A solid-state control system for dynein-based ciliary/flagellar motility

Stephen M. King

Department of Molecular, Microbial, and Structural Biology, University of Connecticut Health Center, Farmington, CT 06030

Ciliary and flagellar beating requires the coordinated action of multiple dyneins with different enzymatic and motor properties. In this issue, Yamamoto et al. (2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201211048) identify the MIA (modifier of inner arms) complex within the *Chlamydomonas reinhardtii* axoneme that physically links to a known regulatory structure and provides a signaling conduit from the radial spokes to an inner arm dynein essential for waveform determination.

Generation of ciliary beating involves multiple different dynein motors acting in concert to specify the waveform and beat frequency (King and Kamiya, 2009; Ishikawa, 2012). Furthermore, these motors must exhibit an integrated response to various signaling factors. This requirement for coordinated action necessitates a complex control mechanism with a response time on the order of 20 ms or less. The fundamental unit of the ciliary axoneme is a 96-nm repeat structure (Fig. 1 a) that in *Chlamydomonas reinhardtii* consists of four outer arms, one inner arm I1/f, multiple additional inner arm dyneins containing single heavy chain motors, a complex structure known as the nexin–dynein regulatory complex (N-DRC) that interacts directly with several inner arm dyneins and acts as a linker connecting adjacent outer doublet microtubules, two complete radial spokes that project toward the central pair microtubule complex and also interact with a calmodulin-containing complex (the calmodulin- and spoke-associated complex [CSC]), and the truncated remnant of a third spoke (Fig. 1, a and b; Nicastro et al., 2006; Bui et al., 2012; Heuser et al., 2012). As isolated demembranated axonemes can be induced to beat in an apparently normal manner by addition of ATP, all the regulatory systems required for generating and propagating a bend must be incorporated within the axonemal superstructure. The dynein motor units within this solid-state system respond to both mechanical cues derived from axonemal curvature (Hayashibe et al., 1997; Patel-King and King, 2009) and other signals, including those from the radial spokes/central pair microtubule complex (Smith and Sale, 1992), changes in phosphorylation status of individual dynein components (Habermacher and Sale, 1995) and the binding of various signaling ligands, such as Ca^{2+} (Bessen et al., 1980), alterations in redox state (Wakabayashi and King, 2006), and the covalent modification of axonemal tubulins (Kubo et al., 2012).

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

There is now very strong evidence to support the idea that the I1/f dynein plays a central role in the control of waveform and potentially also acts as a signal transduction pathway, leading to regulation of outer arm function. Of particular note is the demonstration that a component of this dynein (the IC138 intermediate chain) acts to control I1/f function through reversible phosphorylation by PKA (cAMP-dependent protein kinase), CK1 (casein kinase 1), and the PP1 and PP2A phosphatases (Porter and Sale, 2000; Gokhale et al., 2009). However, the mechanisms by which signals are propagated from the central pair/radial spokes to the I1/f dynein have remained very unclear.

In this issue, Yamamoto et al. define an additional complex (termed modifier of inner arms [MIA]) located on the A-tubule (Fig. 1 a) of the outer doublets that appears to physically interact with the N-DRC and extend toward the base of the I1/f inner arm dynein. This structure consists of two coiled-coil proteins (FAP73 and FAP100), and mutants lacking these components exhibit altered waveform, decreased beat frequency, and abnormal photobehavior that is evidence of defects in flagella control pathways (King and Dutcher, 1997). Furthermore, they observe that the phosphorylation pattern of the key I1/f phosphoregulator IC138 is altered, suggesting that the MIA complex exerts its regulatory effects, at least in part, through the kinases/phosphatases responsible for IC138 modification; most likely by ensuring their correct positioning relative to IC138. Double mutants that lack both the MIA complex and the I1/f dynein exhibit essentially the same phenotype as *mia* mutants alone, providing further support for the idea that signals from the MIA complex propagate their effects through the I1/f dynein. Interestingly, although the MIA complex appears to physically interact with the N-DRC, double mutants lacking both MIA and the N-DRC exhibit a more severe flagella paralysis defect, suggesting that MIA-based signaling is likely independent of the N-DRC; similarly, lack of the MIA complex and several monomeric inner arms also resulted in more severe phenotypes. Another key observation is that mutants defective for the radial spoke head domain (which interacts with the central pair) and MIA exhibit reduced microtubule sliding velocities compared with either single mutant alone, suggesting that the

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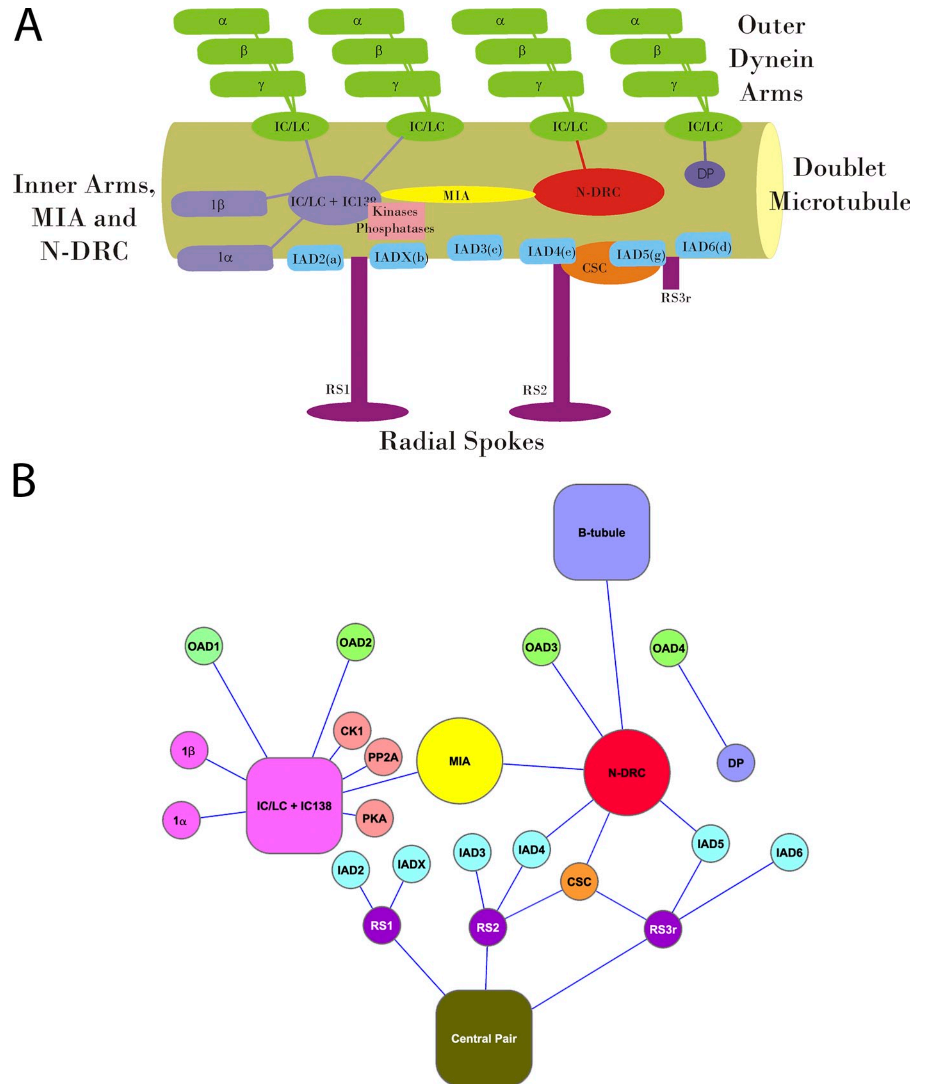
Figure 1. **Overview of the 96-nm axonemal repeat and dynein control pathways.** (A) Cartoon illustrating the approximate location on the outer doublet A-tubule of the various dyneins and regulatory structures within a single 96-nm axonemal repeat. The α, β, and γ heavy chains of the outer arm stack upon each other, with the α heavy chain outermost. (B) This systems level overview of one 96-nm axonemal repeat illustrates the key players and interactions in the solid-state dynein control pathways that are sufficient to generate ciliary/flagellar beating upon addition of ATP to demembranated axonemes. The color code is outer arm dyneins (OAD), light green; inner arm dyneins (IAD), cyan; inner arm I1/f, dark pink; N-DRC, red; MIA complex, yellow; the CSC, orange; radial spokes, purple; central pair microtubule complex, brown; adjacent outer doublet B-tubule, blue; kinases/phosphatases, pink; and distal protrusion (DP), light blue. The monomeric inner arm dynein nomenclature is IAD2, dynein a (DHC6); IADX, dynein b (DHC5); IAD3, dynein c (DHC9); IAD4, dynein e (DHC8); IAD5, dynein g (DHC7); and IAD6, dynein d (DHC2). Note that patterns of linkage between outer arms and other components vary depending on the microtubule doublet and position along the axonemal length, and ATP-dependent interactions between outer arm dyneins and inner arm dyneins with the B-tubule of the adjacent doublet are not illustrated for clarity. IC/LC, intermediate chain/light chain.



MIA complex forms part of an additional signal transduction pathway in addition to that propagated through the radial spokes.

Although the MIA complex is not observed to interact with the outer row of dynein arms, the *mia* mutants do exhibit a significant decrease in flagellar beat frequency, which is a classic sign of reduced outer arm power output (Kamiya and Okamoto, 1985; Mitchell and Rosenbaum, 1985). One clear possibility is that the MIA complex acts as a conduit for a regulatory signal that reaches the outer arms through a series of recently identified linkers present on subsets of doublet microtubules that connect these motors with inner arm I1/f and the N-DRC (Nicastro et al., 2006; Bui et al., 2012). Indeed, the stability of the I1/f dynein appears dependent on both the MIA complex and the outer arms, as mutants lacking both these structures have dramatically reduced amounts of the I1/f motor.

Finally, phylogenetic analysis (Yamamoto et al., 2013) revealed that potential orthologues of *C. reinhardtii* MIA complex components are present in vertebrates, and RNASeq data from the Human BodyMap 2.0 project (http://rnaseq.crg.es/project/HBM) indicate they are highly expressed in ciliated/flagellated tissues, such as testis, ovary, and lung. Furthermore, one potential orthologue is encoded at a locus thought to be required for normal lung function (Soler Artigas et al., 2012), whereas expression of a second is altered in lung carcinoma (Kwon et al., 2012). Thus, the MIA complex represents a highly conserved and essential feature of the axonemal superstructure that occupies a central position in the key signal transduction pathways required for ciliary beating.

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