Molecular motors

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Life implies movement. Most forms of movement in the living world are powered by tiny protein machines known as molecular motors. Among the best known are motors that use sophisticated intramolecular amplification mechanisms to take nanometre steps along protein tracks in the cytoplasm. These motors transport a wide variety of cargo, power cell locomotion, drive cell division and, when combined in large ensembles, allow organisms to move. Motor defects can lead to severe diseases or may even be lethal. Basic principles of motor design and mechanism have now been derived, and an understanding of their complex cellular roles is emerging.

Molecular motors are amazing biological machines that are responsible for most forms of movement we encounter in the cellular world. Three types of cytoplasmic motors are known: myosins, which move on actin filaments, and dyneins and kinesins, which use microtubules as tracks. The mechanism they use to convert chemical energy into mechanical work is both simple and ingenious. In all three motor classes, ATP hydrolysis causes a small conformational change in a globular motor domain that is amplified and translated into movement with the aid of accessory structural motifs. Additional domains outside the motor unit are responsible for dimerization, regulation and interactions with other molecules (Fig. 1).

This modular design of motors has given rise to considerable complexity so that each of the three motors comprises a superfamily whose members may vary appreciably in makeup and function. Today, we can distinguish at least 18 different classes of myosins, 10 different families of kinesins, and 2 groups of dyneins, each with up to several dozen members. The complement of motors varies widely between different organisms. Yeast, for example, gets by with 6 kinesins, 5 myosins and 1 dynein, whereas mammals have genes for over 40 kinesins, 40 myosins and more than a dozen dyneins. These numbers may easily be tripled as a result of post-translational modifications or varied combinations of associated proteins. Many motors are not yet characterized, and clear functions are assigned to only a small subset. Nevertheless, remarkable insights into motor mechanochemistry and function have been gained. This introductory overview highlights recent developments; for a compilation of comprehensive reviews, see ref. 1.

Motor mechanochemistry

Conformational changes

Our understanding of the molecular mechanisms that convert chemical energy into movement is most advanced for representatives of the myosin and kinesin families. High-resolution crystal structures of the motor domain uncovered an unexpected relationship between these two classes of motors: the region surrounding the ATP-binding pocket is virtually identical in structure, although sequence homology is restricted to only a few key residues. The architecture of the active site further revealed a relationship to the G proteins, suggesting that these three classes of molecules are of common evolutionary origin[2]. This notion recently received support from molecular dynamics simulations suggesting that G proteins — usually mediators in signalling pathways — may be able to generate force[3].

Among the various families of kinesins and myosins we find motors that work as monomers, dimers, trimers or tetramers, move to the plus end or the minus end of their track, and take just one or many steps before dissociating. Despite this wide spectrum of behaviours, in all motors the initial events in the generation of movement are similar and can be explained by stepwise amplification (Fig. 2a, b).

The primary event, the loss of the γ-phosphate group from ATP, leaves a space of approximately 0.5 nm, which is thought to cause a rearrangement of conserved structural elements flanking the ATP-binding site. This rearrangement, which represents the first level of amplification, is coordinated with structural changes in the track-binding site. Interruption of this coordination uncouples ATP hydrolysis from track binding[4,5]. The next level of amplification involves communication of the conformational change in the active site to carboxy-terminal structural components that may be viewed as mechanical amplifiers. Here myosins and kinesins differ. In many myosins, the mechanical amplifier is an α-helix of variable length stabilized by light chains. Based on crystal structures in different nucleotide states, this rigid structure acts as a lever that swings through an angle of up to 70° (refs 6, 7). The lever swing is believed to be the ultimate cause for the working stroke[8]. Accordingly, motors with longer necks take larger steps and move faster[9,10]. In conventional kinesins, the amplifier is a short, flexible stretch of ~10 amino acids that can be either docked to the motor core or flexible and free[11]. The mobility of this neck linker, possibly coupled to a rotation of parts of the motor domain[12], is believed to drive kinesin movement. Thus the structural features that sense and transmit hydrolysis-dependent changes are similar in the two motors, but translation into a large-scale conformational change apparently involves rotation of a rigid stalk in myosin and repositioning of a flexible element in kinesin[13].

Mechanistic analysis of the dynein motor is severely hampered by the lack of a high-resolution structure. It is clear though that, based on sequence features, the molecular design of dyneins is fundamentally different from myosins and kinesins. The motor domain of dynein comprises a ring of six AAA-ATPase modules, members of a widespread and highly diverse superfamily of proteins. ATP-dependent conformational changes in the ring of AAA-modules are believed to be transmitted to a stalk that carries the microtubule-binding site at its tip[14]. A swing in the position of this stalk leads to a ~15-nm displacement of the tip (Fig. 2c)[15].

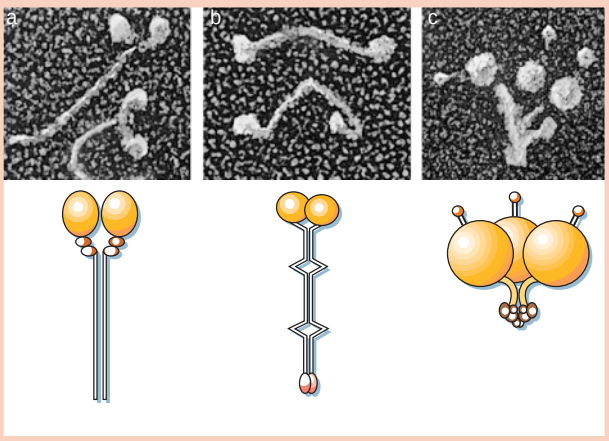

Figure 1 Representative cytoskeletal motors. **a**, Myosin II; **b**, conventional kinesin; **c**, ciliary dynein. The top row shows high-resolution electron micrographs of quick-frozen, rotary-shadowed individual molecules (images courtesy of J. Heuser). Corresponding schematic overviews are shown below. Motor domains are in yellow, associated proteins are shown in brown, and coiled-coil domains are represented by parallel black lines. For detailed overviews of the superfamilies of myosin and kinesin motors, see the myosin home page (http://www.mrc-lmb.cam.ac.uk/myosin/myosin.html) and the kinesin home page (http://www.proweb.org/kinesin//).

Although superficially resembling a swinging lever-arm movement, the structural and molecular basis of this force-generating 'power stroke' differs markedly from the conformational changes in myosins and kinesins.

Stepping

The conversion of these conformational changes into a step (or series of steps) leads us to the next level of complexity. Two fundamentally different behaviours of motors can be distinguished. In one, a single motor molecule can move along the track for long distances without detaching, a behaviour referred to as processivity. In the second, motors lose contact to the track usually after one cycle and therefore are non-processive. These modes of operation are physiological adaptations to different cellular functions. Processive motors are individualists, whereas non-processive motors often work as a team; the former hold on to the track for as long as possible, whereas the latter are optimized for brief, fast interactions.

Conventional kinesin, perhaps the best example of a strictly processive motor, is a dimer that interlaces the reaction cycles of the two heads. One head is tightly bound to the microtubule for at least half of the time of an ATPase cycle, and the two heads are kept out of phase[16]. Most models predict a 'hand-over-hand' cycle where the free head moves towards a new binding site past the bound head, consuming one ATP per step. An alternative model proposes an 'inchworm' type of asymmetrical stepping with a 'front' and a 'back' head[17]. In either model, a phase must exist where both heads are bound to the microtubule. Because a crystal structure of dimeric kinesin places the two motor domains in an unfavourable orientation only 5 nm apart[18], major rearrangements of adjacent domains are required during stepping. As discussed above, these rearrangements may be accommodated by the flexible neck-linker domain[11]. Partial unravelling of the coiled-coil neck may also be involved in some[19], but not all[20], kinesins.

The paradigm for a non-processive motor is muscle myosin II, which uses a lever arm to generate its working stroke. As conventional kinesin it is dimeric, but unlike conventional kinesin, the two heads do not cooperate, and the interaction with the track takes up less than one-tenth of the time of an ATPase cycle. Both factors contribute to its non-processivity. This mode of operation of myosin II makes sense because, in the sarcomeric ensemble, motors that remain bound to actin after their power stroke would slow down the entire system.

There are, however, myosins that possess a rigid lever arm (like myosin II) and combine it with head–head coordination (like kinesin) to operate processively. The best example is myosin V. Its six light-chain-binding sites in the neck create an extraordinarily long lever arm that enables a large step. Indeed, myosin V's step size is ~36 nm, which corresponds to the pitch of the actin helix[21], and its velocity and ATPase activity are consistent with the hydrolysis of one molecule of ATP per 36-nm step[22]. This large stride apparently requires contributions from two different mechanisms: a working stroke of only ~25 nm, and thermally driven diffusion, which contributes the missing 11 nm[23]. A similar 'composite' mechanism also seems to operate in other motors such as myosin VI where the short power stroke serves primarily to bias the reverse directionality of this motor while thermal motion drives its movement[24]. In both motors, the activities of the two heads must be strictly coordinated, which may be achieved via elastic strain exerted on the rear head by the curved neck of the forward head when both heads are bound[25].

Processive movement was generally believed to require dimeric motors. It therefore came as a surprise when monomeric KIF1A kinesin[26], monomeric class IXb myosin[27] and monomeric inner arm dynein[28] were suggested to move processively. But their mode of processivity differs from that of dimeric motors. For example, *in vitro*, monomeric KIF1A diffuses back and forth for several seconds when bound to microtubules, with a net movement towards the microtubule plus end. The key to this behaviour is the presence of a positively charged loop that interacts with the negatively charged C terminus of tubulin. This loop acts as a tether while the power stroke of KIF1A provides the push that biases diffusion towards the microtubule plus end[29]. Performance-enhancing charge interactions may also help to keep dimeric motors 'on track'[30]. Whether charge-dependent tethering is the key to understanding monomer processivity remains in doubt, as other members of the KIF1 family that also possess the ominous K-loop are non-processive[31]. Moreover, KIF1A-like kinesins may actually dimerize under *in vivo* conditions[32], relegating the mode of monomer movement to a mechanistically intriguing, but physiologically irrelevant, *in vitro* phenomenon.

A general conclusion emerging from studies on processive motors is that moving along the track may entail both a mechanical component and a diffusive component, with different motors using different proportions of each. Some motors rely largely on rigid conformational changes and tight coupling, with a relatively small contribution from diffusional searching. Others seem to have a relatively large contribution from diffusion, which alters their manner and form of processivity. In both, the diffusional component is supported by secondary 'tethering' sites that enhance motor performance.

Directionality

Most cell biologists would have been rather comfortable with the idea that a given superfamily of motors moves in one direction only. This comforting thought was shattered with the discovery of minus-end-directed kinesin-like proteins and a minus-end-directed myosin, leaving dynein as the last hope for a unidirectional motor superfamily.

All minus-end-directed kinesins studied so far have the motor domain at the C terminus, as opposed to the N terminus in plus-end motors. The two heads of ncd, for example, are tightly associated with the neck coiled-coil[33], which alters head–neck interaction, a key factor in determining directionality. When motor domains of forward and reverse motors are swapped, the resulting chimaeras adopt the direction of movement specified by the neck[34]. Movement of the chimaeras is usually slow and points to an intrinsic but weak plus-end bias even in a minus-end motor. Convincing evidence for the importance of the neck region in directional determination came from the analysis of a point mutant in the ncd neck that completely lacks directionality, switching stochastically between plus-end and minus-end movement[35].

The reversed polarity of the minus-end-directed myosin VI motor was attributed to a unique insertion of 53 amino acids in the converter

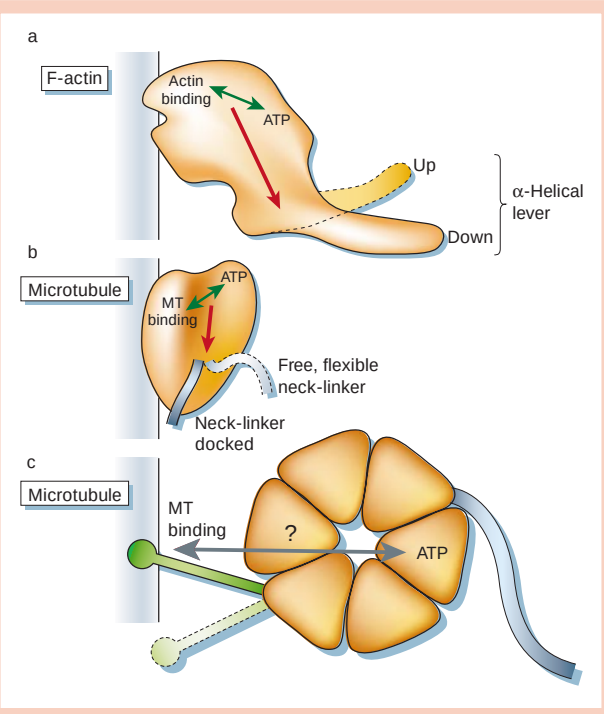

Figure 2 Schematic rendition of the intramolecular communication within one motor domain each of myosin, kinesin and dynein, and translation into a conformational change that leads to movement. In both myosin (**a**) and kinesin (**b**), ATP hydrolysis causes a conformational change to structural elements near the ATP-binding site that is communicated to the track-binding site (green arrow). The information is then relayed (red arrow) via homologous structural elements to a mechanical amplifier. **a**, In myosin the amplifier is a helix stabilized by light chains (not shown) that acts as a swinging lever. **b**, In kinesin the amplifier is a flexible element, the neck linker, that connects the motor domain with the neck helix. This element apparently undergoes a major positional shift, but its precise orientation remains to be determined. **c**, The pathways of intramolecular communication within the dynein motor domain are unknown at present, but the information on ATP hydrolysis is transmitted from one end of the molecule to the stalk that carries the microtubule binding site. The final step apparently involves an angular swing of the stalk.

domain, which is proposed to reverse the direction of the lever-arm swing[36]. This attractive hypothesis was cast in doubt when results from an analysis of several chimaeras between the opposite-polarity motors myosin V and myosin VI suggested that this insertion is neither necessary nor sufficient for minus-end-directed movement[37]. So far, studies have failed to show conclusively where the direction-determining regions reside, although it is hoped that clarification will be obtained upon analysis of the crystal structure of the myosin VI motor domain. It seems, however, that the structural basis of directional reversal is fundamentally different in myosin and kinesin motors.

Forces

The concept of serial amplification of structural rearrangements suggests that a minor change of 0.5 nm set off by the presence of absence of a phosphate group can be enlarged up to 36 nm (in myosin V). How big are the forces involved? To measure these forces, ingenious microdevices were developed that operate with unprecedented precision and sensitivity[38]. Force measurements have been made on only a subset of motors in each superfamily, but they show that the forces developed by kinesin, myosin and dynein motors — about 1–10 pN — are extremely minute by our macroscopic standards. For example, to lift a 5 kg weight, about 10^{13} motors are required. However, in the realm of the cell, these forces are gigantic. A single motor can move an object many times its own size through viscous cytoplasm at near maximum speed. External forces affect the kinesin cycle, suggesting at least one load-dependent transition, most likely associated with ATP binding[39]. Improved force-clamp techniques using laser traps equipped with a feedback control[40] will allow such load-dependent steps to be studied in detail.

Cellular functions

The initial belief that the three types of motors are associated with clearly separate functions (that is, myosin with contraction and movement, dynein with ciliary beating, and kinesin with organelle transport) could not be upheld for long. Now we are aware of, for example, myosins involved in organelle transport, dyneins implicated in vesicle and cell movement, and kinesins required for ciliary function. In addition, we count among their tasks unexpected functions such as signalling, RNA localization and sensory transduction; we are beginning to appreciate their implications in cellular architecture, basic developmental processes and a growing number of diseases; and we know that all three are important in cell division (see review in this issue by Scholey, page 746). This already is an impressive list, but because many motors have not yet been characterized, the full spectrum of cellular roles has yet to be appreciated.

Membrane association and regulation

Members of all three types of cytoskeletal motors are involved in organelle and vesicle transport (for reviews, see ref. 1). To understand these functions, it is essential to determine how motors link up to their cargoes and how transport is regulated. In both processes, non-motor domains and associated proteins have a key role, and a wide spectrum of attachment mechanisms is observed (Fig. 3).

Perhaps the most direct (but seemingly least specific) mechanism of membrane association is linkage to the phospholipid bilayer. Thus, acidic phospholipids are the binding partner for monomeric myosins[41] possessing a basic tail region, whereas a member of the Unc104/KIF1 family of kinesins binds to lipids via a pleckstrin homology domain[42]. This association depends on the presence of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), which promotes clustering of the motor in PtdIns(4,5)P_2-containing rafts. Clustering, in turn, may trigger the onset of transport.

In certain cell types, motors such as conventional kinesin and cytoplasmic dynein can latch onto their cargo via integral membrane proteins. In neurons, the kinesin light chains bind amyloid precursor protein (APP), a transmembrane protein of certain axonally transported vesicles[43]. This link is of potential medical significance as APP has gained fame as the precursor of a proteolytic fragment that gives rise to amyloid plaques in patients with Alzheimer's disease. Impaired APP transport may well contribute to the development of the disease.

Table 1 **Examples of molecular motors involved in disease**

Disease or defect	Motor involved	Reference
Myosin myopathies	Myosin II	88
Griscelli syndrome (pigmentation disorder)	Myosin V	89
Hearing loss	Myosin IIIa (ninaC), myosin VI, myosin VIIa, myosin XVa	90
Retinitis pigmentosa (photoreceptor degeneration)	Cytoplasmic dynein, Kinesin Krp85/95	44 91
Primary ciliary dyskinesia	Axonemal dynein	92
Kartagener syndrome (situs inversus)	Axonemal dynein, kinesin Krp85/95	93
Polycystic kidney disease	Dyneins and kinesins	94
Lissencephaly	LIS1 (dynein/dynactin interactor)	95
Charcot-Marie-Tooth disease type 2A	KIF1B (Unc104 kinesin family)	96
Virus transport	Conventional kinesin, cytoplasmic dynein, myosin	97, 98
Anthrax susceptibility	KIF1C (Unc104 kinesin family)	99
Neurodegenerative diseases	Kinesin Cytoplasmic dynein	100 101

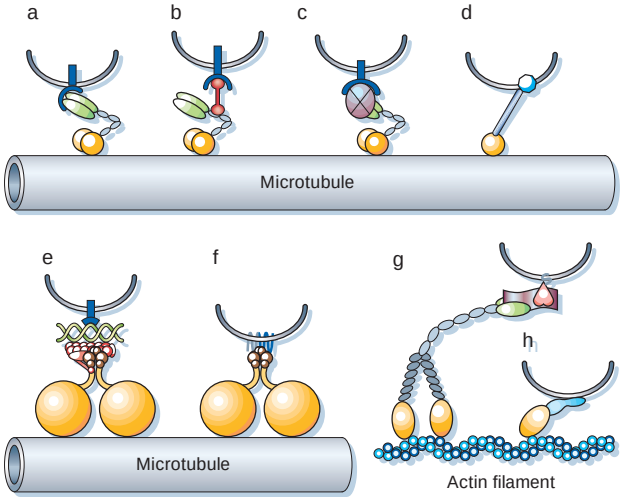

Figure 3 Types of motor-cargo linkage. **a**–**d**, kinesin; **e**, **f**, dynein; **g**, **h**, myosin. **a**, Interaction between a transmembrane receptor (blue) and kinesin light chains (green)[43]. **b**, Interaction between a transmembrane receptor and kinesin heavy chains mediated by a linker protein (red)[47]. **c**, Interaction between a transmembrane receptor and kinesin light chains mediated by a linker complex (purple)[45,46]. **d**, Interaction between membrane phospholipids and a pleckstrin homology domain (blue) in the kinesin-like protein Unc104 (ref. 42). **e**, Interaction between cytoplasmic dynein and an integral membrane protein mediated by the dynactin complex (red) and spectrin (green)[50,51]. **f**, Direct linkage of the Txtex-1 light chain of dynein with an integral membrane protein, rhodopsin[44]. **g**, Linkage of the tail domain of myosin V to membrane-anchored rab27a (red) via melanopholin (purple)[48]. **h**, Direct interaction of the tail domain of myosin I (blue) with acidic phospholipids[41].

In photoreceptor cells, cytoplasmic dynein, which normally requires the dynactin complex for attachment (see below), binds directly to rhodopsin, an integral membrane protein, with its Tctex-1 light chain[44]. This link, too, is significant as certain rhodopsin mutations inhibit this interaction, leading to retinitis pigmentosa.

The most widespread mode of association with integral membrane proteins occurs via linker proteins, often in the form of large assemblies. Work over the past few years has advanced various attachment modes for all three motor types. For example, conventional kinesin, again via its light chains, interacts with Jun kinase-interacting proteins (JIPs), a class of scaffolding proteins that bind components of the JNK signalling pathway[45,46]. JIPs, in turn, bind a transmembrane receptor of the low-density lipoprotein receptor family. Certain other kinesin-like proteins likewise use large linker complexes[47].

Among the myosins, the machinery that links myosin V to cargo is characterized best. In pigment cells, the small GTPase Rab27a and a recently identified Rab-binding protein, melanophilin[48], attach myosin V to melanosomes. The GTPase binds to membranes first and recruits melanophilin, which then binds myosin V. Melanophilin binding is GTP dependent, thus offering a convenient means of regulating motor–cargo association. This Rab-dependent machinery may well be paradigmatic for myosin–cargo association in other systems. Recent discoveries link Rabs and Rab-like effectors not only to several other myosin motors, but also to kinesins and dynein[49], thus opening the possibility of a significant functional interdependence of GTPases, motors and membrane traffic.

Finally, a large protein assembly seems to be involved in linking dynein to membranes (Fig. 4). Through its intermediate chains, dynein interacts with a unique activator complex, dynactin, which has the protein p150^{glued} and a short filament of the actin-related protein Arp1 as its most prominent components[50]. Precisely how the dynein–dynactin complex associates with vesicular cargoes is not understood, although in certain circumstances it binds to membrane-associated spectrin[51], making this the most complex linkage machinery known.

These examples, which represent the proverbial tip of the iceberg, indicate a wide spectrum of attachment mechanisms. Direct association with lipids or transmembrane proteins, linkage via an adaptor, or association mediated by complex protein assemblies all have been found. Given that one motor can interact with several different cargoes, there may well be dozens of specific membrane attachment mechanisms matching the dozens of potential cargoes in a cell.

An important issue arising from studies on cargo association is the question of motor regulation in cellular transport. In principle, motor activity can be regulated at two levels: by turning the motor on or off, and by inhibiting or promoting its association with cargo. Although this is largely uncharted territory, both mechanisms have been encountered in cells. In both, phosphorylation has a significant role, but novel means of regulation exist as well.

Phosphorylation may emerge as a negative regulator of cargo binding of several motors. For example, docking of the globular tail of myosin V onto melanosomes is inhibited by phosphorylation[52], thus holding up melanosome movement, while phosphorylation of the light intermediate chains releases dynein from membranes[53]. There are hints that phosphorylation can also regulate kinesin-based organelle movement[54], but it is unclear whether cargo binding is affected directly.

A radically different mechanism used by kinesin to avert non-productive movement without cargo involves intramolecular folding where the tail inhibits the motor domain. Binding to cargo de-represses tail inhibition and allows the motor to unfold, a process that is critically dependent on a flexible domain in the stalk[55,56]. This is an attractive mechanism because it couples motor regulation and cargo binding. How the tail inhibits the motor is not known, but a tail motif conserved in all conventional kinesins is crucial[57,58]. Thus, intramolecular interactions and phosphorylation may complement each other in the regulation of cargo transport.

Coordinating motors

Research on organelle transport took a completely unexpected twist with the demonstration that some organelles can switch tracks and move on either microtubules or actin filaments[59]. In amphibian melanophores, for example, heterotrimeric kinesin and myosin V cooperate in the dispersion of pigment granules, while during aggregation, myosin V is switched off, presumably by phosphorylation-dependent release from the granules[60]. In vertebrate melanophores[61] or neurons[62], the two classes of motors may act sequentially. Fast, long-range microtubule-dependent transport in the cell body is supplanted by short-range actin-dependent transport in the cell periphery. Here myosin V, with its long neck and large stride, may safely haul its cargo through the tangle of cortical actin filaments, not unlike an ape swaying from branch to branch in a treetop. These examples suggest the intriguing possibility that the deployment of many cell organelles depends on the concerted action of multiple motors. Myosin V and conventional kinesin have been shown to interact directly in their tail domains[63], but it remains to be seen whether physical interaction of motors is the key to their coordination.

Motors in novel contexts

Organelle transport and ciliary movement or contraction are paradigmatic tasks of cytoskeletal motors, but there is more to motors

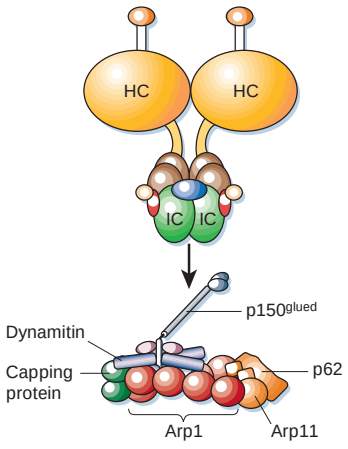

Figure 4 Schematic overview of the dynein–dynactin complex. The dynein molecule, itself a complex of heavy (HC), intermediate (IC) and light chains, interacts with the p150^{glued} subunit of the dynactin complex through its intermediate chains (arrow), although the precise mode of interaction is not known. The most prominent component of the dynactin complex is a short filament of the actin-related protein Arp1.

than meets the microscopist's eye. Some motors are implicated in the transport of messenger RNA or macromolecular complexes. Others are unable to move and yet are indispensable for certain cellular activities (see review in this issue by Howard and Hyman, page 753). Some deletions or mutations of motors can be lethal for multicellular organisms, indicating that these motors are essential for crucial steps in development. In other circumstances, the loss of certain motors leads to debilitating diseases. Finally, motors may participate in cellular homeostasis and cell architecture in ways that extend beyond functions in transport. These are exciting research fields unforeseen only a few years ago.

New on the agenda of motor functions is an involvement in mRNA transport. Restricting mRNA translation restricts the subcellular distribution of the protein product but requires the transport of mRNA to its destination. Depending on the system studied, mRNA transport is accomplished by myosin, kinesin or dynein motors. In yeast, for example, certain mRNAs are transported in a complex with myosin V (ref. 64), whereas in neurons or insect oocytes, microtubule motors are required[65,66]. In all cases, RNA-binding and adaptor proteins integrate the RNA into a ribonucleoprotein transport package, although the precise molecular interactions within this complex have yet to be determined. A paradigm for the extraordinary importance of motor-dependent mRNA localization is the *Drosophila* oocyte where the convergence of *oskar* mRNA and associated proteins at the posterior pole is supported by conventional kinesin[67,68], whereas *bicoid* mRNA-containing complexes are moved by dynein to the anterior pole[69]. Their precise deployment establishes the anterior–posterior axis. There are hints that the establishment of dorsoventrality requires motors as well.

The determination of the left–right axis in mammals also depends on the activity of molecular motors, although the nature of the implication is different. Left–right patterning was suggested to require the transport of a 'morphogen' to the left side of the vertebrate gastrula by cilia of the embryonic node, an organizing structure in the developing embryo. In support of this notion, mutations in a gene encoding a dynein isoform known as left–right dynein[70] and in a member of the Krp85/95 kinesin family[71], both of which are required for ciliary development, inactivate nodal cilia and lead to random positioning of internal organs. The conclusions from these studies met with scepticism, because the mechanism was believed to be too unspecific for such a crucial step in development. However, an artificial flow around the nodal cilia, generated with the use of an ingenious micromechanical device, was shown to influence the positioning of internal organs[72].

This is just one striking example that a motor defect can lead to a pathological condition, situs inversus. Work over the past few years has implicated motors in a growing number of human diseases (Table 1). So far, these can be grouped into five categories: defects associated with contraction that can be traced to myosin II; sensory defects associated with several unconventional myosins; disorders associated with defects in ciliary biogenesis and function that are linked to axonemal dynein and the kinesin-like protein Krp85/95, as described above; intracellular transport deficiencies attributable to defects in cytoplasmic dynein and kinesin organelle transporters; and transport of pathogens. These implications are firmly established in some cases (for example, myopathies or hearing loss) and more tenuous in others (for example, neurodegenerative diseases), but they are tantalizing enough to spur further efforts aimed at the discovery of hidden links between motors and disease.

A final issue concerns an involvement of motors in cell architecture and cytoskeletal remodelling (Fig. 5). We have seen that some motors can be part of large macromolecular complexes and, through their associated proteins, can interact with a wide spectrum of cytoplasmic constituents. A paradigm is the dynein/dynactin machinery, which has been shown to be important not only in organelle transport, but also in cytoskeletal architecture. Dynein associates with adherens junctions of epithelial cells through an interaction with β-catenin and a novel protein, PLAC-24, that binds the dynein intermediate chain[73]. This protein complex may help to tether microtubule ends at sites of cell–cell contact. Additional interactions of cortical cytoplasmic dynein with microtubule plus ends affect spindle orientation, nuclear movement, centrosome positioning and cell polarity[74]. Thus, cortical dynein can profoundly influence the spatial organization of the entire microtubule apparatus, which in turn provides a framework for the organization of cellular membrane systems. In addition, dynein as well as conventional kinesin are required for the assembly and dynamics of the vimentin intermediate filament system[75] and neurofilament transport[76], supporting the long-standing notion of a close spatial relationship between these two cytoskeletal systems. Both motors, or their interacting proteins, can also be part of the microtubule plus-end complex, a large assemblage of proteins associated with growing microtubule ends[77,78].

Motors may link the microtubule and actin systems as well. For example, a class VI myosin interacts with a microtubule plus-end-binding protein[79]; CHO1, a kinesin of the MKLP1 subfamily, possesses an extra domain that interacts with actin filaments[80]; actin reorganization requires a ras-related GTPase that interacts specifically with KIF9 kinesin[81]; a dynein light chain of relative molecular mass 8,000 (DLC8) is also a component of myosin V[82]; and a plant kinesin, KCBP, possesses a myosin tail homology domain, a widespread subdomain of several myosins[83]. Sporadic as they may seem, these findings hint at a system of functional interactions between cytoskeletal systems mediated by molecular motors. Coupled with the observation that microtubule motors help construct large-scale assemblies such as centrosomes[84] or microtubule asters[85], and that myosin tunes viscoelasticity without disrupting filament networks[86], motors are emerging as dynamic modulators of cell architecture.

Outlook

Extrapolating into the future is always challenging and often wrong. Using current work as a guide, four main areas of future research on molecular motors can be identified. First, even though we seem to have a general idea of motor chemomechanics, important details still need to be worked out. Atomic resolution structures will be the guide. In combination with single-molecule techniques of improved spatiotemporal resolution and sensitivity and the rational design of

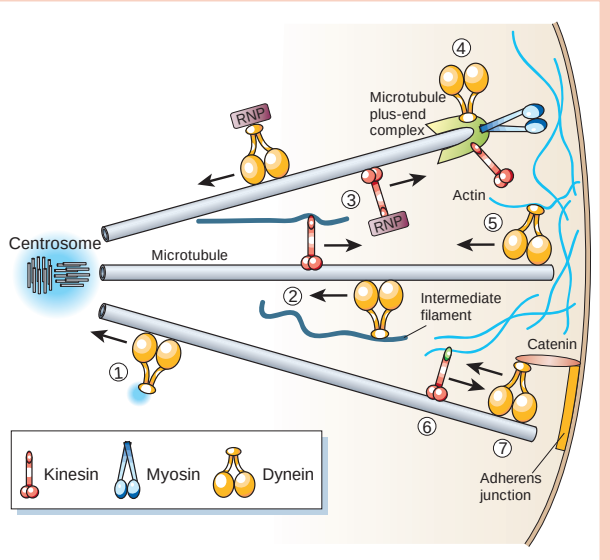

Figure 5 Role of cytoskeletal motors beyond membrane transport. 1, Retrograde transport of centrosomal components[84]. 2, Anterograde and retrograde transport of intermediate filaments[75,76]. 3, Anterograde and retrograde transport of ribonucleoprotein (RNP) complexes[64–66]. 4, Myosin, kinesin and dynein motors interact with components of the microtubule plus-end complex[77–79]. 5, Anchorage of dynein at the actin-rich cell cortex[74]. 6, Interaction of a kinesin-like protein with actin[80]. 7, Catenin-mediated anchorage of dynein at adherens junctions[73].

motor mutants, common principles of motor physiology will emerge. Second, many motors are known only by sequence, particularly in plants, so this is a fertile playground for the cell biological hunter-gatherer. Functional characterization will help answer questions of motor targeting and motor regulation: how does a motor find its cargo, what directs it to the correct target site, and how is its activity regulated in the process? Only partial answers are available at present Third, the implication of motors in disease and developmental defects will attract increasing attention. The questions, and the answers they demand, will undoubtedly be complex, as motor defects will frequently be just one of many factors that contribute to the manifestation of a disease. Fourth, motors are believed to hold promise for use in nanobiotechnological devices, although marketable applications have yet to be achieved.

"Our progress is narrow; it takes a vast world unchallenged and for granted," writes J. Robert Oppenheimer[87]. "This is why we will have to accept the fact that no one of us really will ever know very much. This is why we shall have to find comfort in the fact that, taken together, we know more and more." The field of molecular motors is no exception. □

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