamydomonas*) and also with tubulin located within the A-tubule of the outer doublets. In humans, a point mutation within the last LRR results in an outer arm assembly defect and consequent primary ciliary dyskinesia (Mazor *et al.*, 2011); this mutation disrupts the core packing resulting in a lowered melting temperature leading to aggregation under physiological conditions (King and Patel-King, 2012). RNAi-based knockdowns of LC1 in *Trypanosoma* (Baron *et al.*, 2007) and *Schmidtea* (Rompolas *et al.*, 2010) lead to dramatic defects in motility and in *Schmidtea* to the loss of metachronal synchrony of the ventral cilia. Although no LC1 mutants have been described in *Chlamydomonas*, a series of point mutants designed from structural data yielded dominant negative effects when expressed in a wildtype background (Patel-King and King, 2009); similarly robust phenotypes were observed when point mutants were expressed in *Trypanosoma* (Ralston *et al.*, 2011). Based on these phenotypes, it has been proposed that LC1 represents part of the conformational switch that controls outer arm activity in response to alterations in axonemal curvature (Patel-King and King, 2009). Evidence for such an outer arm-specific system came from experiments where flagella of paralyzed *Chlamydomonas* mutants were induced to beat by mechanical activation; these experiments also revealed that the inner arms respond to mechanical cues derived from the central pair microtubule apparatus and transmitted through the radial spokes (Hayashibe *et al.*, 1997).

The Lissencephaly Protein (Lis1)

For cytoplasmic dynein, the proteins Lis1 and Nde1/Ndel1 have been demonstrated to act as key regulators necessary for the motor to transport particles and function under high load (McKenney *et al.*, 2010). In this system, association of the Lis1 dimer with the motor domain is thought to induce a persistent tight binding state to the microtubule (McKenney *et al.*, 2010). Intriguingly, Lis1 is also present in motile mammalian cilia and a monomeric *Chlamydomonas* homologue has been found to associate with the outer dynein arm (Pedersen *et al.*, 2007). Indeed, recent data suggests that Lis1 transiently associates with outer arm dynein and that levels in the flagellum are controlled in response to imposed changes in flagellar beat parameters (Rompolas and King, in preparation). One might predict, based on the cytoplasmic dynein studies, that this would enhance ciliary/flagellar stiffness under low beat frequency condition and perhaps reflects an adaption to high viscosity environments. This would be especially beneficial when ciliated epithelia are utilized to transport viscous fluids such as mucus in the lung. NudC is an additional component of the cytoplasmic dynein regulatory pathway originally identified in *Aspergillus* (Xiang *et al.*, 1994), and *Chlamydomonas* Lis1 has been observed to interact directly with the mammalian form of this protein (Pedersen *et al.*, 2007). Furthermore, NudC is highly enriched in ciliated epithelia (Gocke *et al.*, 2000) suggesting that it plays a role in Lis1-mediated control of axonemal motility. In contrast, there is no evidence for Nde1/Ndel1 in cilia and indeed orthologues of these proteins are not encoded within the *Chlamydomonas*

genome (Merchant *et al.*, 2007) suggesting that their role is restricted to the canonical cytoplasmic dynein system.

Inter- and Intra-dynein Interactions

In addition to the systems described above, there is also evidence that the IC/LC complex influences motor function. This complex which interacts with the HC N-terminal domains is involved in attachment of the dynein to its correct location within the axonemal superstructure. In general, it consists of two IC proteins containing C-terminal WD-repeat β -propellers and multiple dimeric LCs that stabilize the IC N-terminal regions. It has been clearly demonstrated in many organisms including humans that lack of the entire IC/LC complex due for example to mutations within one of the ICs leads to the complete failure of arm assembly. Removing several of the LCs also leads to assembly defects [*e.g.* lack of LC8 in the *fla14* mutant (Pazour *et al.*, 1998) or less dramatically lack of LC7a in *oda15* (DiBella *et al.*, 2004)]. However, in several cases defective LC assembly can lead to more subtle motility defects without compromising the core assembly process. For example, the *oda6-r88* mutation is an intragenic revertant within the IC2 gene that leads to alteration of a short region near the N-terminus (Mitchell and Kang, 1993). Further analysis revealed that this strain in fact lacks three components of the IC/LC complex (namely LC2, LC6 and LC9) (DiBella *et al.*, 2005). The *oda6-r88* strain has reduced flagellar beat frequency whereas a second intragenic revertant (*oda6-r75*) that retains all the LCs appears essentially wildtype. The *oda6-r88* phenotype is likely due to the loss of either LC2 (a Tctex2-related protein) or LC9 (a Tctex1) as the LC6 protein, which appears to be *Chlamydomonas*-specific, is completely absent in *oda13* which has wildtype beat frequency (DiBella *et al.*, 2005). Although the *oda12-1* strain which has a partial assembly defect and reduced beat frequency was originally described as lacking LC2 (Pazour *et al.*, 1999) it was later found to also be a complete null for LC10 (Tanner *et al.*, 2008) and thus to exhibit a synthetic phenotype due to the loss of both outer arm components.

Genomic analyses have revealed some organisms completely lack either outer or inner arm dynein genes and yet can assemble motile axonemes. For example, the diatom *Thalassiosira* lacks inner arms, radial spokes and the central pair complex whereas the bryophyte *Physcomitrella* does not encode outer arm dynein components. As both organisms make motile flagellated gametes, neither the outer nor inner arm systems are absolutely required for axonemal motility (Merchant *et al.*, 2007; Ginger *et al.*, 2008). However, in most organisms both systems are present and in these cases there must be some communication between the rows of motors in order to coordinate motility. Insights into a potential mechanism came from studies of the ODA7 protein in *Chlamydomonas* which is present in the flagellum and thought to link the outer and inner dynein arms directly (Freshour *et al.*, 2007).

Finally, *in situ* the AAA⁺ rings of the dynein HCs are stacked against each other (Lupetti *et al.*, 2005; Nicastro *et al.*, 2006; Ishikawa *et al.*, 2007; Oda *et al.*, 2007). As the N-terminal linker regions traverse one face of each ring and the C-terminal domain is abutted against the opposite face (Carter *et al.*, 2011; Kon *et al.*, 2011), it seems probable that these two regions from different HCs (rather than the AAA⁺ domains themselves) actually interact directly. However, there has not been a direct demonstration of this nor of its potential consequences.

Integrating Dynein Signaling Pathways – The New Challenge

Although much has been learned about the individual components of axonemal dyneins and their interactions with various signaling ligands, our understanding of the mechanistic and structural consequences of those interactions is much less developed. Our current

understanding of the signaling pathways within in outer arm dynein is illustrated in Figure 2. It has now become clear that different HCs within the complex respond to distinct signaling modules and their effects must be propagated through the structure to result in a coordinated response. For example, alterations in redox state are known to impinge directly on the thioredoxin-related LCs within *Chlamydomonas* outer arm dynein and to alter their interactions with currently unidentified redox-sensitive substrates. However, how those interactions modulate motor function and indeed whether the thioredoxins also form transient disulfides with the HCs remains completely obscure. Similarly, inner arm I1/f is clearly controlled through phosphorylation of the IC138 component and this requires the 1 β HC motor domain (Toba *et al.*, 2011), but what those modifications actually do to alter motor activity is uncertain. As detailed above there is some information on the consequences of Ca²⁺ binding to a LC that leads to conformational changes within the N-terminal region of its associated HC and also to a new interaction with the IC/LC complex that is not observed in the absence of ligand. When combined with tethering of the AAA ring of this same HC by the LC1 protein, the possibility emerges that Ca²⁺ binding converts this HC from an active motor that can undergo a standard mechanochemical cycle to generate force, to a state where ATP hydrolysis and motion are uncoupled such that it can only interact with the B-tubule in an ATP-dependent manner. This hypothesis predicts that in high Ca²⁺ this HC acts as an ATP-dependent brake that in effect limits active inter-doublet microtubule sliding driven by other HCs (King, 2010). This same HC also responds to redox state and is phosphorylated, but how those modifications affect the mechanical and Ca²⁺ signaling mechanisms remains unknown.

In conclusion, we now have considerable information about regulatory systems that are involved in the control of axonemal beating. However, when one considers all the regulatory systems that impinge on the same dynein complex potentially at the same time (*e.g.* Ca²⁺, redox signals, phosphorylation, mechano-sensing, Lis1 on the outer arm), it becomes clear that we are just at the beginning in our efforts to understand the biochemical basis for control of dynein-driven ciliary/flagellar motility.

Acknowledgments

My laboratory is supported by grant GM051293 from the National Institutes of Health.

Abbreviations used

HC	heavy chain
LC	light chain
IC	intermediate chain

References

- Barkalow K, Hamasaki T, Satir P. Regulation of 22S dynein by a 29-kD light chain. *J Cell Biol.* 1994; 126:727–735. [PubMed: 8045936]
- Baron DM, Kabututu ZP, Hill KL. Stuck in reverse: loss of LC1 in *Trypanosoma brucei* disrupts outer dynein arms and leads to reverse flagellar beat and backward movement. *J Cell Sci.* 2007; 120:1513–1520. [PubMed: 17405810]
- Bell CW, Fronk E, Gibbons IR. Polypeptide subunits of dynein 1 from sea urchin sperm flagella. *J Supramol Struct.* 1979; 11:311–317. [PubMed: 161791]
- Benashski SE, Patel-King RS, King SM. Light chain 1 from the *Chlamydomonas* outer dynein arm is a leucine-rich repeat protein associated with the motor domain of the γ heavy chain. *Biochemistry.* 1999; 38:7253–7264. [PubMed: 10353837]

- Bessen M, Fay RB, Witman GB. Calcium control of waveform in isolated flagellar axonemes of *Chlamydomonas*. *J Cell Biol.* 1980; 86:446–455. [PubMed: 6447155]
- Bui KH, Sakakibara H, Movassagh T, Oiwa K, Ishikawa T. Molecular architecture of inner dynein arms *in situ* in *Chlamydomonas reinhardtii* flagella. *J Cell Biol.* 2008; 183:923–932. [PubMed: 19029338]
- Carter AP, Cho C, Jin L, Vale R. Crystal structure of the dynein motor domain. *Science.* 2011; 331:1159–1165. [PubMed: 21330489]
- Carter AP, Garbarino JE, Wilson-Kubalek EM, Shipley WE, Cho C, Milligan RA, Vale RD, Gibbons IR. Structure and functional role of dynein's microtubule-binding domain. *Science.* 2008; 322:1691–1695. [PubMed: 19074350]
- Casey D, Inaba K, Pazour G, Takada S, Wakabayashi K, Wilkerson C, Kamiya R, Witman G. DC3, the 21-kD subunit of the outer dynein arm-docking complex (ODA-DC), is a novel EF-hand protein important for assembly of both the outer arm and the ODA-DC. *Mol Biol Cell.* 2003a; 14:3650–3663. [PubMed: 12972554]
- Casey D, Yagi T, Kamiya R, Witman G. DC3, the smallest subunit of the *Chlamydomonas* flagellar outer dynein arm-docking complex, is a redox-sensitive calcium-binding protein. *J Biol Chem.* 2003b; 278:42652–42659. [PubMed: 12920131]
- DiBella LM, Gorbatyuk O, Sakato M, Wakabayashi K, Patel-King RS, Pazour GJ, Witman GB, King SM. Differential light chain assembly influences outer arm dynein motor function. *Mol Biol Cell.* 2005; 16:5661–5674. [PubMed: 16195342]
- DiBella LM, Sakato M, Patel-King RS, Pazour GJ, King SM. The LC7 light chains of *Chlamydomonas* flagellar dyneins interact with components required for both motor assembly and regulation. *Mol Biol Cell.* 2004; 15:4633–4646. [PubMed: 15304520]
- Elam CA, Wirschell M, Yamamoto R, Fox LA, York K, Kamiya R, Dutcher SK, Sale WS. An axonemal PP2A B-subunit is required for PP2A localization and flagellar motility. *Cytoskeleton.* 2011; 68:363–372. [PubMed: 21692192]
- Freshour J, Yokoyama R, Mitchell DR. *Chlamydomonas* flagellar outer row dynein assembly protein Oda7 interacts with both outer row and I1 inner row dyneins. *J Biol Chem.* 2007; 282:5404–5412. [PubMed: 17194703]
- Gaillard A, Diener D, Rosenbaum J, Sale W. Flagellar radial spoke protein 3 is an A-kinase anchoring protein (AKAP). *J Cell Biol.* 2001; 153:443–448. [PubMed: 11309423]
- Garbarino J, Gibbons I. Expression and genomic analysis of midasin, a novel and highly conserved AAA protein distantly related to dynein. *BMC Genomics.* 2002; 3:18. [PubMed: 12102729]
- Gibbons BH, Gibbons IR. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. *J Cell Biol.* 1972; 54:75–97. [PubMed: 4261039]
- Gibbons IR, Fronk E. A latent adenosine triphosphatase form of dynein 1 from sea urchin sperm flagella. *J Biol Chem.* 1979; 254:187–196. [PubMed: 214440]
- Ginger ML, Portman N, McKean PG. Swimming with protists: perception, motility and flagellum assembly. 2008; 6:838–850.
- Gocke C, Osmani S, Miller B. The human homologue of *Aspergillus* nuclear migration gene *nudC* is preferentially expressed in dividing cells and ciliated epithelia. *Histochem Cell Biol.* 2000; 114:293–301. [PubMed: 11131094]
- Gokhale A, Wirschell M, Sale WS. Regulation of dynein-driven microtubule sliding by the axonemal protein kinase CK1 in *Chlamydomonas* flagella. *J Cell Biol.* 2009; 186:817–824. [PubMed: 19752022]
- Goodenough UW, Heuser JE. Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. *J Cell Biol.* 1985; 100:2008–2018. [PubMed: 2860115]
- Habermacher G, Sale WS. Regulation of flagellar dynein by an axonemal type-1 phosphatase in *Chlamydomonas*. *J Cell Sci.* 1996; 109:1899–1907. [PubMed: 8832412]
- Habermacher G, Sale WS. Regulation of flagellar dynein by phosphorylation of a 138-kD inner arm dynein intermediate chain. *J Cell Biol.* 1997; 136:167–176. [PubMed: 9008711]
- Hamasaki T, Barkalow K, Richmond J, Satir P. cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22S dynein arm regulates microtubule translocation velocity

- and swimming speed in *Paramecium*. Proc Natl Acad Sci USA. 1991; 88:7918–7922. [PubMed: 1654550]
- Harrison A, Sakato M, Tedford HW, Benashski SE, Patel-King RS, King SM. Redox-based control of the γ heavy chain ATPase from *Chlamydomonas* outer arm dynein. Cell Motil Cytoskeleton. 2002; 52:131–143. [PubMed: 12112141]
- Hayashibe K, Shingyoji C, Kamiya R. Induction of temporary beating in paralyzed flagella of *Chlamydomonas* mutants by application of external force. Cell Motil Cytoskeleton. 1997; 37:232–239. [PubMed: 9227853]
- Hook P, Mikami A, Shafer B, Chait BT, Rosenfeld SS, Vallee RB. Long range allosteric control of cytoplasmic dynein ATPase activity by the stalk and C-terminal domains. J Biol Chem. 2005; 280:33045–33054. [PubMed: 16030013]
- Howard DR, Habermacher G, Glass DB, Smith EF, Sale WS. Regulation of *Chlamydomonas* flagellar dynein by an axonemal protein kinase. J Cell Biol. 1994; 127:1683–1692. [PubMed: 7798320]
- Hyams J, Borisov G. Isolated flagellar apparatus of *Chlamydomonas*: characterization of forward swimming and alteration of waveform and reversal of motion by calcium ions *in vitro*. J Cell Sci. 1978; 33:235–253. [PubMed: 31367]
- Ishikawa T, Sakakibara H, Oiwa K. The architecture of outer dynein arms *in situ*. J Mol Biol. 2007; 368:1249–1258. [PubMed: 17391698]
- Kagami O, Kamiya R. Translocation and rotation of microtubules caused by multiple species of *Chlamydomonas* inner-arm dynein. J Cell Sci. 1992; 103:653–664.
- Kamiya R, Okamoto M. A mutant of *Chlamydomonas reinhardtii* that lacks the flagellar outer dynein arm but can swim. J Cell Sci. 1985; 74:181–191. [PubMed: 4030906]
- King SJ, Dutcher SK. Phosphoregulation of an inner dynein arm complex in *Chlamydomonas reinhardtii* is altered in phototactic mutant strains. J Cell Biol. 1997; 136:177–191. [PubMed: 9008712]
- King SM. Sensing the mechanical state of the axoneme and integration of Ca^{2+} signaling by outer arm dynein. Cytoskeleton. 2010; 67:207–213. [PubMed: 20186692]
- King, SM., editor. Dyneins: Structure, Biology and Disease. Elsevier, Inc; Waltham, MA: 2012.
- King, SM.; Kamiya, R. Axonemal dyneins: assembly, structure and force generation. In: Witman, GB., editor. The *Chlamydomonas* Source Book, 2nd Edition. Volume 3: Cell Motility and Behavior. Vol. III. San Diego: Elsevier; 2009. p. 131–208.
- King SM, Patel-King RS. Identification of a $\text{Ca}^{(2+)}$ -binding light chain within *Chlamydomonas* outer arm dynein. J Cell Sci. 1995; 108:3757–3764. [PubMed: 8719882]
- King SM, Patel-King RS. Functional architecture of the outer arm dynein conformational switch. J Biol Chem. 2012; 287:3108–3122. [PubMed: 22157010]
- King SM, Witman GB. Multiple sites of phosphorylation within the a heavy chain of *Chlamydomonas* outer arm dynein. J Biol Chem. 1994; 269:5452–5457. [PubMed: 7508939]
- Kon T, Sutoh K, Kurisu G. X-ray structure of a functional full-length dynein motor domain. 2011; 18:638–642.
- Lupetti P, Lanzavecchia S, Mercati D, Cantele F, Dallai R, Mencarelli C. Three-dimensional reconstruction of axonemal outer dynein arms *in situ* by electron tomography. Cell Motil Cytoskeleton. 2005; 62:69–83. [PubMed: 16106450]
- Mastroratte DN, O'Toole ET, McDonald KL, McIntosh JR, Porter ME. Arrangement of inner dynein arms in wild-type and mutant flagella of *Chlamydomonas*. J Cell Biol. 1992; 118:1145–1162. [PubMed: 1387403]
- Mazor M, Alkrinawi S, Chalifa-Caspi V, Manor E, Sheffield V, Aviram M, Parvari R. Primary ciliary dyskinesia caused by homozygous mutation in *DNALI1*, encoding dynein light chain 1. Am J Human Genet. 2011; 88:1–9.
- McKenney RJ, Vershinin M, Kunwar A, Vallee RB, Gross SP. LIS1 and NudE induce a persistent dynein force-producing state. Cell. 2010; 141:304–314. [PubMed: 20403325]
- Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R, Dutcher S,

- Fernandez E, Fukuzawa H, Gonzalez-Ballester D, Gonzalez-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riano-Pachon DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, Dubchak I, Goodstein D, Hornick L, Huang YW, Jhaveri J, Luo Y, Martinez D, Ngau WCA, Otilar B, Poliakov A, Porter A, Szajkowski L, Werner G, Zhou K, Grigoriev IV, Rokhsar DS, Grossman AR. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science*. 2007; 318:245–250. [PubMed: 17932292]
- Mitchell DR, Kang Y. Reversion analysis of dynein intermediate chain function. *J Cell Sci*. 1993; 105:1069–1078. [PubMed: 8227195]
- Mocz G, Gibbons IR. Phase partition analysis of nucleotide binding to axonemal dynein. *Biochemistry*. 1996; 35:9204–9211. [PubMed: 8703926]
- Nicastro D, Schwartz C, Pierson J, Gaudette R, Porter ME, McIntosh JR. The molecular architecture of axonemes revealed by cryoelectron tomography. *Science*. 2006; 313:944–948. [PubMed: 16917055]
- Oda T, Hirokawa N, Kikkawa M. Three-dimensional structures of the flagellar dynein-microtubule complex by cryoelectron microscopy. *J Cell Biol*. 2007; 177:243–252. [PubMed: 17438074]
- Ogawa K, Takai H, Ogiwara A, Yokota E, Shimizu T, Inaba K, Mohri H. Is outer arm dynein intermediate chain 1 multifunctional? *Mol Biol Cell*. 1996; 7:1895–1907. [PubMed: 8970153]
- Padma P, Hozumi A, Ogawa K, Inaba K. Molecular cloning and characterization of a thioredoxin/nucleoside diphosphate kinase related dynein intermediate chain from the ascidian, *Ciona intestinalis*. *Gene*. 2001; 275:177–183. [PubMed: 11574167]
- Patel-King R, Gorbatyuk O, Takebe S, King S. Flagellar radial spokes contain a Ca^{2+} -sensitive nucleoside diphosphate kinase. *Mol Biol Cell*. 2004; 15:3891–3902. [PubMed: 15194815]
- Patel-King RS, Benashki SE, Harrison A, King SM. Two functional thioredoxins containing redox-sensitive vicinal dithiols from the *Chlamydomonas* outer dynein arm. *J Biol Chem*. 1996; 271:6283–6291. [PubMed: 8626422]
- Patel-King RS, King SM. An outer arm dynein light chain acts in a conformational switch for flagellar motility. *J Cell Biol*. 2009; 186:283–295. [PubMed: 19620633]
- Pazour GJ, Koutoulis A, Benashki SE, Dickert BL, Sheng H, Patel-King RS, King SM, Witman GB. LC2, the *Chlamydomonas* homologue of the *t* complex-encoded protein Tctex2, is essential for outer dynein arm assembly. *Mol Biol Cell*. 1999; 10:3507–3520. [PubMed: 10512883]
- Pazour GJ, Wilkerson CG, Witman GB. A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). *J Cell Biol*. 1998; 141:979–992. [PubMed: 9585416]
- Pedersen L, Rempel P, Christensen S, Rosenbaum JL, King SM. The lissencephaly protein Lis1 is present in motile mammalian cilia and requires outer dynein arm for targeting to *Chlamydomonas* flagella. *J Cell Sci*. 2007; 120:858–867. [PubMed: 17314247]
- Pfister KK, Fay RB, Witman GB. Purification and polypeptide composition of dynein ATPases from *Chlamydomonas* flagella. *Cell Motil*. 1982; 2:525–547. [PubMed: 6220806]
- Piperno G, Luck DJ. Axonemal adenosine triphosphatases from flagella of *Chlamydomonas reinhardtii*. Purification of two dyneins. *J Biol Chem*. 1979; 254:3084–3090. [PubMed: 155062]
- Piperno G, Luck DJ. Inner arm dyneins from flagella of *Chlamydomonas reinhardtii*. *Cell*. 1981; 27:331–340. [PubMed: 6460559]
- Ralston K, Kisalu N, Hill K. Structure-function analysis of dynein light chain 1 identifies viable motility mutants in bloodstream-form *Trypanosoma brucei*. *Eukaryotic Cell*. 2011; 10:884–894. [PubMed: 21378260]
- Roberts AJ, Numata N, Walker ML, Kato YS, Malkova B, Kon T, Ohkura R, Arisaka F, Knight PJ, Sutoh K, Burgess SA. AAA⁺ ring and linker swing mechanism in the dynein motor. *Cell*. 2009; 136:485–495. [PubMed: 19203583]

- Rompolas P, Patel-King RS, King SM. An outer arm dynein conformational switch is required for metachronal synchrony of motile cilia in planaria. *Mol Biol Cell*. 2010; 21:3669–3679. [PubMed: 20844081]
- Sakato M, Sakakibara H, King SM. *Chlamydomonas* outer arm dynein alters conformation in response to Ca^{2+} . *Mol Biol Cell*. 2007; 18:3620–3634. [PubMed: 17634291]
- Samsó M, Koone M. 25 Angstrom-resolution structure of a cytoplasmic dynein motor reveals a seven-member planar ring. *J Mol Biol*. 2004; 340:1059–1072. [PubMed: 15236967]
- Shimizu T, Kimura I. Effects of N-ethylmaleimide on dynein adenosinetriphosphatase activity and its recombining ability with outer fibers. *J Biochem (Tokyo)*. 1974; 76:1001–1008. [PubMed: 4281424]
- Shpetner HS, Paschal BM, Vallee RB. Characterization of the microtubule-activated ATPase of brain cytoplasmic dynein (MAP 1C). *J Cell Biol*. 1988; 107:1001–1009. [PubMed: 2971069]
- Tanner C, Rompolas P, Patel-King RS, Gorbatyuk O, Wakabayashi K, Pazour GJ, King SM. Three members of the LC8/DYNLL family are required for outer arm dynein motor function. *Mol Biol Cell*. 2008; 19:3724–3734. [PubMed: 18579685]
- Toba S, Fox LA, Sakakibara H, Porter ME, Oiwa K, Sale WS. Distinct roles of 1 α and 1 β heavy chains of the inner arm dynein II of *Chlamydomonas* flagella. *Mol Biol Cell*. 2011; 22:342–353. [PubMed: 21148301]
- Wakabayashi K, King SM. Modulation of *Chlamydomonas reinhardtii* flagellar motility by redox poise. *J Cell Biol*. 2006; 173:743–754. [PubMed: 16754958]
- Wakabayashi K, Misawa Y, Mochiji S, Kamiya R. Reduction-oxidation poise regulates the sign of phototaxis in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA*. 2011; 108:11280–11284. [PubMed: 21690384]
- Watanabe T, Flavin M. Nucleotide-metabolizing enzymes in *Chlamydomonas* flagella. *J Biol Chem*. 1976; 251:182–192. [PubMed: 397]
- Wickstead, B.; Gull, K. Evolutionary biology of dyneins. In: King, SM., editor. *Dyneins: Structure, Biology and Disease*. Waltham, MA: Elsevier, Inc; 2012. p. 89-121.
- Wirschell M, Pazour G, Yoda A, Hirono M, Kamiya R, Witman G. Oda5p, a novel axonemal protein required for assembly of the outer dynein arm and an associated adenylate kinase. *Mol Biol Cell*. 2004; 15:2729–2741. [PubMed: 15064350]
- Wu H, Maciejewski MW, Marintchev A, Benashski SE, Mullen GP, King SM. Solution structure of a dynein motor domain associated light chain. *Nature Struct Biol*. 2000; 7:575–579. [PubMed: 10876244]
- Xiang X, Beckwith SM, Morris NR. Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans*. *Proc Natl Acad Sci U S A*. 1994; 91:2100–2104. [PubMed: 8134356]
- Yagi T, Uematsu K, Liu Z, Kamiya R. Identification of dyneins that localize exclusively to the proximal portion of *Chlamydomonas* flagella. *J Cell Sci*. 2009; 122:1306–1314. [PubMed: 19351714]
- Yamamoto R, Yanagisawa H, Yagi T, Kamiya R. A novel subunit of axonemal dynein conserved among lower and higher eukaryotes. *FEBS Lett*. 2006; 580:6357–6360. [PubMed: 17094970]
- Yamamoto R, Yanagisawa H-a, Yagi T, Kamiya R. Novel 44-kilodalton subunit of axonemal dynein conserved from *Chlamydomonas* to mammals. *Eukaryotic Cell*. 2008; 7:154–161. [PubMed: 17981992]
- Yang P, Fox L, Colbran RJ, Sale WS. Protein phosphatases PP1 and PP2A are located in distinct positions in the *Chlamydomonas* flagellar axoneme. *J Cell Sci*. 2000; 113:91–102. [PubMed: 10591628]
- Yang P, Sale WS. Casein kinase I is anchored on axonemal doublet microtubules and regulates flagellar dynein phosphorylation and activity. *J Biol Chem*. 2000; 275:18905–18912. [PubMed: 10858448]

Figure 1. Composition and Organization of Outer Arm Dynein

The composition and organization of the *Chlamydomonas* outer dynein arm is diagramed. This complex enzyme is built around three AAA⁺ motors (α, β, and γ) which associate directly with a series of LCs (LCs 1, 3, 4, 5) that transduce mechanical, Ca²⁺ and redox signals. The HCs in turn associate with a complex consisting of two WD-repeat ICs which contain N-terminal regions to which a series of LCs (LCs2, 6, 7a, 7b, 8 and 10) bind. One of these LCs (LC7a) interacts with a component of the trimeric docking complex that anchors the structure within the axoneme. The ODA5 protein is also required for assembly but its associations are uncertain (Wirschell *et al.*, 2004). Finally, the ODA7 protein may mediate association of the outer arm with inner arm I1/f (Freshour *et al.*, 2007).

Figure 2. Integrated Signaling Pathway for Outer Arm Dynein

This diagram illustrates the known signals that impinge on *Chlamydomonas* outer arm dynein (green), the effector molecules (pink), the distinct HC motor components (light blue) and the functional output (yellow/red). Of note is that each HC acts to integrate a distinct set of signals: redox, Lis1 and phosphorylation (α HC), redox (β HC), and Ca^{2+} , mechanical sensing, phosphorylation, and redox state (γ HC). The lack of individual motor units also leads to distinct phenotypic results which likely derive from both alterations in motor function and disruption of particular signaling modalities.