Kinesin Assembly and Movement in Cells

Kristen J. Verhey, Neha Kaul, and Virupakshi Soppina

1Department of Cell and Developmental Biology, 2Department of Mechanical Engineering, University of Michigan, Ann Arbor, Michigan 48109; email: kverhey@umich.edu, nehakaul@umich.edu, vsoppina@umich.edu

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Abstract
Long-distance transport in eukaryotic cells is driven by molecular motors that move along microtubule tracks. Molecular motors of the kinesin superfamily contain a kinesin motor domain attached to family-specific sequences for cargo binding, regulation, and oligomerization. The biochemical and biophysical properties of the kinesin motor domain have been widely studied, yet little is known about how kinesin motors work in the complex cellular environment. We discuss recent studies on the three major families involved in intracellular transport (kinesin-1, kinesin-2, and kinesin-3) that have begun to bridge the gap in knowledge between the in vitro and in vivo behaviors of kinesin motors. These studies have increased our understanding of how kinesin subunits assemble to produce a functional motor, how kinesin motors are affected by biochemical cues and obstacles present on cellular microtubules, and how multiple motors on a cargo surface can work collectively for increased force production and travel distance.
INTRODUCTION

Microtubules are cylindrical cytoskeletal filaments whose polymerization from heterodimeric α- and β-tubulin subunits results in a structural and functional polarity of the two ends. Microtubules and their associated proteins carry out a variety of important functions in cells, from structural support to cell division to movement within and of cells. Movement along microtubule tracks is driven by molecular motors in the kinesin and dynein families (Figure 1), and abnormalities in motor-driven transport have been linked to a wide range of human diseases including neurodegenerative diseases, polycystic kidney disease, and cancer (46, 117, 153).

Kinesin motors are enzymes that convert the chemical energy of ATP hydrolysis into mechanical work along microtubule tracks. Most of our understanding of kinesin mechanics comes from work on kinesin-1 (formerly conventional kinesin or KIF5) (88), the founding member of the kinesin superfamily. Minimal kinesin-1 motors, which contain only the motor domain and dimerization sequences, move processively (maintain an interaction with the microtubule for many rounds of catalysis) toward the plus end of the microtubule in 8-nm steps (the distance between adjacent β-tubulin subunits) and generate speeds of 0.6–0.8 μm s\(^{-1}\). Processivity is due to a hand-over-hand stepping mechanism in which the two motor domains alternate their catalytic cycles in order to maintain the motor-microtubule interaction (reviewed in References 45 and 133).

Molecular, genetic, and biochemical analyses of organisms from fungi to animals have revealed a superfamily of proteins that have in common a kinesin motor domain (≈350 amino acids, with ≈40% amino acid identity within the superfamily). On the basis of homology between motor domains, the kinesin-related proteins have been placed in 14 families (kinesin-1 to kinesin-14) (63, 88, 98). One of the biggest surprises in studying the mechanics of kinesin motors is that the catalytic motor domain has been adapted over evolution for functions other than movement to microtubule plus ends. For example, kinesin-14 motors undergo directional motility to the minus ends of microtubules. And in kinesin-8 and kinesin-13 motors, catalytic activity results in destabilization or depolymerization of microtubules. Outside the motor domain, each kinesin family contains distinct sequences for cargo binding, oligomerization, and regulation (Figure 2).

Motor-dependent transport along microtubules takes place with remarkable efficiency despite the crowded environment in cells. Indeed, a major challenge in the field is to understand how the in vitro motility properties of kinesin motors relate to their cargo transport properties in vivo. In this review, we focus on recent work on the assembly and movement of kinesin motors that drive anterograde trafficking events. The reader is referred to recent reviews on kinesin motors that participate in mitosis (48, 146) and cytoplasmic dynein (45, 74).
Figure 1
Intracellular transport in interphase mammalian cells. Microtubules are polarized cytoskeletal filaments polymerized from heterodimeric α- and β-tubulin subunits. In most cell types, microtubules (dark blue lines) are organized with their plus (growing, +) ends extending into the cell periphery and their minus (anchored, −) ends in the middle of the cell. Transport cargoes (purple) such as vesicles, organelles, and protein complexes are carried along microtubule tracks by kinesin (green) and dynein (red) motors, which read and respond to microtubule polarity to carry out directed motility. In general, kinesin motors carry out long-distance transport to the plus ends of microtubules in the cell periphery (anterograde transport), whereas cytoplasmic dynein carries cargoes in the retrograde direction, toward the minus ends of microtubules in the cell center.

SUBUNIT COMPOSITION
AND ASSEMBLY

The Kinesin-1 Family: A Heterotetrameric Motor

The kinesin-1 holoenzyme is a heterotetramer consisting of two kinesin heavy-chain (KHC) and two kinesin light-chain (KLC) subunits (Figure 2). The KHC subunit contains an N-terminal kinesin motor domain followed by a neck domain, a coiled-coil stalk, and a globular tail domain (Figure 2). The neck domain consists of a neck linker and a neck coil, which are critical for motor processivity and homodimerization, respectively. The coiled-coil stalk contains hinge segments that enable the motor to adopt a folded, autoinhibited state (see below) and also impart flexibility necessary for multiple motors to work collectively when attached to the same surface (10, 25, 26, 42, 70). The globular tail domain participates in regulation of motor activity and cargo binding. The KLC subunit contains a heptad repeat region for oligomerization with KHC followed by six tetratricopeptide repeat (TPR) motifs that participate in cargo binding (3, 63, 135).

In mammals, the KHC subunit is encoded by three genes (KIF5A, KIF5B, and KIF5C), whereas the KLC subunit is encoded by four genes (KLC1–4). It seems likely that all kinesin-1 transcripts can undergo alternative splicing. Thus, there is the potential to generate many biochemically distinct kinesin-1 motors by differential association of the gene products. Recent work using well-defined antibodies that specifically recognize the different kinesin-1 gene products demonstrated that both KHC and KLC polypeptides exclusively form homodimers and that each KHC homodimer can associate with each KLC homodimer (30). The biochemically distinct kinesin-1 motors assembled from homodimers and/or splice forms can have functionally distinct roles in cells (18, 21, 41, 51, 147).
Figure 2
Subunit composition of kinesin motors. Schematic of the subunit composition and organization of kinesin-1, kinesin-2, and kinesin-3 motors. All these kinesins contain a kinesin motor domain (dark green oval) at their N terminus for ATP-dependent processive motion toward the plus ends of microtubules. These kinesins also have a neck domain (neck linker and neck coil) and varying amounts of coiled-coil stalk regions for oligomerization. Several of these kinesins have protein-protein or protein-lipid interaction domains such as TPR, Armadillo, FHA, and PH domains. Note that the kinesin-3 motor KIF1A is depicted as a dimeric molecule as this appears to be the state of the processive motor, although it is still unclear whether KIF1A motors are monomeric or dimeric in solution.

Abbreviations: FHA, forkhead associated; KAP, kinesin-associated protein; KHC, kinesin heavy chain; KIF, kinesin family; KLC, kinesin light chain; PH, pleckstrin homology; TPR, tetratricopeptide repeat.

Although the kinesin-1 motor is generally considered a heterotetramer, there is evidence that the KHC gene product can exist biochemically and act functionally in the absence of KLC. KHC motors lacking KLC can be isolated during purification of kinesin-1 from the brain, although this was thought to be due to degradation of the KLC subunits (54). In fungi, the kinesin-1 motor lacks KLC subunits (126), whereas in cultured cells, the kinesin-1 pool contains both tetrameric molecules and KHC dimers (32, 52). Functionally, KHC can have distinct roles that do not require the presence of the KLC subunit, including (a) transport of mRNA granules (13, 73, 94), (b) mitochondrial transport (47), (c) cytoplasmic streaming (106), and (d) microtubule-microtubule sliding (72, 128).

The Kinesin-2 Family: Heterotrimeric and Homodimeric Motors
The kinesin-2 family contains two subfamilies that are either heterotrimeric or homodimeric assemblies. The heterotrimeric kinesin-2 motors are composed of two motor-domain-containing subunits from the KIF3 family and a nonmotor accessory protein known as kinesin-associated protein (KAP) (Figure 2). The homodimeric subfamily includes...
CeOSM-3 (*Caenorhabditis elegans*), MmKIF17 (*Mus musculus*), and TtKin5 (*Tetrahymena thermophila*) (Figure 2). Both kinesin-2 subfamilies have been implicated in a wide variety of transport events, from transport along axonemal microtubules during the construction and maintenance of cilia and flagella, to transport along cytoplasmic microtubules of organelles, melanosomes, mRNA granules, and membrane-bound vesicles (63, 118). Whereas the assembly of heterotrimeric kinesin-2 motors has been studied (below), much less is known about the assembly of homodimeric kinesin-2 motors, although recent work has shown that the neck coil is sufficient for homodimerization of KIF17 (58).

KIF3 polypeptides are composed of a kinesin motor domain, a neck domain, a coiled-coil stalk, and a globular tail domain (Figure 2). Three KIF3 genes have been identified in mammals, KIF3A, KIF3B, and KIF3C. Whereas KIF3A and KIF3B are widely expressed, KIF3C is expressed mainly in neural tissues. A variety of studies have shown that KIF3A forms heterodimers with either KIF3B or KIF3C (8, 27, 99, 109, 112, 151), although KIF3C may also exist in a homodimeric complex (99). The KAP subunit, composed largely of armadillo repeats (Figure 2), binds only to the KIF3A/KIF3B heterodimer (14, 36, 150).

How do the KIF3 subunits assemble via heterodimerization and avoid the formation of homodimeric molecules? A unique feature of the KIF3A and KIF3B polypeptides is the highly charged segments at the proximal region of the stalk (Figure 2). As KIF3A exhibits opposite charges of KIF3B and KIF3C (Figure 2), these charged segments were proposed to specify heterodimer formation. Using synthetic peptides in solution, Chana et al. (19, 20) showed that the KIF3A neck coil is capable of homodimer formation and that the charged segments drive heterodimer formation. In contrast, De Marco et al. found that the C-terminal region of the *Xenopus laevis* Xklp3A and Xklp3B stalk domains is critical for heterodimerization based on coimmunoprecipitation of truncated motors (27) and on solution studies of a stalk fragment (28). Thus, although it seems plausible that both the charged region and the C terminus of the stalk can contribute to heterodimer formation, the exact mechanisms and contribution of each segment remain to be clarified.

Why nature combined two different polypeptide chains to create a heterodimeric motor is still unclear. In general, both native and recombinant kinesin-2 motors display processive motility in microtubule gliding assays or when attached to beads in motility assays (8, 9, 14, 23, 100, 107, 109). That heterodimeric kinesin-2 motors are processive indicates that both motors in the complex are contributing to motility. Indeed, when expressed as monomeric motor domains, the CeKLP-20 (homolog of MmKIF3A and SpKRP85) and CeKLP-11 (homolog of MmKIF3B and SpKRP95) motors display similar microtubule binding properties and ATP hydrolysis rates (14), indicating that both motors have equivalent enzymatic activities. Kinesin-2 motors have a shorter run length than do kinesin-1 motors (8, 9, 100), and recent work has shown that this is due to an extended neck linker sequence in kinesin-2 that relieves the strain between the two motor domains (100, 120). Because of the shorter run length, kinesin-2 motors may be better adapted to participate in bidirectional motility with cytoplasmic dynein.

To dissect the contribution of the KIF3A and KIF3B polypeptides to the motility of kinesin-2, several groups have engineered dimeric constructs that contain two KIF3A heads or two KIF3B heads. Engineered homodimeric molecules of murine KIF3A/KIF3A and KIF3B/KIF3B (100) or *C. elegans* KLP-20/KLP-20 and KLP-11/KLP-11 (107) were found to have motility properties similar to those of heterodimeric wild-type motors, indicating that the ability of the motor to take multiple steps before detaching does not require the presence of two different motor domains. Yet there does appear to be functional differences between the two nonidentical motor domains of the kinesin-2 heterotrimer. Pan et al. (107) found that an engineered *C. elegans* KLP-11/KLP-11 homodimer drives the
rotation of microtubules in a motility assay, indicating that the KLP-11 subunit can produce torque. In addition, Brunnbauer et al. (14) determined that engineered homodimeric C. elegans KLP-11/KLP-11 molecules have limited activity in ATPase and motility assays, suggesting that the KLP-11 motor exhibits different kinetic properties depending on the identity of its partner head. Differences between these studies, such as the linkages used to engineer homodimeric molecules and the species of KIF3 polypeptide, will need to be resolved to thoroughly understand the contribution of the two different motor domains to the motility of heterotrimeric kinesins.

The Kinesin-3 Family: Monomeric and/or Dimeric Motors

The kinesin-3 family was first identified in C. elegans because of mutations in the unc-104 gene that result in a severe defect in axonal transport of synaptic vesicles, and the mouse homolog, KIF1A, was characterized soon thereafter. Further work identified a variety of gene products across species with homology to the motor domains of CeUNC-104/MmKIF1A, and the kinesin-3 family now comprises five subfamilies (KIF1, KIF13, KIF14, KIF16, and KIF28), named for the corresponding muring gene products (98).

Sequence analysis demonstrated that kinesin-3 motors contain only short α-helical segments with a weak propensity for coiled-coil formation (Figure 2) (37, 59, 104, 111, 121, 148). Indeed, oligomerization of kinesin-3 motors has been experimentally challenging, and thus the monomer/dimer state of kinesin-3 motors has been controversial. The initial characterization of MmKIF1A suggested that it is a globular protein whose behavior in biochemical and biophysical assays is most consistent with a monomeric molecule (104).

The first model for kinesin-3 motility used analysis of engineered MmKIF1A motor domains and showed that, in single-molecule assays, KIF1A moves by a biased diffusion mechanism in which a basic stretch on the KIF1A motor domain (the K-loop) interacts with the acidic C-terminal tails of α- and β-tubulin (the E-hooks) to maintain the motor-microtubule interaction after ATP hydrolysis (75, 101–103).

Although this elegant and convincing work provided a molecular mechanism for motility, the speeds of the engineered constructs were too slow (140 nm s⁻¹) compared with the fast motilities of kinesin-3 constructs in multiple-motor gliding assays and kinesin-3 cargoes in vivo (91, 104, 109, 155). In addition, other kinesin-3 family members have shorter K-loops and do not show diffusive motion (4, 109, 131). Thus, motility via a biased diffusion mechanism may not be a general feature of kinesin-3 motors.

A second model was put forth that kinesin-3 motors must work in teams to produce processive motility (109, 115, 131). Indeed, forced dimerization of CeUNC-104 resulted in a motor with high velocity and processivity (131). Teamwork on a vesicle surface could be accomplished by multiple monomeric motors cooperating during motility or by dimerization of motors at high local concentrations. Interestingly, CeUNC-104/MmKIF1A motors contain a lipid binding pleckstrin homology (PH) domain at their C terminus (Figure 2) that could recruit motors to the vesicle surface. Klopfenstein et al. (76, 77) provided strong support for this model by showing that the PH-domain-dependent recruitment of CeUNC-104 motors to vesicles or liposomes produces fast motilities in vitro and in vivo. Fast motility is likely due to dimerization on the membrane surface as mutation of the neck coil abolished processive motility but not membrane binding (76).

More recently, the possibility has been raised that kinesin-3 motors may indeed be homodimeric molecules. Analysis of MmKIF1A demonstrated that the molecule can exist in a dimeric state in mammalian cells, but the tightly folded globular state of the molecule...
makes its oligomeric state difficult to assess (59). This work is consistent with reports that other members of the kinesin-3 family, HsGAKIN/KIF13B, HsKIF1C, DdUNC-104, and NcKin3 (1, 37, 111, 148), exist as homodimeric molecules. Thus, the common classification of kinesin-3 motors as the monomeric family of kinesins does not appear to be widely applicable.

Dimerization now appears to be critical for the fast processive motility of metazoan kinesin-3 motors. While both monomeric and dimeric MmKIF1A motors can undergo one-dimensional diffusion along the microtubule surface, only dimeric motors undergo processive motility at fast velocities (59). Biochemical and structural analyses have shown that dimerization requires not only the neck coil but also several residues of the hinge segment (1, 5, 59, 121). Do dimeric kinesin-3 motors utilize a hand-over-hand mechanism of motility? Analysis of metazoan kinesin-3 motors has not been carried out, but recent work has shown that a dimeric fungal kinesin-3, NcKin3 (Neurospora crassa), detaches from the microtubule after one ATPase cycle and is therefore unable to step hand-over-hand to generate processive motility (2, 4). Clearly, further work on the molecular mechanisms that drive kinesin-3 assembly and motility are likely to be informative and interesting.

**AUTOINHIBITION IN THE ABSENCE OF CARGO**

Motor activity must be precisely regulated in cells to avoid futile ATP consumption and to ensure the proper subcellular localization of motors and their cargoes. In recent years, a general model has emerged that kinesin motors, when not bound to cargo, are kept in an inactive state by an autoinhibition mechanism. Autoinhibition allows motor activation to occur with precise spatial and temporal control.

Autoinhibition as a control mechanism was first described for kinesin-1, and to date, this remains the best-studied motor for autoinhibitory control (3, 135). Kinesin-1 motors exist in two conformations: an extended conformation in the active state and a folded conformation in the inactive state. Folding is due to hinge 2, which interrupts the long coiled-coil stalk of kinesin-1 (Figure 2) and allows C-terminal regions to interact with and inhibit the N-terminal motor domain (15, 25, 33, 42). A critical component of the autoinhibitory mechanism is the KHC tail domain, which interacts directly with a key enzymatic helix in the KHC motor domain and blocks intrinsic and microtubule-stimulated release of ADP from the nucleotide binding pocket (15, 25, 33, 42, 53, 55, 56, 127, 136, 139, 144). A second intramolecular mechanism for inhibition involves the KLC subunit. Studies on full-length kinesin-1 molecules expressed in bacteria and mammalian cells have shown that the KLC subunit suppresses motor activity, perhaps by physically separating the two motor domains (15, 25, 42, 136). However, recent studies with recombinant KHC and KLC fragments provide a contradictory view and suggest that the KLC subunit contributes to motor activation by blocking interactions of the KHC tail with both the KHC motor and the microtubule (145), although many of these interactions, in particular the tail-microtubule interaction, are based on ionic interactions and are thus sensitive to buffer conditions (139).

An autoinhibitory control mechanism has also been described for kinesin-3 motors. Full-length MmKIF1A and CeUNC-104 motors are inactive in motility assays (59, 104, 109), likely because of intramolecular interactions within the globular conformation of kinesin-3. The C-terminal half of CeUNC-104 or MmKIF1A is dispensable for autoinhibition as motors that are truncated after coil 2 remain inactive in single-molecule motility assays (59, 109). Rather, autoinhibition involves intramolecular interactions within the N-terminal half of kinesin-3 motors, in particular between the neck coil and coil 1 and between the FHA domain and coil 2 (5, 90, 148), suggesting that, like kinesin-1, kinesin-3 motors utilize dual autoinhibitory mechanisms. A potential mechanism for how the neck coil–coil 1 interaction functions in autoinhibition has been suggested on
the basis of biochemical and structural work. Motor constructs containing both the neck coil and coil 1 of MmKIF1A are monomeric and not processive, whereas removal of coil 1 results in dimeric processive motors (59, 90). Structural analysis demonstrated that the neck coil and coil 1 of CeUNC-104 can either self-fold in the monomer state or engage in intermolecular interactions in the dimeric state (5). Thus, neck coil–coil 1 interactions contribute to the regulation of both the autoinhibited state and the monomer-to-dimer transition, although a functional role in the full-length motor remains to be verified.

Homodimeric members of the kinesin-2 family are also regulated by autoinhibition. Full-length CeOSM-3 and HsKIF17 motors are inactive but can be activated by mutation of hinge 2 in the coiled-coil stalk domain that causes a corresponding shift to a more extended conformation (58, 68). The inactive folded conformation suggests that C-terminal regions could interact directly with and inhibit the N-terminal motor domain. Indeed, two intramolecular interactions that contribute to regulation were identified. First, the C-terminal tail interferes with microtubule binding of the motor domain, and second, coil 2 blocks processive motility (58). Thus, like autoinhibition of kinesin-1, regulation of homodimeric kinesin-2 motors utilizes two intramolecular mechanisms that collectively maintain the motor in an inactive state.

It remains largely unclear how the activity of heterotrimeric members of the kinesin-2 family is regulated. Purified native and recombinant forms of kinesin-2 are active in microtubule gliding assays and when attached to beads, yet they show no motility in single-molecule assays (8, 9, 14, 100, 109, 149), suggesting that kinesin-2 motors are regulated by autoinhibition. Recent work showed that autoinhibition is intrinsic to the motor-containing KIF3 subunits, as CeKLP-20/KLP-11 motors undergo few motility events even when attached to beads (14). Autoinhibition may involve the adoption of a folded conformation as purified sea urchin SpKRP85/KRP95/KAP (Strongylocentrotus purpuratus) molecules undergo a salt-dependent shift to a more extended conformation (140).

Given the autoinhibited state of kinesin motors, the simplest model of motor activation posits that cargo binding relieves autoinhibition. Indeed, several motors are activated by binding of cellular cargo proteins. The first demonstration of kinesin activation was for kinesin-1. As two intramolecular interactions contribute to autoinhibition of kinesin-1 (the KHC globular tail and the KLC subunit), binding partners for each of these regions were required for activation of heterotetrameric motors (11). In the absence of KLC, only the KHC binding partner is needed for activation (22, 56). A kinesin-3 motor, GAKIN/KIF13B, is also activated by binding of a cargo protein (148). Although kinesin motors can additionally be regulated by mechanisms such as phosphorylation (135), these results provide a basis for understanding how kinesin activity is coupled to cargo binding.

**MOVEMENT ALONG HETEROGENEOUS CELLULAR MICROTUBULES**

The elegant biochemical and biophysical approaches that have elucidated the mechanics of kinesin motors have been carried out mostly with recombinant truncated proteins in dilute buffer conditions and along homogeneous populations of microtubules that are taxol stabilized and free of microtubule-associated proteins (MAPs). Such experimental systems lack relevant cargo and associated proteins and operate far from the physical and physiological constraints of the cytoplasm. Thus, how do the motile properties of kinesin motors in vitro compare to those in vivo?

The first step in understanding how kinesin motility is affected by cellular conditions is to compare the motile properties of single motors in vitro and in cells. In this regard, two approaches have been taken. Truncated kinesin motors either have been purified in recombinant form, labeled with quantum dots, and...
then introduced into cells via osmotic lysis of pinocytic vesicles or lipid-mediated transfection (24, 152), or kinesin motors have been genetically tagged with fluorescent proteins (FPs) and expressed endogenously in the cell of interest (16, 17). Overall, the motile properties of kinesin-1 in cells are similar to those observed previously in vitro, indicating that the basic movement of kinesin-1 along cellular microtubules is neither hindered by the crowded conditions in cells nor upregulated by unknown factors.

Further work used single-molecule imaging to compare the motile properties of kinesin-1 (KIF5C), kinesin-2 (KIF17), and kinesin-3 (KIF1A) motors in mammalian cells (16). In each case, the overall speed and processivity of the motor in cells were similar to its previously determined in vitro properties (16). However, differences between the motors were found in how they responded to the cellular complex of microtubules. The first difference observed was the ability of the motors to navigate the heterogeneous populations of microtubules generated by the cell. Kinesin-1 was observed to be a selective motor, preferentially utilizing stable microtubules marked by specific posttranslational modifications (PTMs). In contrast, kinesin-2 and kinesin-3 motors were not selective and utilized both stable and dynamic microtubules (16). The second difference observed between the motors was that kinesin-2 and kinesin-3 motors appear to tip-track on the plus ends of growing microtubules (16). For KIF17, this is due likely to interactions with plus-end-tracking proteins (+TIPs) (71). These results indicate that there are family-specific differences in how kinesin motors respond to the heterogeneous microtubule tracks that exist in cells.

Which cellular factors on microtubules could differentially influence kinesin motors? At least three differences in microtubule structure and/or composition can be envisaged. The first is tubulin isotype. In most organisms, multiple genes encode for α- and β-tubulins, resulting in a variety of isotypes (96). Most of the differences between isotypes are found in the C-terminal tails, which provide chemical diversity on the microtubule surface and could thus influence the motor-microtubule interaction, although little work has been done to address this possibility.

The second factor that could influence kinesin motors is the PTMs of α- and β-tubulin subunits that mark subsets of microtubules in cells. To date, the best-studied PTMs of tubulin are acetylation of Lys40 in α-tubulin, detyrosination of α-tubulin, and polyglutamylation and polyglycylation of the C-terminal tails of α- and β-tubulin (Figure 3) (67, 134, 143). Kinesin-1 appears to be most sensitive to the

![Figure 3](https://example.com/figure3.png)

Microtubule posttranslational modifications (PTMs). Within the microtubule lattice, α- and β-tubulin subunits can be modified by various PTMs. The best-studied modifications are illustrated on the crystal structure of a single αβ-tubulin heterodimer (top, PDB code 1TUB). Acetylation of Lys40 (K40) of α-tubulin occurs in the lumen of the microtubule, whereas the other modifications occur on the microtubule surface. The C-terminal tyrosine (Y) residue of α-tubulin can be removed to generate detyrosinated tubulin. Glutamate residues in the C-terminal tails of both α- and β-tubulin can be modified by chains of glutamate (E) or glycine (G) residues to generate polyglutamylated and polyglycylated tubulin, respectively.
presence of these tubulin PTMs. Kinesin-1 binds with higher affinity to microtubules marked by acetylation and detyrosination (39, 80, 93, 113), and changing the levels of these modifications can alter kinesin-1 transport events in cells (35, 43, 60, 80, 113). Acetylation and detyrosination may provide two separate cues to kinesin-1. For example, in polarized hippocampal neurons, detyrosination but not acetylation can regulate the axon-specific targeting of kinesin-1 (60, 80). A molecular mechanism for the ability of kinesin-1 to distinguish acetylation and detyrosination cues was provided by Konishi & Setou (80), who showed that a tyrosine-glutamate-arginine-phenylalanine TERF sequence in the kinesin-1 (KIF5C) motor domain is critical for the recognition of detyrosinated but not acetylated microtubules.

Kinesin-2 and kinesin-3 motors appear less sensitive to acetylation and detyrosination marks on microtubules (16, 80). Rather, kinesin-3 motors may be more sensitive to polyglutamylation. In ROSA22 mice that lack functional PGs1, a subunit of α-tubulin-selective polyglutamylase, there was a decrease in the localization of kinesin-3 (KIF1A) motors to neurites and synaptic vesicle cargoes to axon terminals (66). Taken together, there exists significant evidence that PTMs of cellular microtubules can serve as kinesin-specific traffic signals and regulate intracellular trafficking events.

The third factor that could influence kinesin motors in cells is obstacles on microtubule tracks. For example, the MAPs that are believed to organize or stabilize microtubules (31) may pose roadblocks for kinesins. Most studies have focused on the neuronal MAP tau, but several studies have shown that MAP2C negatively influences the interaction of kinesin-1 motors with microtubules, whereas ensconsin promotes the recruitment of kinesin-1 to microtubules (6, 119, 129). In neuronal cells, overexpression of tau impairs kinesin-dependent transport with a specific reduction in attachment rates but not velocity of transport (40, 125, 132). Similar effects were found in single-molecule assays where tau reduced the attachment rate of truncated kinesin-1 motors but had no effect on velocity (34, 119, 137). Whether tau affects the run length of kinesin-1 is less clear as different effects have been found in vivo and in vitro (119, 132, 137). When present at higher concentrations, tau can also regulate the motor off-rate as single kinesin-1 motors detached from the microtubule when they encountered patches of tau protein (34). Using beads coated with varying numbers of kinesin-1 motors, it was found that tau has a greater effect under multiple-motor conditions as tau-dependent decreases were observed in the attachment rate, run length, force production, and detachment rate of kinesin-1 (137). The finding that filamentous tau results in a decrease in the speed of vesicle transport in isolated squid axoplasm is another example of tau effects in a multimotor situation (86). Thus, although the effects of tau on kinesin-1 cannot always be directly compared between studies due to differences in tau isoform, concentration, and ability to form filaments, the general view is that tau negatively affects kinesin-1 motility, with the majority of studies noting an effect on attachment and detachment rates.

Other obstacles on microtubule tracks can also influence kinesin-1 motility. Using single-molecule imaging, Telley et al. (130) found that kinesin-1 motors detach when microtubules are crowded with active or nonmotile mutant kinesin motors, although others (38) have observed kinesin pausing and bypassing such obstacles. In similar assays, Korten & Diez (81) found that kinesin-1 motors stopped and/or paused when encountering streptavidin obstacles on biotinylated microtubules and that about half of the kinesin-1 molecules were able to overcome this obstacle. When faced with larger obstacles such as intersecting microtubules, single kinesin-1 motors were more likely to pass the obstacle (i.e., they stayed on the same track) than to detach (116). It was proposed that the presence of multiple motors on a bead or cargo would enable kinesin-1 to better navigate obstacles, and indeed, kinesin-1-coated beads were more likely to continue on the same track or cross to the intersecting
track than to detach (116). Thus, the data available to date indicate that there are a variety of microtubule-associated factors in cells that can influence kinesin motility whether by directing transport to specific microtubule tracks or by altering the motor-microtubule interaction. It appears the effects of tau may be a specific biological function of this MAP, as kinesin-1 motors detach in the presence of tau but are able to navigate around other and larger obstacles. The specific influence of each factor on kinesin motors in the single and multiple motor states is a topic that is likely to occupy investigators for many years to come.

COORDINATION OF MULTIPLE MOTORS

Coordination Between Multiple Motors of the Same Type

The motile properties of cellular cargoes are due largely to the collective action of multiple motors present on the cargo surface. These can be multiple motors of the same type (e.g., multiple plus-end-directed motors or multiple kinesin-1 motors) or multiple motors of different directionality (e.g., kinesin-1 and dynein). Research on understanding how multiple motors of the same type coordinate their motions to work collectively has focused mostly on kinesin-1 and understanding the effects on intrinsic motor properties (e.g., velocity, processivity, and force generation), although there is also evidence that step size can be altered in the multimotor situation (89).

Whether kinesin-1 motors cooperate to increase the speed of motion has been controversial. In now-classic experiments using microtubule gliding assays, the speed of microtubule translocation was independent of the number of kinesin-1 motors at saturating ATP concentration and under no load (64). Even under load conditions, multiple kinesin-1 motors do not increase the speed of movement beyond that of a single motor (65, 79, 97, 138). It is thought that the resistive load applied by an optical trap, or a cargo moving in the cytoplasm, is distributed among the motors such that each motor experiences a negligible load (83). That cargo velocity is insensitive to motor number is supported by an analysis of lipid droplets in Drosophila embryos where the average velocity of lipid droplet motion was largely unaffected by a genetic 50% reduction in kinesin-1 concentration (123) and live-cell imaging of intrflagellar transport particles in Chlamydomonas flagella (85). Yet a thorough understanding of how multiple motors coordinate their activities requires precise control over motor number. In this respect, it is exciting to see recent studies using linear DNA duplexes to link exactly two kinesin-1 motors. In this case, a two-motor construct showed no change in velocity at low load but could maintain single-motor velocities at high load (69, 114), again arguing that multiple motors of the same type do not cooperate to increase the speed of their collective motion.

However, several studies tracking vesicles or organelles in live cells have shown that the velocity histograms contain regularly spaced peaks (62, 84, 122, 154). Although the similarity of velocity profiles across transport events, cells, and species argues that these peaks reflect a shared biological phenomenon, a description of the molecular events that contribute to this phenomenon is yet unclear. One possibility is that the stepwise changes between velocity peaks indicate the addition or removal of active motors from the cargo surface (62, 84, 92, 122). A second possibility is that the velocity peaks occur when motors work in the viscous cytoplasmic environment, as similar distinct peaks in the instantaneous velocity histogram were identified when kinesin-1 worked against a load in microtubule gliding assays (44). A third possibility is that motor activity can be regulated by cellular systems such that motors are capable of faster speeds in vivo than in vitro. While single truncated motors display motile properties in vivo similar to those in vitro (16, 17, 24, 152), the motile properties of cargo-attached motors can be modulated...
by binding partners to produce faster speeds and/or higher forces (95). A fourth possibility is that the faster speeds in vivo result from motors and cargoes piggy-backing on microtubules that are themselves moved as cargoes along other tracks. Strong support for this possibility comes from experiments in Drosophila S2 cells where a photoswitchable tubulin construct was used to specifically mark and track microtubules (72). A final possibility is that the velocity peaks indicate transport events carried by different motors. Support for this possibility comes from the fact that genetic ablation of the kinesin-3 motor CeUNC-104 specifically decreased the fast velocities of dense core vesicle transport events in neurons (154). In addition, studies on intraflagellar transport particles in the sensory cilia of C. elegans chemosensory neurons have shown that fast (homodimeric CeOSM-3) and slow (heterotrimeric kinesin-2) motors can cooperate to produce intermediate velocities both in vivo in the initial segment of cilia and in vitro in microtubule gliding assays (105, 108). Similar in vitro assays using fast (wild-type kinesin-1) and slow (mutant kinesin-1) motors found that even a small number of fast motors are able to dominate motility and shift the balance in their favor (87).

Cellular cargoes generally move for longer distances along microtubule tracks than single motors do in vitro, most likely due to cooperation between motors that results in an increase in run length. This was first shown by Block et al. (12), who used kinesin-1-coated beads in optical trapping experiments to reveal that beads driven by two kinesin-1 motors moved longer distances than those driven by single motors. Following up on these experiments, the Gross lab determined detailed statistics for motor number and run length for kinesin-1 motors attached to beads (137). In particular, these results showed that beads driven by two motors of the same type can move more than 8 μm, much longer than the average run length of single kinesin-1 motors. Interestingly, recent experiments using two kinesin-1 motors linked by a 50-nm DNA duplex found that the average run length of two linked motors is only ~1.5-fold higher than that of a single kinesin-1 motor (114). These latter experiments are more in line with theoretical work that predicted a relatively small increase in run length for cooperating motors of the same type (78). The differences between these studies can be explained by the arrangement of the motors. It seems that reattachment to the microtubule track is more likely to occur for motors nonspecifically adsorbed to a bead surface, and perhaps also for motors attached to vesicular cargoes in cells, than for a group of clustered motors. Thus, in contrast to the effects of motor coordination on speed, there is good agreement that multiple motors of the same type can work collectively to increase the distance traveled.

There is also good agreement that multiple motors can cooperate for increased force generation, particularly for multiple motors attached to beads and analyzed in vitro (137). Force measurements of lipid droplets in Drosophila embryos showed that the forces exerted by multiple kinesin-1 motors are nearly additive (123), in agreement with theoretical work (83). Whether individual motors generate equivalent forces in vitro and in vivo is less clear. Soppina et al. (124) measured the average force exerted by motors on endosomes in Dictyostelium and concluded that the total force exerted by kinesin-3 (DdUnc104) in vitro and in vivo is similar. In contrast, force measurements of lipid droplets in Drosophila embryos showed that the force exerted by a single motor in vivo was smaller than that determined in vitro (123). Clearly, more work needs to be done to better understand the relationship between motor number and force production on a vesicle surface as well as the influence of parameters intrinsic to cellular systems such as motor-cargo linker stiffness, cellular viscosity and other resistive forces, and stochastic effects such as load sharing (82).

### Coordination Between Motors of Different Types

Cooperation between motors of different types (e.g., kinesin-1 and dynein) results in
Models for bidirectional cargo transport. Many cargoes in eukaryotic cells contain both kinesin and dynein motors and thus move in a bidirectional manner where periods of persistent motion are interrupted by reversals in directions. Two models have been put forth to explain bidirectional transport: the regulated switching model and the tug-of-war model. In the regulated switching model, the activity of kinesin and dynein motors is regulated to ensure that only one type of motor is active at any time. In the tug-of-war model, the two types of motors generate force against each other such that one motor wins to ensure a reversal in its favor.

Bidirectional motility. In some cases, the cargo moves for long distances in each direction before undergoing a remodeling and/or switching event to generate movement in the opposite direction. For example, intraflagellar transport in cilia and flagella is carried out by kinesin-2 and cytoplasmic dynein motors, with the change in motor activity, and thus cargo direction, occurring at the distal end of the cilium (118). Recent imaging in live Chlamydomonas cells showed that these motors are reciprocally coordinated for exclusive transport in one direction (85).
In other cases, bidirectional movement involves short periods of directed motion interrupted by reversals in direction. Two popular models have been put forth to explain reversals: regulated switching of motor activity or tug-of-war between opposite motors (Figure 4) (49, 141). A complete understanding of bidirectional transport will require methods to determine the number of active motors in real time. However, as both models have considerable experimental support, it appears that regulated switching and tug-of-war mechanisms are employed in cells in a cargo-specific manner.

In the regulated switching model, minus-end- and plus-end-directed motors alternate their activities due to regulatory mechanisms that rapidly activate and inactivate motors in a mutually exclusive manner. In some cases of bidirectional transport, inhibition or removal of one motor abolishes transport in both directions rather than enhances transport in the opposite direction (142). This indicates, first, that the motors are not competing with each other and, second, that both motors are required for the regulatory systems to operate. The regulatory systems, such as the dynactin complex, have been envisioned to involve specific cargo proteins that regulate individual motors (29, 50, 57, 110).

The tug-of-war model requires that motors of opposite directionality pull against each other and that one class of motor wins to effect a reversal in its favor. Experimental support for this model has come from studies on endosomes purified from Dictyostelium cells (124). During motility events, the endosomes were observed to elongate in morphology and slow in speed as they underwent reversals in direction due to a tug-of-war between motors with opposite directionality. Further support has come from recent work using endosomal vesicles purified from mouse brain. Quantitative analysis determined that low numbers of kinesin and dynein motors are stably attached to the vesicles and drive bidirectional motility via force-dependent interactions that exert a tug-of-war on the vesicle (61). Evidence that bidirectional transport is intrinsic to the motors themselves comes from analysis of peroxisome transport in Drosophila S2 cells. By knocking down kinesin-1 and cytoplasmic dynein motors and replacing them with various plus-end- or minus-end-directed motor constructs, Ally et al. (7) determined that the specific linkage of the motor to the organelle is irrelevant, that the specific plus-end- or minus-end-directed motor is irrelevant, and that the presence of active motors capable of ATP-dependent motility is required for bidirectional motility. These results demonstrate that bidirectional motility is not due to motorspecific sequences or regulators but rather that mechanical tension between opposite-polarity motors is sufficient for activation of motility in both directions.

CONCLUSIONS

In this review, we have highlighted recent work on how the assembly and motility properties of kinesin motors give rise to intracellular transport events, particularly in metazoan cells. Future work is likely to continue to yield important and exciting information on the motile properties of kinesin motors. First, it is still unclear whether the hand-over-hand mechanism that drives kinesin-1 motility is generally applicable to kinesin motors that drive intracellular trafficking events. Quantitative and qualitative analyses of various kinesin motors in traditional in vitro assays are likely to continue to provide important information on how the kinesin motor domain has been adapted over evolution to provide specific chemomechanical functions. Second, it will be important to understand how multiple motors present on the same cargo coordinate their motilities. Third, the influence of biochemical cues on the microtubule tracks will continue to provide mechanisms for cells to segregate and guide cargo transport. Finally, how these mechanisms are integrated in cells is the ultimate challenge in understanding intracellular trafficking events.
SUMMARY POINTS

1. The kinesin motor domain has been expanded and adapted over evolution to generate families of kinesins with distinct oligomerization states, cargo-carrying capabilities, and regulation mechanisms.

2. The presence of two motor domains in a dimeric kinesin molecule allows for processive motility.

3. In the absence of cargo, kinesin motors are regulated by an autoinhibition mechanism in which intramolecular interactions maintain the motor in an inactive state.

4. Kinesin motors can read and respond to biochemical cues on microtubule tracks, including PTMs and MAPs, in order to segregate and direct trafficking events.

5. Most cargoes are carried by multiple motors that work collectively to increase force and run length but not velocity of movement.

6. Bidirectional cargo transport can result from regulated switching between plus-end- and minus-end-directed motors or from tug-of-war, in which directionality is determined by the dominant motor.

FUTURE ISSUES

1. Mechanisms of motility need to be determined for members of the kinesin-2 and kinesin-3 families. For heterotrimeric kinesin-2 motors, the requirement for two different motor domains is unclear, whereas for kinesin-3 motors, the oligomerization state and mechanisms of processive motility remain to be determined.

2. How kinesin motors navigate the complex cellular environment is an area of active research. The contributions of tubulin isotypes, PTMs, MAPs, and other obstacles to the regulation and direction of motor transport need to be worked out. These factors may work together or may provide conflicting cues to the motor.

3. Future work is also needed to understand how multiple motors cooperate to increase transport fidelity without interfering with each other’s motion. The contribution of regulated switching and tug-of-war models to the transport of various cargoes needs to be solved. And other models need to be developed for transport events that are not adequately described by these models.

DISCLOSURE STATEMENT

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