WALKING ON TWO HEADS: THE MANY TALENTS OF KINESIN

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The gallop of a race horse and the minute excursions of a cellular vesicle have one thing in common: they are based on the directional movement of proteins termed molecular motors — many trillions in the case of the horse, just a few in the case of the cell vesicle. These tiny machines take nanometre steps on a millisecond timescale to drive all biological movements. Over the past 15 years new biochemical and biophysical approaches have allowed us to take a giant step forward in understanding the molecular basis of motor mechanics.

"Where there's life, there's movement." This catch phrase, which could just as well be reversed, epitomizes the fundamental realization that movement is one of the most characteristic features of life. Most biological movements are accomplished by ingenious protein machines termed molecular motors[1]. Some motors may occur in large ensembles as in our skeletal muscles, whereas others may operate as single molecules. Some undergo linear motion along a substrate, and others rotate about their axis (BOX 1). Some of the linear motors drive subcellular transport ranging from just a few micrometres up to several metres in certain neurons of large animals. Notwithstanding their diversity, all molecular motors have in common the fact that they undergo energy-dependent conformational changes that result in unidirectional movement.

Among the best-studied molecular motors are those that use cytoskeletal fibres as a track. Three classes of cytoskeletal motors are known: myosin, which interacts with actin filaments, and two types of microtubule motors, dynein and kinesin (FIG. 1). All cytoskeletal motors possess a catalytic motor domain, also referred to as the 'head', characterized by the presence of two binding sites, one for ATP and one for the track. This domain is surprisingly small in the kinesins (about 350 amino acids), of intermediate size in the myosins (about 800 amino acids), and large in the dyneins (over 4,000 amino acids). Outside the motor domain the

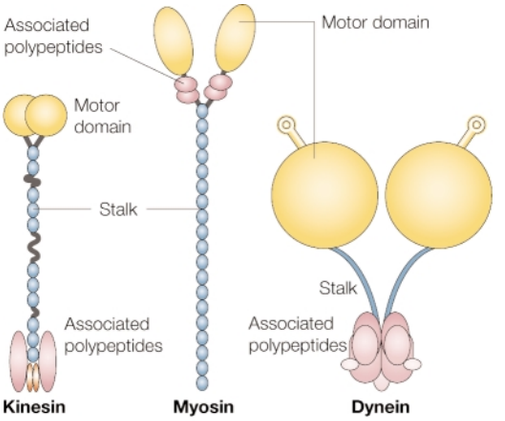

Figure 1 | **Overview of three molecular motor 'prototypes'.** The actin-based motor skeletal muscle myosin in the centre is flanked by the microtubule motors conventional kinesin on the left and cytoplasmic dynein on the right. All three motors consist of a dimer of two heavy chains whose catalytic domains are shown in yellow, whereas the stalks, which form extended coiled-coils in both myosin and kinesin, are shown in blue. Associated polypeptides (four light chains in skeletal muscle myosin, two light chains in conventional kinesin, and a complex set of intermediate, light-intermediate and light chains in dynein) are shown in purple. The 'antennae' extending from the dynein heads contain the microtubule binding site, which in myosin and kinesin is part of the compact head. (Drawn roughly to scale.)

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Box 1 | A motor by any other name...

Many protein machines undergo ordered conformational changes to execute vectorial processes. Ion pumps, translocation pores that move proteins across membranes, ribosomes, DNA helicases, nuclear pores — in a sense, they all are motors. Even though the design principles differ and certainly do not suggest a common origin of these machines, some properties may be shared by otherwise distinct devices. Therefore conventional kinesins and many DNA or RNA polymerases have in common the ability to move along their respective tracks (microtubules or DNA) for long distances without dissociating, taking hundreds or even thousands of 'steps' in the process[38,80,83–85]. This characteristic feature is termed 'processivity' (see main text). In the case of polymerases, contact with the track is facilitated by protrusions that clamp around the DNA strand [86,87] (see the review by Hingorani and O'Donnell on page 22 of this issue). A different type of movement results when the molecules that constitute the 'track' are arranged in a circular fashion and the motor rotates in the centre. Such is the case in ATP synthase, where a single γ-subunit rotates against a surrounding cylinder of three α- and three β-subunits, a process driven by a proton gradient[88]. This is the smallest known 'rotary' motor. A larger but even more remarkable rotary machine is the bacterial flagellar motor, where a corkscrew-like flagellum is attached to a rotating ring-shaped assembly inserted into the membrane. This complex is powered by proton or sodium gradients, and although it is only composed of about 20 proteins, it can rotate at rates exceeding 1,000 Hz, propelling a bacterium at speeds of several hundred micrometres per second[89,90].

A 'motor' of completely different design is used by certain intracellular pathogenic bacteria or viruses. They exploit the complex cellular machinery normally used for lamellipodial extension during cell migration and modify it to create an intracellular 'rocket propulsion' system[91]. By nucleating the assembly of a network of actin filaments and actin-associated proteins near their surface to generate a cushion of crosslinked fibres, the intruder is pushed through the cytoplasm[92,93]. This machinery differs from the other motors described here in that it is composed of a massive three-dimensional cytoskeletal network rather than a compact macromolecule.

P-LOOP-TYPE ATP-BINDING SITE 'Phosphate-binding loop'; a nucleotide-binding consensus motif (GXXXXGKT/S) at the ATP-binding site.

three classes of motors differ considerably, suggesting that functional diversity is in part embodied in the non-motor domains.

The discovery of numerous motors over the past 10–20 years has led to the realization that all three motors constitute superfamilies with dozens of members. However, functional characterization has not kept up with the pace of discovery and, as a result, many are known only as a piece of sequence or a twig on a phylogenetic tree. To appreciate the diversity of motors, consider the fact that a mammalian organism harbours at least 40 kinesin motors[2,3], probably just as many myosins, and at least half a dozen dyneins. Consider, in addition, that some of these motors may have associated polypeptides that help specify function. For example, animal conventional kinesins are associated with light chains, of which several isoforms are known[4,5], leading to further functional diversification. If a similar scenario applies to other types of motors, eukaryotic cells may easily harbour hundreds of motor complexes with highly specific functions.

In this review we discuss the molecular basis of motor protein function, using kinesin as a model. We centre on two of the most fascinating properties of kinesins: the capacity to decide which way to move along the track, and the ability to move long distances without dissociating (termed processivity). Wherever possible, we include comparisons with other cytoskeletal motors to emphasize common principles of action.

Different makes, same engine

On the basis of phylogenetic analysis of the motor domain, the kinesin superfamily comprises at least ten families, some of which can even be divided into distinct subfamilies (FIG. 2). Many motors cannot be assigned to any of the existing groups and therefore are referred to as 'orphans'[6,7]. New members are still being found, but with the advances in genome sequencing projects, the pace of discovery of new motors is slowing down.

The common denominator of kinesin motors is the catalytic motor domain, which shows at least 35% sequence identity among all the kinesins found so far. It possesses a P-LOOP-TYPE ATP-BINDING SITE and a number of signature sequences that are found only in kinesins[8,9]. Some of the latter are now known to be responsible for the interaction with microtubules, and others are of unassigned function. As also shown in FIG. 2, the size of kinesin motors may differ widely, even among members of the same family, whereas other families are more conserved in size. Likewise, the speed of motors may vary considerably, indicating a high degree of functional adaptation.

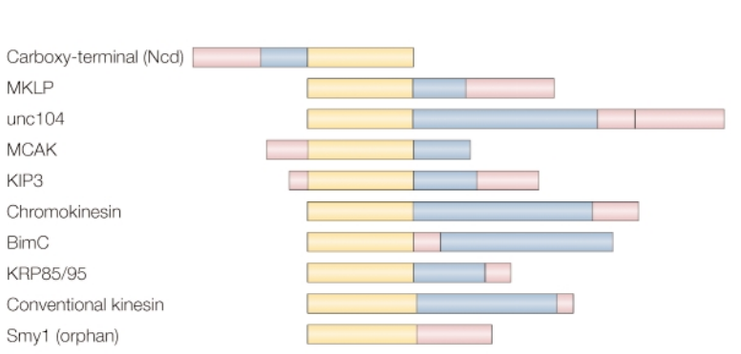


Figure 2 | **Overview of the domain organization, heavy chain molecular weight, polarity of movement and velocity of the main kinesin families.** The abbreviations of the diverse families, which are named after certain prototypes of motors characteristic of each family, correspond to the nomenclature of the kinesin home page. The conserved motor domain is shown in yellow, domains that include coiled-coil segments are shown in blue, and predominantly globular domains with family-specific and presumably varied functions are shown in purple. The velocity of MCAK, which actually may be a microtubule depolymerizer rather than a motor, is controversial. Only one example of an 'orphan' kinesin (Smy1) is shown. (For further characteristics of the kinesin families see the kinesin home page.) (ND, not determined).

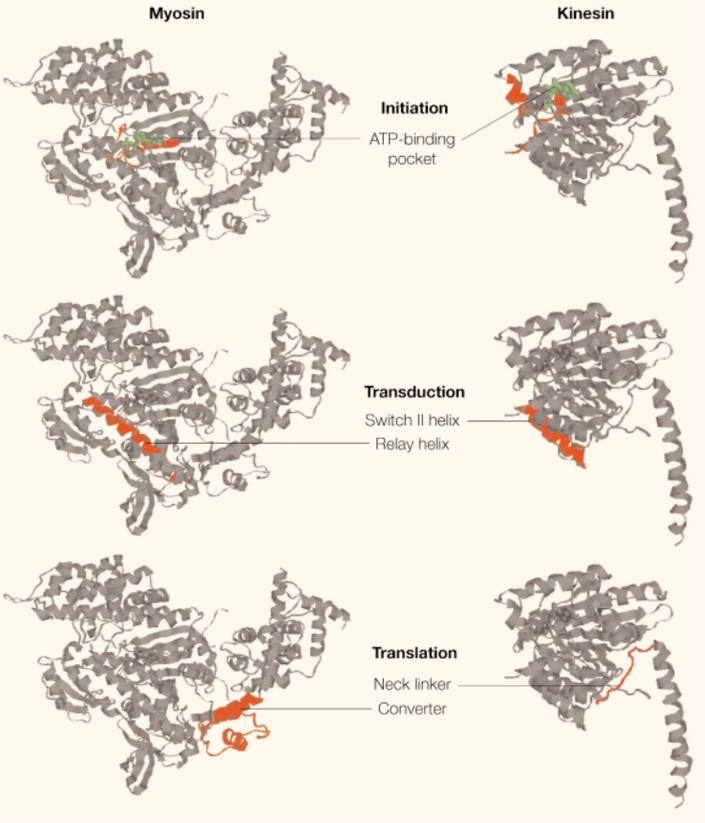
The regions outside the motor domain are family specific and share little, if any, sequence homology. They often include one or more segments predicted to form a coiled-coil that may facilitate oligomerization. The diversity of these non-motor regions correlates with the range of biological functions for kinesin motors, which include not only the transport of membrane-bound organelles, protein assemblies and messenger RNA, but also cell division, chemosensory and signal transduction functions, microtubule dynamics, neuronal plasticity and embryonic development (for recent reviews, see REFS 3,10). Interaction with their respective cargoes is probably mediated through the non-motor domains but, with few exceptions, little is known about these interactors and how they link up with the motor.

Getting started

Members of the conventional kinesin family have been extensively used for the study of motor mechanisms. Conventional kinesin derives its designation from the fact that it was the first kinesin to be identified and purified from cell extracts. It has developed into a 'gold standard' with which the properties of other kinesins, discovered later, are compared. Conventional kinesin is a homodimer of two heavy chains, each of which possesses an amino-terminal motor domain, a long (60–80 nm) stalk with alternating flexible and coiled-coil segments, and a small globular tail domain (FIG. 3). The atomic structure of the kinesin motor domain[11] reveals a completely unexpected structural homology with myosin and small G-proteins, which are also P-loop nucleotidases. This suggests that, in these three classes of proteins, the catalytic core has a similar function as an engine that exploits the energy of ATP hydrolysis to drive a conformational change. In kinesin motors this is controlled by the interaction with the microtubule. Free in solution, the ATPase is inactive and the molecule rests in its ADP-bound form. The hydrolytic cycle can only be initiated upon binding to the microtubule, a process resembling the activation of myosin by actin or the regulation of G-proteins by activators and exchange factors[12,13].

However, if it were for this feature alone, the catalytic domain would be no more than an allosteric enzyme.

Box 2 | Force generation in kinesin and myosin



Kinesin and myosin have long been considered to be unrelated molecules because they use different tracks, possess motor domains of different size, and show no marked sequence homology. Therefore the least expected outcome of the crystallographic studies[11] was a striking structural homology between the kinesin motor domain and the core of the myosin head. The core structural elements, a set of seven β-sheets sandwiched between three α-helices on either side, are essentially superimposable. In addition, several amino acids around the nucleotide-binding site are conserved positionally, indicating that both classes of motors may originate from a common ancestral nucleotidase[94]. The structural similarities in the catalytic cores of kinesin and myosin indicate that the conformational changes upon ATP hydrolysis are initiated in the same way in what seem to be homologous domains. However, the (much larger) conformational changes that lead to a step along the respective tracks are executed in domains carboxy-terminal to the catalytic core. In skeletal muscle myosin, an α-helix stabilized by two associated light chains and based in a domain termed the 'converter' undergoes a nucleotide-dependent angular rotation. In conventional kinesin, conformational changes in a flexible subdomain, the 'neck linker', which alternates between a mobile state and a docked position on the catalytic core, are associated with the forward motion of the kinesin head[45]. The communication between the nucleotide-binding site and the mechanical element at the carboxyl terminus of the head is mediated by analogous elements in kinesin and myosin: an α-helix that contacts the catalytic site at one end passes by the polymer binding face and ends near the mechanical amplifier, the converter in myosin or the neck linker in kinesin. So myosin and kinesin have developed different ways of converting small ATP-dependent conformational changes (on a scale of ångströms) into large changes of conformation (on a scale of nanometres) of an associated mechanical element. Nevertheless, the sequence of events in the respective motor domains follows remarkably similar pathways. The figure shows the sequence of events during force generation in the myosin (left) and kinesin (right) heads. In both motor domains, a conformational change is initiated by the binding of ATP (green) in the catalytic site (top row). This information is transmitted through analogous elements, the relay helix in myosin and the switch II helix in kinesin (middle row), to a mechanical element. There, the initially small conformational change is translated by structurally unrelated elements, the converter in myosin and the neck linker in kinesin (bottom row), into a much larger conformational change.

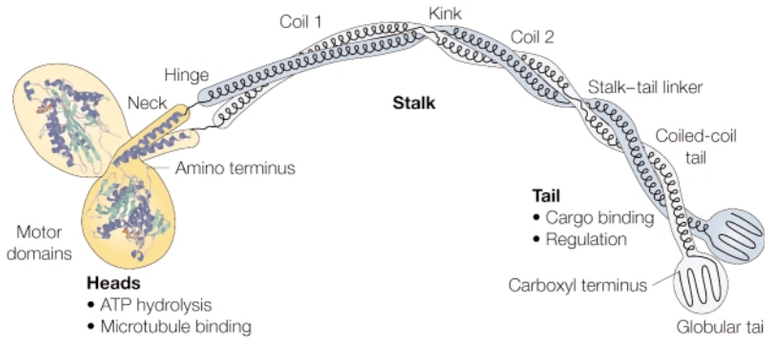


Figure 3 | **Domain organization of the conventional kinesin heavy-chain dimer, showing the crystal structure of the catalytic domains and the neck[96]**. The structure of the stalk and tail are inferred from electron microscopic images and coiled-coil prediction analyses. Regions predicted to form coiled-coils (neck, coil 1, coil 2, coiled-coil tail) and flexible regions (hinge, kink, stalk–tail linker) are indicated.

Further domains are required to turn the enzyme into a motor. In conventional kinesins, the neck and neck linker, and probably also the hinge (FIG. 3), translate the small conformational change that is generated in the ATP-binding site into a much larger mechanical movement. Studies on truncated and mutated kinesins show that both the velocity and the ability to stay on track are affected when these domains are deleted or changed[14–16]. Because motility is not entirely abolished in these artificial molecules, neck and neck linker seem to amplify events in the core motor domain to produce physiological behaviour. In this respect kinesin resembles myosin, where small conformational changes in the catalytic site are translated into a large conformational change of a mechanical element, the lever arm (BOX 2).

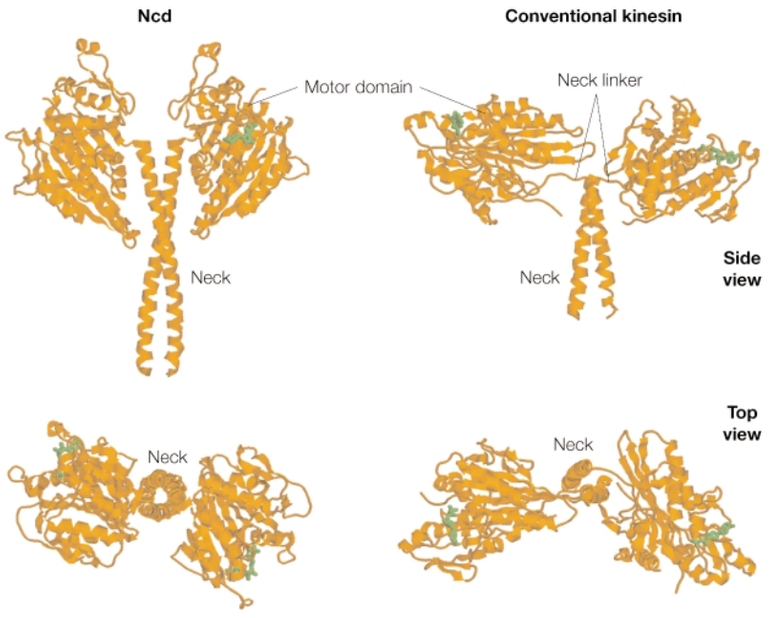


Figure 4 | **Crystal structures of dimeric Ncd and conventional kinesin.** Ncd[23] (left) and conventional kinesin[96] (right) are shown in side view (top) and top view (bottom). These views clearly show the different positions of the catalytic motor domains relative to the neck regions in these two motors, which move in opposite directions along microtubules. ADP, bound in the active site, is shown in green.

Knowing which way to go

Considering the similarity between the core motor domains of different kinesins, you might assume that, except for variations in speed and processivity, all members of the kinesin superfamily would behave similarly. This is not the case. One kinesin family shows a particularly surprising feature — its members move in the opposite direction to conventional kinesin.

Microtubules are intrinsically polar assemblies of α/β-tubulin dimers (BOX 3). Conventional kinesins, and most other kinesins, move towards the plus end (FIG. 2). So the discovery of a minus-end-directed kinesin, non-claret disjunctional (*ncd*), named after a long-known spindle mutant of the fruitfly *Drosophila melanogaster*, came as a surprise[17,18]. Because other members of the ncd family of motors also move towards the minus end[19], and all of these motors have the motor domain at the carboxyl terminus, these two features are probably linked.

The finding of 'reverse' motility immediately challenged our understanding of how kinesin motors generate movement. It raised the question of where the 'gear and transmission' are that revert the direction of stepping, and which protein domains are important for this movement. To answer these questions, artificial chimeric motors combining parts of plus-end-directed conventional kinesins with those of ncd have been generated[20–22]. Using the motor domain of ncd attached to a conventional kinesin stalk, it was possible to reverse ncd's physiological minus-end motility, an indication that regions outside the catalytic core confer directionality. The reverse experiment, making kinesin move backwards, turned out to be more difficult but finally revealed regions that were responsible for minus-end directionality in the ncd neck.

So the neck linker and neck regions emerge as being primarily responsible for directional determination. Although the common kinesin core seems to possess a subtle intrinsic bias that is sufficient for very slow plus-end motility[16], the conserved helix preceding the motor core of 'reverse' motors is able to override this bias, forcing the molecule to move towards microtubule minus ends. Conversely, the neck and neck linker of conventional kinesin amplify the intrinsic bias into robust and fast plus-end motility.

How can neck and neck-linker domains cause opposite movement? A look at the three-dimensional structures of dimeric conventional kinesin and ncd reveals that the respective necks position the two motor domains differently (FIG. 4). In ncd, hydrogen bonds cause the heads to lie close to the neck coiled-coil, generating a 180° rotational symmetry around its axis in a shape that resembles two oppositely oriented 'P's[23]. With only a small angular variation, this crystallographic structure can be fitted into three-dimensional images of motor-decorated microtubules obtained by electron microscopy. Conventional kinesin, in contrast, dimerizes through a neck that points away from the core motor domains, and the two heads include an angle of about

Box 3 | Microtubule structure

Microtubules are built from α/β-tubulin dimers that are stacked in linear arrays termed protofilaments, 13 of which form the wall of a microtubule in most cell types. Owing to the stereotyped stacking of subunits, these protofilaments (and therefore the microtubules) possess an intrinsic molecular polarity, with one end exposing the α-subunit, and the other the β-subunit. Although the end exposing the α-subunit, called the minus end, is usually anchored near the centrosome, the cell's microtubule-organizing centre, a microtubule can grow or shrink rapidly at the end exposing the β-subunit, called the plus end. The motor domain of kinesin possesses a microtubule-binding face that interacts with tubulin dimers (mostly the β-subunit) in the microtubule wall in always the same orientation, thereby recognizing (and exploiting) the intrinsic molecular polarity of microtubules.

120°. This spatial arrangement is incompatible with electron microscopic images[24,25]. Although cryo-electron microscopic reconstructions visualize the bound head, they do not clearly resolve the second head[26–28]. This may be due to disorder (or movement) of the second head, but variations in the pattern of kinesin decoration cannot be excluded[29]. Whatever the reason, there is a clear difference in the binding patterns of conventional kinesin and ncd, resulting from differences in the neck region, which helps to establish a structural asymmetry and, as a consequence, generates a directional bias.

The mechanism by which the bound head is moved in the right direction along microtubules — plus end for conventional kinesin, minus end for ncd — is largely unknown. One important point to consider is that, in the molecular world, brownian motion generates a large positional noise on a nanometre scale and causes the tethered head to fluctuate around an average position. A motor protein might just limit these fluctuations of the unbound head to a location near a new microtubule-binding site and allow it to 'find' this site by a diffusive mechanism. Alternatively, the neck may actively push the unbound head towards the next binding site. These models represent two possible principles for understanding molecular motility. In the first, translocation would essentially be driven by biased diffusion, with the motor acting as a molecular ratchet. The second model explains directional force generation by conformational changes of rigid mechanical elements. These models are not mutually exclusive and allow for a combination of both mechanisms during stepping (see online animation).

Which mechanisms are used by the other two classes of molecular motors, myosin and dynein, to decide which way to go? As shown by the studies using the kinesin–ncd pair, it is extremely useful if representatives that move in opposite directions are available. Such a myosin that 'goes the other way' has recently been found. So how does it work? Recall that in skeletal muscle myosin, the structural change initiated in the catalytic core is translated into a swing of the lever arm whose motion is coordinated by the converter domain[30] (BOX 2). Theoretically, by changing the connectivity at the base of the lever, you should be able to engineer a myosin whose mechanism of transduction from the active site is unchanged, but whose converter region causes the lever to swing in the opposite direction[31].

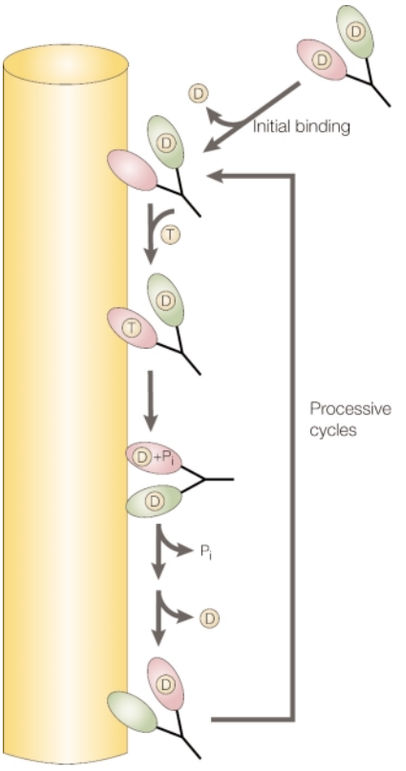


Figure 5 | **Processive catalysis of conventional kinesin.** After initial binding, kinesin is able to 'walk' along the microtubule without dissociating. It is thought that a two-headed motor is necessary for this processive behaviour because at each time point, the dimeric molecule needs to remain tethered to the microtubule through one head. This is achieved by the coordinated catalysis in the two heads: throughout the entire catalytic cycle, one of the heads is kept in the tight microtubule-bound form (containing either ATP or no nucleotide), while the other head is in transit in an ADP-bound form. During a brief phase of the cycle both heads may be bound to the microtubule. D and T inidicate bound ADP and ATP, respectively.

Nature has apparently done just that. The 'reverse' myosin, a member of the myosin VI family, moves along actin filaments in the opposite direction because the lever swings the other way[32]. The only main difference from the motor domain of muscle myosin is in the sequence of the converter region, which contains a large insertion. Because all members of the class VI myosins possess this insertion, it is conceivable that they all move in the opposite direction.

A dynein that goes the other way has not been isolated, but there are reasons to believe that 'reverse' dyneins may also exist. Dynein is the motor that powers ciliary and flagellar beating[33], but cytoplasmic isoforms involved in mitosis and organelle transport also exist[34]. All cytoplasmic dyneins characterized so far move towards the microtubule minus end, but in the cell processes of an unusual giant amoeba[35], the characteristics of ATP-driven organelle transport along uniform bundles of microtubules are dynein-like in both directions[36]. Another good place to look for a reverse dynein should be the

Box 4 | Duty ratio and processivity

The velocity of a molecular motor is restricted by the velocity of the catalytic events that supply the energy. Many kinesins, myosins and dyneins presumably move in a stepwise manner along their respective tracks. If one step is coupled to one ATP hydrolysis event, as generally believed[81,82], the stepping frequency cannot be higher than the ATPase rate.

To calculate the gliding velocity from ATPase rates, you must consider the working distance per step. For conventional kinesin it is about 8 nm, so given an ATPase rate of 20–40 ATP per second per head, we obtain a gliding velocity of 320–640 nm s^{-1} for a two-headed kinesin[42–44]. In the case of muscle myosin, the ATPase rate of about 20 s^{-1} and a step-size of 5.5 nm gives 110 nm s^{-1} — a value 80-fold too small to explain the observed velocity of movement. The answer to this paradox lies in the concept of the duty ratio. It is defined as the fraction of time that a motor remains attached to its track during one full CROSSBRIDGE CYCLE[95]. All molecular motors are proposed to undergo a working stroke during the attached phase, and then to recover their initial conformation in a detached (or weakly bound) phase. Conventional kinesin has a high duty ratio, meaning that the attached phase is long (over half of the full cycle), allowing a single molecule to transport a microtubule for several micrometres without falling off. Skeletal muscle myosin, on the other hand, has a low duty ratio, as shown by its inability to work as a single molecule. Rather, several tens of molecules are required to generate continuous movement. This seems to be an adaptation to the arrangement in sarcomeres, where myosin filaments interdigitate with large ensembles of actin filaments, ensuring the close proximity of many potential binding sites for myosin heads.

The duty ratio is intimately linked to the ability of a motor to operate processively, that is, to undertake many steps in succession without dissociating from the track. For kinesin, a high duty ratio and high processivity are thought to be an adaptation to its function as a single-molecule motor that transports cargo over long distances along a microtubule. Processivity is believed to require two heads that move in a 'hand-over-hand' fashion where the chemo-mechanical coordination between the duty cycles of the two motor domains ensures that there is always one head bound.

axoneme of eukaryotic cilia and flagella, a complex microtubule-based motile machinery. Axonemes may contain as many as a dozen dyneins, few of which have been characterized. It is pure speculation, but it would not be surprising if axonemes were to require 'reverse' motors for coordination of their intricate behaviour.

Staying on track

Kinesin moves along microtubules in steps that bridge the distance between adjacent tubulin subunits and can take many steps from one dimer to the next without falling off [37,38]. This form of movement is dubbed processive and is linked to the DUTY RATIO (BOX 4). The ability to stay on track allows a single kinesin molecule to move a microtubule in a GLIDING ASSAY. In contrast, for skeletal muscle myosin to move an actin filament, several molecules (probably more than 20) need to cooperate.

Processivity of conventional kinesin requires two motor domains that are linked by heavy-chain dimerization. A motor that possesses just one head, but otherwise has an unchanged neck and stalk region, is still active in multiple-motor gliding assays, but it fails to operate as a single molecule because it seems to fall off after a single step[39–41]. Therefore it is thought that conventional kinesin motility relies on a precise coordination between the two motor domains where one head proceeds to the next binding site while the motor remains tethered to the microtubule through the other, attached head. Conventional kinesin can move processively because, at each time point, at least one head is microtubule bound. How is this amazing natural clockwork synchronized? How does a head 'know' when to hold on and when to let go?

The answer is still open, though kinetic models have set a framework for processivity models. According to the 'alternating site model', kinesin uses a nucleotide-dependent change in its affinity for the microtubule to regulate the behaviour of the two heads[42–44] (FIG. 5). In solution, a kinesin dimer contains one ADP per head. Upon microtubule binding, only one head (say, head A) locks onto the microtubule and loses its ADP. Head A can detach again only if it binds and hydrolyses a new ATP molecule. During this hydrolysis process, head A allows head B to find the next microtubule binding site, where it loses its ADP and holds on tight. How head A acts on head B is crucial for an understanding of kinesin's motility mechanism, but precisely when and how force is being produced still remains an open issue. After the attachment of head B, head A finishes hydrolysis in a weakly bound ADP state and detaches from the microtubule while head B holds on. At this point, the heads have exchanged their roles (see online animation). So processive kinesin movement is achieved by three steps: first, a modulation of microtubule affinity through ATP hydrolysis, second, a mechanism that keeps the two heads out of phase and, last, a 'power stroke' linked to the hydrolysis cycle. In conventional kinesin, the power stroke entails conformational changes in the neck linker region[45], whereas in myosin it is the swing of the lever arm in conjunction with the converter region (BOX 2).

Stepping of other motors

The concept of how nucleotide binding and hydrolysis are linked to molecular motion was first developed for skeletal muscle myosin[46]. However, a single myosin molecule is not processive and seems to hop along its track, making contact with the actin filament for only a short period of time (BOX 4), in contrast to the behaviour of conventional kinesin. Does that imply that kinesins are, in general, processive, and myosins are not?

An answer to this question requires single-molecule studies of other motors. The few that have been looked at indicate that generalizations may be premature. For example, the dimeric kinesin-like motor Ncd is not processive[47–49]. It is not known whether other members of the family of 'reverse' kinesins are, or whether minus-end motility is incompatible with processivity; this is a question worth pursuing. On the other hand, at least one dimeric myosin of class V has now been shown to be processive[50]. In analogy to conventional kinesin, myosin V may drive vesicular transport as a single dimeric molecule[51,52]. On the basis of an OPTICAL TRAP ASSAY[53] and electron microscopic visualization[54], myosin V appears to move in large steps (thanks to a long neck region) of about 36 nm, even against an opposing force exerted by the trap[50]. So, physiologically, myosin V is more similar to conventional kinesin than to its close relative, muscle myosin.

More surprising is the observation that a single-headed motor can be processive as well. The monomeric

CROSSBRIDGE CYCLE
The sequence of structural changes of a myosin head coordinated with the hydrolysis of one molecule of ATP.

DUTY RATIO
The fraction of time that a motor molecule remains attached to the track during one full ATP hydrolysis cycle.

GLIDING ASSAY
Optical assay for the movement of cytoskeletal filaments over a 'lawn' of motor molecules attached to a coverslip.

OPTICAL TRAP ASSAY
A focused laser beam that traps refractile particles (for example, polystyrene beads) with attached motor molecules, allowing determination of step size and force per step.

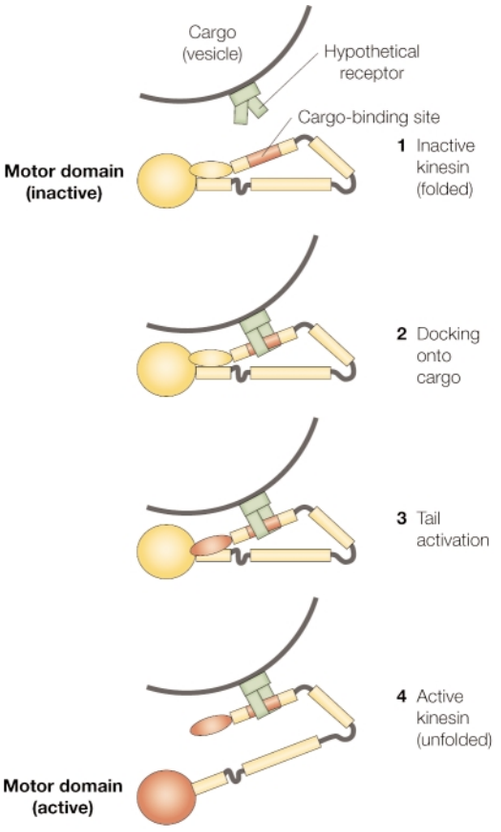


Figure 6 | **Model for how cargo binding might be linked to motor activation.** When not bound to cargo, the globular tail and the adjacent cargo-binding site (red segment) are located close to the motor domain (step 1). Docking onto cargo activates the cargo interaction site in the tail coiled-coil (step 2). Cargo docking is proposed to be transmitted to the globular tail domain (step 3) by as yet unknown mechanisms, initiating a conformational change (step 4) that relieves the inhibition of the motor domain. This sequence of events is indicated by red coloration of the domains involved. Whether this model leads to complete unfolding of the motor, as is generally assumed, or where it allows the motor to retain a modified folded conformation, remains to be shown.

mouse kinesin KIF1A, implicated in axonal transport[55], may promote processive motility despite being a monomer[56]. Its motile behaviour, however, differs from that of conventional kinesins in that KIF1A shows phases of back-and-forth movement with a net directional bias. Contact with the microtubule surface is apparently maintained by a positively charged surface loop in the head that interacts with the negatively charged carboxyl terminus of tubulin[57]. This interaction may allow for one-dimensional diffusion along microtubules during the weak binding state, though the mechanism that sustains the directional bias towards the microtubule plus end remains unclear. Given this diffusional step, it should be interesting to see whether the motor is still processive when it moves against a RETAINING FORCE.

Dyneins have also been analysed for processivity, and they show unusual behaviour. Single dynein molecules from *Tetrahymena* cilia move in 8-nm steps and are processive at low ATP concentrations, therefore resembling kinesin[58]. Unlike kinesin, however, the motor shows frequent backward steps, and at higher ATP concentrations becomes nonprocessive altogether — a behaviour not found in any other motor so far. A single-headed dynein of *Chlamydomonas reinhardtii* flagella[59] has also been reported to be processive, but this motor poses a riddle: it behaves as a motor with a low duty ratio, which is incapable of processive movement in gliding assays, but in a laser trap it behaves as a single motor that can take eight or nine continuous steps, even against a weak retaining force. The mechanism is unclear, but this behaviour may, in principle, result from the coordination of two independent microtubule-binding sites within the large dynein head. So processivity can be based, it seems, on mechanisms distinct from the strict 'hand-over-hand' coordination model of dimeric kinesin.

Getting (in)activated

Kinesin's enzymatic activities and cellular functions are probably regulated at several levels[60], including associated light chains, phosphorylation, binding to cargo and, as a more recent addition, intramolecular folding. Light chains, which interact with the heavy chains near the globular carboxyl terminus, have long been suspected to mediate kinesin function. Mutations in light chains result in the same phenotype as heavy-chain mutations[61,62], indicating that both molecules may cooperate in the same cellular pathway. This idea is supported by experiments with antibodies against light chains, which interfere with kinesin binding to vesicles[63] and organelle movements *in vitro*[64,65], albeit in both directions. Precisely how light chains affect kinesin function is not yet known, but one study indicates that they may inhibit binding of the heavy chains to microtubules[66], possibly in a phosphorylation-dependent manner[67,68]. They may also be involved in cargo targeting and cargo binding, because in one study a splice variant of light chains has been found to be associated specifically with mitochondria[69].

Perhaps the most basic level of kinesin regulation occurs within the kinesin dimer itself and involves an intramolecular interaction of the head and tail that is mediated by folding[70,71]. In the compact conformation, which prevails at physiological ionic strength, the ATPase activity of the motor domain is inhibited[72]. Self-inhibition requires neither associated proteins nor post-translational modifications[73] but critically depends on the presence of the flexible kink in the middle of the stalk[74–76]. Upon cargo binding — even an artificial cargo such as silica beads[77] — tail inhibition is relieved. The ability of unloaded kinesin to bind to, and move along, microtubules is not abolished[75], but movement is initiated less frequently and terminated earlier. The pronounced inhibition of the ATPase activity of a folded motor is due to the selective inhibition of the initial productive interaction with a microtubule[78]. Once bound, subsequent processive cycles are not strongly inhibited[75].

At the molecular level, folding requires an interaction between a domain near the carboxyl terminus and a region near the motor domain[73,76]. In the folded state,

RETAINING FORCE
Force exerted by a laser trap on a motor-carrying bead, moving along a microtubule.
molecule of ATP.

the globular tail domain is placed in close proximity to the catalytic motor domain. A conserved motif in the globular tail[79] may be directly involved in modulating the ATPase activity of the motor domain[73,76], whereas a cargo-binding region has been located in a coiled-coil next to the globular tail domain[76]. Its binding partner on the cargo is not known, but it is important that this domain is adjacent to the regions thought to act during folding and regulation. A model of how cargo binding might be linked to motor activation is presented in FIG. 6.

The tail inhibition model offers a reasonable explanation for the behaviour of conventional kinesins *in vitro* and *in vivo*, and may represent the most basic level of regulation. The reduction of the spontaneous activity of folded kinesin that the model proposes helps to explain how excessive movements of unloaded motor are prevented. However, because folding does not suppress movement completely, other factors are also likely to contribute.

Conclusions and outlook

This year kinesin celebrates its fifteenth birthday, but the field has advanced far past the 'puberty' stage and has reached a degree of maturity previously unforeseen. From atomic structures, the realization has come that the actin- and microtubule-based motors, myosin and kinesin, are closely related[9,11,92]. This finding has led to the now widely held view that largely homologous conformational changes in the catalytic site are translated into motion by a diverse set of structural elements in different motors[30,32,45] and result in steps of different size, duration or direction[23,30,56]. Because only a handful of motors have been looked at in detail so far, analyses of other motor classes may reveal more variations on the theme of mechanical amplification. Tremendous advances have been made in the development of techniques for single-molecule analysis, contributing information on step size, duty cycle, force generation and energy consumption per step[37,80,81,82]. Many valuable contributions to the study of motor mechanics have also been made possible through ingenuity (and intuition) in the design of mutant motors and their expression in suitable host cells.

Nevertheless, uncharted territory still lies ahead. The diversity of kinesin motors, reflected in the existence of at least ten kinesin families, has not yet been fully exploited to learn more about different ways, or unexpected subtleties, of force generation. Mutant motors generated by rational design and/or random mutagenesis will continue to help in this endeavour. Although the likely binding site for tubulin on the kinesin surface has been mapped and models of kinesin docking onto microtubule exist, details of this mechanism, in particular possible repercussions of the tubulin docking site on conformational changes in the motor head, have not yet been explored.

Possibly the largest gap in our knowledge of kinesin function is in the way that motors are attached to their respective cargoes. The diversity of kinesin tails suggests a similar range in cargo attachment mechanisms. *In vitro* assays of cargo binding will probably yield answers, but reliable assays do not yet exist. The question of cargo binding is intimately linked to the mechanism of its regulation, and the regulation of kinesin activity in general. Folding and tail inhibition are of basic importance, but as means of motor regulation they are, so far, restricted to conventional kinesins. Other regulatory factors must exist, and some of them are known, but they are likely to constitute only the tip of the iceberg. The existence of a complex regulatory machinery would be in line with the fact that all vital cellular processes are controlled by complex regulatory networks. Consequently, the elucidation of motor regulation may constitute the biggest challenge for years to come.

Links

DATABASE LINKS myosin | dynein | kinesin | *ncd* | myosin VI | myosin V | *KIF1A*

FURTHER INFORMATION Kinesin home page | Structure and function of microtubules | Online animation: Kinesin stepping

ENCYCLOPEDIA OF LIFE SCIENCES Dynein and kinesin | Cytoskeleton | Intracellular transport | ATP-binding motifs

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