JCB Comment

Breathing down the neck of Unc104

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The Unc104/Kif1A class of kinesins transports synaptic vesicle precursors along microtubules with high speed and processivity that has been proposed to depend on reversible dimerization between two poorly motile monomers. In this issue, Al-Bassam et al. (2003) discover a structural basis for regulation of motility by reversible dimerization.

Kinesins represent a large superfamily of molecular motors specialized for unidirectional transport of cargo along cellular microtubule arrays. Members of the kinesin superfamily of microtubule-stimulated ATPases are identified by high sequence identity within a highly conserved ~40-kD core motor domain. However, kinesin subfamilies have very different styles of motility that are related to their functional specialization. Some kinesins are fast and can travel processively for long distances (many ATP hydrolysis cycles) without letting go of the microtubule. Some are slow and meander over the surface of the microtubule, letting go easily. Most travel toward the plus ends of microtubules, but some travel in the opposite direction. Others depolymerize microtubules from both ends. A simple but effective classification scheme for the kinesin superfamily based on the position of the motor domain within the primary amino acid sequence was originally proposed by Vale and Fletterick (1997). This scheme was based on a consistent correlation that was noted by researchers studying the motility of different kinesins. All kinesins identified thus far that exhibit anterograde (plus end–directed) motility along microtubules possess a motor domain at the NH_2 terminus of the protein (Kin N), whereas all known retrograde kinesins possess a COOH-terminal motor domain (Kin C). Those kinesins possessing an internally located motor (Kin I) exhibit microtubule-depolymerizing activity. Focus turned to the motor domain as a potentially important predictor of basic function. Yet, as it turned out, the crystal structures of the core motors for a Kin N versus a Kin C kinesin were surprisingly similar, despite fundamental differences in motile characteristics (Kull et al., 1996; Sablin et al., 1996). Later studies demonstrated that subclass-specific functional refinements, such as directionality and processivity, are conferred upon the motor domain by the "neck" domain (Henningsen and Schliwa, 1997; Endow and Higuchi, 2000). The neck is the region just outside of the core motor domain as defined by the crystal structure of the human ubiquitous kinesin heavy chain (KHC) motor (KNS1) (Kull, et; al., 1996). The neck can be found either NH_2 terminal or COOH terminal to the core motor (historically referred to as the "head") and is highly conserved within kinesin subfamilies. Vale and Fletterick (1997) noted that the conserved neck domains are probably the most likely determinant of kinesin functional specialization and used this sequence conservation to further classify kinesin family members.

What exactly is the neck? If the neck were functionally defined as the minimal region necessary for full-strength motility in combination with the core motor, this would require that the poorly conserved "hinge" domain be included in the definition of the neck for *Syncephalastrum racemosum* KHC (Grummt et al., 1998). Also, it has been observed for Ncd that velocity decreases linearly with the length of the coiled coil, which extends for ~150 aa (Yun et al., 2003), suggesting that the entire coiled-coil region of Ncd be included to satisfy this definition of the neck. Until all the functional data is in, it is safer to restrict the neck to the most highly conserved domains adjacent to the core motor. Following this convention, the KHC neck consists of a "neck linker" and "neck coiled coil". The small, mobile neck linker (connecting the core motor with the rest of the neck) is predicted to be capable of cyclic interactions with the motor domain in a nucleotide-dependent manner that serves to coordinate hand-over-hand motility (Rice et al., 1999). The rest of the KHC neck (neck coiled coil) confers processivity by mediating dimer formation, thus allowing inter-head communication (Huang et al., 1994), and by tethering the motor electrostatically to the microtubule surface (Thorn et al., 2000). However, necks predict functional specialization, so they vary between kinesin subclasses and do not all contain the same consistent motifs. Kin I kinesin necks may only serve a tethering purpose (Ovechkina et al., 2002). Necks of other kinesins can directly interact with the motor domain, influencing directionality (Endow and Higuchi, 2000) and affecting the ATP hydrolysis cycle (Schafer et al., 2003). All of these activities have been demonstrated to be essential for efficient

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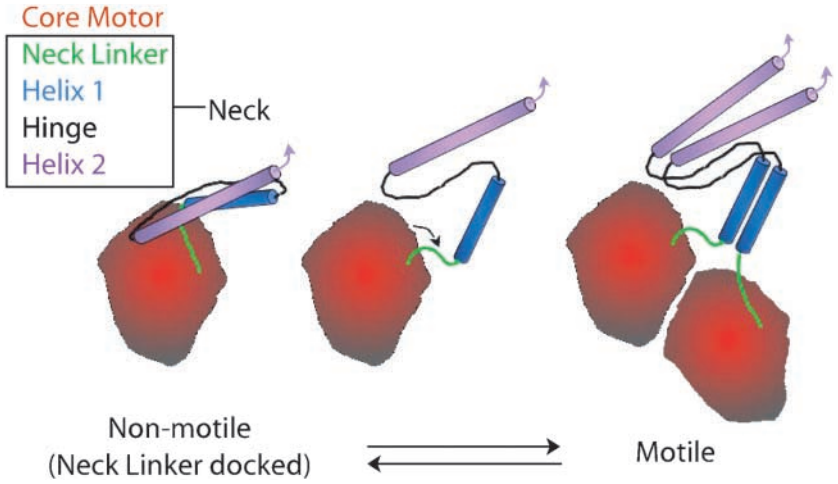

Figure 1. **A model for the conversion of Unc104 from an autoinhibited, monomeric state to motile, dimeric state.** Docking of the neck linker in the ATP-bound state promotes the self-association of the neck, stabilizing the monomer. Physiologically, this monomer–dimer transition may occur when the motors become clustered on a membrane surface.

motility in kinesins. Removal or inactivation of the neck via mutations severely cripples motility for all kinesins for which this has been tested. Precisely because of the high level of conservation of necks within functional subclasses of kinesins and its role as the "fundamental engine" for motility, the neck is a ripe target for the regulation of motility in vivo.

The Unc104/Kif1A class of motile Kin N kinesins has a most interesting, large, and controversial neck. Unc104/Kif1A consists of a neck linker that resembles KHC followed by two α-helices with a weak propensity for coiled-coil formation separated by a hinge. Full-length Unc104 and Kif1A are monomers in solution. A single, monomeric molecule of mouse Kif1A trimmed down so that it consists of only the core motor plus neck linker can travel processively at ~0.28 μm/s via directionally biased diffusional motility that is dependent on electrostatic tethering between the negatively charged surface of the microtubule and a positively charged lysine loop within the core motor (Okada and Hirokawa, 2000; Okada et al., 2003). This is an astounding observation and has much to tell us about the contribution of diffusion to kinesin motility. However, single Unc104 monomers have never exhibited this type of processive motility (Pierce et al., 1999; Tomishige et al., 2002). Instead, rapid processive motility (~2.4 μm/s), which is the hallmark of the Unc104/Kif1 class of motors, is only observed when the motor is aggregated on liposomes or artificially dimerized. Mutational disruption of the weak neck coiled coil eliminates rapid, processive motility (Tomishige et al., 2002). This paradox leads to the hypothesis that the Unc104/Kif1A class of motors associates to form weak dimers that are undetectable under solution conditions and that this dimer is the unit of fast processive movement. Tomishige et al. (2002) illustrated the functional potential of positive regulation by dimerization by artificially converting the nonprocessive Unc104 monomer to highly motile processive dimers. This highly processive dimer cannot be fully substituted for by a collection of monomers working together. Now a fascinating structural study by Al-Bassam et al. (in this issue) suggests a model for the structural regulation of reversible dimerization.

Because many kinesin necks are mobile and relatively small, they are invisible in most structural studies (Kull et al., 1996; Sablin et al., 1996; but see Turner et al., 2001 for an exception). Unexpectedly, Al-Bassam et al. (2003) have observed a highly ordered (and visible!) folded neck structure in the ATP-bound state of Unc104 monomers using cryo-EM. The authors cleverly demonstrate that the highly folded neck represents a parallel coiled coil that forms between neck helix 1 and neck helix 2 (Fig. 1). In contrast, an unfolded neck would be invisible by cryo-EM irrespective of whether it was promoting dimerization. No evidence of dimer formation is seen in the ATP-bound state of Unc104 when the neck is visible, suggesting that the folded neck is correlated with the monomeric state. This is an important observation because it suggests that the folded neck is fostering monomerization. If dimerization were at all possible, then it would undoubtedly be promoted by this experimental technique. Cryo-EM and 3D reconstructions are performed, by necessity, by binding the motor to the microtubule under saturating conditions. Saturating the microtubule with motors has previously been shown to promote dimerization in situations in which the motor is monomeric in solution (Hoenger et al., 2000). However, it is worth noting that there is a situation in which dimerization can be missed by cryo-EM. If a dimeric motor is bound to microtubules under less than saturating conditions, dimerization can be missed if the neck linking the two kinesin motor domains is flexible enough to allow strong binding of the core motors to two adjacent tubulin dimers. This has been reported to occur with rat KHC dimers (Hoenger et al., 2000). Because the ATP-bound Unc104 neck is visible and folded, it is highly unlikely that the cryo-EM image represents a dimer strongly bound to two tubulin dimers. One small caveat is that when the authors delete the hinge region that mediates folding, the neck disappears as expected but without the concurrent appearance of a second head. This is interesting because the hingeless construct is still capable of fairly robust motility that would likely be associated with dimerization. Yet the authors do not observe a second head associating with the hingeless motor. Therefore, either the high affinity of the construct for microtubules resulted in strong binding of two dimerized heads to adjacent dimers or the dimer is too weak to be detectable.

The neck linker of Unc104, which resembles that of KHC, may undock from the motor in the ADP state, as predicted by Rice et al. (1999). This undocking occurs commensurately with the disappearance of the rigid folded neck and the ap-

pearance of a second head, indicating dimerization of Unc104 in the ADP state (Fig. 1). This is the first time that reversible dimerization of Unc104 has been directly confirmed, although it had been detected biochemically when the Unc104/Kif1 family member mouse Kif1C was overexpressed in cultured mammalian cells (Dorner et al., 1999). Although Dorner et al. (1999) did not correlate dimerization with motility, they did suggest that reversible dimerization might represent a mechanism of regulation. The monomeric version of Unc104 with tightly folded neck described by Al-Bassam et al. (2003) suggests a structural model for inactivating Unc104. A nonmotile monomeric conformation might be promoted by self-association within the neck domain when the concentration of Unc104 is low. Clustering of Unc104 would favor dissociation of the intraneck coiled coil in favor of interneck interactions, promoting dimerization and efficient motility. The visualization of these conformations under saturating conditions is, like many seminal observations, a lucky accident probably stemming from the response of the neck linker to the ATP state (Fig. 1). It is highly unlikely that the protein would transition from monomer to dimer with each ATP hydrolysis cycle. Furthermore, kinesins in solution, with tightly bound ADP, would not necessarily automatically assume this inactive conformation. Rather, the authors propose that the monomeric conformation with tightly folded neck is likely to be promoted by other components. A candidate for modulation of the neck is the intriguing forkhead-associated (FHA) domain COOH terminal to the neck. Loss of the FHA domain disrupts worm movement in vivo and suspiciously increases microtubule affinity of Unc104 in vitro without much loss in MT gliding velocities. It would be interesting to use cryo-EM to see whether the FHA domain is in a structurally coherent location when Unc104 is inactivated. Also, it is essential to determine what protein the Unc104 FHA domain binds to in the cell. In this way, the activation of Unc104 can be understood within the context of cell signaling. FHA domains are protein modules that preferentially recognize phosphothreonine epitopes potentially linking the inactivation/activation of Unc104/Kif1 motility into the richly diverse cellular kinase signaling pathways.

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References

Al-Bassam, J., Y. Cui, D. Klopfenstein, B.O. Carragher, R.D. Vale, and R.A. Milligan. 2003. Distinct conformations of the kinesin Unc104 neck regulate a monomer to dimer motor transition. *J. Cell Biol.* 163:743–753.

Dorner, C., A. Ullrich, H.U. Haring, and R. Lammers. 1999. The kinesin-like motor protein KIF1C occurs in intact cells as a dimer and associates with proteins of the 14-3-3 family. *J. Biol. Chem.* 274:33654–33660.

Endow, S.A., and H. Higuchi. 2000. A mutant of the motor protein kinesin that moves in both directions on microtubules. *Nature.* 406:913–916.

Grummt, M., G. Woehlke, U. Henningsen, S. Fuchs, M. Schleicher, and M. Schliwa. 1998. Importance of a flexible hinge near the motor domain in kinesin-driven motility. *EMBO J.* 17:5536–5542.

Henningsen, U., and M. Schliwa. 1997. Reversal in the direction of movement of a molecular motor. *Nature.* 389:93–96.

Hoenger, A., M. Thormahlen, R. Diaz-Avalos, M. Doerhoefer, K.N. Goldie, J. Muller, and E. Mandelkow. 2000. A new look at the microtubule binding patterns of dimeric kinesins. *J. Mol. Biol.* 297:1087–1103.

Huang, T.G., J. Suhan, and D.D. Hackney. 1994. *Drosophila* kinesin motor domain extending to amino acid position 392 is dimeric when expressed in *Escherichia coli*. *J. Biol. Chem.* 269:16502–16507.

Kull, F.J., E.P. Sablin, R. Lau, R.J. Fletterick, and R.D. Vale. 1996. Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature.* 380:550–555.

Okada, Y., and N. Hirokawa. 2000. Mechanism of the single-headed processivity: diffusional anchoring between the K-loop of kinesin and the C terminus of tubulin. *Proc. Natl. Acad. Sci. USA.* 97:640–645.

Okada, Y., H. Higuchi, and N. Hirokawa. 2003. Processivity of the single-headed kinesin KIF1A through biased binding to tubulin. *Nature.* 424:574–577.

Ovechkina, Y., M. Wagenbach, and L. Wordeman. 2002. K-loop insertion restores microtubule depolymerizing activity of a "neckless" MCAK mutant. *J. Cell Biol.* 159:557–562.

Pierce, D.W., N. Hom-Booher, A.J. Otsuka, and R.D. Vale. 1999. Single-molecule behavior of monomeric and heteromeric kinesins. *Biochemistry.* 38: 5412–5421.

Rice, S., A.W. Lin, D. Safer, C.L. Hart, N. Naber, B.O. Carragher, S.M. Cain, E. Pechatnikova, E.M. Wilson-Kubalek, M. Whittaker, et al. 1999. A structural change in the kinesin motor protein that drives motility. *Nature.* 402: 778–784.

Sablin, E.P., F.J. Kull, R. Cooke, R.D. Vale, and R.J. Fletterick. 1996. Crystal structure of the motor domain of the kinesin-related motor ncd. *Nature.* 380:555–559.

Schafer, F., D. DeLuca, U. Majdic, J. Kirchner, M. Schliwa, L. Moroder, and G. Woehlke. 2003. A conserved tyrosine in the neck of a fungal kinesin regulates the catalytic motor core. *EMBO J.* 22:450–458.

Thorn, K.S., J.A. Ubersax, and R.D. Vale. 2000. Engineering the processive run length of the kinesin motor. *J. Cell Biol.* 151:1093–1100.

Tomishige, M., D. Klopfenstein, and R.D. Vale. 2002. Conversion of Unc104/Kif1A kinesin into a processive motor after dimerization. *Science.* 297: 2263–2267.

Turner, J., R. Anderson, J. Guo, C. Beraud, R. Fletterick, and R. Sakowicz. 2001. Crystal structure of the mitotic spindle kinesin Eg5 reveals a novel conformation of the neck-linker. *J. Biol. Chem.* 276:25496–25502.

Vale, R.D., and R.J. Fletterick. 1997. The design plan of kinesin motors. *Annu. Rev. Cell Dev. Biol.* 13:745–777.

Yun, M., C.E. Bronner, C.-G. Park, S.-S. Cha, H.-W. Park, and S. Endow. 2003. Rotation of the stalk/neck and one head in a new crystal structure of the kinesin motor protein, Ncd. *EMBO J.* 22:5382–5389.