Molecular motors: structural adaptations to cellular functions

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Molecular motors are protein machines whose directed movement along cytoskeletal filaments is driven by ATP hydrolysis. Eukaryotic cells contain motors that help to transport organelles to their correct cellular locations and to establish and alter cellular morphology during cell locomotion and division. The best-studied motors, myosin from skeletal muscle and conventional kinesin from brain, are remarkably similar in structure, yet have very different functions. These differences can be understood in terms of the 'duty ratio', the fraction of the time that a motor is attached to its filament. Differences in duty ratio can explain the diversity of structures, speeds and oligomerization states of members of the large kinesin, myosin and dynein families of motors.

There is no let-up in the pace at which new molecular motors are being discovered. In the past ten years, the founding members of the three families of motor proteins—myosin from skeletal muscle, axonemal dynein from sperm, and conventional kinesin from brain—have been joined by hundreds of new-found relatives whose amino-acid sequences contain regions of high similarity to the force-generating 'motor domains' of the founding proteins (see reviews for kinesin[1,2], myosin[3,4], and dynein[5]). The extent of molecular diversity can be appreciated on realizing that the several isoforms of myosin II that are found in skeletal, smooth and cardiac muscle, as well as in virtually all non-muscle cells, all fall within one subfamily—and yet there are at least ten other myosin subfamilies. Many of these motor-related proteins have been shown to move *in vitro* (they really are motors) and 'knockout' experiments (in which expression of a motor protein is prevented by targeted disruption of the gene encoding it) have revealed a remarkable diversity of cellular functions[1–5] (Table 1). Some members in each family function in large macromolecular assemblies (myosin II in muscle, axonemal dynein in sperm, and cytoplasmic dynein and various kinesin-related proteins in the mitotic spindle), whereas others function independently or in small numbers to move membrane-bound vesicles and organelles (conventional kinesin, kinesin II, cytoplasmic dynein and myosin V). In addition to being motors, some members of the myosin I, III and IX subfamilies may also be signalling molecules[6,7]. Mutations in myosins cause human diseases such as familial cardiac hypertrophy[4] and Usher's syndrome, which is associated with vision and hearing loss[3].

Meanwhile, the understanding of the molecular mechanisms of force generation by motor proteins has proceeded rapidly with the solution of the atomic structures of skeletal muscle myosin[8] and conventional kinesin[9], and with the recent development of optical trapping, atomic-force microscopic and fluorescence techniques, which allow the visualization and manipulation of single motor molecules as they move along filaments in the presence of ATP[10–13].

Now is a good time to take stock and ask what we have learned so far about the molecular mechanisms of force generation. Can we find common principles in the workings of muscle myosin and conventional kinesin that will help us understand the function and mechanisms of the other proteins within the motor families? I will argue that the new results have forced us to revise some of our fundamental assumptions about how motors, especially myosin, work, but they have also given us new insight into the divergent structures and cellular roles of motor proteins in general.

Conventional kinesin and muscle myosin

A superficial comparison of muscle myosin and conventional kinesin presents a puzzle: the structures are so similar yet the motor functions and mechanisms appear to be so different. Electron microscopy reveals two motors with a common domain organization: each has two identical globular 'heads', which dimerize

Table 1 Motor speeds *in vivo* and *in vitro*

Motor	Speed* *in vivo* μm s^{-1}	Speed† *in vitro* μm s^{-1}	ATPase‡	Function
Myosins				
Myosin IB	?	0.2	6	Acanthamoeba motility[71,72]
Myosin II	6	8	20	Rabbit psaos muscle[73]
Myosin II	0.2	0.25	1.2	Avian smooth muscle[74,75]
Myosin V	0.2	0.35	5	Vesicle transport in yeast and mice[66,67]
unknown	60	60	?	*Nitella* cytoplasmic streaming[76]
Dyneins				
Axonemal	7	4.5	10	Sea urchin sperm[77,78]
Cytoplasmic	1.1	1.25	2	Retrograde axonal transport[64,79,80]
Kinesins				
Conventional	1.8	0.8	44	Anterograde axonal transport[12,27,79]
Fla10/KinII	2.0	0.4	?	Anterograde transport in flagella[81,82]
Kip1/Eg5	0.018	0.06	2	Mitosis and meiosis[83–85]
Ncd	?	0.09	1	Meiosis and mitosis[86,87]

* *In vivo* speed applies to the motion of the motor relative to the filament without external load.
† *In vitro* speed is that of purified motor and filament at high ATP concentration.
‡ ATPase is the maximum filament-activated rate of hydrolysis per head per second measured in solution at high ATP and filament concentrations.

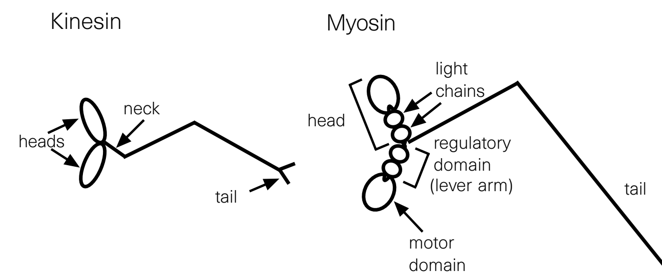

Figure 1 Structures of conventional kinesin and muscle myosin based on electron microscopy and sequence analysis. The kinesin homodimer has two heads, each about 8 nm long, which are joined at the coiled-coil neck, a dimerization domain. The neck connects to a long coiled coil that terminates in the tail region, thought to bind to the organelle cargo. Myosin's heads are about twice the size of kinesin's and are composed of two major domains, the motor domain which binds actin and nucleotides, and the regulatory domain, an 8-nm-long α-helix that is stabilized by two calmodulin-like light chains. The regulatory domain, which is thought to act as a lever arm, then connects to a long coiled-coil rod, which in muscle oligomerizes to form the thick filament.

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through long coiled-coil dimerization regions that terminate in a 'tail' (Fig. 1). The heads are the crossbridges between the thin and the thick filaments of muscle[14] and between organelles and microtubules in neurons[15] and they contain the motor domains that bind both the nucleotide and the filament. Except for the dozen or so amino acids that bind the nucleotide, the head domains of myosin and kinesin contain no amino-acid sequence similarity. Thus it came as a surprise that the core 180 amino acids within the myosin and kinesin head structures have the same fold[9]. Furthermore, the preserved sequence direction and order of the α-helices and β-sheets within the core suggest that these motors evolved from a common ancestor.

Yet despite these structural similarities, there are profound functional differences between kinesin and myosin. Conventional kinesin operates alone or in small numbers to transport membrane-bound organelles over large distances (up to a millimetre) along microtubules[16], whereas muscle myosin operates in huge arrays of up to a billion molecules in a large muscle fibre and moves relatively short distances (up to about one micron) along actin filaments[14].

These functional differences are inherent to the motor proteins themselves, rather than being due to different accessory proteins in the different tissues. Surfaces sparsely coated with purified kinesin support the gliding of microtubules, and quantitative dilution studies show that a single kinesin molecule is sufficient for motility: one motor can walk up to several microns along the surface of a microtubule without falling off[17]. This property is an adaptation for kinesin's role as an organelle motor, and kinesin is said to be a 'processive' enzyme, by analogy to the DNA and RNA polymerases which move along and polymerize up to thousands of bases during each encounter with the DNA strand. By contrast, myosin assays do not work at low density, and it is estimated that a minimum of tens to hundreds of myosin heads are needed for the continuous movement of an actin filament in a gliding assay[18,19]. Although any propensity of an organelle motor for dissociating from its filament would pose a transport problem, it is not a problem in muscle, in which there are 300 myosin molecules per thick filament[20] and where the sarcomere is reinforced to ensure that the muscle does not fall apart even when it is relaxed and none of the crossbridges is engaged.

High-resolution single-molecule experiments confirm that kinesin is processive whereas myosin is not. Using optical tweezers[10,21] or glass fibres[12] to impose loads and photodiode detectors to measure displacements with nanometre sensitivity, it has been shown that a single molecule of kinesin can move hundreds of nanometres along a microtubule even against an opposing force of up to 5 piconewtons. By contrast, a single myosin molecule makes only transitory interactions with the actin filament and is unable to progress to the next binding site even when unloaded[11,22,23].

The duty ratio concept

The functional differences between kinesin and myosin can be understood using the concept of the 'duty ratio', the fraction of the time that a motor domain spends attached to its filament. The concept first arose (initially called the duty cycle[24]) to explain the high force generated by smooth muscle, an issue to which I will return. In order to move along a filament through a distance that is large compared to molecular dimensions, each crossbridge must cycle repetitively between attached and detached phases (Fig. 2a). In the attached phase, of duration τ_{on}, the head or crossbridge undergoes the 'working' stroke, and in the detached phase, of duration τ_{off}, it undergoes the 'recovery' stroke, which returns the crossbridge to its initial conformation (Fig. 2b). We define the working distance (δ) as the distance that the distal, cargo-binding end of the crossbridge moves relative to the proximal filament-binding end (Fig. 2b). By recovering during the detached phase, the motor avoids stepping back, and so progresses through the working distance during each cycle. We formally define the duty ratio, *r*, as the fraction of the time that each head spends in its attached phase:

$$r = \frac{\tau_{on}}{\tau_{on} + \tau_{off}} = \frac{\tau_{on}}{\tau_{total}} \quad (1)$$

Unlike a two-stroke internal combustion engine, which has a duty ratio of 0.5 because the working stroke (the expansion stroke) and the recovery stroke (the compression stroke) are constrained to be of equal duration, the duty ratio of a crossbridge can in principle be large (~1) if it spends most of its time attached, or small (~0), if it spends most of its time detached. Because a two-headed molecule of conventional kinesin is able to maintain continuous attachment to the microtubule, its duty ratio must be at least 0.5, otherwise there will be times when neither head is attached and the motor will diffuse away from the filament. On the other hand, because skeletal muscle myosin must be in large assemblies with at least 10 to 100 crossbridges to move, its duty ratio must be small, ~0.01 to 0.1, the reciprocal of the minimum number of heads needed for continuous motility[19,25].

In addition to explaining why some motors can operate alone but others must work in assemblies, the duty ratio also provides the crucial link between the chemical speed of a motor (its ATPase rate) and the mechanical speed (its velocity[26]). Consider a filament moving at constant speed, *v*, over an array of fixed motor proteins, as occurs during filament sliding in muscle or in an *in vitro* gliding assay. We suppose that there are enough heads interacting with the filament to ensure continuous motility: that is, at least one kinesin molecule or at least 10 to 100 myosin molecules. If each head is attached for time τ_{on} and moves through the working distance δ, then $v = \delta/\tau_{on}$. On the other hand, because the cycle is driven by ATP hydrolysis, we expect that the total cycle time $t_{total} = 1/V$, where *V* is the rate at which each head hydrolyses ATP. Substituting these expressions for τ_{on} and t_{total} into equation (1), we obtain another expression for the duty ratio:

$$r = \frac{\delta \cdot V}{v} \quad (2)$$

It is important to note that this argument assumes that exactly one

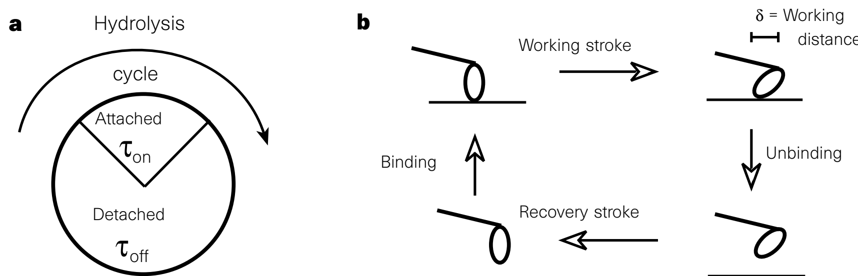

Figure 2 The crossbridge cycle. **a**, It is proposed that a crossbridge, or 'head', makes a working stroke during the attached phase (of duration τ_{on}) and makes a recovery stroke during the detached phase (of duration τ_{off}). **b**, By recovering its initial conformation while detached, the motor avoids stepping backwards and so progresses by a distance equal to the working stroke during each cycle. We define the duty ratio as the fraction of the time that the motor is bound and in its working phase.

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ATP is hydrolysed per mechanical cycle: although there is indirect evidence that this is true for kinesin at low load[27–29], a pressing goal of the motor field is the direct measurement of the coupling stoichiometry at high and low loads.

The value of this analysis is that it explains some very puzzling kinetic differences between myosin and kinesin as simply being due to differences in duty ratios. The puzzle is that the unloaded gliding speed of fast skeletal muscle myosin is ten times greater than that of conventional kinesin, even though myosin's ATPase rate is only one half that of kinesin's (Table 1). In other words, myosin moves some twenty times farther than kinesin for each ATP that it hydrolyses. This leads to a paradox that generated much controversy in the myosin field[30,31]: during the 50 ms it takes for each myosin head to hydrolyse a molecule of ATP, the actin filament moves through a distance of $\sim$400 nm (8,000 nm $s^{-1} \times$ 50 ms), a distance much larger than the dimension of the myosin head itself. The resolution of the paradox is that myosin has a low duty ratio: with $r = 0.01$ and an ATPase rate of 20 per head per second, a speed of 8,000 nm s^{-1} can be reached with a working distance of only 4 nm (equation (2)), well within the dimension of the crossbridge. The crucial point is that each of the hundred or so heads moving the filament contributes only 4 of the 400 nm moved while it hydrolyses one molecule of ATP; then, while this motor is detached, the other heads sweep the filament along the rest of the way. By contrast, because kinesin has a high duty ratio, it needs a high ATPase rate to attain even moderate speeds: a working distance of 8 nm (see below), a speed of 800 nm s^{-1}, and an ATPase rate of 50 s^{-1} implies that $r = 0.5$, consistent with the duty ratio needed to account for kinesin's processivity.

Structural basis for the duty ratio

The definition of the duty ratio in terms of attached and detached times (equation (1)) gives the misleading impression that the duty ratio can equal any number between zero and one, depending on the biochemical 'on' and 'off' rate constants. However, this is not the case. In fact, the duty ratio can take on only a discrete set of values owing to the discrete sites on the filament to which the crossbridges can bind. To see why this is so, let d be the minimum distance between the motor's consecutive binding sites on the filament, the 'stepping stones'. We call this distance the path repeat distance, or simply the path distance: it depends not only on the structure of the filament, but also on the path that a particular motor follows on the surface of the filament. Now again consider a filament gliding over a fixed array of motor proteins. If the working distance, δ, is smaller than the path distance, d, then it is clear that each individual crossbridge must spend a significant time detached while other (attached) crossbridges move the filament and the next binding site forward. In this case, the duty ratio must be less than one, and continuous motility will require an assembly of crossbridges. In other words, the duty ratio ought to equal the fraction of the distance to the next binding site that the working stroke takes the crossbridge:

$$r = \frac{\delta}{n \cdot d} \qquad (3)$$

where the integer n can be greater than one if the crossbridge skips over one or more of the stepping stones. Equation (3) represents the crucial steric constraint that is present in a moving, motor-filament system, but absent when isolated heads are freely diffusing in solution (in which case the path has no meaning). I will argue that this equation, which is both new and likely to generate some controversy, is of great use in understanding the diverse structures and biological functions of motors.

The first question to ask is whether this structural definition of the duty ratio accords with the previous definitions in terms of fractional binding and ATPase-to-speed ratios. The answer comes from recent single-molecule experiments which have elucidated the paths trodden by conventional kinesin and muscle myosin, as well as the working distances. Most cytoplasmic microtubules are composed of 13 protofilaments that run parallel to the axis of the microtubule: these microtubules do not rotate when they glide over a kinesin-coated surface, whereas specially synthesized 12- and 14-protofilament microtubules whose protofilaments supertwist about the microtubule axis[32] do rotate with the pitch and handedness of the supertwist[33]. Thus kinesin's path is parallel to the protofilaments, and, because there is just one kinesin binding site per tubulin dimer[34,35], the path distance must be a multiple of 8 nm, the length of the dimers that form the protofilament (Fig. 3a). High-resolution tracking experiments of single (dimeric) kinesin molecules show that its steps are probably equal to 8 nm[10,28,36], although alternating 7-nm and 9-nm steps, which would be expected if kinesin were to 'waddle' along adjacent protofilaments, have not been ruled out. These data are not quite complete enough to derive a definitive value of the duty ratio, because there is still no direct measure of kinesin's working distance. However, they are quite consistent with a duty ratio of 0.5 if we take the working distance to be 8 nm (the step), the path distance to be 8 nm (the tubulin dimer length), and $n = 2$ in equation (3), because the second head must jump over the tubulin dimer to which the other head is bound (Fig. 3a).

The path trodden by myosin is also parallel to the axis of the actin filament (Fig. 3b). Actin filaments rotate little[37] or not at all[38] as they glide over surfaces coated with myosin: this shows that myosin does not move along the two-stranded helix as this would cause one rotation per 72 nm, the pitch of this helix. Now it is possible that the crossbridge is so flexible that it binds equally well to almost any actin subunit around the circumference of the filament. Alternatively, if the crossbridge has limited flexibility then it will bind only to correctly oriented subunits, and the path distance will be equal to 36 nm, the half-pitch of the helix (Fig. 3b). The latter, 36-nm path distance is directly supported by high-resolution single molecule experiments in which an actin filament is suspended between two beads that are each held in an optical trap and in which the filament is 'bowed' past a fixed myosin crossbridge: binding is only observed at multiples of about 35 nm[39].

Although the finding that kinesin moves parallel to the axis of the microtubule is not surprising (for example, this is the shortest path from one end to the other), the conclusion that myosin follows a path parallel to the actin filament is. Some earlier models assumed a parallel path[40], but it has more commonly been considered, especially in model diagrams, that myosin follows the two-stranded helix of actin[41,42], presumably because the consecutive binding sites would be considerably closer (only $\sim$5.5 nm). However, the new conclusions, based on *in vitro* data, do accord with other biological and structural considerations. If a motor treads upon an angled path, a torque will be generated[33]: this will disrupt the arrays of the filaments found in muscle or sperm, or it will cause transported organelles to spiral about their filaments, creating serious steric problems, especially if there are two filaments within an organelle-diameter of each other, as is usually the case in the cytoplasm.

The working distance of a single skeletal muscle crossbridge has been directly measured using high-resolution single-molecule techniques. In the absence of an external load, it is 4 to 6 nm (refs 22, 43). There has, however, been considerable controversy about the interpretation of these experiments, and some groups have inferred much larger working distances in the range of 10 to 20 nm (refs 11, 23, 44), although these high values are likely to have been inflated by the brownian motion of the actin filaments held in the soft optical traps[45]. The smaller value is consistent with mechanical measurements in muscle showing that the tension in an active muscle is reduced to zero by a rapid shortening of the muscle by only 4–6 nm per half sarcomere[46,47]. Considering that 2–3 nm is accounted for by the shortening of the actin filaments[48,49], the remaining 2-3 nm then corresponds to the average strain in the attached crossbridges (which will range from zero to the full 4–6 nm).

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The small working distance of 4–6 nm compared to the large path distance of 36 nm is the structural reason why the duty ratio of skeletal muscle myosin is so small. The duty ratio can be no larger than about 0.1 to 0.2, and could be much less if myosin skips over neighbouring binding sites. Thus the structural data predict a low duty ratio, in accordance with the motility and biochemical data.

A low duty ratio of only ~10–20% for skeletal muscle myosin was originally deduced by electron paramagnetic resonance spectroscopy, which showed that only about 20 per cent of the heads in an active muscle were ordered, as would be expected if they were bound to the actin filament[50]. But this conclusion met a lot of resistance from physiologists. The problem was that the stiffness of an activated muscle that is prevented from shortening (an 'isometrically contracting' muscle), or that is shortening only very slowly, is about 80% of that of a rigor muscle, in which most of the heads are attached[51]. If it is assumed that the stiffness is proportional to the number of attached heads, then the duty ratio must be close to one. However, the discovery that the actin filaments contribute about half the compliance of muscle[48,49,51,52] means that the stiffness is not proportional to the number of attached heads; a low duty ratio is therefore not inconsistent with the stiffness measurements, especially if the rigor heads are themselves somewhat less rigid than the active heads. On the other hand, a high duty ratio in muscle leads to a mechanical inconsistency: if all the crossbridges are bound and contributing to the stiffness of muscle, then the individual crossbridges cannot be very stiff and so will perform only a small amount of work per mechanical stroke[47]. This means that each crossbridge must make many working strokes for each ATP that it hydrolyses as muscle has an efficiency[20] of 50%. However, multiple steps are not observed in the single-molecule recordings. The discrepancy is resolved if the duty ratio is low[53], because in this case the individual crossbridges are nearly an order of magnitude stiffer[42] and so generate more force and work. The higher stiffness of the crossbridges also reinforces the view that they lack the flexibility to bind anywhere along the two-stranded actin helix. Finally, there is direct evidence that the low duty ratio in muscle is due to steric rather than

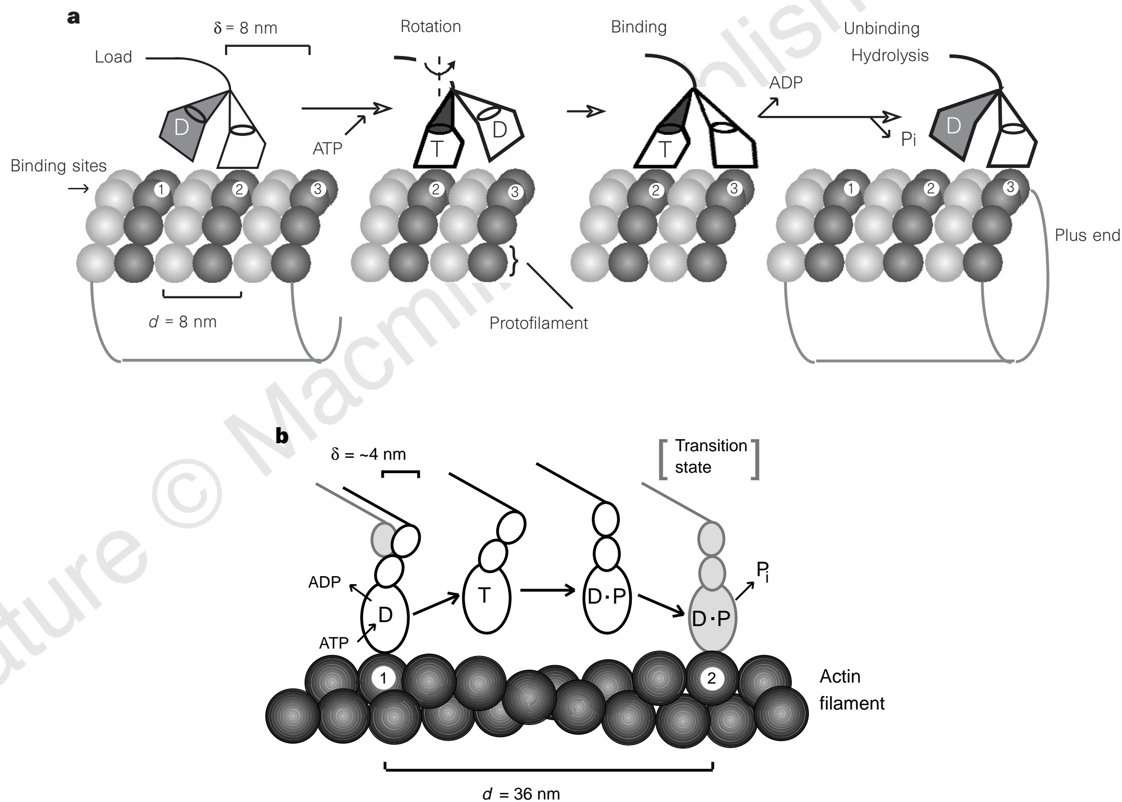

Figure 3 Structural and chemical models for the movement of **a**, kinesin, and **b**, myosin. **a**, The motion of conventional kinesin along the microtubule surface. Kinesin's path follows one of the protofilaments. As there is one binding site per tubulin dimer (α- and β-subunits shown as light and dark spheres, respectively), the path distance, *d*, is 8 nm, the dimer's length. The working distance is also 8 nm, the length of the step made by the distal, cargo-binding end of the motor during the hydrolysis of a molecule of ATP. Note that the rear head, which was initially bound at site 1 (left panel), skips over the other head attached at site 2, and binds at site 3 to become the new leading head (right panel). The middle panels show the chemical and structural transitions that probably drive the motion: the binding of ATP[57,60] (T) to the bound head is thought to produce a rotation in this head that swings the second head towards its next binding site[36]. The second head then binds and releases its ADP (D). In order to be processive, it is essential that kinesin does not dissociate until after this second head is bound; this could be achieved if the tight binding associated with ADP release were to give the kick necessary to dislodge the first, now-trailing head. Perhaps this is the role for the tilting motion seen by cryoelectron microscopy upon ADP dissociation[88]. The high fidelity of the coordination would be maintained if the hydrolysis were contingent upon dissociation of the first head. If the two-heads-bound state is highly strained, then it is likely to be short-lived and can be considered as a transition state. In this case, the motor spends most of its time with just one head bound, and therefore the duty ratio is ~0.5. **b**, The motion of skeletal muscle myosin along the actin filament surface. In contrast to the microtubule, the two 'protofilaments' of the actin filament twist around the filament axis; as a result, myosin's consecutive binding sites are separated by $d = 36$ nm, and are on different protofilaments. Note that whereas kinesin's working stroke is large enough to span the 8 nm to the next binding site, a single myosin head cannot span the 36 nm to its next binding site: as a result the collaboration of at least ~10 myosin crossbridges in a macromolecular assembly is required to reach the next binding site. For convenience, only one head is shown–it is possible that the other head runs along a different actin filament. The exchange of ADP (D) for ATP (T) causes the head to unbind from the actin filament. The rebinding catalyses the release of phosphate (P) which initiates the working stroke.

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biochemical constraints. If there were no steric limitations, then it would be expected that by decreasing the ATP concentration and therefore increasing the attached time, τ_{on} (see below), the number of attached crossbridges would increase (by equation (1)) and the force should increase. But the force actually decreases a little[54]. Thus, in a muscle fibre only a small fraction of the myosin heads can be attached at the one time in force-generating states, even at very low ATP concentration, consistent with there being a steric constraint to binding.

Transitions that drive the working stroke

Identifying the chemical and conformational changes that drive the working stroke remains a central aim in the motor field. Solving the atomic structure of skeletal muscle myosin was a tremendous step forward. It revealed that the regulatory domain contains an 8-nm-long α-helix which is stabilized by two calmodulin-like light chains[8], suggesting that this domain acted as a 'lever arm' that might amplify a much smaller conformational change in the nucleotide-binding pocket[41]. For example, a 30° rotation would provide the requisite 4 nm working distance. As reviewed in refs 55 and 56, the 'swinging lever-arm' hypothesis gains support from *in vitro* motility experiments showing that the speed is proportional to the length of the arm, and from electron microscopic and spectroscopic evidence for a swing. According to the standard biochemical model[20] shown in Fig. 3b, the rotation of the lever is driven by the release of phosphate from the actin–myosin–ADP state. The on time (τ_{on}) is determined primarily by the lifetime of this state: in a contracting muscle the lifetime is only about 1 ms, but this is long enough for the swing to be completed. After ADP dissociates, ATP binds and rapidly dissociates the actin–myosin complex. It is during the long-lived myosin–ATP and myosin–ADP-P_i states (which determine τ_{off}) that the motor is detached; but the actin filament continues to move owing to the action of other motors bringing the first motor to its next binding site, a multiple of 36 nm away, so that another cycle can begin. Thus the motor is carried through the full path distance even though it has contributed only about one tenth the distance with its own swing.

For kinesin, the postulated working stroke of 8 nm leads to a problem. How can the kinesin head, shown by crystallography to be only ~8 nm across, possibly span the 8 nm to the next binding site? One hypothesis is that kinesin's two heads undergo a coordinated motion so that movement within the attached head is amplified by the second, detached head[35,36,57]—in this way the second head acts as the 'lever arm', rather like the regulatory domain of myosin. This would account for the slow speeds of single-headed kinesin fragments[58] in which the proposed lever is absent. A coordinated motion of the two heads also accounts for processivity if the release of the trailing head is contingent upon the binding of the leading head, analogous to the 'hand-over-hand' climbing of a rope[17,59]. Such an alternating-head model is shown in Fig. 3a. The key idea is that a conformational change associated with ATP binding to the bound head[57,60] leads to the motion of the second head so that it can bind to the next dimer and release its bound ADP. In this scheme, τ_{on} corresponds primarily to the microtubule–kinesin–ATP state and τ_{off} to the kinesin–ADP state: the coordination implies that for each head $\tau_{on} \cong \tau_{off}$, giving a duty ratio of 0.5.

The outstanding questions for both kinesin and myosin are how conformational changes associated with nucleotide hydrolysis generate force, and conversely, how mechanical force influences the rate constants of the chemical steps. For example, both myosin and kinesin slow down and eventually stall when the load is increased[12,20,21,61]. If the duty ratio is to remain bounded by the mechanical constraints as discussed, then for equation (2) to remain valid at high external force, one or more of the following occurs: the ATPase rate decreases (Fenn effect[20]); the effective working distance decreases owing to premature dissociation (slippage[11,25]); or the stoichiometry of the coupling of the ATPase to the mechanical steps decreases[21]. This is the next set of questions to be answered by single-molecule techniques.

Biological implications of the duty ratio

Let us now return to our original question, which was to see whether there are design principles that have emerged from the study of conventional kinesin and muscle myosin that can be used to understand the structures, functions and mechanisms of the more recently discovered motor proteins.

I have argued that the sizes of the working stroke and the distance between consecutive binding sites on the filament place a constraint on the biochemistry of motors—the fraction of time that a motor spends bound can be no larger than the ratio of the working distances and the path distances. Because the binding-site separation is so large, about 36 nm, the head of skeletal muscle myosin can spend no more than about 10–20% of its time bound to the actin filament, so the duty ratio is small. On the other hand, the relative proximity of the binding sites for kinesin, 8 nm, allows kinesin a high duty ratio; an obvious functional adaptation of a high duty ratio is that a single motor molecule suffices to transport organelles. Is there a functional reason why muscle myosin has a low duty ratio? A possible adaptation is for speed. Skeletal muscle myosin must be able to respond quickly if the load it is pulling on begins to yield: a low duty ratio ensures that there is a pool of primed heads that can come 'on line' after a small shortening as previously unreachable actin binding sites come into striking range. The muscle does not have to wait for all the heads to go around another cycle before it begins to shorten.

This is not to say that the biochemical rates cannot be tuned according to a motor's function. For example, the duty ratio of maximally shortening skeletal muscle is only about 0.01 (the stiffness is one fifth that of isometric muscle[62], implying that the fraction of attached heads is only one tenth that of isometric muscle, considering the actin filament compliance mentioned above). Presumably the rebinding rate is so slow and the muscle contracting so fast that the heads are jumping over as many as ten potential binding sites. This is clearly an adaptation for higher efficiency because low loads require little work, and so a low duty ratio minimizes the number of ATP-consuming cycles. The differential tuning of biochemical rates can also explain the differences between axonemal dynein, which appears to have a low duty ratio[63], and cytoplasmic dynein, which ought to have a high duty ratio if it is to function as an organelle motor[64]. If the duration of the detached state of axonemal dynein were ten times that of cytoplasmic dynein, then the duty ratio would be one tenth that of cytoplasmic dynein, explaining the approximately tenfold increase in speed of the axonemal over the cytoplasmic motor (Table 1). Likewise, it would seem fairly easy to make a non-processive kinesin, as might be the case for the kinesin-related protein Ncd[65], which functions in a large assembly—the meiotic spindle.

Finally it must be considered whether myosin could be made processive. This is particularly relevant to the myosin Vs, which are thought to act as vesicle transporters in yeast[66] and mice[67]. To increase the duty ratio above 0.1–0.2, it is necessary to increase the working stroke or to decrease the binding-site separation. The only way to decrease the binding site separation would be for the motor to follow a different path but, as argued above, this would lead to spiralling of the organelle cargo around the actin filament, which is untenable as actin filaments are usually found close together. On the other hand, there are at least two ways of increasing the working stroke. One is to lengthen the neck. This is precisely what has happened with myosin V: each of its two heads has six light chains and a neck that is seen to be ~25 nm long by electron microscopy[67] (Fig. 4). This is almost long enough to reach the next actin binding site. So duty ratio considerations would say that the long neck is for processivity rather than for high speed—indeed, myosin V is a slow motor *in vitro* and *in vivo* (Table 1). Several other

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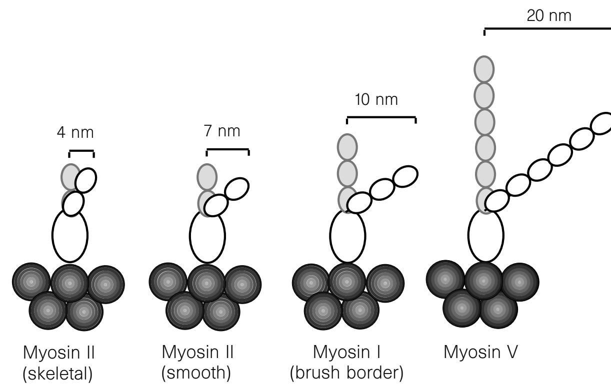

Figure 4 Ways in which myosin might increase its working stroke. By increasing the angle of the swing from ~30 deg to ~60 deg, it is possible to increase the working distance from as little as ~4 nm for skeletal muscle myosin II (left), to ~7 nm for smooth muscle myosin II. By increasing the length of the lever arm by increasing the number of light chains in the regulatory domain to three or six, it is possible further to increase the working distance to ~10 nm for brush-border myosin I, and to ~20 nm for myosin V. The larger the working distance, the larger the duty ratio and, as argued in the text, the fewer crossbridges are required to reach the next binding site. In the extreme case of myosin V, it is possible that just two heads in a dimer could reach to the next actin binding site 36 nm away, giving myosin V the capacity for processivity (as might be expected, given its biological function as a vesicle transporter).

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members of the myosin superfamily also have 4–6 light chains, such as myosin VII, IX and XI, giving them potential for higher duty ratios. A second way to increase the working stroke is to increase the angle of the swing (Fig. 4): both smooth-muscle myosin[68] and brush-border myosin[69] Ib have an additional swing of about 30° associated with ADP release, bringing the total swing up to ~60°. This could double the working distances of smooth muscle myosin to ~8 nm and myosin Ib to ~12 nm (as it has three light-chain domains). Such a mechanism explains the higher force generated by smooth-muscle myosin over skeletal-muscle myosin: the larger the working distance, the higher the duty ratio, and therefore, as postulated when the duty ratio concept was first formulated[24], the greater the fraction of the heads that are attached and generating force. An argument has recently been made against this mechanism[44]; however, as mentioned earlier, these workers may have misinterpreted their single-molecule data.

Discussion

We have learnt a lot in the past several years about how motor proteins work. This is partially due to the new information that has come from the atomic structures of actin, myosin and kinesin, as well as the development of new single-molecule techniques that are beginning to revolutionize our understanding of molecular motors just as the patch clamp technique has revolutionized our understanding of ion channels[70]. But perhaps as important has been the insight obtained by comparing the structures and functions of kinesin and myosin. In a sense, they have acted as controls for each other: our confidence that myosin is a low-duty-ratio motor is greatly increased by knowledge of how a high-duty-ratio motor like kinesin behaves in biochemical and motility assays; and whereas kinesin has given a clearer insight into the role of the filament and its discrete binding sites, myosin has given us a clearer look at the working stroke itself.

The unifying concept to emerge from this comparison of conventional kinesin and muscle myosin is that of the duty ratio—the fraction of the time that the motor spends in its attached, working state. The fundamental link between the structure and function of motor proteins is that the duty ratio can be no larger than the fraction of the distance that the working stroke moves the motor towards its next binding site. Understanding these constraints then gives insight into the structure and function of other motors, and determines whether the motor can move independently or whether motility is contingent on oligomerization into a macromolecular assembly. The low duty ratio of muscle myosin explains why it must function in large macromolecular assemblies, while the high duty ratio of kinesin permits this motor to function on its own to transport organelles. The different duty ratios are not simply due to different biochemical rate constants governing attachment and detachment; instead, the duty ratios are constrained by the sizes of the conformational changes that the motors make, as well as the paths that the motors tread on the surfaces of their respective filamentous tracks.

The biochemistry can be tuned to decrease the duty ratio, and this may prove important to increase the speed of skeletal muscle over smooth muscle, or cilial beating over organelle transport. On the other hand, the structure can be tuned to increase the duty ratio by lengthening the lever arm or increasing its swing; with a larger working stroke the distance to the next binding site can be more easily spanned. Application of single-molecule and protein engineering techniques to the diverse family of molecular motors promises to reveal a trove of design principles that might one day prove useful for building our own little machines. □

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