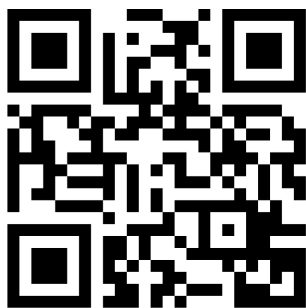REVIEW

The kinesin–tubulin complex: considerations in structural and functional complexity

Zachary T Olmsted
Andrew G Colliver
Janet L Paluh

State University of New York Polytechnic Institute, Colleges of Nanoscale Science and Engineering, College of Nanoscale Science, Nanobioscience Constellation, Albany, NY, USA

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Correspondence: Janet L Paluh
State University of New York Polytechnic Institute, Colleges of Nanoscale Science and Engineering, 4424 NanoFab East, 257 Fuller Road, Albany, NY 12203, USA
Tel +1 518 956 7047
Fax +1 518 437 8687
Email jpaluh@sunycnse.com/
jpaluh@albany.edu

Abstract: The ability of cells to respond to external cues by appropriately manipulating their internal environment requires a dynamic microtubule cytoskeleton that is facilitated by associated kinesin motor interactions. The evolutionary adaptations of kinesins and tubulins when merged generate a highly adaptable communication and infrastructure cellular network that is important to understanding specialized cell functions, human disease, and disease therapies. Here, we review the state of the field in the complex relationship of kinesin–tubulin interactions. We propose 12 mechanistic specializations of kinesins. In one category, referred to as sortability, we describe how kinesin interactions with tubulin isoforms, isotypes, or posttranslationally modified tubulins contribute to diverse cellular roles. Fourteen kinesin families have previously been described. Here, we illustrate the great depth of functional complexity that is possible in members within a single kinesin family by mechanistic specialization through discussion of the well-studied Kinesin-14 family. This includes new roles of Kinesin-14 in regulating supramolecular structures such as the microtubule-organizing center γ-tubulin ring complex of centrosomes. We next explore the value of an improved mechanistic understanding of kinesin–tubulin interactions in regard to human development, disease mechanisms, and improving treatments that target kinesin–tubulin complexes. The ability to combine the current kinesin nomenclature along with a more precisely defined kinesin and tubulin molecular toolbox is needed to support more detailed exploration of kinesin–tubulin interaction mechanisms including functional uniqueness, redundancy, or adaptations to new roles upon cell specialization, and to thereby accelerate applications in human health.

Keywords: microtubule, cellular network, cell functions, sortability, transport, disease, signaling

Introduction

Three decades ago when "conventional" kinesin was discovered, the incredible diversity of kinesin–tubulin interactions along with their fundamental roles in a range of cellular processes and multicellular development was not foreseen. The molecular nature of the kinesin–tubulin interface is highly evolved and includes combined kinesin and tubulin sequence specializations that enable unique functional capabilities. Fourteen kinesin families have been identified.[1] In addition, a variety of microtubule networks exist that are made of multiple tubulin family isoforms and isotypes. Posttranslational modifications of kinesins and tubulins provide additional spatial and temporal control.[2,3] This review evaluates progress in understanding the underlying molecular mechanisms of kinesin–tubulin interactions. Specifically, we focus on those mechanistic features of kinesins and tubulins that enable their contribution to diverse cellular processes such as mitosis, neurophysiology, immunology, and intracellular transport, which impact cell–cell communication networks

and human pathologies when disrupted. Here, we use the general term kinesin to refer to both conventional kinesin (Kinesin-1 family) and kinesin-like proteins (Kinesin-2 through Kinesin-14 families) or otherwise provide specific kinesin family or member names. While 14 kinesin families have been proposed,[1] the functional diversity that is achieved within families through sequence and domain specializations extends further. We illustrate this point through discussion of a subset of well-studied Kinesin-14 family members with emphasis on key roles in mitosis. We discuss recently identified kinesin–tubulin interactions that direct the architecture and function of the supramolecular centrosome, cilia, and flagella structures, and how incorporating the knowledge of kinesin–tubulin interactions will improve biomedical understanding, bioinformatic modeling, and biomimetic design.

Deciphering kinesin–tubulin functions by evaluation of their molecular toolbox

Kinesins are most often thought of in terms of motility. After all, it is this function that led to the initial discovery of conventional kinesin as a novel protein translocator of organelles on microtubules of the squid giant axon.[4] Indeed, motility and transport are of high importance in the functional repertoire of kinesin–tubulin interactions; however, an exclusive focus on this function would neglect the extensive capabilities designed into this complex. Modeling kinesins into phylogenetic trees[5,6] and families[1] paired with experimental research identifies some general functional patterns. Briefly, kinesins perform the following functions: mediate the transport of numerous cellular components that include organelles (Kinesin-1 through Kinesin-3[7]), RNA (Kinesin-1[8]), and chromosomes (Kinesin-4 and Kinesin-10[9]) along microtubule tracks; provide control of microtubule organization by cross-linking or sliding parallel or antiparallel microtubules (Kinesin-5,[10] Kinesin-6,[11] and Kinesin-14[12]); and regulate microtubule depolymerization (Kinesin-8 and Kinesin-13[13]), microtubule growth (Kinesin-7 and Kinesin-10[14]), and microtubule flux (Kinesin-5 and Kinesin-13[15–19]). Newly recognized functions include control of microtubule nucleation from the γ-tubulin ring complex (γ-TuRC) of human centrosomes and yeast spindle pole bodies (Kinesin-14 and Kinesin-5[20]) and regulation of mother centriole stability that impacts ciliogenesis (Kinesin-13[21]). Kinesins can also cross-bridge microtubules with other cytoskeleton networks that are intermediate filaments[22] and actin.[23] The overall complexity

of kinesin–tubulin interactions currently prevents the ability to rapidly predict all functional roles. An advanced understanding of underlying molecular mechanisms will assist in addressing this challenge.

For a comprehensive view of kinesin–tubulin functions, we must discuss how mechanistic specialization of kinesins and tubulins is achieved, as outlined in Figure 1 and Figure 2. This includes the following: monomeric or oligomeric state; force generation; kinesin step size; directional motility, processivity, and speed; tethered diffusion; microtubule dynamics; cross-linking and sliding of microtubules; cooperative kinesin–tubulin interactions; cross-cytoskeleton bridging; sortability; and cargo, transport, and signaling. This further illustrates why a highly detailed mechanistic analysis of each kinesin is indispensible to create a map of functional kinesin–tubulin interactions that reflects their scope and incredible diversity.

Kinesin monomeric or oligomeric state

Kinesins typically bind microtubule polymers as monomeric, dimeric, or tetrameric forms by utilizing an adenosine triphosphate (ATP)-dependent motor domain. The oligomeric state of partnering kinesins may be as homodimers or homotetramers or instead be heteromeric, and it is enabled by the electrostatic binding of amino acids in a coiled coil "stalk" domain. The stalk includes a "breathable" terminal neck linker region that connects to the motor domain and interacts with this domain during ATP hydrolysis. Dimeric or tetrameric kinesins have different capabilities to organize microtubules. For example, a tetrameric kinesin with motor domains positioned at each end in the complex can both cross-link and slide parallel or antiparallel microtubule arrays. Oligomeric kinesins participate in microtubule organization for such functions as bipolar spindle assembly, axon transport, immunological synapse formation, and other specialized cellular needs.[24,25] Homodimer forms of Kinesin-2, Kinesin-3, Kinesin-4, Kinesin-6, Kinesin-7, Kinesin-8, Kinesin-10, Kinesin-12, Kinesin-13, and Kinesin-14 members exist. However, alternate oligomeric states are also observed for kinesins in several of these families,[26] including a heterodimeric Kinesin-14 in which one partner has an ATP-independent motor domain. The ability of some kinesins to move between oligomeric states in vivo has also been reported, such as *Caenorhabditis elegans* Kinesin-3 Unc104 in which the transition between monomeric to dimeric state regulates its potential for motility.[27]
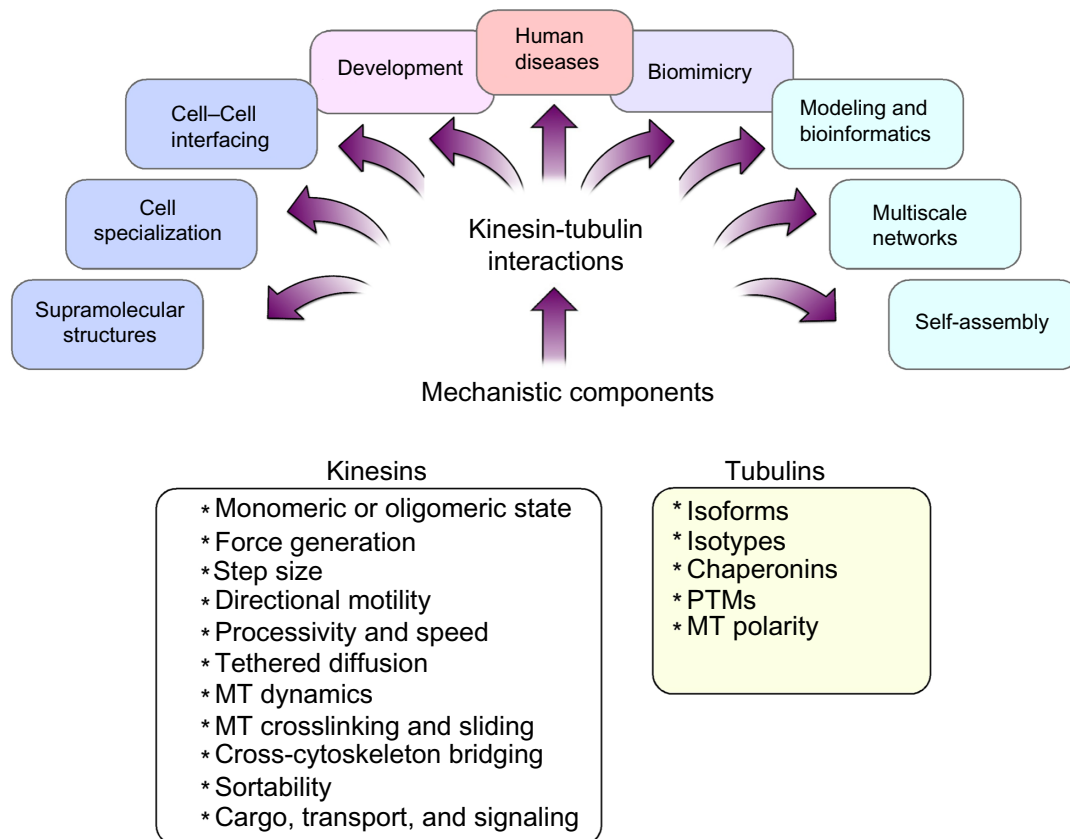
Figure 1 Mechanistic components of kinesin and tubulin.
Notes: The diagram summarizes the mechanistic components that lead to functional specializations present in kinesins and tubulins. The broader impact on functions at the single-cell, multicellular, and developmental stages is indicated as well as the ability of changes at any of these levels to contribute to human diseases. Bioinformatic modeling as well as biomimetic systems that help to understand self-assembly and multiscale networks is expected to be important along with experimental approaches toward understanding mechanistic specializations of kinesin–tubulin interactions, functional integration, and human disease.
Abbreviations: PTMs, posttranslational modifications; MT, microtubule.

Force generation

The ~360-amino acid globular motor domain of kinesin is its defining feature that contains conserved HYPIR and LAGSE sequence elements along with an ATP-binding catalytic site.[28] In the kinesin–tubulin ATPase cycle, the binding of the motor domain to microtubules stimulates the ATP turnover rate by 1,000-fold.[29] The affinity of the motor domain to the microtubule is altered upon ATP hydrolysis and release of Mg-ADP. This action generates a molecular switch that can couple ATP hydrolysis with neck linker rotation and affect speed and motility. The rate of ATP hydrolysis is determined by ATPase activity and ADP plus Pi release, and affects the force generated and speed of translocation.[30] The force can be applied toward directed motility, to generate a ratcheting motion for depolymerizing microtubule ends, or allow release and recycling of kinesins from α/β-tubulin heterodimers.

Kinesin step size

A microtubule in vivo has a typical diameter of 25 nm that contains 13 protofilaments composed of α/β-tubulin heterodimer building blocks.[31,32] The typical kinesin step size on microtubules is 8 nm[33] that corresponds to the spacing of β-tubulin proteins within the longitudinal protofilament. Our understanding of the mechanics of the kinesin steps and their size comes largely from work with the dimeric non-mitotic cargo transporter, Kinesin-1.[34–36] The asymmetric binding of the dimeric motor heads to microtubules along with asymmetric ATP binding, hydrolysis, and ADP plus Pi release and rotation of the neck linker generates a hand-over-hand stepping motion.[37] A recently revised model of the mechanochemical cycle of kinesin on microtubules is shown in Figure 3A, along with the structure of a single ADP-bound kinesin motor head of Kinesin-1 KIF5B docked to an α/β-tubulin heterodimer in Figure 3B. KIF5B binds primarily to 8 nm spaced β-tubulin subunits in the microtubule protofilament, near helices 11 and 12 in the structure.[38–40] Kinesins can also bind to γ-tubulin, using residues conserved between β- and γ-tubulin helix 11, indicated in Figure 3C. The kinesin–microtubule ATPase cycle is well studied by many groups and includes more recently a higher
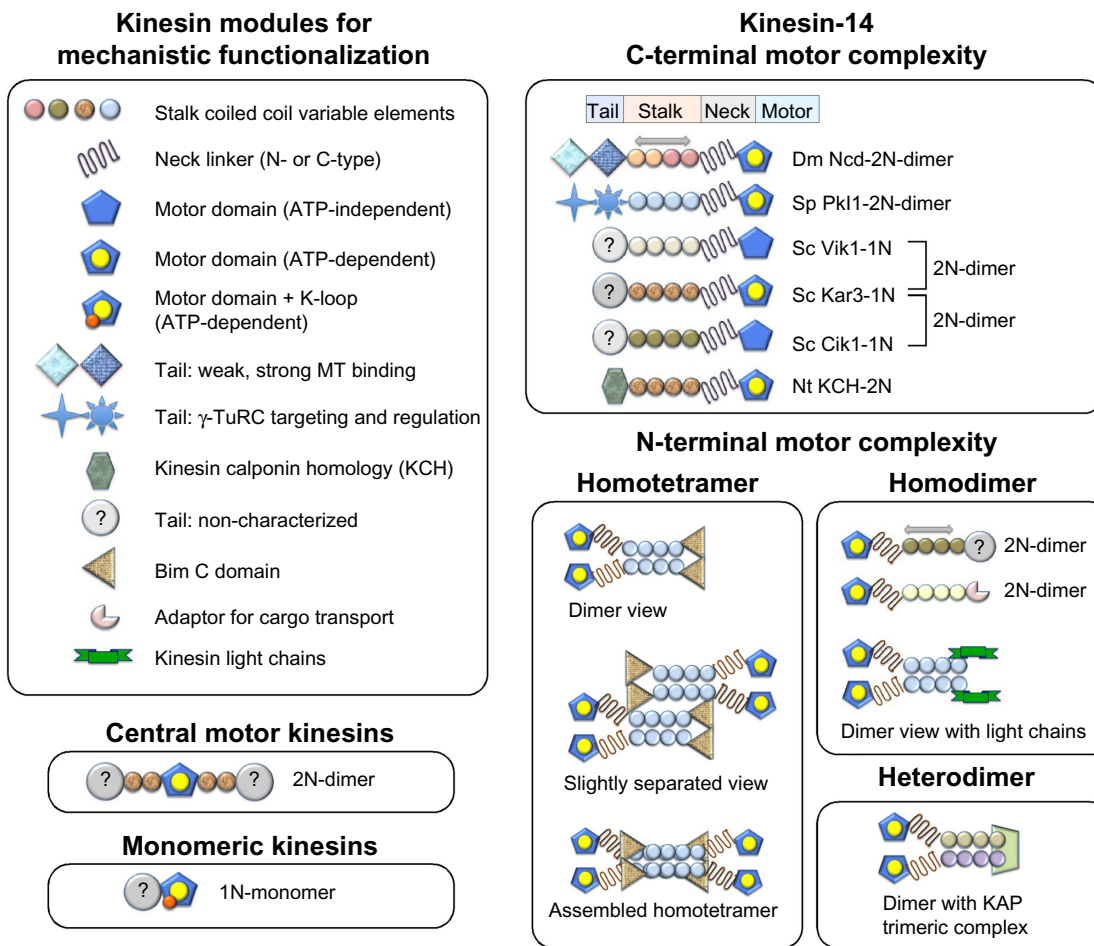
Kinesin modules for mechanistic functionalization

Stalk coiled coil variable elements

Neck linker (N- or C-type)

Motor domain (ATP-independent)

Motor domain (ATP-dependent)

Motor domain + K-loop (ATP-dependent)

Tail: weak, strong MT binding

Tail: γ-TuRC targeting and regulation

Kinesin calponin homology (KCH)

Tail: non-characterized

Bim C domain

Adaptor for cargo transport

Kinesin light chains

Central motor kinesins

2N-dimer

Monomeric kinesins

1N-monomer

Kinesin-14 C-terminal motor complexity

Tail | Stalk | Neck | Motor

Dm Ncd-2N-dimer

Sp Pkl1-2N-dimer

Sc Vik1-1N ⎫ 2N-dimer

Sc Kar3-1N ⎭ ⎫ 2N-dimer

Sc Cik1-1N ⎭

Nt KCH-2N

N-terminal motor complexity

Homotetramer

Dimer view

Slightly separated view

Assembled homotetramer

Homodimer

2N-dimer

2N-dimer

Dimer view with light chains

Heterodimer

Dimer with KAP trimeric complex

Figure 2 Modular kinesins.

Notes: The functional repertoire of kinesins is not yet predictable through sequence alone. The diagram illustrates organization of kinesins as monomeric or oligomeric with defining mechanistic components that can confer functional specializations. The diversity of the Kinesin-14 family members among different eukaryote model organisms is presented to illustrate the elegant complexity possible within a single kinesin family and emphasize the continued need for detailed structure–function studies of all kinesins. Dimeric kinesins are shown as single-polypeptide chains for simplicity with oligomeric state indicated by 2N-dimer or 1N-monomer. Heterodimer pairings for Kinesin-14 members are indicated by brackets.

Abbreviations: ATP, adenosine triphosphate; MT, microtubule.

resolution reevaluation of structural changes upon stepping[39] and a better understanding of molecular adaptations within motor domains that affect ATPase activity and directional motility.[41] For monomeric kinesins, a short charged sequence element referred to as the K-loop is present. The K-loop binds to the C-terminal tails of microtubule tubulins at the interface of the α/β-tubulin heterodimers. This interaction provides a second microtubule-binding site during stepping that prevents complete dissociation of the monomeric kinesin from the microtubule upon ATP hydrolysis.[42,43] Nonequivalent stepping of dimeric heads by Kinesin-14 Kar3/Vik1 heterodimer has also been reported[44] as well as the ability of Kinesin-8 Kip3 to take a larger lateral step onto side-by-side protofilaments.[45] The latter would permit the ability to bypass obstacles, such as other motor proteins, avoiding traffic on microtubules. Advances in imaging

technology[26] continue to reveal new findings in regard to step size and trafficking.

Directional motility, processivity, and speed

Not all kinesins are motile. Among motile kinesins, the retention time on microtubules before dissociation, referred to as processivity, and the rate of motion may differ. Processivity can be understood in terms of the number of ATP hydrolyses (number of steps) per initial microtubule-binding event. Highly processive kinesins, such as Kinesin-1, may take over 100 steps per encounter with a microtubule, hydrolyzing a single ATP per step.[33,46] For non-processive kinesins, such as *Drosophila* Kinesin-14 Ncd and human Kinesin-14 KIFC1, only a single step or ATP hydrolysis occurs before the kinesin dissociates from
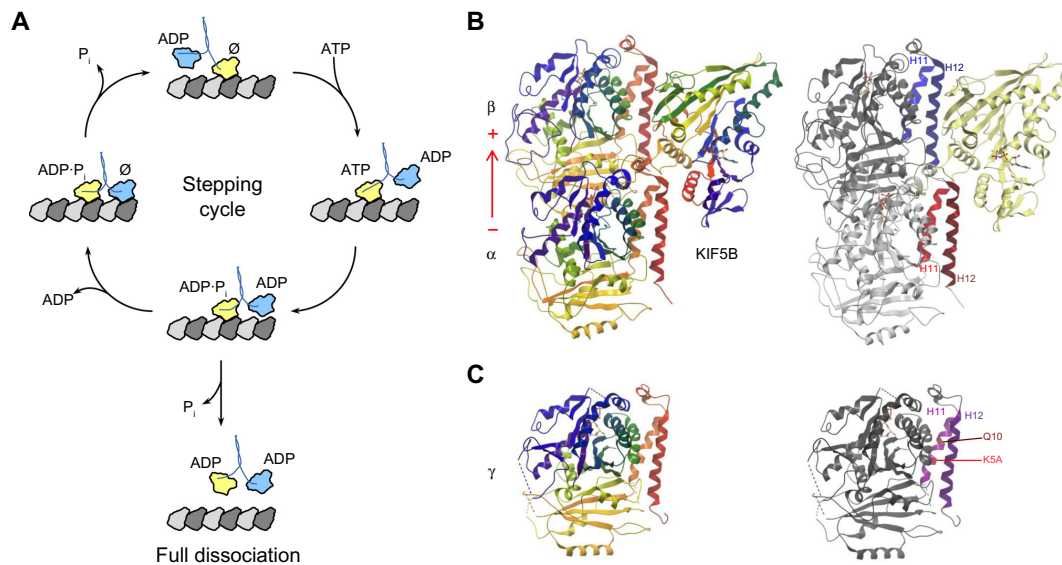
Figure 3 The kinesin–tubulin complex.

Notes: (**A**) The ATP mechanochemical cycle of a processive dimeric kinesin motor is described in detail in the text. Microtubule binding of the kinesin head stimulates ATPase activity in the motor domain. The kinesin dimer is shown initially with one-head bound in an apo (empty) or ATP-waiting state. It is attached to the microtubule without nucleotide in its active site (Ø), while the second tethered but non-bound motor head is shown free from the microtubule with ADP in its active site. ATP binding to the bound head induces partial docking of the neck linker with this motor head, biasing the tethered head forward. Subsequent hydrolysis of ATP to ADP·P_i completes neck linker docking and results in one of two outcomes. The once free tethered head may also bind adjacently on the microtubule and release ADP resulting in a forward step. The subsequent release of P_i returns kinesin to the ATP-waiting state, thus completing the cycle. Alternatively, the bound head may prematurely release P_i that would result in entire dissociation of the kinesin molecule from the microtubule. Adapted with permission from Milic B, Andreasson JOL, Hancock WO, Block SM. Kinesin processivity is gated by phosphate release. *Proc Natl Acad Sci USA*. 2014;111(39):14136–14140. Available from: http://www.pnas.org/content/111/39/14136.[169] (**B**) Single motor domain head of Kinesin-1 (KIF5B) without a docked neck linker (PDB ID: 1BG2[170]) fit into the 9 Å cryo-electron microscopy map of nucleotide-free kinesin (Ø) complexed with α/β-tubulin. (**C**) Structure of human γ-tubulin (PDB ID: 1Z5W).[171] Two residues in helix 11, K5 and Q10, shown by mutation to affect kinesin motor binding to γ-tubulin in fission yeast are indicated.

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; MT, microtubule.

the microtubule.[47] The degree of processivity of a kinesin on a microtubule is not yet fully predictable. It is influenced by sequence elements in both the neck linker and motor domain that interact upon microtubule binding and ATP hydrolysis. Removal of the neck linker region diminishes processivity,[48] while the addition of positive or negative charges to the neck linker region increases or decreases processivity, respectively.[49] Directional motility is primarily associated with the kinesin neck domain.[50] In fact, altering conserved elements in the neck sequence of *Drosophila* Kinesin-14 Ncd allowed directional reversal of motility from minus-end to plus-end directed.[51] Within a kinesin polypeptide that has an N-terminal motor domain, the neck sequence follows, whereas the neck sequence precedes a C-terminal-positioned motor domain (Figure 2). Neck linker sequences differ between kinesins in regard to N-terminal or C-terminal motor domain position and along with the motor confer plus- or minus-end directionality, respectively. Motile members of the Kinesin-14 family are minus-end directed. This paradigm helps to classify motor proteins into kinesin families but is not predictive of processivity or rates of motility that can be very different.[52] Some tubulin modifications can selectively affect kinesin rate of motility. The polyglutamylation of the C-terminal tails of α/β-tubulins

has been shown to enhance the motility of members of Kinesin-1 and Kinesin-2 but not Kinesin-13.[53]

Tethered diffusion

Nonmotile or low-processivity kinesins have the capability to track along microtubules by tethered diffusion in a one-dimensional random walk.[30] That is, the initial three-dimensional diffusion to find microtubules is reduced in dimensionality to one dimension, following the initial contact with microtubules. In the case of the central motor domain kinesins, Kinesin-13 XKCM1 (MCAK) and XKIF2,[13,54] one-dimensional tethered diffusion directs these kinesins to microtubule ends where the ATPase cycle is used in depolymerization of microtubules.[13] In single-molecule microscopy assays, MCAK on average diffused 0.83 seconds with a diffusion coefficient of 0.38 μm^2/s.[55] Strong binding to the microtubule occurs in the ATP-bound state, whereas diffusion occurs during the weakly bound ADP state. For MCAK, tethered diffusion increased the concentration of kinesin at microtubule plus- or minus-ends over 100-fold compared to free diffusion in three dimensions.[55] Two Kinesin-14 dimeric family members also exhibit tethered diffusion, that for *Drosophila* Ncd is unbiased[56] and for *Schizosaccharomyces pombe* Pkl1 is biased[57] diffusion along the microtubule.

Microtubule dynamics

Microtubules exhibit dynamic instability[58] that is an inherent ability to transition between periods of stable growth or rapid disassembly of subunits, referred to as polymerization and depolymerization, respectively.[31] Microtubules also undergo a period of attenuated dynamics or a pause state during which they neither grow nor shorten appreciably.[59,60] Heterodimeric α/β-tubulin subunits assemble as polarized longitudinal protofilaments that become linked laterally as a sheet that closes to form a tube.[60,61] The polar microtubule lattice terminates in α-tubulin at minus-ends and is stabilized by a GTP-β-tubulin cap. The hydrolysis of this cap to GDP-tubulin triggers rapid depolymerization[31] that can be regulated by kinesins and other microtubule-associated proteins. Depolymerization mechanisms are not fully understood and may generate either small or large changes to microtubule length. Kinesin-13 mediates bending of individual protofilaments away from the longitudinal face of the microtubule and generates large changes in microtubule length, while Kinesin-8 either suppresses dynamics or removes just one or two heterodimers and may not deform protofilaments.[13] Certain members of the Kinesin-4 family also regulate dynamics. Kinesin-4 Kif7 regulates microtubule length in the cilium by depolymerization that is also linked to Hedgehog signaling.[62] The Sonic Hedgehog pathway is critical for vertebrate organogenesis in the developing embryo, including brain development. Microtubule plus-end proteins Kinesin-10 Nod and Kinesin-7 CENPE both promote microtubule plus-end elongation[14] but either do so by an ATP hydrolysis-independent pathway, as for Nod,[63] or require an ATP turnover for polymerizations, as for CENPE.[64] Multicellular eukaryotes encode genes for Kinesin-4, Kinesin-7, Kinesin-8, Kinesin-10, and Kinesin-13 families, while only Kinesin-8 is present in fission and budding yeasts.[13,65] The translocation of α/β-tubulin heterodimers within microtubule protofilaments during mitosis is referred to as flux.[66] Coupling plus-end addition of α/β-tubulin subunits with minus-end loss of subunits during flux can generate minus-end-directed transport of microtubule-bound proteins. The flux mechanism is unknown but requires Kinesin-5 in *Xenopus*, although not in human cells.[67,68] Microtubule dynamics facilitate rapid organization and reorganization of microtubule architectures, regulate multiple microtubule-dependent signaling pathways, and provide forces to push and pull as needed for chromosome capture and alignment during mitosis.

Cross-linking and sliding of microtubules

The ability to cross-link and/or slide parallel or antiparallel microtubules is a classical role of dimeric or tetrameric kinesins that drives the assembly of larger microtubule arrays. These actions are integral to ubiquitous processes such as spindle assembly and anaphase B elongation in dividing cells as well as cell specialization such as transport in neuronal axons and dendrites that contain microtubule bundles of opposite orientation. Tetrameric Kinesin-5 members help to drive bipolar mitotic spindle assembly and anaphase B spindle elongation by cross-linking and sliding antiparallel microtubules extending from opposing spindle poles.[69] In fission yeast, the Kinesin-6 Klp9 is the anaphase B motor and is regulated by the mitotic Cdc2/cyclin B kinase.[70] Cross-linking without sliding can be used to generate a molecular brake to maintain integrity of bipolar microtubule arrays. For example, *C. elegans* Kinesin-5 BMK-1 resists outward sliding during anaphase B to prevent spindle collapse.[71] A similar braking mechanism is proposed to regulate neuronal process outgrowth by Kinesin-5 and Kinesin-12 members.[72,73] The homodimer *Drosophila* Kinesin-14 Ncd cross-links microtubules via its motor domain and tail region that contains ATP-independent tubulin-binding elements and allows Ncd to span two adjacent microtubules.[74,75] The positively charged tubulin binding elements in the Ncd tail bind to negatively charged residues in the C-terminus of α/β-tubulins, which extend from the microtubule surface. As with tetrameric kinesins, Ncd can either drive antiparallel microtubule sliding or act as a brake on cross-linked microtubule arrays.[12] Kinesin-5 and Kinesin-14 families in *Drosophila* provide balanced antagonistic forces that enable spatial and temporal control of mitotic spindle assembly.[76] This antagonistic relationship is now considered ubiquitous in all eukaryotes. It also likely extends to nondividing cells, such as neurons for the organization of axon and dendrite microtubule arrays, in which Kinesin-5 is already known to play a role.[72]

Cooperative kinesin–tubulin interactions

The ability of multiple copies of a single kinesin protein bound on microtubules to uniformly coordinate their actions requires cooperation. It is of particular importance to such processes as antiparallel microtubule sliding in which coordinated effort may be needed for large force-requiring events, such as anaphase B, and where uncoordinated efforts can result in braking and stuttered movements. A model of cooperative action of *Drosophila* Kinesin-14 Ncd proteins on microtubules is proposed based on differing electrostatic interactions in its coiled coil stalk, a structural domain that participates in dimerization.[77] The Ncd stalk

contains differing coiled coil regions. Weak electrostatic interactions are adjacent to its tail domain, followed by a strongly dimerizing region.[78,79] In the Ncd cooperativity model, the weak coiled coil segment will collapse under force when non-synchronized with force-generating Ncd proteins. In addition, for both *Drosophila* Ncd and *S. pombe* Pkl1, the diffusive component of movement is reduced with increasing motor numbers that may facilitate cooperation.[57,77] Continued studies are needed to determine detailed mechanisms through which collective motor coordination contributes to microtubule organization and other processes such as intracellular transport.

Cross-cytoskeleton bridging

In addition to classical microtubule cross-linking, kinesins can also bridge microtubules to additional cytoskeleton networks, which is often referred to as cytoskeletal intersections.[80] Two studies using the squid conventional kinesin, Kinesin-1, indicate an ability to link microtubules to intermediate filaments via light chains that are a feature of this kinesin family.[81–83] The kinesin attachment to the intermediate filament is static, but motile on the microtubule, and contributes to the assembly of large intermediate filament networks that are important for directional protrusion of lamella and cell migration.[84] Kinesins can also cross-bridge microtubules to actin. In the plant *Nicotiana tabacum*, cell division requires Kinesin-14 cross-bridging of actin to microtubules that uses an internal kinesin calponin homology domain (KCH).[23] In budding yeast *Saccharomyces cerevisiae*, the Kinesin-1-like Smy1 colocalizes with myosin V motor Myo2, binds the Myo2 tail, and is able to suppress myosin defects.[85–87] Smy1 has also been shown to have a role in actin cable assembly by binding to the FH2 domain of the formin Bnr1 to decrease the rate of actin filament elongation and enable proper actin architecture and dynamic function.[88] Although a distant kinesin relative, any microtubule-based roles are yet to be defined. No kinesin-facilitated tubulin–actin interactions in metazoans have been identified, although non-motor adaptor proteins like the actin–microtubule linker protein ACF7 that is found primarily in neural network, muscle, and lung could possibly assist in cell-specific interactions.[80]

Sortability

Here, we define the ability to direct kinesin–tubulin interactions to specific cellular locations or to promote or prevent interactions. Three primary strategies enable separation of kinesin–tubulin interactions that are kinesin adaptor proteins, kinesin specialization or tubulin diversity, and

modifications. Kinesin adaptor proteins and specialized intrinsic kinesin targeting domains both frequently involve the tail regions and are exemplified in the Kinesin-14 family described in a later section. The restriction of kinesin–tubulin interactions by specific tubulin isoforms, isotypes, or post-translational modifications (PTMs) is sometimes referred to as the tubulin code.[89] These strategies are expanded in multicellular eukaryotes through additional kinesin families and members and an extended tubulin superfamily and PTMs.[53,90] In yeasts, the limited tubulin diversity includes only three tubulin isoforms that are α, β, and γ and two α-tubulin isotypes. In contrast, the human genome has seven genes that code for α-tubulin, eight for β-tubulin,[91] two genes for γ-tubulin,[92] as well as genes encoding other tubulin members.[90,93,94] Tubulin PTMs further contribute to sortability and compartmentalized functions as exemplified in a recent review in regard to neural physiology[3] as well as a comprehensive bioinformatic analysis of the C-terminal tail of tubulins. The tubulin tail is where most PTMs occur,[94] except for acetylation.[95] The other PTMs found in tubulins include phosphorylation, tyrosination, polyglutamylation, and polyglycylation that can selectively serve as a code for specific cell functions.[89] Examples of use of the tubulin code include Kinesin-1 KIF5 that navigates to axonal but not dendritic microtubules by differences in tyrosination[96] and inability of Kinesin-13 MCAK and Kif2A to depolymerize neuronal microtubules that are detyrosinated at the α-tubulin C-terminal tail and stabilized.[13]

Cargo, transport, and signaling

Kinesin-mediated transport along microtubules has been studied extensively due to the wide scope of cargo. This includes RNA, proteins, chromosomes, vesicles, organelles, small multiprotein complexes, and other microtubules.[97] Multiple Kinesin families participate in intracellular transport with cargo-specific domains and include Kinesin-1, Kinesin-2, Kinesin-3, Kinesin-4, Kinesin-7, and Kinesin-10 members.[25,98] Tight spatiotemporal control of transport by kinesins is achieved by both auto-inhibition and phosphorylation-dependent mechanisms acting on kinesins as molecular switches.[26] Numerous kinesin–microtubule transport functions contribute to such critical processes as assembly and functional dynamics of the mitotic spindle,[99] establishment of correct microtubule length and architecture of cilia and flagella via intraflagellar transport,[100] and neuronal function.[101] Detailed mechanisms for cargo release and handoff at a given destination are largely unknown. Thus, it is unclear whether the kinesin is recycled by active retrograde transport with dynein, simple diffusion, or used singly and

then eliminated through proteasome degradation pathways. For example, experimental analysis of human Kinesin-1 Kif5 coupled with mathematical modeling supports diffusional recycling after cargo transport and release at neurite tips.[102] In mitosis, Kinesin-7 CENPE transports misaligned chromosomes to plus-ends at the metaphase plate, switching from microtubule lateral association to plus-end tracking that couples dynamic microtubule ends to kinetochores.[103–105] This action also links CENPE to spindle assembly checkpoint signaling that monitors chromosome bipolar attachment and tension.[106] Other examples linking cargo, transport, and signaling include *Drosophila* Kinesin-4 Cos2 that transports the transcription factor Ci required for Hedgehog signaling[107,108] and Kinesin-1 that transports SMAD2 in the TGF-β signaling pathway.[109] Members of a single kinesin family may regulate the same signaling pathway in different ways between eukaryotes. Mammalian Kinesin-4 KIF7/KIF27 regulates the Hedgehog signaling pathway in cilia by controlling cilia length through microtubule depolymerization at plus-ends and not by participation in microtubule-based transport as does *Drosophila* Kinesin-4 Cos2.[62]

Kinesin-14-tubulin paradigm for functional specialization

Only one kinesin family, Kinesin-14, is capable of minus-end-directed motility. This may explain why its diverse functions include all mechanistic specializations reviewed so far as well as incorporation into supramolecular machines discussed in the following section. This detailed appreciation of Kinesin-14 member capabilities comes from their analysis by site-directed mutations, partial deletions, chimeric kinesins, cross-species functional studies, single-molecule and motility assays, and structural and bioinformatic modeling. Specialization of tail domain sequences differs between *S. pombe* Pkl1 and *Drosophila* Ncd allowing these proteins to sort to and function at γ-TuRC or on microtubules, respectively.[12,74,110] Pkl1 is nonmotile but actively diffuses along microtubules to spindle poles.[57] The Ncd tail has two specialized elements for ATP-independent microtubule binding, including a lysine-rich so-called E-Hook-binding sequence that binds negatively charged residues in the tubulin C-terminal tails referred to as the E-Hook.[74] With microtubule-binding capabilities at both ends, Ncd can cross-link parallel or antiparallel microtubule arrays and slide microtubules. As mentioned previously, specialization of the Ncd stalk is proposed to coordinate the action of multiple Ncd proteins on microtubules.[79] The budding yeast *S. cerevisiae* Kinesin-14 Kar3 is best studied in regard to its heterodimeric partnering with Cik1 and Vik1

proteins.[111–115] The ATP-independent motor domains of both Cik1 and Vik1 generate an alternate stepping mechanism with the Kar3 ATP-regulated motor head. The Cik1 and Vik1 partners also provide sortability by targeting Kar3/Vik1 to the spindle pole body protein Spc72 during meiosis or Kar3/Cik1 to microtubules during mitosis.[113,116] Typically, eukaryotes have two to three Kinesin-14 members, except for the single Ncd in *Drosophila*.[1] Multifunctional properties often exist in Kinesin-14 members that are not fully understood mechanistically. Pkl1 participates in spindle assembly checkpoint signaling.[117,118] Ncd has additional described roles in meiosis with γ-tubulin.[119,120] Human Kinesin-14 HSET cross-links microtubules in human cells[121,122] but binds to γ-TuRC in cross-species studies in *S. pombe*.[110] Kinesin-14 members currently share no sequence similarities outside of conserved neck linker and kinesin motor elements. Thus, understanding separation of function either among multiple members between eukaryotes or within a single eukaryote continues to require detailed functional knowledge aided by understanding of specialized domains.

Kinesin–tubulin integration into supramolecular machines and cell specialization

Kinesin–tubulin interactions have important conserved roles in multiprotein complexes to coordinate function with signaling. Required mitotic complexes include spindle kinetochores for chromosome attachment,[123] γ-TuRC for microtubule nucleation from spindle poles,[20,124] and passenger proteins that transfer from kinetochores to a spindle midzone for temporal control of anaphase.[105,125] Orientation of the spindle axis with the cell cortex by astral microtubules also uses cytoplasmic microtubule plus-end complexes.[105] The following are well studied in cell specialization: intraflagellar trafficking to control microtubule length, and architecture and function in cilia and flagella;[100,126,127] neurophysiology of axon architecture and regeneration as well as neuronal growth cone formation and turning;[101] cell–cell interfacial communication through adherens junctions;[128] and developmental roles such as cell migration[84] and regulation of nodal flow.[126] Here, we discuss briefly the newly emerging roles in centrosomal complexes as well as summarize the now extensive studies on cilia and flagella, also reviewed in detail elsewhere.[98,100,126,129]

The centrosome is a massive microtubule-generating and microtubule-organizing center and hub for intracellular signaling.[130] A pair of centrioles within a pericentriolar material helps to organize this complex matrix that

includes numerous γ-TuRC sites that nucleate microtubules. The microtubule polarity enabled by centrosomal γ-TuRC contributes to classical roles such as mitosis and meiosis, as well as specialized functions in neurophysiology and immune cell targeting via the immunologic synapse. Centriolar biogenesis is a semiconservative process in which a daughter centriole is generated from a mother template. The Kinesin-13 Kif24 has been shown to asymmetrically bind to mother centrioles of the centrosome in S/G2 phases of the cell cycle to assist in remodeling of centriolar microtubules.[21] Kif24 does not alter cytoplasmic microtubules or arrays. Kinesin-14 and Kinesin-5 were recently shown to antagonistically bind to γ-TuRC to regulate microtubule nucleation.[20,124] The γ-TuRC provides a 13-protofilament ring template for microtubule growth and minus-end attachment.[131] The kinesin motor domains bind γ-TuRC through γ-tubulin helix 11 (Figure 3C), predicted to be similar to kinesin–β-tubulin microtubule interactions.[132] Additional targeting to γ-TuRC, blocking of microtubule nucleation, and antagonism of Kinesin-14 by Kinesin-5 require specialized tail domain elements in Kinesin-14 Pkl1[110,124] and the BimC domain in Kinesin-5 Cut7.[20] Pkl1 tail elements alone are both necessary and sufficient to block microtubule nucleation by γ-TuRC in human breast cancer cells.[20,110,124] Incorporation of kinesin–tubulin roles at centrosome centriolar and nucleation sites may underlie needs for precise coupling to spindle mechanisms and non-mitotic centrosome functions.

Eukaryotic cilia and flagella microtubule-based organelles serve multiple roles in chemo-, thermo-, and mechano-sensation, cell motility, and fluid flow rate. Immotile primary cilia are found in eye rod photoreceptor cells and olfactory receptors, while motile cilia line fluid compartments. The latter includes lining of the trachea that protects the lungs from fluid accumulation and the fallopian tubes to facilitate transport of the ovum to the uterus. An axoneme structure in primary cilia has a ring of nine microtubule doublets (9+0), while motile cilia and flagella have two additional microtubule singlets within the ring for a 9+2 arrangement. In *Caenorhabditis reinhardtii*, Kinesin-13 is positioned at flagellar microtubule ends where it controls microtubule assembly and disassembly in this organelle.[133] Kinesin mechanisms for microtubule depolymerization in ciliary, flagellar, and centriole length control are reviewed elsewhere.[13] A heterotrimeric Kinesin-II complex that is composed of a heterodimeric KIF3A/KIF3B and KAP protein regulates intraflagellar cargo transport in multiple species. Polarized cargo transport from the cell body to the developing cilium assists ciliogenesis by participating in

assembly and regeneration.[127] Patterning in mammalian and fly embryos uses kinesin–tubulin regulation of Hedgehog pathway signaling, as described earlier, in transport and depolymerization roles affecting cilia architecture and function.[62,107,134–136] These roles are needed during mesodermal induction and neurogenesis.[126,137]

The kinesin–tubulin complex in development, disease, and human health

Mutations in kinesins and tubulins as well as inappropriate up- or downregulation of these proteins in specific cell or tissue types have been linked to cancer, transport-based neuropathologies, plasticity-related neuropathologies, memory, liposomal diseases, and developmental defects (Table 1). Deciphering the cause of disease requires an understanding of kinesin–tubulin mechanistic functions. Although a cellular process such as transport may be affected, the precise point where the mechanism fails is not always evident. Transport-based failures that lead to disease may occur via a variety of onslaughts to kinesin or tubulin proteins that alter any of the mechanistic functions described in this review as well as protein stability and concentration. Here, we highlight both mechanistic functions and additional considerations in the kinesin–tubulin complex as it relates to development, disease, and potential therapies to improve human health.

Although mechanisms underlying kinesin–tubulin interactions are often studied in regard to a particular cell process, functional diversification can exist. We define that as the ability of a kinesin to participate in multiple processes in different cell types using all or a subset of its mechanisms. Functional diversification often exists in multicellular eukaryotes that must be considered in human disease treatment strategies. In cancers, the important roles that mitotic kinesins play in spindle function and genomic stability are apparent when disrupted due to their impact on tumorigenesis and cancer progression.[138–140] In this regard, further disrupting mitotic kinesins or spindle microtubule dynamics are effective anticancer therapies[139,141] by promoting apoptotic cell death.[142,143] However, current microtubule poisons such as paclitaxel in clinical use that bind tubulins and alter microtubule dynamics,[141,144,145] while effective, can also interfere with microtubule function of nondividing differentiated cells such as neurons.[146] Similarly, Kinesin-5 Eg5 drugs that block bipolar spindle assembly[147] that are in Phase I clinical development must contend with non-mitotic roles of this kinesin. The ability of Kinesin-5 to slide microtubules is

Table 1 Kinesin–tubulin interactions in human diseases

Disease	K–T mechanistic component	Organism in study	References
Neuromuscular and muscular defects			
Charcot–Marie–Tooth disease (type 2)/hereditary spastic paraplegia	N256S mutations in the Kinesn-1 KIF5A motor domain decrease MT gliding velocity but not MT affinity; additional mutations in K253N and R280S prevent motor–MT binding (bioinformatic patient data source)	Human, *Drosophila*	153,154,171
Charcot–Marie–Tooth disease (type 2)	Loss-of-function Q98L mutation in the ATP consensus of the Kinesin-3 KIF1B motor domain prevents ATPase activity	Mouse	156
Congenital fibrosis of extraocular muscle (types 1 and 3)	Mutations in the Kinesin-4 KIF21A stalk domain (bioinformatic patient data; K–T mechanistic component unknown)	Human	159,172
Huntington's disease	JNK3 phosphorylation of KIF5 at S175 reduces kinesin force generation but not ATPase activity, MT binding, or processivity	Human	151,173,174
Memory			
Alzheimer's disease	KIF5 light chain-dependent transport of amyloid precursor protein (K–T mechanistic component unknown); GSK3β phosphorylates KIF5 light chains and releases membrane-bound organelles (K–T mechanistic component unknown); amyloid beta accumulation disrupts Kinesin-5 Eg5 neuronal function (K–T mechanistic component unknown)	Human, mouse	151,175,176
Brain development			
Microcephaly–lymphedema–chorioretinopathy	Mutations identified in the Kinesin-5 Eg5 motor, stalk, and tail domains disrupt normal CNS development (bioinformatic patient data; K–T mechanistic component unknown)	Human	148
Intellectual disability (with epilepsy, microcephaly, or cortical malformation)	Exon-skipping mutations in Kinesin-4 KIF4A and the Kinesin-1 KIF5C Q237K motor domain mutation interrupt the balance between excitatory and inhibitory synaptic excitability (bioinformatic patient data; K–T mechanistic component unknown)	Human	161
Brain signaling			
Schizophrenia	Truncating mutation in Kinesin-2 KIF17 was discovered in one schizophrenia patient (bioinformatic patient data; K–T mechanistic component unknown)	Human	162
Epilepsy	Knockout of KIF5A inhibits transport of GABA$_A$ receptor protein (K–T mechanistic component unknown)	Mouse	160
Organ patterning and functioning			
Polycystic kidney disease	Tissue-specific inactivation of KIF3A in renal tubular epithelial cells (K–T mechanistic component unknown)	Mouse	177
Kartagener's syndrome	KIF3A/KIF3B knockouts disrupt embryonic left–right axis determination (K–T mechanistic component unknown)	Human	178
Cancer* (lymphomas, leukemias, tumors)	Eg5 motor domain loop 5-directed inhibition causes release of ADP and destabilization of the K–T interaction	Human, mouse	139,179,180
Cancer (solid tumors)	Inhibition of the Kinesin-7 CENPE motor domain disrupts MT-stimulated ATPase activity, and stabilizes the K–T interaction	Human	181

Note: *Altered expression (as opposed to mutation) of multiple kinesin family members is associated with a variety of different cancers (reviewed in Rath and Kozielski[139]).
Abbreviations: K, kinesin; T, tubulin; CNS, central nervous system; MT, microtubule; ATP, adenosine triphosphate; ADP, adenosine dophosphate.

utilized in neurons to promote axonal outgrowth by focusing of microtubule arrays.[72] The impact of altered function of Kinesin-5 on neural health may be significant as revealed by Ostergaard et al.[148] In this study, 14 mutations in the *KIF11* gene encoding human Kinesin-5 Eg5 were identified that map to the stalk region and occur in 15 patients with autosomal-dominant microcephaly accompanied by lymphedema and/or chorioretinopathy. Stalk mutations including the neck linker may alter the kinesin–tubulin interface by affecting the kinesin oligomeric state, step size, or directional motility parameters. The ability to target a specific cell process by affecting kinesin–tubulin interactions, without also affecting

other processes, remains challenging and requires knowledge on the scope of functional diversification.

During human development, kinesin–tubulin interactions participate in both more confined short-range processes such as mitosis and cell–cell communication as well as in extended and complex systemic architectures possible with the human neural network. For proper neurophysiological functions, microtubule networks in neuronal axons that transport organelles and synaptic vesicles can extend to a meter long and must be maintained and repaired to prevent neuronal death and impaired neural function.[149] Accumulation of materials in neuronal compartments is a characteristic of Alzheimer's

and Huntington's disease.[150,151] Mutations in Kinesin-1 KIF5A, Kinesin-3 KIF1B, and Kinesin-4 KIF21A, as well as in tubulin isotypes, have been reviewed in regard to neuronal disease contributions[3,149] along with detailed studies of the KIF5 family in transport-based accumulation diseases like Alzheimer's[150,152] and KIF13B in cholesterol homeostasis.[8] Mutations to the KIF5A motor or stalk that impair axonal transport are linked to Charcot–Marie–Tooth disease 2 (CMT2)[153] as well as hereditary spastic paraplegia in humans.[153,154] CMT2 also results from mutations in KIF1B that reduce transport of vesicle proteins.[155,156] Mutations in KIF21A underlie atrophy of ocular motor muscles and the onset of congenital fibrosis of the extraocular muscle (referred to by the acronyms CFEOM1 and CFEOM3).[157–159] In mice, the absence of *Kif5A* leads to development of epilepsy due to the failed transport of the $GABA_A$ receptor protein.[160] Mutations in tubulin proteins that inhibit neuronal migration have been linked to brain development. This includes insufficient brain folds and grooves seen in lissencephaly, as well as microcephaly that is characterized by a small brain size.[3]

Changes to higher order functions such as memory are also linked to kinesin–tubulin interactions due to effects on transport for synaptic function, neural architecture, and neuroplasticity. A recent patient study by Willemsen et al[161] found that a disruptive mutation in Kinesin-4 KIF4A (exon skipping) was linked to mild intellectual disability (ID) and epilepsy, whereas a patient with a missense mutation in Kinesin-1 KIF5C (Glu-237-Lys) exhibited severe ID, epilepsy, microcephaly, and cortical malformation. A truncating mutation in the synaptic Kinesin-2 KIF17 was identified in one patient with Schizophrenia during a bioinformatic screen.[162] Altered kinesin functions in these patients may interrupt the balance between excitatory and inhibitory synaptic excitability required for normal brain function and normal development.

Animal studies of kinesin–tubulin interactions and functions extend studies in model eukaryotes and have focused primarily on the brain. These studies include generation of knockout genes in mice, analysis of brain sections, and establishment of primary cultures and explants. During development, mitotic-cycling cells stop dividing, exit the cell cycle, and differentiate. In this transition, kinesins typically thought to be mitotic specific may be repurposed into non-mitotic roles, or alternate family members may perform these roles. The story is expected to be complex, since in murine brain development, as many as 38 kinesin superfamily proteins (KIFs) may be required.[163,164] Analysis of murine KIFs may offer solutions for human diseases that build off of kinesin–tubulin knowledge from model eukaryotes. For example, strategies for enhancing regeneration of adult axons via inhibition of Kinesin-5.[165]

Future directions

Nature has coevolved both kinesins and tubulins to generate extensive functional capabilities. The complex nature of kinesin–tubulin interactions includes not only mechanistic specializations but also functional diversification. The latter is particularly evident in multicellular eukaryotes in which cells exit the cell cycle and differentiate to accommodate new cellular and systemic roles, yet may take advantage of kinesin–tubulin interactions originally only needed for mitotic cell division. The ability of kinesin–tubulin interactions to contribute to human development and higher order functions such as memory, and impact these through diseases can be used as a powerful tool to help identify the molecular basis of these processes. Structural studies continue to provide an insider's view at the molecular scale but currently remain incomplete in resolution detail and provide static time points. Computational bioinformatics will help to bridge the gap between experiment, structure, and in vitro models as has been done to understand the role of tubulin C-terminal tails,[94] microtubule dynamics of tubulin polymerization/depolymerization,[60] mesoscale communication networks,[166] and in vitro models to test kinesin–microtubule functional and organizational plasticity.[52,167] As well, biomimicry is important to test and apply our understanding of kinesin–tubulin interactions. Biomimicry takes its template from biology to incorporate functionality or design from nature,[168] including copying, repurposing, or developing novel emerging functions. The ability to copy nature is not trivial, and so hybrid biosynthetic systems are likely to emerge first. Manipulating the kinesin–tubulin interaction has been attempted in vitro and informs on nature's design but still pales in contrast to the dynamic adaptability of the natural system.[52] While we have only begun to scratch the surface of kinesin–tubulin complexity and its impact on cellular functions, multicellular development, eukaryotic diversity, and human diseases, a new excitement exists in the cytoskeleton field to unlock these secrets.

Disclosure

The authors report no conflicts of interest in this work.

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