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Keeping an Eye on I1: I1 Dynein as a Model for Flagellar Dynein Assembly and Regulation

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Among the major challenges in understanding ciliary and flagellar motility is to determine how the dynein motors are assembled and localized and how dynein-driven outer doublet microtubule sliding is controlled. Diverse studies, particularly in *Chlamydomonas*, have determined that the inner arm dynein I1 is targeted to a unique structural position and is critical for regulating the microtubule sliding required for normal ciliary/flagellar bending. As described in this review, I1 dynein offers additional opportunities to determine the principles of assembly and targeting of dyneins to cellular locations and for studying the mechanisms that regulate dynein activity and control of motility by phosphorylation. Cell Motil. Cytoskeleton 64:569–579, 2007.   © 2007 Wiley-Liss, Inc.

INTRODUCTION AND OVERVIEW

Genetic, proteomic, and computational analyses have recently shed much light on the importance of cilia and flagella in human health [El Zein et al., 2003; Avidor-Reiss et al., 2004; Li et al., 2004; Pazour, 2004; Snell et al., 2004; Pazour et al., 2005]. Failure to assemble these structures or failure in their motility can lead to severe consequences in development or adult functions in many organs [Snell et al., 2004; Davenport and Yoder, 2005; Pan et al., 2005; Quarmby and Parker, 2005; Davis et al., 2006; Christensen et al., 2007]. Therefore, understanding how cilia and flagella assemble and the processes that drive motility—particularly the dynein motors—are of great interest. Here, we focus on a conserved dynein motor, inner arm dynein I1, and how studies, primarily in *Chlamydomonas*, have revealed new features of dynein assembly and regulation of axonemal motility by phosphorylation.

Chlamydomonas is a unicellular, biflagellate, green alga that is an excellent model system for the discovery of conserved genes required for flagellar assembly and function [Silflow and Lefebvre, 2001] and in particular for study of the flagellar dyneins-the microtubule motors that drive flagellar motility [DiBella and King, 2001; Kamiya, 2002; Oiwa and Sakakibara, 2005]. Structural and biochemical studies of wild-type and mutant *Chlamydomonas* have revealed the axoneme bears at least seven different inner arm dyneins that differ in composi-

tion and structural location [DiBella and King, 2001]. For example, in the axoneme, I1-dynein is a trilobed structure originally identified as the ''triad'' [Goodenough and Heuser, 1985a]. Mutations affecting certain I1 subunits often result in a complete failure in I1-dynein assembly [*ida1, ida2, ida7*-See Table I for nomenclature, Kamiya et al., 1991] leaving a gap in the I1 position that repeats every 96-nm and does not affect the assembly of the other flagellar dynein isoforms [Piperno et al., 1990; Kamiya et al., 1991; Porter et al., 1992; Smith and Sale, 1992b; Perrone et al., 1998]. Thus I1-dynein must be targeted to a unique position in the axoneme by specialized proteins or domains that repeat every 96-nm.

Phenotypic analysis has revealed that the inner arm dyneins are responsible for determining the size and shape of the flagellar bend, parameters that define waveform [Brokaw and Kamiya, 1987; Kamiya et al., 1991; Kamiya, 1995]. In some cases, mutations that affect I1-dynein assembly suppress paralysis in a central pair mutant [Porter et al., 1992]. Also, I1-dynein assembly mutants exhibit defects in phototaxis—a behavioral response to a light stimulus that requires precise control of flagellar bending indicating I1 is important, although not absolutely required, in this process [King and Dutcher, 1997; Okita et al., 2005]. These phenotypic data demonstrate that I1 dynein is a critical component of a complex system, involving multiple axonemal dyneins, which controls axonemal bending. Extensive in vitro functional and pharmacological data also indicate I1 dynein is required for control of axonemal sliding [Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Hendrickson et al., 2004], and that the mechanism of regulation involves reversible phosphorylation of the I1 subunit IC138 in a process that also may be controlled by calcium [Hennessey et al., 2002; Smith, 2002a; Dymek and Smith, 2006].

Phylogenetic analyses have revealed that I1-dynein proteins are highly conserved, thus the study of I1 in *Chlamydomonas* is relevant to a wide range of organisms. For example, not only is I1-dynein activity regulated by phosphorylation in *Chlamydomonas*, recent data suggests that I1 activity plays a role in regulating motility in *Tetrahymena thermophila* [Hennessey et al., 2002; Deckman and Pennock, 2004].

In this commentary, we discuss mechanisms for assembly and transport of I1, anchoring I1-dynein to the doublet microtubule, and localizing I1-dynein within the 96-nm repeat. This review also incorporates very recent and exciting unpublished work that has been presented in Abstract form at the 2006 American Society for Cell Biology annual meeting. We describe a model for how localized control of I1-dynein phosphorylation operates to locally control microtubule sliding and adjust axonemal waveform. In particular, we summarize a model in which I1 dynein locally inhibits microtubule sliding driven by other dynein isoforms.

ORGANIZATION OF THE INNER ARMS: THE 96-nm REPEAT AND SUBUNIT COMPOSITION OF I1 DYNEIN

Compared to the outer dynein arms, the inner arm dyneins are heterogeneous with respect to their organization [Porter and Sale, 2000; DiBella and King, 2001; Kamiya, 2002]. Analysis of mutant phenotypes, biochemical identification of the inner arm dynein subtypes, and eloquent ultrastructural studies have helped to define the composition and arrangement of the complex inner dynein arm system, particularly the I1-dynein. The complexity of composition and structure has led to different nomenclatures, but on the basis of heavy chain (HC) number, there appear to be two types of inner arm dyneins. I1-dynein, also called f-dynein, contains two HCs, whereas the inner arm dyneins containing one HC are designated a, b, c, d, e, and g, and were initially thought to be organized in structures referred to as I2 and I3 [Piperno et al., 1990; Kagami and Kamiya, 1992, 1995].

The inner arm dyneins are organized around a regular repeating pattern with a 96-nm repeat length (Fig. 1). This pattern has been elucidated in exquisite detail by rapid freeze-deep etch microscopy [Goodenough and Heuser, 1985b], analysis of inner arm mutants [Piperno et al., 1990], image analysis of thin sections [Mastronarde et al., 1992; Porter et al., 1992; Perrone et al., 1998], and most recently by cryoEM tomography of axonemes [Nicastro et al., 2005, 2006]. The I1 dynein is located in the proximal position of the 96-nm repeat, in close proximity to radial spoke S1, and is found along the entire length of the axoneme (Fig. 1). Although it is presumed that I1 dynein is found on all nine outer doublets, this has not yet been determined by direct localization at the ultrastructural level. Based on analysis in a number of organisms, the organization of the 96-nm repeat is a highly conserved feature of the axoneme [Goodenough and Heuser, 1985b; Woolley, 1997; Nicastro et al., 2005, 2006]. Additional features of the 96-nm repeat module are the single headed inner arm dyneins, two radial spokes (S1 and S2), and the dynein regulatory complex (DRC)-structures that are critical for regulation of dynein function [Gardner et al., 1994; Piperno, 1995; Smith and Yang, 2004], as well as novel structures connecting I1 dynein and the DRC to specific outer dynein arms within the 96-nm module [Nicastro et al., 2006]. The novel structures linking I1-dynein to the outer arm may be composed of the Oda7 protein [Freshour et al., 2006; Nicastro et al., 2006].

TABLE I. Inner Dynein Arm Components and Mutations that Affect I1-Dynein Assembly or Function[a]

Subunit	M_r	Mutant[a,b]	Mutant phenotype	Predicted function	References
1αHC	523	ida1 (pf9, pf30)	Lacks I1, slow-swimming	Motor activity; Altered waveform, phototaxis	Kamiya et al., 1991; Porter et al., 1992; Okita et al., 2005; Piperno et al., 1990; Brokaw and Kamiya, 1987
1βHC	511	G41a	Truncated motor domain	Required for I1 assembly	Myster et al., 1999
		ida2	Lacks I1, slow-swimming; Altered waveform, phototaxis	Motor activity	Kamiya et al., 1991
IC140	140	D11	Truncated motor domain	Required for I1 assembly	Perrone et al., 2000
		ida7	Lacks I1, slow-swimming; Altered waveform, phototaxis	Required for I1 assembly	Yang and Sale, 1998; Perrone et al., 1998
IC138	138	5A	Truncated IC140	Not required for I1 assembly	Perrone et al., 1998
		bop5-1	Truncated IC138, I1 lacks LC7b; Slow-swimming	Not required for I1 assembly	Hendrickson et al., 2004; Dutcher et al., 1988
?	?	mia1, mia2	Hyperphosphorylated IC138	Not required for I1 assembly	King and Dutcher, 1997
IC97	97[c]	–	–	Not required for I1 assembly	Wirschell et al., 2006
TcTex1	13	–	–	Cargo binding	Harrison et al., 1998
TcTex2b	13.7	pf16-D2::PF16	Slow-swimming	Not required for I1 assembly; May modulate motor function; Stability of I1	DiBella et al., 2004b
LC8	10	ftla4	Short immotile flagella,	Stable assembly of I1 found in outer arms, inner arms, radial spokes, and the retrograde IFT motor	Pazour et al., 1998
LC7a	14	oda15	Partial outer arm assembly defect	Stable association of LC7b in I1	DiBella et al., 2004a
LC7b	11	–	Missing in bop5-1 mutant	Not required for I1 assembly; Interacts with IC138; May modulate I1 function	Hendrickson et al., 2004; Hendrickson et al., 2004; DiBella et al., 2004a
?	?	ida3	Lacks I1, slow-swimming; Altered waveform, phototaxis		Kamiya et al., 1991

[a] Mutations affecting the inner dynein arm system generally are referred to as *ida* mutants (Kamiya et al., 1991). Exceptions to this system of nomenclature include mutants designated as *pf* (*p*aralyzed *f*lagella), *bop* (*b*ypass *o*f *p*aralysis), or *mia* (*m*odifiers of *i*nner *a*rms); named either prior to the establishment of the *ida* nomenclature or prior to the recognition that the mutation affected inner arm dyneins.

[b] Individual alleles at each locus are not detailed here.

[c] In some reports using different SDS-PAGE markers, IC97 is reported to migrate at 110 kDa and is cited as IC110 (Dibella and King, 2001; King and Dutcher, 1997; Porter et al., 1992).
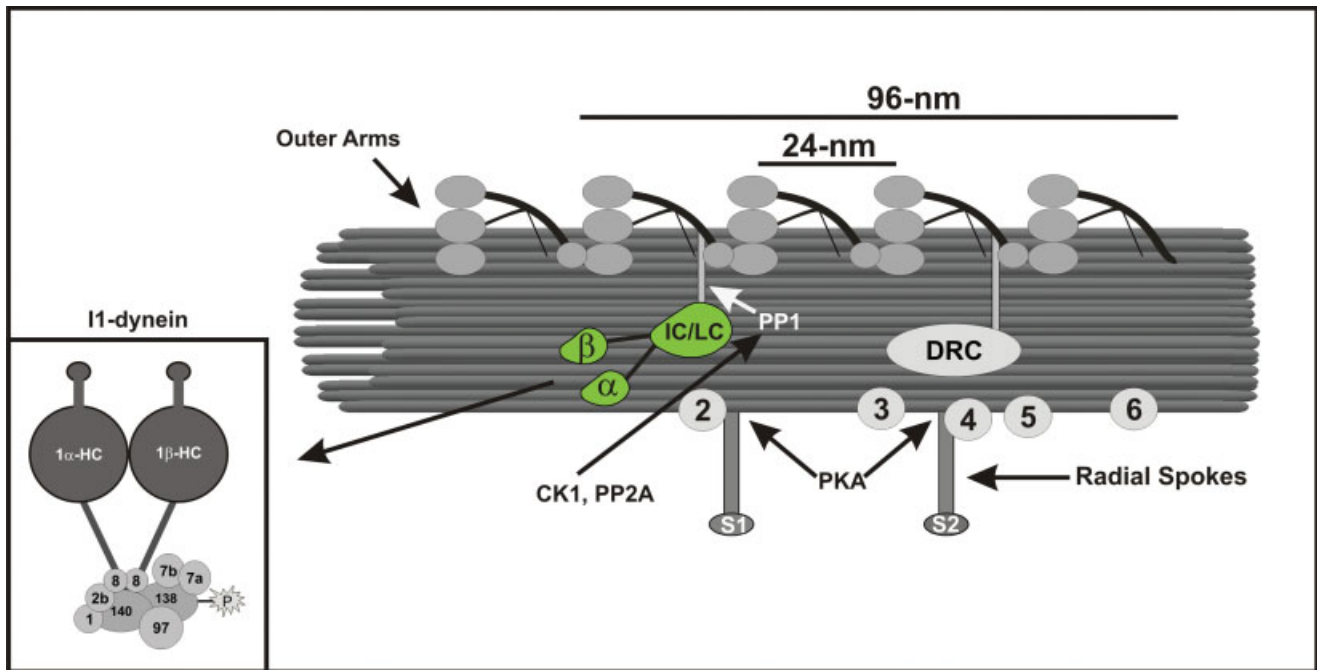
Fig. 1. Schematic of the 96-nm axonemal dynein repeat. The arrangement of the inner dynein arms, outer dynein arms, radial spokes, and DRC on the A-tubule is shown [adapted from Porter and Sale, 2000; Nicastro et al., 2006]. These structures repeat every 96-nm along the length of the axoneme (the outer dynein arms repeat every 24-nm). The proximal end (closest to the cell body) faces left. I1-dynein is the trilobed inner arm just proximal to radial spoke S1 in each repeat (green); it is followed by a series of single headed inner arms (labeled 2–6). PKA is thought to be localized at the base of the radial spokes [Gaillard et al., 2006]; CK1 and PP2A are predicted to be anchored near I1 dynein; a fraction of PP1 located on the outer doublets. The inset shows a diagram of the I1-dynein and its individual subunits [Harrison et al., 1998; Perrone et al., 1998; Yang and Sale, 1998; Porter and Sale, 2000; DiBella et al., 2004a,b; Hendrickson et al., 2004]. I1-dynein is composed of two motor subunits (1α- and 1β-HC), three intermediate chains (IC140, IC138, and IC97), and five LC subunits (Tctex1, Tctex2b, LC8, LC7a, and LC7b). IC138 is a phosphoprotein in I1 and is one of the key regulatory subunits critical for I1-dynein activity.

I1-deficient axonemes reveal that I1 is localized to a unique position in the 96-nm repeat and that failure in I1 assembly does not affect assembly of other axonemal structures [Piperno et al., 1990; Kamiya et al., 1991; Porter et al., 1992; Smith and Sale, 1992b; Nicastro et al., 2006]. This result is important in several ways. First, the other axonemal dyneins assemble normally indicating mutations in I1 assembly do not disrupt assembly of other dynein isoforms. Second, the results also imply that I1-dynein is localized in the axoneme by a docking mechanism that resides near the S1 radial spoke, at the proximal end of the 96-nm repeat (Fig. 1). Consistent with this prediction, in vitro reconstitution of I1-deficient axonemes results in a restoration of functional I1 structures to the original position in the 96-nm repeat [Smith and Sale, 1992b; Yamamoto et al., 2006]. However, to date, no docking complex has been identified.

I1 is the only inner dynein arm with two HCs, designated 1α and 1β, [Piperno et al., 1990]. I1 dynein forms a two headed structure as revealed by electron microscopy of isolated I1 particles [Goodenough et al., 1987; Smith and Sale, 1991; Sakakibara et al., 2006].

Along with the HCs, are three intermediate chains (IC140, IC138, and IC97), and five known light chains (LC) (Tctex1, Tctex2b, LC8, LC7b, and LC7a) [Porter et al., 1992; Harrison et al., 1998; Perrone et al., 1998; Yang and Sale, 1998; DiBella et al., 2001, 2004a,b; Hendrickson et al., 2004; Wirschell et al., 2006] (Fig. 1 inset and Table I). The HCs form stem domains which connect to an IC/LC complex that forms the cargo-binding domain of the dynein motor as well as globular head domains that possess the motor activity. For dyneins in general, the intermediate and LCs play important roles in motor assembly and regulation [Perrone et al., 1998; Yang and Sale, 1998; DiBella et al., 2001, 2004a,b]. Several of the LCs are not unique to I1, notably LC8, LC7a, LC7b, and Tctex1. Rather, they are also LCs in other dyneins and additional enzymes and structures [Pazour et al., 1998; DiBella et al., 2004a,b]. These LCs may have common functions in stable assembly of dynein structures (LC8), cargo binding (Tctex1-rhodopsin), or regulation (LC7b) [King et al., 1996; Harrison et al., 1998; Pazour et al., 1998; Tai et al., 1999; Hendrickson et al., 2004].

I1-DYNEIN MUTANTS REVEAL NEW FEATURES OF ASSEMBLY AND DOCKING

Mutations that disrupt specific I1 subunits or specific domains of those subunits often result in assembly of incomplete, or partial, I1 dynein complexes (Table I). For example, mutant strains containing truncated HC genes, lacking the motor domains, still assemble an I1 complex [Myster et al., 1999; Perrone et al., 2000]. These results are consistent with previous studies indicating that N-terminal fragments between 140- and 160-kDa are capable of complex assembly [Sakakibara et al., 1993; Koonce and Knecht, 1998; Iyadurai et al., 1999]. Importantly, electron microscopy of these HC truncations revealed the location of the globular motor domains of the 1α and 1β-HCs in the axoneme [Myster et al., 1999; Perrone et al., 2000]. They also indirectly reveal the location of the IC/LC complex at the base of I1 dynein where it is anchored to the A-tubule (Fig. 1). The assembly of I1 dynein complexes that lack one of the HC motor domains provides an opportunity to dissect the individual roles of the 1α- and 1β-HCs [Fox et al., 2006; Toba et al., 2006].

The ICs typically contain WD repeats and are integral structural components of dynein motors. This indeed seems to be the case for the IC140 subunit (see below). Like the ICs of the outer arm, IC140 is required for assembly of the I1 dynein complex and thus is implicated in anchoring of I1 to the axoneme [Perrone et al., 1998; Yang and Sale, 1998]. In support of this, the C-terminus of IC140 interacts with the axoneme directly [Yang and Sale, 1998] and thus may interact with components that localize I1 in the 96-nm axonemal repeat structure. However, while IC140 is required for I1 assembly in the axoneme, the N-terminal region is not critical for its assembly function (Table I-strain *5A*, [Perrone et al., 1998]). Alternatively, in the absence of IC140, I1-dynein components in the cytoplasm may be unstable and degraded, thus contributing to the failure of I1-dynien to assemble in the axoneme.

The *bop5-1* mutant expresses a truncated IC138 protein that lacks the C-terminal WD repeat domain (Table I). All I1 subunits assemble in this mutant, except for LC7b indicating that LC7b and IC138 interact [DiBella et al., 2004a; Hendrickson et al., 2004]. A second IC138 mutation indicates that, unlike IC140 and the outer arm ICs, IC138 is not required for assembly of the entire I1-dynein complex, but may be required for assembly of the IC97 and LC7b subunits [Hendrickson et al., 2004; Bower et al., 2006]. These studies indicate that the primary role of IC138 is not in assembly and is consistent with the known regulatory role of IC138 in control of microtubule sliding (see below). The results also reveal that IC97 is not required for assembly of I1, but they do not reveal the exact function of IC97 within I1. This mutation further indicates that IC140 along with the N-terminal HC domains are principally responsible for targeting and anchoring I1 to its specific location in the 96-nm repeat.

Based on crosslinking studies, IC138 and IC140 interact directly with axonemal proteins [Yang and Sale, 1998; Hendrickson et al., 2004] and at least some of these interactions may reflect interactions with tubulin (Wirschell, Hendrickson, and Sale, unpublished observations). IC97 interacts directly with both α- and β-tubulin subunits [Wirschell et al., 2006]. Given that all three ICs may interact directly with tubulin, anchoring of I1 dynein to the doublet microtubule involves multiple interactions. However, IC-tubulin interactions cannot explain the exact placement of I1 within the 96-nm repeat. One idea is that I1 interacts with an intrinsic component of the outer doublet that establishes the 96-nm repeat structure that governs placement of the inner dynein arms, radial spokes, and DRC. Proteins that might be part of such a complex could include the tektins [Norrander et al., 1996; Yanagisawa and Kamiya, 2004; Setter et al., 2006]. Consistent with this idea, tektins may act as a molecular ruler that defines the axonemal 96-nm repeat [Setter et al., 2006] and in *Chlamydomonas*, defects in tektin assembly are associated with defective inner dynein arm assembly [Yanagisawa and Kamiya, 2004].

Structural analyses of axonemes from dynein mutants have demonstrated that each dynein isoform is targeted to its own unique position along the length and around the circumference of the A-microtubule of most outer doublets. Anchoring of the outer dynein arm is mediated in part through additional complexes called outer dynein arm–docking complexes (ODA-DC and the Oda5 complex) [Takada and Kamiya, 1994; Wakabayashi et al., 2001; Casey et al., 2003; Wirschell et al., 2004]. Moreover, additional mutations have revealed the outer dynein arm is transported into the axoneme specifically through an interaction with the intraflagellar transport component IFT46 and Oda16p—a novel non-dynein transport factor required for outer dynein arm assembly [Ahmed and Mitchell, 2005; Hou et al., 2007]. Evidence suggests that single-headed inner arms are also transported into the flagellum by IFT [Piperno et al., 1996]. Given that other dyneins are transported by IFT, it is likely that I1 is as well. However, to date, similar IFT transport mechanisms have not been defined for I1 dynein. Predictably, recovery of additional mutants that fail to assemble I1 will reveal new genes required for assembly, transport, targeting, or docking of I1 dynein. The *ida3* mutant may be an example. The *ida3* mutation causes a complete I1 assembly defect, but the *IDA3* gene product remains unknown (Table I).

FUNCTIONAL ROLE OF I1 IN REGULATION OF FLAGELLAR BENDING: A MODEL FOR LOCAL REGULATION OF MICROTUBULE SLIDING BY PHOSPHORYLATION

Among the major challenges in understanding the mechanism of ciliary motility is to determine how dynein-driven microtubule sliding is regulated. Based on models, oscillatory bending and bend propagation require mechanical feedback mechanisms that control the timing and location of active sliding [Brokaw, 1985, 1994; Lindemann, 2002]. However, the experimental evidence for the identity and nature of such a mechanical feedback mechanism is lacking.

In contrast, direct experimental evidence, through genetic and in vitro functional studies using *Chlamydomonas* axonemes, has revealed I1-dynein is important for control of microtubule sliding and is likely designed for modulating the size and shape of the axonemal bend [Brokaw and Kamiya, 1987]. We summarize data indicating I1-dynein is important for control of flagellar waveform and that the mechanism involves regulation of phosphorylation of the I1-dynein subunit IC138 and control of microtubule sliding [Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Hendrickson et al., 2004]. We also discuss a model in which localized inhibition of microtubule sliding [Smith, 2002b] is mediated by I1-dynein and IC138 phosphorylation and is required for normal control of bending behavior.

Mutations in I1-dynein subunits or mutations that alter I1 phosphorylation result in failure of control of normal axonemal waveform and phototaxis in *Chlamydomonas* [Brokaw and Kamiya, 1987; King and Dutcher, 1997; Okita et al., 2005]. For example, the *mia* mutants were isolated in a screen for phototaxis mutations and the mutant axonemes exhibit hyperphosphorylated IC138 [King and Dutcher, 1997]. Based on microtubule sliding assays and pharmacological analysis, dynein-driven sliding is inhibited in *mia* mutant axonemes. However, addition of kinase inhibitors rescues the microtubule sliding defects. The data revealed a correlation between IC138 phosphorylation and regulation of dynein; phosphorylated IC138 inhibited microtubule sliding and dephosphorylation rescued microtubule sliding (Fig. 2). Okita et al. have further examined the role of I1 in phototaxis and suggest that I1 may be involved in one of many pathways required for generating phototactic behavior [Okita et al., 2005]. Additionally, mutations in I1 subunits can suppress paralysis in a central pair mutant, indicating a functional regulatory interaction between I1-dynein activity and the central pair-radial spoke structures for control of microtubule sliding and axonemal motility [Porter et al., 1992]. Thus, I1
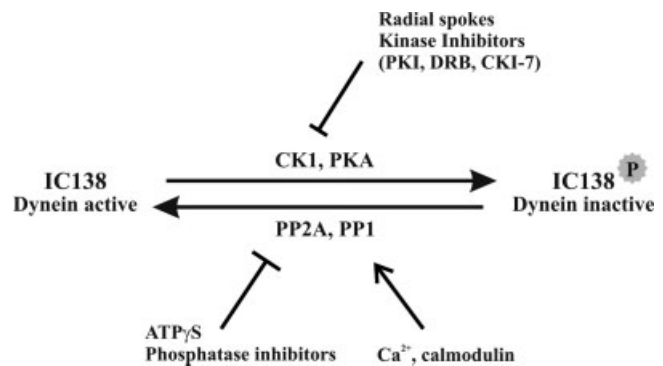

Fig. 2. Regulation of IC138 phosphorylation. IC138 is a critical component of I1 involved in the radial spoke-central pair mechanism for regulating I1 dynein activity [adapted from Habermacher and Sale, 1997; Porter and Sale, 2000]. This regulatory process involves changes in IC138 phosphorylation such that in the phosphorylated state, I1 is inactive; when IC138 is dephosphorylated, I1-dynein activity is restored. The critical axonemal kinases and phosphatases involved in this pathway are indicated [Porter and Sale, 2000; Smith and Yang, 2004].

dynein appears to regulate axonemal motility through a mechanism that controls microtubule sliding.

Consistent with the idea that I1-dynein plays a role in control, in vitro functional assays, using isolated axonemes and microtubule sliding, have also revealed that I1-dynein plays a central role in regulation of microtubule sliding and that the mechanism depends upon changes in phosphorylation of IC138. Figure 2 illustrates the role of CK1 and PKA in phosphorylation of IC138. Interestingly, a mating induced elevation in intraflagellar cAMP [Hasegawa et al., 1987; Pasquale and Goodenough, 1987] levels is presumably responsible for quiescence of flagella during mating (observations by Ursula Goodenough, personal communication). An interesting and testable idea is that the increased intraflagellar cAMP levels affect IC138 phosphorylation and thus globally inhibits microtubule sliding leading to cessation or slowing of flagellar motility during the mating response. Moreover, dynein-driven microtubule sliding in paralyzed axonemes, lacking either the radial spokes or the central pair, is inhibited presumably throughout the axoneme [Smith and Sale, 1992a]. The inhibition of microtubule sliding in these paralyzed mutant axonemes can be rescued by addition of kinase inhibitors indicating abnormal phosphorylation in the axoneme inhibits microtubule sliding [Howard et al., 1994; Habermacher and Sale, 1995, 1997; Yang and Sale, 2000; Smith, 2002b]. The results are consistent with the model shown in Fig. 2 indicating that inhibition of dynein in the paralyzed mutants is due, in part, to phosphorylation of IC138 and that dephosphorylation rescues microtubule sliding [Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Smith, 2002b; Hendrickson et al., 2004]. However, the in vitro experiments were performed in a paralyzed flagella

Fig. 3.   A model for localized regulation of I1 and axonemal bending. The axoneme is shown in cross section illustrating the 9 + 2 arrangement of the axoneme and the asymmetric central pair apparatus, the radial spokes, and the dynein arms [Smith, 2007]. The black line indicates the plane of bending [Mitchell, 2003b; Mitchell and Yokoyama, 2003]. The current model for regulation of I1-dynein is that chemical and/or mechanical signals from the central pair are transmitted through the radial spokes to affect IC138 phosphorylation on specific outer doublets (e.g., outer doublets N, N + 1, N − 1). Thus, phosphorylation of IC138 in an asymmetrical manner is predicted to result in local inhibition of sliding. This in turn would alter the form of the bend.

mutants, lacking either the central pair or radial spokes, in which axonemal kinase activity is misregulated, revealing a predicted global change in dynein activity [Gaillard et al., 2001, 2006; Gokhale et al., 2006]. The same in vitro measurements of microtubule sliding in wild-type axonemes have not been informative, most likely because the axonemal kinases are tightly and locally controlled. Thus, a major challenge is to determine how I1-dynein operates in wild-type axonemes for control of movement.

One idea, illustrated in Fig. 3 is that in wild-type axonemes, I1-dynein is locally and asymmetrically regulated in response to chemical or mechanical signals that impinge on the central pair apparatus. In this model, signals originating from the central pair, an inherently asymmetric structure, are directed to specific doublet microtubules through the radial spokes [Smith and Yang, 2004; Smith, 2007]. The idea is that an asymmetric signal, for example from one of the central pair microtubules, is directed at doublet ''N'' (Fig. 3) and locally induces phosphorylation of IC138 on doublet ''N''. In contrast, signals from the other central pair microtubule would have a different affect on the phosphorylation of IC138 on other outer doublet microtubules. Thus, following local changes in phosphorylation, sliding between doublets ''N'' and ''N + 1'' will be different relative to sliding between other doublets, therefore altering bending. As discussed before [Yang et al., 2004], the input for the asymmetric central pair-radial spoke signal could be a second messenger, such as calcium impinging upon the central pair [Wargo et al., 2005; Dymek and Smith, 2006], and/or a mechanical interaction with the radial spoke [Warner and Satir, 1974; Smith and Yang,

2004; Yang et al., 2004]. The output signal, presumably mediated by a radial spoke on one doublet microtubule, would be the activation of axonemal kinases located near I1-dynein [Yang et al., 2004; Gaillard et al., 2006].

Consistent with these ideas, the central pair apparatus is asymmetric in structure and composition [Mitchell and Sale, 1999; Mitchell and Yokoyama, 2003; Mitchell, 2003a; Wargo and Smith, 2003; Smith and Yang, 2004; Lechtreck and Witman, 2007] and the position of the central pair correlates with sites of active sliding [Wargo and Smith, 2003; Wargo et al., 2004] and the position of doublets relative to the bend [Mitchell, 2003a; Smith and Yang, 2004]. The results have also revealed a functional role for kinases (PKA, CK1) and phosphatases (PP1, PP2A) that are physically localized in the axoneme [Habermacher and Sale, 1996, 1997; King and Dutcher, 1997; Porter and Sale, 2000; Yang et al., 2000; Gaillard et al., 2001, 2006; Gokhale et al., 2006]. Much effort is underway to identify molecular mechanisms for targeting otherwise ubiquitous signaling molecules to precise locations in the axoneme [Gaillard et al., 2001, 2006]. Further tests of these ideas will require new assays linking radial spoke function to the direct control of axonemal kinases located on the outer doublet microtubule.

Related challenges include determining the mechanism for how IC138 can alter microtubule sliding. The implication of the model is the I1-dynein locally operates to inhibit microtubule sliding [Smith, 2002b]. Consistent with this idea, isolated I1-dyneins generate relatively slow microtubule translocation indicating that I1 dynein is unlike the outer arm dyneins or other inner arm dyneins [Smith and Sale, 1991; Kagami and Kamiya, 1992;

Sakakibara and Nakayama, 1998; Sakakibara et al., 1999; Yagi et al., 2005]. Although it is possible that the I1-dynein is not well suited to such in vitro assays that involve absorption to inert surfaces, recent advances in the K. Oiwa lab (Kobe Advanced ICT Research Center, Kobe, Japan) have confirmed that I1-dynein generates only slow microtubule translocation compared to other dyneins [Sakakibara et al., 2006; Toba et al., 2006].

When IC138 is phosphorylated, microtubule sliding is inhibited in the axoneme [Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Hendrickson et al., 2004]. One hypothesis is that phosphorylation of IC138 inhibits I1-dynein motor activity. However, using purified I1-dynein and in vitro motility assays, it has been determined that motor activity of isolated I1 dynein is not altered by IC138 phosphorylation, yet in the axoneme microtubule sliding is inhibited by the same changes in IC138 [Sakakibara et al., 2006]. Thus further progress will require measurements of single I1 motor characteristics including assessment of processivity and duty cycle as has been done for other inner arm dyneins and further structural analyses of I1 [Sakakibara et al., 2006, 1999]. Additionally, our collaborative studies with M. Porter (University of Minnesota), Toba, Oiwa, Sakakibara, and colleagues (Kobe Advanced ICT Research Center, Kobe, Japan) are addressing models of I1-dynein mechanism using single motor analysis and dyneins isolated from wild-type and I1-mutant axonemes [Myster et al., 1999; Perrone et al., 2000; Fox et al., 2006; Toba et al., 2006]. The goal is to further test the hypothesis that I1 dynein acts as a ''brake'' to locally inhibit microtubule sliding driven by other dynein isoforms and thus control axonemal waveform.

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