Malleable folding of coiled-coils regulates kinesin-3 dimerization

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Microtubules (MTs) form polarized intracellular fibers inside eukaryotic cells that organize the cytoplasm, form tracks for long-range vesicle transport, and generate forces to align and segregate chromosomes during cell division. The discovery of conventional kinesin (termed kinesin-1) over three decades ago revealed a vast superfamily dedicated to transporting a variety of intracellular cargos or generating forces in a variety of cellular functions (1). Kinesins are now organized into 14 subfamilies which share class-conserved motor domains that promote directional motility or MT dynamic polymerization/dynamoregulation by adenosine 3,5 triphosphate (ATP) hydrolysis-driven cyclical binding and release from the MT lattice (2, 3). The majority of kinesin subfamilies oligomerize into dimers or tetramers via coiled-coil folding of an α-helical region named the neck coil (NC), which lies in close proximity to the motor domain (4, 5). The NC dimerizes motor domains and couples their conformational changes driven by ATP hydrolysis via a connecting element termed the neck linkers (NLs) (6). Kinesins utilize these elements to couple dual-motor domains to undertake alternating steps in a persistent hand-over-hand walking mechanism along MTs (7). In the kinesin-1 subfamily, cargo-binding domains located at the opposite end of the polypeptide directly inhibit the motor domains to prevent wasteful motility and ATP hydrolysis in the absence of cargo (8).

Although the majority of kinesins are oligomeric, the diverse kinesin-3 subfamily represents the clear exception by being monomeric (9). The kinesin-3 subfamily includes Caenorhabditis elegans UNC-104 and its mammalian homologs KIF1A/B/C, KIF13A/B, KIF16A/B, and KIF26A/B (5). Native KIF1A and recombinant UNC-104 can be purified as globular compact monomers (9, 10). The kinesin-3 subfamily shares a conserved and specialized motor domain that includes a lysine-rich insert in loop 12 (K-loop), located near its MT binding interface. The kinesin-3 subfamily also shares a conserved α-helical NC with poor propensity for coiled-coil folding, which is followed by two α-helical regions [coiled-coil 1 (CC1) and CC2] that exhibit high coiled-coil folding propensity and flank a conserved forhead-associated (FHA) homology domain between them (Fig. 1). The kinesin-3 subfamily includes a conserved membrane-associated guanylate kinase homolog binding stalk (MBS) and binds diverse cargos through different types of C-terminal Plekstrin (PH), Phox (PX), or Cap-Gly homology domains (Fig. 1) (5, 11). Among the best-studied kinesin-3 proteins are UNC-104 and its ortholog, KIF1A, which transport presynaptic vesicles in neuronal axons (9, 12). Their defects lead to a decrease in synaptic signaling in neurons, producing an uncoordinated phenotype in C. elegans or severe disturbances in knockout mice, which die at birth (13, 14). Mutations inactivating human KIF1A are linked to a neurological disorder characterized by cognitive impairment, neuropathy, and paraplegia (15, 16), while mutations in KIF1B are linked to the neurological disease Charcot-Marie-Tooth type 2B (17).

KIF1A was initially thought to undergo motility as monomers by skating diffusively along the MT lattice, mediated via the weak interactions of the K-loop with the tubulin C termini (18). Together with the poor coiled-coil dimerization capacity of the NC and CC1, KIF1A motility was suggested to be different from hand-over-hand motility exhibited by dimeric kinesins (18). However, subsequent studies have demonstrated that the monomeric kinesin-3 state is a self-inhibited conformation, primarily suppressing premature dimerization and preventing motility without cargo (11, 19). Increasing kinesin-3 loading onto cargo, mediated via PH, PX, and Cap-Gly cargo-binding domains, activates its dimerization, leading to rapid and persistent hand-over-hand motility (19, 20). Consistently, this kinesin-3 self-regulation mechanism can be overcome either by artificially increasing solution concentration of kinesin-3 or by placing sequences with high coiled-coil folding capacity after CC1 to create artificially stable kinesin-3 dimers (20). Once dimerized, kinesin-3 motors undergo persistent hand-over-hand walking motility similar to kinesin-1 (11, 20). The kinesin-3 motor domain, NC, CC1 α-helices, and the FHA domain are critical to form this monomeric

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self-inhibitory state, which down-regulates motor activity and ATP hydrolysis, resulting in weak diffusion along MTs (11, 20, 21). Low-resolution cryoelectron microscopy structures of UNC-104 with analogous elements bound to MTs suggest that CC1 forms an intramolecular coiled-coil with the NC, which is rigidly anchored onto the motor domain. Dimeric kinesin-3 states were observed bound to MTs at higher UNC-104 concentration, but only in the absence of ATP (21). Despite extensive study, the nature of kinesin-3’s self-regulated monomeric state and how these conserved elements modulate kinesin-3 dimerization have remained mostly out of reach due to a lack of high-resolution structural information.

In PNAS, Ren et al. (22) finally solve this puzzle utilizing a combination of biochemistry and X-ray crystallography. The authors studied the mechanism of kinesin-3 self-regulated dimerization by determining structures of KIF13B self-folded monomeric and dimerized conformations (Fig. 1). Utilizing two KIF13B constructs that differ by either the presence or absence of CC1, they show that CC1 is essential to form the kinesin-3 monomeric state in the presence of the NC, NL, and motor domains, producing a poorly ATP-hydrolyzing motor, even in the presence of MTs. Deletion of CC1 led to spontaneous kinesin-3 dimerization and increased MT-activated ATP hydrolysis. Ren et al. (22) present X-ray structures of the monomeric and dimeric kinesin-3, revealing the long-sought role of CC1 in the transition to dimerization. In the monomeric state, CC1 and NC α-helices form an antiparallel intramolecular coiled-coil assembly. However, only the N-terminal section of CC1, termed CC1a, folds onto the NC. The authors discovered that the C-terminal section of CC1, termed CC1b, surprisingly folds onto the kinesin-3 motor domain, binding class-conserved residues extending from elements such as β4, α3, and loop 8 that face away from the MT surface (Fig. 1). Thus, CC1b forms the equivalent of a safety belt, further stabilizing the kinesin-3 monomeric CC1-NC folded state and inhibiting its disassembly (22).

Furthermore, the base of the NC-CC1a antiparallel coiled-coil is seen to be anchored rigidly onto the motor domain via a network of ionic and hydrophobic interactions with the kinesin-3 specialized β5-β6 hairpin. In the dimeric state, the kinesin-3 NC forms a stable parallel dimeric coiled-coil in the absence of CC1, leading to a homodimeric motor with an organization generally similar to kinesin-1. In full-length kinesin-3 proteins, the release of CC1a and CC1b from the kinesin-3 monomeric state likely promotes CC1 homodimerization, up to the FHA domains, as demonstrated previously (Fig. 1) (23), likely further stabilizing the newly dimerized kinesin-3 motor. Comparison of the NC conformation in the two states reveals its 90° rotation toward the motor domain in the self-folded state, preventing the NC and NL from assisting
motility, and its dissociation in the dimeric state freeing its ability to dimerize (22). The comparison also reveals that ionic- and hydrophobic-specific interactions, in some cases mediated by the same NC amino acid residues that participate in forming both states by interacting either with elements of the dimeric NC or with residues in CC1a. However, there are many more specialized ionic and hydrophobic interactions in the monomeric kinesin-3 state involving residues in the NC, CC1a, CC1b, and motor domains, making this state clearly dominant and more stable (Fig. 1) (22).

With such a detailed understanding of amino acid interactions stabilizing the self-folded monomeric state, Ren et al. (22) next sought to understand whether disrupting those interactions can autoactivate kinesin-3 dimerization and motility. Thus, the authors studied the biochemical and in vivo motility activities of a slew of structure-based KIF13B mutants in which some or many of these interactions were disrupted, by creating the mutants of KIF13B in living cells and biochemically probing their dimerization and ATP hydrolysis activities. These studies reveal clear support for the importance of this network of residue interactions in regulating kinesin-3 dimerization and activity (22). This study highlights the malleability of kinesin-3 coiled-coil elements and their coevolution in promoting this form of self-regulation. In combination with previous studies, this work cemented the importance of these conserved molecular features in regulating spontaneous kinesin-3 dimerization as a means of avoiding wasteful ATP-hydrolysis (Fig. 1). Despite the diversity of kinesin-3 cargo-binding domains, cargoS seem sufficient to concentrate motors to overcome this self-regulated inhibition (11).

Important questions remain regarding the nature of this kinesin-3 transition. How does the self-inhibited monomeric conformation become destabilized in the presence of cargo and the MT lattice? Does the transition occur in solution or does the MT lattice form a platform for the transition? Does the diffusion of self-regulated monomers on the MT lattice play a role in the transition? Are there any posttranslational modifications, such as phosphorylation or binding proteins, that stabilize either the self-inhibited or the dimeric conformation? Future studies should also aim to tease out the differences in the regulation between the different forms of kinesin-3 motors and how the FHA domain, CC1, CC2, and MBS may influence the cooperativity of the transition to dimerization. The role of MTs and cargo in kinesin-3 activation may also be elucidated.

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