Kinesins are motor proteins that move cargoes such as vesicles, organelles and chromosomes along microtubules. They are best known for their role in axonal transport and in mitosis. There is a diverse family of kinesins, members of which differ in composition and functions. Roles of kinesins in diseases typically involve defective transport of cell components, transport of pathogens, or cell division.

Kinesin motor proteins generate motion along microtubules. During the past 17 years more than 1600 publications have appeared on kinesin that reveal an increasing variety of members in this superfamily of proteins with many possessing correspondingly diverse functions. Major functions include:

- Movement of cargoes through the cytoplasm on microtubule tracks – cargoes include vesicles (e.g. Golgi-derived vesicles), organelles (mitochondria, peroxisomes, lysosomes), cytosolic components (e.g. mRNAs, proteins, elements of the cytoskeleton such as intermediate filaments, or virus particles) and membrane-associated complexes (e.g. rafts or intraflagellar transport particles).
- Organization of the mitotic spindle – focusing of microtubules on spindle poles, attachment and movement of chromosomes, antiparallel sliding of microtubules during spindle elongation, regulation of microtubule dynamics.

This review focuses on aspects of kinesin functions and interactions that are related to human diseases. Consistent with the motor and mitotic functions of kinesins, most disease-related aspects can be broadly classified as cases where physiological cargoes are not delivered appropriately (e.g. clogging of axonal transport); cases where non-physiological cargoes make use of the transport system (e.g. viruses); and cases where kinesins participate in mitosis and are therefore drug targets in cancer chemotherapy.

Kinesin structure, composition and nomenclature

The defining criterion for a kinesin is its ‘motor domain’, ≈320 residues in size, which binds and hydrolyses ATP (the energy source for movement) and binds to microtubules (the ‘tracks’ for movement; Fig. 1). The folding of the motor domain produces a core similar to that of other motors (e.g. the actin-dependent motor myosin) or signalling G-proteins [1]. The motor domain is usually coupled to additional domains with structural or regulatory roles. These in turn can attach to other cofactors, adaptor proteins or interaction modules [2]. The founding member, ‘conventional’ kinesin (alias KIF5/kinesin-I), contains an N-terminal motor domain, an α-helical stalk and a C-terminal tail domain (Fig. 1).

KHC tail binds to a ‘kinesin light chain’ (KLC) as a cofactor, thus forming a heterotetramer. The stalk can bend in a hairpin fashion so that the tail interacts with the motor and inactivates it. This is one method of avoiding futile consumption of ATP while the motor is not needed [6]. The light chains contain tetrastricopeptide repeat protein-interaction motifs (TPRs), which can dock onto adaptors or cargo receptors, linking kinesin to the cargo to be transported. There are many variations of this theme: other kinesins can be monomeric (KIF1); heterotrimeric (KIF3/kinesin-II), containing two distinct heavy chains, KIF3A and KIF3B, and one light chain, KAP3; or homotetrameric (two antiparallel pairs of heavy chain dimers, KIF11/End5). The interaction partners and docking complexes are only partially known, but this is an area of rapid progress [2,7]. The variability in composition explains why a limited number of kinesins can perform the wide variety of cellular transport tasks.

There are three principal types of kinesins, with the motor domain located at the N-terminus, at the C-terminus, or at an internal or middle position of the polypeptide chain, hence the nomenclature Kin-N, Kin-C and Kin-I – or N-type, C-type and M-type (for middle) kinesin [1,8]. In this framework the heterotrimeric conventional kinesin-I and heterotrimeric kinesin-II are both N-type kinesins (and have transport functions), whereas Kin-I proteins (KIF2/MCAK) are microtubule disassembly factors. Other kinesins are abbreviated ‘KLP’ (kinesin-like protein) or ‘KRP’ (kinesin-related protein), or have names derived from mutant phenotypes (e.g. unc-104, fla-10). This has led to a bewildering variety of names, but the recent elucidation of various genomes allows a classification of kinesins and simplification of terminology [9,10]. The 45 mammalian kinesins of the kinesin superfamily have been subdivided into 14 classes (11 N-type, termed N-1 to N-11; 1 M-type; 2 C-type, C-1, C-2) containing 19 families [10]. The proteins are numbered KIF1 to KIF26 (with variants denoted as A, C, B), which includes the N-type and M-type motors. By contrast, the C-type motors are

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proteins.

Kinesins are not motors in the strict sense but cause movement along microtubules (towards the cell periphery or synapse), whereas C-type motors move to the minus-end (cell interior, microtubule-organizing center, MTOC). However, the directionality is not dictated directly by the position of the motor in the chain, but by crucial residues immediately outside the core domain (the ‘neck’). In addition, the combination of motors as dimers or higher-order ensembles can have a strong influence on motor properties such as processivity and even directionality [11,12]. By contrast, M-type kinesins are not motors in the strict sense but cause microtubule protofilaments to fray and thus destroy the cylindrical structure [13,14].

Compared with the motor–microtubule interaction, much less is known about attachment of the motor to cargo; the emerging evidence shows a remarkable diversity [2,7,15]. Apart from the motor (kinesin), one can distinguish adaptors or scaffolding proteins, cargo receptors, and cargo (vesicles, organelles, transport complexes). Receptors can be firmly integrated into the cargo, for example by a membrane anchor, whereas adaptor proteins can be more loosely associated either with the receptor or with the motor. The light chains of KIF5 or KIF3 (KLC and KAP3) are examples of adaptors tightly bound to their motor. The interactions with adaptors or cargo are often mediated by domains typical for protein–protein interactions, such as α-helical coiled-coil (as in KIF5), TPR (in KIF3), armadillo repeat (in KIF3), pleckstrin homology (PH) domain

denoted as KIFC1 to KIFC3 (note that KIF1C is distinct from KIFC1, etc.). In this system, conventional kinesin-I is KIF5, M-type kinesin (Kin-I) is KIF2, heterotrimeric kinesin-II is KIF3, and monomeric kinesin Unc-104 is KIF1.

Fig. 1. Kinesin domain structure and associated proteins. The diagram shows some known molecular structures in backbone representation (without side chains). The top structure represents a protomer of a microtubule, the ‘track’ for kinesin motors, containing alternating α- and β-tubulin subunits (light and dark green, ~450 residues each), oriented so that the plus-end points to the right, towards the cell periphery for most microtubules or towards the kinetochore in mitotic spindles (Protein Data Bank code 1jff.pdb) [57]. The blue and red structure in the middle represents a dimeric kinesin heavy chain (KHC) as found in KIF5B, the conventional kinesin-I from rat brain (3kin.pdb) [58]. Each heavy chain contains a motor domain (‘head’, α-helices red, β-strands blue) that binds to ATP and microtubules, a neck linker (cyan) whose conformation changes during the ATPase cycle, an α-helical neck and a stalk (red) that causes dimerization by a coiled-coil interaction. In KIF5B, this domain contains ~570 residues, only ~20% of these are represented here. The stalk is interrupted by several nonhelical hinges (only one shown here) that allow the heads to swivel and the stalk to bend over in a hairpin-like fashion, generating a ‘folded’ conformation that is inactive when kinesin is not moving [6]. The globular tail domain (~50 to 130 residues) is structurally not known and represented as a red sphere roughly in scale to its expected size. The domain contains determinants (JAK motif) for folding kinesin over into an inactive conformation. The tail of the KIF5/kinesin-I dimer binds to two light chains (KLC, ~570 residues, yellow). Their N-terminal ~250 residues are engaged in coiled-coil formation (~80 residues predicted coiled coil), linking the light chains to the heavy chains. The C-terminal ~320 residues dock to the cargo or scaffolding proteins of the transport vesicle – e.g. JIP, amyloid precursor protein (APP), kinasein or others. This domain contains six tetratricopeptide repeats (TPRs: each containing 34 residues in two antiparallel α-helices), which overlap with two DnaJ-motifs, which are typically involved in interacting with chaperones such as hsp70 [59,60]. The kinesin light chain structure is not known, the montage shown here was taken from the known TPR domain of protein phosphatase PPS (1a17.pdb) [61]. In the case of the heterotrimeric KIF3/kinesin-II structure, the two heavy chains are distinct (KIF3A and B) but otherwise have broadly similar domains as KIF5/kinesin-I. However, instead of two light chains, there is only one, KAP3 (shown, magenta), which is largely α-helical and contains 11 ‘armadillo’ repeats [62]. Each repeat contains ~42 residues, folded into units of three α-helices – adapted here from the structure of β-catenin (2bdt.pdb) [63]– flanked by ~150 residue N- and C-terminal domains (indicated by spheres). Note that both TPR and armadillo repeats form shallow curved surfaces suitable for variable protein–protein interactions.

Box 1. Supplementary information on websites

The major source of information about kinesin is the kinesin homepage www.proweb.org/kinesin/ [a]. It contains all aspects of kinesin structure, biochemistry and cell biology, as well as links to other sites. Information on kinesin structure and biophysics, illustrated with movies, can be found at www.mpasmb-hamburg.mpg.de (Mandelkow laboratory), www.scripps.edu/milligan (Milligan laboratory), valelab.ucsf.edu (Vale laboratory), mc11.mcri.ac.uk/Motorhome.html (Cross laboratory). Kinesin family trees are shown at the kinesin homepage (see above) and at cb.m.u-tokyo.ac.jp (Hirokawa laboratory).

Reference

(in KIF1), PDZ (in KIF17) or other domains with analogous functions.

How could motors be involved in human disease? Considering the many forms of motor proteins, the implications for human diseases reported so far are fairly small. Lessons from transgenic mice, fruit flies or worms suggest several explanations: (1) some motor-dependent traffic is essential, and disruption is lethal – this applies particularly to long-distance transport in elongated cells such as neurons; (2) for most cells the diffusion of small molecules, proteins or other cell components is reasonably efficient, even in the absence of organized traffic; (c) there is redundancy in the motor systems so that one type of kinesin can be replaced by another (or even by myosin-based traffic). On this basis, the disease-related roles of kinesin can be grouped into a few categories:

(1) Problems with long-distance traffic, especially in neurons or other asymmetric cells where diffusion would be too inefficient. This applies to various types of kinesin, but especially to KIF5, KIF1 and KIF3.

(2) Problems with ciliary or flagellar function. The absence or malfunction of cilia can cause a variety of disease conditions, often traced to KIF3 and its cofactors because this kinesin is responsible for the intraflagellar transport complex [16].

(3) Uncontrolled cell division and cancer. Various types of kinesin are involved in mitosis, and their malfunction can cause undesirable cell proliferation. Conversely, kinesins of transformed cells represent targets for drug intervention.

(4) Kinesins and toxins. Certain (environmental) toxins inactivate kinesins; this is most apparent in the failure of axonal transport and the resulting axonopathies.

(5) Entry and exit of pathogens. Viruses and parasites and their components are too large for diffusion, and thus their movement in and out of the cell is dependent on motor proteins.

**Diseases caused by disruption of long-distance transport**

Neurodegenerative diseases are often accompanied by aggregation of proteins in the cell body or cell processes, interruption of axonal transport, and eventually neuronal ‘dying-back’ or axonopathy. Examples are amyotrophic lateral sclerosis (ALS) with prominent neurofilament aggregates, or Alzheimer’s disease with aggregates of the Aβ-peptide derived from amyloid precursor protein (APP) or of the microtubule-associated protein tau [17]. The ‘traffic jams’ observed in the dystrophic neurites are reminiscent of jams that can be generated by disrupting kinesin functions [18].

However, considering the likely importance of kinesin-based transport, the reports on the role of kinesin in diseases are surprisingly scarce. The accumulations in neurons have mostly been ascribed to genetic defects in non-motor proteins, such as superoxide dismutase in ALS, or APP and presenilin in Alzheimer’s disease. Nevertheless, there is an intriguing relationship between KIF5/kinesin-I and proteins implicated in Alzheimer’s disease: APP interacts (through its C-terminal tail) with the light chains of kinesin that forms insoluble aggregates in Alzheimer’s disease. It is thought that APP acts as a cargo receptor, interacting with the tetratricopeptide repeats (TPRs) of kinesin light chains [2]. Neuronal microtubules are coated by tau protein, which promotes neurite outgrowth and stabilizes microtubules. However, in Alzheimer’s disease, tau aggregates into pathological neurofibrillary tangles. (b) When tau becomes elevated (in this case by transfection), the transport pathways become clogged, most APP disappears from the neurites and remains in the cell body. The same applies to mitochondria, peroxisomes or other cell components, and the neurites become vulnerable. Similar effects can be generated by blocking the tracks, inactivating the kinesin motor domain, or disrupting the motor-cargo interaction. Adapted from Ref. 21. Bar, 10 μm.

**Fig. 2.** Disease-related proteins in axonal traffic. (a) Differentiated N2a neuroblastoma cell shows vesicles carrying amyloid precursor protein (APP, labelled by a fluorescent marker). They are transported by kinesin along microtubule tracks. APP plays a neurotrophic role, but its abnormal cleavage generates a peptide (Aβ) that forms insoluble aggregates in Alzheimer’s disease. It is thought that APP acts as a cargo receptor, interacting with the tetratricopeptide repeats (TPRs) of kinesin light chains [2]. Neuronal microtubules are coated by tau protein, which promotes neurite outgrowth and stabilizes microtubules. However, in Alzheimer’s disease, tau aggregates into pathological neurofibrillary tangles. (b) When tau becomes elevated (in this case by transfection), the transport pathways become clogged, most APP disappears from the neurites and remains in the cell body. The same applies to mitochondria, peroxisomes or other cell components, and the neurites become vulnerable. Similar effects can be generated by blocking the tracks, inactivating the kinesin motor domain, or disrupting the motor-cargo interaction. Adapted from Ref. 21. Bar, 10 μm.
inhibit motors on the microtubule tracks, which could lead to transport disruption of numerous cargoes, including APP vesicles, mitochondria and peroxisomes; the latter would explain the energy deprivation and sensitivity to oxidative stress in Alzheimer’s disease neurons [21] (Fig. 2).

A second example is the human neuropathy of the Charcot–Marie–Tooth (CMT) type 2A. The origins of CMT are quite heterogeneous, but one case involves a mutation in the motor domain of KIF1Bβ [22]. This variant of the monomeric motor KIF1 is responsible for the transport of synaptic vesicle precursors, thus helping to explain the phenotype of the mutations in humans and transgenic mice (progressive atrophy of distal muscles due to the degeneration of peripheral axons).

Other examples of disease related to kinesins concern the function of the heterotrimeric motor KIF3/kinesin-II and its role in supplying material for building cilia and flagella [16,23,24]. The diseases can have diverse phenotypes, depending on the type of cilia affected. For example, defects in sensory cilia in the kidney can cause polycystic kidney disease [25]. The defect need not be in the motor itself, but could reside in an accessory factor in the transport complex. Similarly, the transport of material between the inner and outer segments of photoreceptors depends on KIF3 motors, and defects can cause retinitis pigmentosa [26]. Kartagener’s syndrome describes a combination of defects that appear as abnormalities in sperm flagella, bronchial cilia and nodal cilia, all caused by malfunctioning of the KIF3-dependent transport complex. Thus, situs inversus (inverted positioning of organs) is due to the failure of nodal cilia to move morphogens during gastrulation [27,28].

Kinesin as a drug target

The cases discussed above involve traffic being obstructed because the motor was impaired or detached from its cargo, or the tracks were blocked, resulting in disease. But there is also the opposite situation where kinesin functions normally and the cell abuses this for a disease-causing process. This is the case in cancer where cell division is no longer properly regulated. Considering the number of kinesins involved in mitosis they should be natural targets for cytostatic drugs. This use of drugs would be analogous to the more traditional drugs aimed at blocking DNA replication or microtubule dynamics. Several compounds have been described that inactivate the kinesin motor domain. On example is the compound adociasulfate-2 from a marine sponge that blocks kinesin-dependent motility and mitosis and has served as a proof of principle [29]. Taking this further, the potential binding sites of kinesin inhibitors were probed by a docking study of several classes of compounds expected to be important for the motor functions of kinesin [30]. Apart from the obvious nucleotide-binding pocket, other sites that are sensitive to mechanochemical switching were considered as targets. These expectations were verified experimentally, and several compounds had binding constants in the low micromolar range, proving their potential as drug inhibitors of kinesin.

A further step was the demonstration of a screening method that revealed inhibitors of kinesins involved in mitosis. Because KIF11/Eg5 takes part in the separation of spindle poles, its blockage results in a monopolar aster and failure of cell division, and the appropriately named ‘monastrol’ is a prototype Eg5 inhibitor [31]. Equivalent results are expected of other mitotic kinesins; for example, interfering with microtubule dynamics has long been recognized as a tool to disrupt the cell cycle. This can be achieved by drugs binding to tubulin directly (such as taxol or nocodazol, which over- or understabilize microtubules, respectively), or, in principle, by drugs that inactivate KIF2/MCAK. This M-type kinesin depolymerizes microtubules from their ends, which is important for remodelling microtubules during mitosis [13]. The drugs mentioned above are directed against the conserved motor domain of kinesins. This raises the concern that a given inhibitor would block other vital kinesins as well. However, experience with other conserved enzymes (e.g. protein kinases) shows that even slight structural differences can be exploited to generate monospecific drugs [32].

The first drug targeted to kinesin was discovered as a substance from a sponge whose only defence against predators is its toxicity, pointing to the possibility that other toxins might also interfere with kinesin functions. Remarkably, local anaesthetics (e.g. lidocaine) can stop anterograde axonal traffic by inhibiting the movement of kinesin but not its ATPase activity, possibly by blocking the coordination between the motor heads at the neck junction [33]. This mode of action is distinct from that of adociasulfate-2, which blocks both microtubule binding and ATPase activity [29], and illustrates that there are diverse points where a toxin could attack kinesin. Similarly, neurotoxic agents such as acrylamide or ethylene oxide block kinesin-based anterograde transport and cause the ‘dying-back’ of axons [34].

Another possible mode of toxin action would be at the level of cargo attachment. In fact, given the diversity of cargo adaptors and receptors, this approach should yield better tailor-made compounds interfering with specific kinesin-dependent cellular processes. There are no published data on drugs operating this way, but their potential is illustrated by the fact that mutation or overexpression of proteins involved in cargo attachment (but lacking the motor function) can disrupt axonal traffic. Examples are SYD/JIP-3, a protein otherwise known to interact with JNK, which interacts with the TPR motifs of KIF5 in fast anterograde transport of membranous cargoes [35]; and one of the cofactors in the multiprotein complex transported by KIF3 in ciliogenesis, defects in which cause autosomal recessive polycystic kidney
Other disease-related roles of kinesins

Microtubule-dependent transport is involved in the intracellular transport of certain viruses, bacteria or parasites. This can take place at several levels, such as docking, and transport towards the nucleus or back to the cell membrane [37]. In the case of vaccinia virus, KIF5 is needed to transport the viral protein A36R, which interacts with the TPR domain of the light chains [38]. The interaction of retroviral Gag proteins with KIF4 is a prerequisite for viral capsid assembly [39]. The transport of viruses or parasites towards the nucleus makes use of dynein, as expected from its minus-end-directed polarity (e.g. adenovirus [40]), but, to dock on the cell surface before entry, receptors must be assembled, which in turn depends on kinesin, as documented for the case of Chlamydia [41]. Kinesin-dependent transport is essential in the case of neurotrophic herpes viruses, which have to be transported over long distances from the cell body to the axon terminals of dorsal root ganglion neurons, and a direct interaction between KIF5 and the viral capsid protein US11 has been demonstrated [42]. Some viruses have the additional effect of destroying the microtubule tracks. An interesting case is that of HIV Rev protein, mimicking an M-type kinesin that depolymerizes microtubules by bending the protofilaments and induces tubulin rings similar to KIF2/MCAK [14, 43]. A similar depolymerizing effect on microtubules is observed for HIV Tat protein, causing the rearrangement of the cytoskeleton and mislocalization of kinesin [44].

Several reports mention kinesin in the context of tumour generation or suppression. For example, a variant of KIF1B is downregulated in some neuroblastomas, and has the potential to function as a tumour suppressor [45]. Recently, KIF5B has been identified as part of a complex with two neurofibromatosis tumour suppressors, NF1 and NF2 (neurofibromin and merlin) [46]. Similarly, a truncated form of KIF1B, lacking the motor domain but containing the PH domain, was considered a tumour suppressor in neuroblastomas [47]. In this case, it is possible to speculate that the truncated protein blocks the assembly of motile KIF1 docking complexes on membranes; this would be reminiscent of the expression of nonfunctional light chains of KIF5, which cause the displacement of cargo from motors [12, 48]. In the treatment of pancreatic cancer with retinoic acid, KIF11/Eg5 (one of the mitotic kinesins) was downregulated, consistent with its essential role in cell division [49]. In epithelial cancers, there is crosstalk between the antineoplastic drugs taxol and cisplatin, mediated by KIF5: kinesin is required for the growth arrest induced by taxol, but cisplatin preferentially damages the gene encoding KHC, thus reducing the efficacy of taxol [50]. In a related context, rheumatoid arthritis is caused by transformed synovial fibroblasts that destroy articular cartilage. One of the key genes upregulated in these cells is KIF10/CENP-E, a kinetochore-based mitotic motor responsible for chromosome segregation and known previously as an autoantigen in systemic sclerosis [51].

Eukaryotic cells respond to genotoxic factors (e.g. UV irradiation) by inducing growth arrest and DNA damage-inducible (GADD) proteins that protect differentiated cells against apoptosis. The GADD proteins are coordinately induced via the MAP (microtubule-associated protein) kinase pathway that mediates the stress response. It appears that their function involves the interaction with KIF3A [52]. This relationship is reinforced by the observation that stress-signalling kinases of the MAP kinase pathway (MLK2, JNK) occur in a complex with KIF3 subunits [53]. The role of the motors in the complex is not well defined, but some clues come from the fact that proinflammatory cytokines such as TNF (tumour necrosis factor) cause the phosphorylation of the light chains of KIF5/kinesin-I by the p38 stress-response kinase [54]. This in turn releases the motor from its cargo and leads to the retraction of mitochondria into the cell interior, similar to what is observed by blocking microtubule tracks with tau protein [55]. Glycogen synthase kinase 3β (GSK-3β), another kinase involved in signalling pathways, has also been implicated recently in the discharge of cargo through the phosphorylation of KIF5 light chains [56]. It remains to be seen whether similar regulation applies to the light chains of other motors.

Concluding remarks

There is as yet no clinically approved drug that is targeted specifically against kinesin, but a phase I clinical trial has just been announced by Cytokinetics (see www.cytokinetics.com). The examples mentioned above suggest several opportunities. The most obvious ones relate to the role of kinesin in mitosis, which could be exploited by kinesin inhibitors in cancer chemotherapy. The same strategy would apply to other diseases, for example arthritis, where the disease features pathological proliferation of certain cell types. A third example is the inhibition of the movement or assembly of pathogens, particularly viruses. They make use of the normal cellular transport machinery, which must be spared, but an obvious target would be the binding site of the viral capsid proteins with the kinesin motor or its adaptor proteins. The strategy for neurodegenerative diseases is less clear-cut because, in principle, kinesin would need to be activated to overcome the ‘traffic jams’ caused by pathological protein aggregates. Here, the major goal would be to remove the aggregates, without destroying the cell, to open the passage for motors. In cases where kinesin is disease [25]. Analogous examples exist for the accessory docking proteins of dynein, notably LIS1, which causes lissencephaly, a developmental brain disorder caused by defects in migration of neuronal progenitor cells [36].
inactivated by premature discharge of the cargo, it might be possible to inhibit the responsible protein kinases. Independently of these considerations, the microtubule-based transport system represents an excellent tool for the analysis of mechanisms, drugs or toxins, particularly for diseases affecting neurons because of their exquisite dependence on motors.

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