Jump-starting kinesin

David D. Hackney

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213

When it is not actively transporting cargo, conventional Kinesin-1 is present in the cytoplasm in a folded conformation that cannot interact effectively with microtubules (MTs). Two important and largely unexplored aspects of kinesin regulation are how it is converted to an active species when bound to cargo and the related issue of how kinesin discriminates among its many potential cargo molecules. Blasius et al. (see p. 11 of this issue) report that either binding of the cargo linker c-Jun N-terminal kinase–interacting protein 1 (JIP1) to the light chains (LCs) or binding of fasciculation and elongation protein ζ1 (FEZ1) to the heavy chains (HCs) is insufficient for activation but that activation occurs when both are present simultaneously. A related paper by Cai et al. (see p. 51 of this issue) provides structural insight into the conformation of the folded state in the cell obtained by fluorescence resonance energy transfer analysis.

Conventional kinesin (designated Kinesin-1 in the standard nomenclature for the kinesin superfamily; Lawrence et al., 2004) is a motor protein that is responsible for movement of a wide range of cargoes along MTs. Kinesin from animals is a heterotetramer that contains two HCs and two LCs, as indicated in Fig. 1 for *Drosophila melanogaster* Kinesin-1. A large number of proteins has been shown by proteomic methods to interact with kinesin (Adio et al., 2006; Gindhart, 2006), and many of these are likely to be cargo molecules or regulators. The tetratrico peptide repeat (TPR) domains of the LCs are a major site for the binding of cargo/scaffold proteins such as JIP1, which links kinesin to vesicles (Verhey et al., 2001). Other cargo molecules have been shown to bind directly to the HCs, and the binding sites for several have been mapped to Coil-4a,b, which also plays a critical role in fungal kinesins (Seiler et al., 2000). Under physiological conditions, the soluble kinesin heterotetramer is in a compact, inhibited conformation that is produced by the interaction of a region in the tail with the head/neck region. The region in the tail that is required for folding has been localized to Coil-4c (aa residues 910–930) and the adjoining positively charged region 928–937, but the downstream conserved IAK region is also required for full inhibition of MT-stimulated ADP release (Hackney and Stock, 2000). Although LCs are not required for folding, the LCs would be in close proximity to the motor domains in the folded conformation, where they could play a role in modulating the properties of the complex. The addition of LCs both shifts the salt dependence of unfolding (Hackney et al., 1992) and reduces the MT affinity of the HCs to such a great extent that tight binding to MTs is not observed even in the presence of AMPPNP at pH 7.2–7.4 (Verhey et al., 1998).

An attractive model for how the inhibited folded conformation could be activated was for cargo binding to shift the equilibrium toward the active unfolded conformation. However, any cargo-induced changes would have to be indirect, as the cargo-binding regions on both the LCs and HCs are physically separate from the region in the tail of the HC that binds to the head/neck to produce the folded conformation (Fig. 1). Blasius et al. (2007) have now shown that even such an indirect effect is not likely to be a major factor because cargo binding alone is insufficient for activation, at least for the major LC cargo JIP1.

What activates the folded species, if not cargo binding? Posttranslational modification such as phosphorylation does play a role in the detachment of cargo (Morfini et al., 2004), but its role in the activation of kinesin is not as well established. The study by Blasius et al. (2007) provides a mechanism for activation through the simultaneous binding of JIP1 to the LCs and FEZ1 to the HCs. *Drosophila* UNC-76 is the homologue of FEZ1, and it had previously been shown to bind to the tail region of the HC (Gindhart et al., 2003). Blasius et al. (2007) have now further shown that FEZ1 is unlike most other potential cargoes or regulators that interact with the tail of kinesin in that the binding site for FEZ1 likely includes part of the region that binds to the head/neck region. Specifically, they have shown that mutation of the positively charged cluster (aa 929–938 for *Drosophila* HC in Fig. 1 and aa 908–917 in the rat HC (KIF5C) used by Blasius et al. [2007]) between Coil-4c and the IAK region prevents interaction with the head/neck region in a yeast two-hybrid assay. Because inclusion of the positively charged cluster in the tail is required for folding (Stock et al., 1999), the binding of FEZ1 could potentially produce unfolding by direct competition with the head/neck region. However, FEZ1 alone is also not sufficient for activation. It will be of interest to see whether this requirement for the dual activation by cargo and a potential direct disruptor of folding will become the general pattern for both HC and LC cargoes.

Correspondence to David D. Hackney: ddh@andrew.cmu.edu

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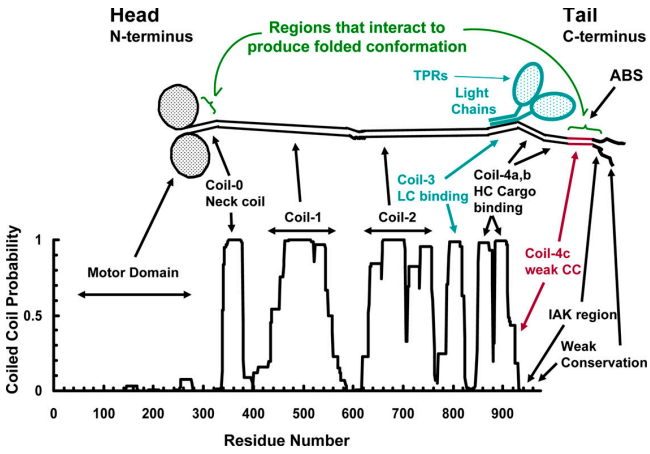

Figure 1. **Coiled-coil prediction for the HC of *Drosophila* Kinesin-1 and corresponding schematic representation of the domain organization.** The two motor domains are connected to the neck coil by the neck linker and are followed by the long coiled-coil stalk composed of coil-1 and coil-2 (de Cuevas et al., 1992). The coiled-coil region near the N terminus of the LCs binds to coil-3 of the HCs (Diefenbach et al., 1998) to anchor the cargo-binding TPR domains of the LCs to the HCs. Coil-4a,b is a site for the binding of at least some cargoes to the HC, as first indicated by its importance for cargo transport in *Neurospora crassa* (Seiler et al., 2000) and later by the direct mapping of cargo-binding sites for animal kinesins. The whole region between aa 850–930 (aa 828–908 in rat and human kinesin) is highly conserved in animal kinesins and is predicted to be in a coiled-coil conformation when calculated with a window size of 28 residues, but with the aa 910–930 region (Coil-4c) more weakly predicted and in a different heptad frame. At the more stringent window size of 21 residues shown here, Coil-4c is not well predicted. Coil-4c is followed by a region with an excess of positive charge that is critical for both MT and head/neck interaction and by the highly conserved IAK (Stock et al., 1999) region that is required for the inhibition of ATPase in the folded conformation. The C-terminal region beyond the IAK domain is not well conserved and is likely to be unstructured.

One possible explanation for the requirement of both JIP1 and FEZ1 is that the binding of each separately destabilizes the folded conformation, but their combined action is needed to produce sufficient activation to register in assays using lysates from cells that express tagged proteins. The lysate method has the advantage that normal cellular processes are involved, but it needs to be complemented with reconstitution studies using purified components to allow better quantification and to distinguish direct from indirect effects. For example, either the addition of LCs or an increase in pH favors unfolding, as indicated by a decrease in the salt concentration required to produce 50% unfolding (Hackney et al., 1992). A simple model in which unfolding alone is responsible for activation would predict that the addition of LCs or higher pH should increase activation, yet the opposite result is observed (Verhey et al., 1998). The observation by Cai et al. (2007) of two different conformational transitions in the folded species may provide a mechanism to account for these complexities. An additional consideration is that HC dimers are active in the assay used by Blasius et al. (2007), as defined by their ability to bind to MTs at pH 7.2 in the presence of AMPPNP, but they are inactive in vitro as defined by their negligible MT-stimulated ATPase and affinity for MTs in the presence of ATP (Hackney and Stock, 2000).

The kinesin HC has both a nucleotide-dependent MT-binding site in the motor domain and a nucleotide-independent auxiliary binding site (ABS) in the tail region (Navone et al., 1992). The tail ABS has been localized to the same aa 910–937 region that interacts with the head/neck region to produce the folded conformation (Hackney and Stock, 2000; Yonekura et al., 2006). Full-length folded kinesin has negligible affinity for MTs in the presence of ATP and, thus, cannot bind tightly to the MT through either the motor domains or the tail ABS. Dimers of HCs that are truncated at position 937 are more weakly folded than longer constructs and bind to MTs even more strongly than short, unfolded dimers (Hackney and Stock, 2000). This high affinity for MTs likely results from the combined affinity of the heads and the ABS that can occur when the ABS is unmasked in this weakly folded construct. Further truncation to position 927 removes the key positively charged region of the ABS with consequent loss of tight MT binding and superactivation. This role of the ABS in the superactivation of kinesin suggests another possible layer of complexity in the effects of FEZ1. The binding of FEZ1 to the aa 910–937 region may not only perturb the interaction of this region with the head/neck region but may also modulate the interaction of the ABS with MTs. A complete understanding of regulation will require determination of the complex balance between a number of interacting components.

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