Despite the overall structural similarities between motor proteins, especially within the individual motor families, there are striking functional differences. Different motors go in different directions and at different speeds; some go in huge arrays and some go alone (see Table 13.1). In this chapter, I argue that the speed of a motor as well as the number of motors necessary for movement can be understood using the concept of the duty ratio, the fraction of the time that a motor domain spends attached to its filament. Motors with high duty ratios can maintain continuous attachment to their filaments and so can function on their own. Motors with low duty ratios must oligomerize into large assemblies in order to produce continuous motility. Differences in duty ratio account for much of the variation in speeds of different motors.

The Speeds of Motors in Vivo

Motor proteins move at a variety of speeds inside cells. For example, during the unloaded contraction of rat extensor muscle, one of the fastest vertebrate skeletal muscles, the tissue shortens in length by 20% in only 10 ms (37°C; Close, 1964). This corresponds to a movement of the myosin crossbridges along the actin filaments at a speed of 25,000 nm/s. At the other extreme, chromosomes move along microtubules during anaphase of mitosis at a speed of only 5 to 10 nm/s (Nicklas, 1983). Table 13.1 shows the in vivo speeds of several motor proteins from the myosin, dynein, and kinesin families. The range of speeds is remarkable. Even within a motor family the range is large. The quickest myosins move over one hundred times faster than the slowest myosins, those responsible for the transport of organelles and the contraction of smooth muscle. Similarly, the quickest kinesins...
port, move one hundred times faster than mitotic motors. The speed of a specific motor may also vary depending on physiological conditions. For example, imposing a large enough load on a muscle will stall the myosins. In this chapter we consider the unloaded speeds of motors, deferring to Chapter 15 consideration of how load affects speed.

**Rowers and Porters**

Some motors, like muscle myosin and axonemal dynein, operate in huge arrays. For example, a large muscle fiber may have as many as a billion myosin molecules. Even within a sarcomere, the anatomical unit of muscle contraction (see Figure 1.1C), there are thousands of myosin-containing thick filaments in parallel. Because each thick filament contains ~600 crossbridges, it can be likened to a galley ship with the crossbridges forming oars that row each side of the thick filament into the array of actin filaments. On the other hand, conventional kinesin operates alone or in small numbers: Electron micrographs show at most a few crossbridges between vesicles and microtubules (see Figure 12.1; Miller and Lasek, 1985; Ashkin et al., 1990). For this reason, kinesin has been likened to a porter (Leibler and Huse, 1993).

Interestingly, there are members of the myosin and dynein families that function alone or in small numbers to move membrane-bounded vesicles and organelles. Myosin V and cytoplasmic dynein are examples of such motors. And conversely, there are kinesin-related proteins that function in macromolecular assemblies such as the mitotic spindle. The oligomerization state has been indicated by the shading in Table 13.1.

**In Vitro Motility Assays**

The study of motor proteins was revolutionized by the development of in vitro motility assays in which the motility of purified motor proteins along purified cytoskeletal filaments is reconstituted in cell-free conditions. An important milestone in this development was the visualization of fluorescent beads coated with purified myosin moving along actin cables in the cytoplasm of the alga *Niuella* (Sheetz and Spudich, 1983). This was quickly followed by the first completely reconstituted assay in which motor-coated beads were shown to move along oriented filaments made from purified actin that had been bound to the surface of a microscope slide (Spudich et al., 1985). Though “threads” of actin and myosin had been known to contract in the presence of ATP (Szent-Györgyi, 1941), this contraction was very slow. The significance of the new findings was that it proved that myosin (together with actin) was sufficient to produce movement at rates consistent with the speeds of muscle contraction and cell motility. At about the same time, technical developments in light microscopy allowed the visualization of individual microtubules by differen-
tial interference contrast microscopy (Allen et al., 1981, 1985) and individual actin filaments by fluorescence microscopy (Yanagida et al., 1984), greatly facilitating the in vitro assays and allowing the speed and direction of many motors to be measured. Further refinement led to visualization of movement by single-motor molecules (Howard et al., 1989). The combination of these assays with increasingly sophisticated optical and mechanical techniques has allowed measurement of the conformational changes of individual motor molecules as they generate force (Chapter 15). These techniques have now been applied to several other molecular motors, such as RNA polymerase (Yin et al., 1995; Wang et al., 1998), ATP synthase (Noji et al., 1997; Yasuda et al., 1998), and DNA polymerase (Wuite et al., 2000).

There are two geometries used in in vitro motility assays: the bead assay and the gliding assay. In the bead assay, filaments are fixed to a substrate, such as a microscope slide, and motors are attached to small plastic or glass beads with typical diameters of 1 μm. The motion of the beads along the filaments in the presence of ATP can then be visualized using a light microscope (Figure 13.1A). In the gliding assay, the motors themselves are fixed to the substrate, and the filaments are observed to diffuse down from solution, attach to, and glide along the motor-coated surface (Figure 13.1B). Visualization of the beads, actin filaments, and microtubules is readily accomplished using fluorescence microscopy; beads and microtubules can also be seen using other light-microscopic techniques such as differential-interference contrast and darkfield microscopy (Spencer, 1982). With improved fluorescence sensitivity, it is even possible to image individual fluorescently labeled motors (Funatsu et al., 1995) and to watch them move along filaments (Vale et al., 1996; Pierce et al., 1997). The motions can be recorded on videotape and the speed measured by tracking the centroid of the bead or the leading edge of the filament from frame-to-frame (see Scholey, 1993 for detailed methods). There is good overall agreement between the speed of a motor protein in vitro and the speed of the cellular motion that is attributed to the motor (see Table 13.1).

![Figure 13.1 In vitro motility assays](image)

(A) Bead assay. (B) Gliding assay. A motor that moves the bead toward the plus end of the filament will cause a filament to glide with its minus end leading.
The direction of movement of a motor along its associated polar filament can be ascertained readily in the in vitro motility assay. For example, the minus end of a microtubule can be fluorescently labeled to distinguish it from the plus end. In gliding assays using kinesin, such “polarity-marked” microtubules always move with their minus ends leading (Figure 13.2), showing that the kinesin motors try to move toward the plus end but, because they are fixed to the substrate, the microtubule moves the other way (see Figure 13.1B). Using these assays, it was discovered that the kinesin-related proteins Ncd (Walker et al., 1990) and Kar3 (Endow et al., 1994) were minus-end-directed motors. These discoveries were very surprising at the time because of the high degree of sequence similarity between these motors and kinesin. However, recently a myosin VI has been shown to have reversed directionality, and, as explained in the last chapter, these discoveries have led to the notion of a converter domain adjacent to the motor domain that can act like a gearbox to switch the direction of movement.

In vitro motility assays have also provided biochemical information about motor proteins. For all motor proteins studied, removing the nucleotides from solution results in a very strong rigor attachment between the motor and filament. Consistent with this, movement in the in vitro motility assay ceases. In the case of kinesin (but not myosin), AMP-PNP, a nonhydrolyzable analogue of ATP, also promotes rigor-like binding in motility assays (Lasek and Brady, 1985). This finding led to the discovery of kinesin (Brady, 1985; Vale et al., 1985). In vitro assays can also be used to estimate rate constants of individual molecules, providing the information that would traditionally be obtained using stopped-flow methods of enzyme kinetics. For example, the rate constants

![Figure 13.2 Polarity assay](image)

Gliding of polarity-marked, fluorescent microtubules over a dense lawn of kinesin. The two images are taken 15 seconds apart. The minus ends of four microtubules are brightly labeled (two with arrows), and in all cases the minus end leads, indicating that kinesin attached to the surface is actually moving toward the microtubules' plus ends. (From Howard and Hyman, 1993.)
for the unbinding of kinesin from microtubules can be estimated from measurement of the average time that a bead coated with just one kinesin molecule remains attached to microtubules (Hancock and Howard, 1999). These measurements show that the unbinding of kinesin from a microtubule is accelerated by hydrolysis and phosphate release.

The mechanical loads in these assays are very small. The viscous drag on a 20-μm-long microtubule moving at 1 μm/s through a solution with the viscosity of water (~1 mPa·s) is only ~0.14 pN (Hunt et al., 1994; Chapter 6). Yet a single kinesin molecule can move such a long microtubule just as quickly as it can move a 1-μm-long microtubule (Hunt et al., 1994), indicating that even a single kinesin molecule is capable of producing forces much larger than 0.1 pN. The drag in the bead assay is even smaller because the beads are typically only ~1 μm in diameter. Furthermore, in typical motility assays there are often a large number of motors moving the one filament or bead. Thus in both the gliding and bead assays, the loads are usually minute. In Chapter 15 we will consider the influence of large loads on the motor mechanism.

**Processive and Nonprocessive Motors**

In vitro motility assays have shown that conventional kinesin is a processive motor: A single molecule of kinesin can move continuously along the surface of a microtubule for up to several microns (Howard et al., 1989; Block et al., 1990). This distance corresponds to hundreds of 8 nm steps (Chapter 15). The evidence for processivity is that motion can be observed at a very low density of kinesin on a surface (<1 molecules/μm²), that gliding microtubules swivel about a single point on the surface (at which the motor is presumed to be located), and that the rate at which microtubules bind and begin to move across a kinesin-coated surface is linearly proportional to the density of motors on the surface. Subsequent force measurements (Hunt et al., 1994; Svoboda and Block, 1994; Meyhöfer and Howard, 1995) discussed in Chapter 15 establish this processivity almost beyond doubt by showing that there is an indivisible force-generating unit that produces ~6 pN of force. The only remaining possibility, that this motor unit is a small, constant-sized aggregate of kinesins, is unlikely because the formation of such an aggregate would be too slow at the concentration of kinesin used to coat the surfaces (Howard et al., 1989). Biochemical experiments also confirm that kinesin is processive: kinesin hydrolyzes on average about 125 molecules of ATP following its initial binding to the microtubule (Hackney, 1995), consistent with the motor taking 125 8 nm steps (1 step/ATP) before dissociating. Some kinesin-related proteins are also processive (NkIn, Crevel et al., 1999; KIP1A, Okada and Hirokawa, 1999); others are not (Ncd, deCastro et al., 1999).

In contrast to conventional kinesin, muscle myosin II is not a processive motor. A threshold density of myosin on the surface is required for continuous motility of actin filaments. Below the threshold, the filaments slide.
video microscopy. The threshold density is ~4000 molecules/µm² to move actin filaments longer than ~0.040 µm (Harada et al., 1990) and ~600 HMM molecules/µm² to move actin filaments longer than 1.1 µm (Toyoshima et al., 1990). If each myosin head can reach 30 nm to contact an actin filament, then there are respectively 48 and 84 myosin molecules on average able to interact with these filaments at these densities. High-resolution displacement measurements also show that many myosin molecules are required for continuous motility: In the presence of ATP, actin filaments make only transient attachments to surfaces sparsely coated with myosin and move distances less than 20 nm before dissociating (Finer et al., 1994; Molloy et al., 1995; Chapter 15). Thus skeletal muscle myosin II is not processive. But myosin V, a vesicle transporter like conventional kinesin, is processive (Mehta et al., 1999; Rief et al., 2000; Sakamoto et al., 2000; Walker et al., 2000).

Like the other motors, some dyneins are processive and some are not. Densities of outer-arm dynein of ~1000/µm² are required for continuous gliding of microtubules (Vale and Toyoshima, 1988; Sale et al., 1993; Hamasaki et al., 1995), suggesting that outer-arm dynein also requires large assemblies for continu- ous motility. This is supported by high-resolution mechanical studies showing that outer-arm dynein is not processive at the high ATP concentrations found in cells (Hirakawa et al., 2000); interestingly, though, it is processive at low ATP concentrations. By contrast, high-resolution assays provide evidence that inner-arm dynein is processive even at high ATP concentrations (Shingyoji et al., 1998; Sakakibara et al., 1999). Cytoplasmic dynein is processive (King and Schroer, 2000); and its processivity is increased (i.e., it moves further before dissociating) by the dynactin complex, which is the dynein receptor thought to anchor cytoplasmic dynein to its membranous cargo.

Processivity, or the lack thereof, is reflected in the dependence of the speed of movement on the number of participating motors. The speed of kinesin is independent of the density: One motor can move a microtubule as quickly as ten or a hundred motors (Figure 13.3A). The speed is also independent of filament length (Howard et al., 1989). Because the load in these in vitro assays is small, this is analogous to a group of people carrying a pole: Provided that the pole is light enough, one person can carry it as quickly as a dozen people, irrespective of its length. This analogy illustrates another point: If many people are carrying a pole, a certain amount of coordination is required; otherwise, the speed may decrease as the number of helpers increases. Thus measurements of speed and motor number constrain models for the interaction of motors. When the density of myosin or dynein exceeds a certain threshold, the speed is also independent of density and filament length, as found for kinesin. However, in the case of myosin, it is possible to observe actin gliding at densities below threshold. This is done by adding methyl cellulose to the buffer solution. This highly elongated polymer inhibits diffusion of actin filaments perpendicular (but not parallel) to their long axes; it thereby promotes motility at low motor densities by slowing the diffusion of the filaments away from a surface during brief moments when all the motors are unbound. In the presence of methyl cellulose, the speed of actin filaments of fixed length increases with the myosin density (Figure 13.3B; Uyeda
Figure 13.3 Dependence of speed on motor density
(A) The speed of microtubules as a function of the density of kinesin. At a nominal density of 6 molecules/μm², it is estimated that on average one motor molecule is interacting with each microtubule. Average microtubule length is 2.5 ± 1.4 μm. (B) The speed of 2-μm-long actin filaments as a function of the density of myosin moving in buffer solution containing methyl cellulose. (A after Howard et al., 1989, 1993; B after Uyeda et al., 1990.)

et al., 1990). The speed reaches half maximum when the number of heads interacting with the actin filament equals about 50 (assuming a reach of 30 nm), again consistent with myosin not being processive.

The Hydrolysis Cycle and the Duty Ratio

The functional differences between different motors—their speeds and processivity—can be understood using the concept of the duty ratio, the fraction of the time that each motor domain spends attached to its filament. The concept first arose (initially called the duty cycle) to explain the higher force generated by smooth muscle compared to skeletal muscle (Dillon and Murphy, 1982). The development of this concept requires the notion that motor proteins have mechanical and chemical cycles.

An important, early insight into how motor proteins work arose from a simple observation: Motor proteins move along their filaments through distances that are large compared to molecular dimensions (e.g., Huxley, 1980). For example, the thick filament can slide up to 0.7 μm along the thin filament as a muscle contracts (see Figure 1.1), and individual microtubules glide for many microns over a kinesin-coated surface (e.g., see Figure 13.2). Because these distances are much larger than the crossbridges, the motor reaction must be a cyclic one in which the motor repetitively binds to and unbinds from the filament. During each crossbridge cycle, we imagine that a motor domain spends an average time attached to the filament.
Figure 13.4 The crossbridge cycle
(A) During one cycle of ATP hydrolysis, each head, or crossbridge, spends time $\tau_{on}$ attached to the filament and time $\tau_{off}$ detached from the filament. (B) It is hypothesized that a crossbridge makes a working stroke during the attached phase and makes a recovery stroke during the detached phase. By recovering to its initial conformation while detached, the motor avoids stepping backward, and so progresses a distance equal to the working stroke during each cycle.

stroke and an average time detached from the filament, $\tau_{off}$, during which it makes its recovery stroke. In this way it returns to its initial conformation for the beginning of a new cycle (Figure 13.4). By recovering during the detached phase, the motor avoids taking a step backwards, and thus progresses through a distance along the filament equal to the working distance.

We define the duty ratio, $r$, as the fraction of the time that each head spends in its attached phase

$$r = \frac{\tau_{on}}{\tau_{on} + \tau_{off}}$$

(13.1)

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.

Differences in duty ratio account for the finding that some motors are processive and others are not. The minimum number of heads required for continuous movement, $N_{min}$, is related to the duty ratio by

$$r \equiv \frac{1}{N_{min}}$$

(13.2)

as this guarantees that there will usually be at least one head bound to the filament (Harada et al., 1990; Uyeda et al., 1990; Leibler and Huse, 1993). 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 for each head; otherwise, there will be times when neither head is attached and the motor will diffuse away from the filament. Likewise, two-headed myosin V and cytoplasmic dynein must have duty ratios $\geq 0.5$ because they, too, are processive. On the other hand, because skeletal muscle myosin and outer-arm dynein must be in large assemblies with at least 50 to 100 crossbridges to move, their duty ratios must be small, ~0.01 to 0.02, the reciprocal of the minimum number of heads needed for continuous motility.
If there is one-to-one coupling between mechanical cycles (binding, working stroke, unbinding, and recovery) and chemical cycles (the ATP hydrolysis reaction), we expect that the speed of a motor, \( v \), is equal to

\[
v = k_{\text{ATPase}} \Delta
\]

(13.3)

where \( \Delta \) is the distance traveled by each head relative to the filament per mechanical cycle, and \( k_{\text{ATPase}} \) is the rate at which each head hydrolyzes a molecule of ATP. The speed is easy to measure in a motility assay. The ATPase rate is more difficult to measure because in a motility assay not all the motors are moving (e.g., only a small fraction of the motors on a surface are interacting with filaments). For this reason, the ATPase rate is usually measured with motors in solution (Figure 13.5A). For all motors studied so far, addition of filaments to the solution increases the ATPase rates (e.g., see Figure 13.5B), showing that the chemical cycle is coupled to the mechanical cycle. Conversely, the speed of movement increases with the ATP concentration, showing that the mechanics is coupled back to the chemistry. The maximum ATPase rate measured in solution (\( k_{\text{cat}} \)) is often assumed to correspond to the ATPase during motility. The \( k_{\text{cat}} \) values of several motor proteins are shown in Table 13.1.

It might have been expected that the different speeds of different motors could be explained by differences in ATPase rates. But Table 13.1 shows that this is not true in general. For example, skeletal muscle myosin moves 40 times faster than myosin IB, yet consumes ATP only three times faster. The difference is even more dramatic when comparing skeletal muscle myosin with kinesin; myosin moves ten times faster but has only half the ATPase rate. However, within subfamilies, such as the myosin II subfamily, there is a closer correlation between the speed and the ATPase rate. For example, Bárány (1967)
showed that the contraction speed of 16 types of muscles varies 100-fold, yet, with one exception, the ratio of the speed to the ATPase rate varies by less than a factor of two.

Another unexpected finding is that some motors move a very large distance during the time they take to hydrolyze a molecule of ATP. For example, dividing the speed by the ATPase rate gives a distance of 400 nm/ATP for skeletal muscle myosin and 450 nm/ATP for outer-arm dynein! These findings are confirmed by more well-controlled experiments in which the ATPase rate and the speed are measured in similar buffers and geometries: Muscle myosin HMM molecules in vitro move 190 nm/ATP (Toyoshima et al., 1987), and the crossbridges in myofibrils move 140 nm/ATP (Ma and Taylor, 1994). These distances are perplexing because they are an order of magnitude larger than the dimensions of the crossbridges! These findings generated much controversy in the myosin field (Harada et al., 1990; Uyeda et al., 1990); they led to the step-size paradox and the suggestion that myosin might take multiple mechanical steps for each ATP that it hydrolyzed (Yanagida et al., 1985; Kitamura et al., 1999).

The duty ratio concept provides a simple explanation for the step-size paradox. 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 50–100 myosin heads. If each head is attached for time $\tau_{on}$ and moves through the working distance $\delta$, then

$$\nu = \frac{\delta}{\tau_{on}}$$

(Harada et al., 1990; Uyeda et al., 1990; Pate et al., 1993). On the other hand, because the cycle is driven by ATP hydrolysis, we expect that the total cycle time, $\tau_{total} = \frac{1}{k_{ATPase}}$, where $k_{ATPase}$ is the ATPase rate. Substituting these expressions for $\tau_{on}$ and $\tau_{total}$ into Equations 13.1 and 13.4 we obtain another expression for the duty ratio:

$$r = \frac{\tau_{on}}{\tau_{total}} = \frac{\delta \cdot k_{ATPase}}{\nu} = \frac{\delta}{\Delta}$$

Hence the resolution of the paradox is that myosin has a low duty ratio: With $r = 0.02$ and an ATPase rate of 20/s per head, the attached time is only 1ms and a speed of 5000 nm/s can be reached with a working distance of only 5 nm (Equation 13.4), well within the dimension of the crossbridge. The crucial point is that each of the hundred or so crossbridges moving the filament contributes only 5 of the 250 nm moved while it hydrolyzes one molecule of ATP; while this motor is detached, the other myosins sweep the filament along the rest of the way. Because each half of a thick filament contains 300 crossbridges (Bagshaw, 1993), a duty ratio of only 2% in a rapidly contracting muscle still means that at all times there will be 6 or so crossbridges maintaining contact between the thin and thick filaments.

The low duty ratio of myosin II contrasts with that of processive motors such as conventional kinesin, myosin V, and cytoplasmic dynein. Because
kinesin has a high duty ratio it needs a high ATPase rate to attain even moderate speeds: A working distance of 8 nm, a speed of 800 nm/s, and an ATPase rate of 50/s implies that \( r = 0.5 \), consistent with the duty ratio required to account for kinesin's processivity. Likewise, using values from Table 13.1, the duty ratios for NkIn and myosin V are ~0.5. However, values in Table 13.1 suggest that the duty ratio of cytoplasmic dynein is \(<1\), inconsistent with its processivity (King and Schroer, 2000); a likely explanation is that the ATPase activity measured in solution underestimates the ATPase activity during motility.

Because both the distance moved per ATP, \( \delta \), and the duty ratio have been measured, it is possible to use Equation 13.5 to estimate the working distance, \( \delta \). For myosin, \( \delta = 200 \) to 400 nm, which gives a working stroke distance in the range of 2 nm to 8 nm if the duty ratio is 0.01 to 0.02. This is in good agreement with direct measurement of the working distance using single-molecule techniques (Molloy et al., 1995a; see also Chapter 15). This working distance is no larger than the molecular dimension of the heads and is therefore consistent with myosin making just one mechanical cycle per chemical cycle of ATP hydrolysis. This important conclusion is confirmed by experiments using single-molecule fluorescence to detect ATP hydrolysis and optical tweezers to detect steps: There is a one-to-one coupling of the mechanical and chemical cycles of myosin (Ishijima et al., 1998), though some uncorrelated events have been interpreted differently. In the case of kinesin, careful comparison of the distance moved per ATP (see Table 13.1, Figure 13.4; Coy et al., 1999b; Iwata et al., 1999) with the step size of 8 nm (see Chapter 15) shows that the coupling is also tight. There is exactly one step for each molecule of ATP that is hydrolyzed. Thus there is now good evidence that under the low loads characteristic of these motility assays, both kinesin and myosin are tightly coupled motors.

The coupling has not been directly measured at high loads. However, high loads decrease both the speed of contraction and the ATPase activity of muscle (Fenn, 1924; Kushmerick and Davies, 1969), as expected for tight coupling; though the maintained ATPase activity of isometrically contracting muscle—fully activated muscle held so it cannot shorten—shows that the coupling is not one-to-one at the very highest loads. High loads decrease the speed of kinesin (Hunt et al., 1994; Svoboda and Block, 1994b; Meyhöfer and Howard, 1995) by decreasing the rate of stepping and (and therefore the rate of completion of mechanical cycles). Statistical analysis of the timing of the steps at high forces suggests that kinesin remains tightly coupled under load (Visscher et al., 1999), though a definitive statement must wait for an ATPase measurement at high load.

*The Yanagida lab argues that the working distance of myosin (the distance moved while it is attached to the actin filament) is large compared with the size of the head (7.1 nm in Saito et al., 1994; 11-30 nm in Kitamura et al., 1999). However, in the former paper, the number of heads in the synthetic filaments may have been underestimated. Thus their value for the duty ratio, 0.16, may be an overestimate, which would lead to an overestimate of the working distance. The results in the latter paper, which cannot be reconciled with a 5 nm working stroke, are not con-
The analysis of molecular motion in terms of duty ratios and working strokes is a useful way of synthesizing a large body of experimental data on the speeds of different motors. However, this analysis is necessarily a simplification of the motor reaction. I have not attempted to account for the force and energetics of motors, nor for the likely dependence on strain of the binding and unbinding of motor filaments, nor the more detailed interactions between motors moving the one filament. I defer a more complete analysis to Chapter 16.

**Analogies to Internal Combustion Engines and Animal Locomotion**

The rotating crossbridge is analogous to a two-stroke internal combustion engine. In a two-stroke engine, the fuel mixture is injected into the cylinder and compressed during the compression stroke, creating a high chemical energy state at top dead center. Following ignition, the chemical energy is converted to heat, which creates the high pressure that drives the expansion of the gases during the power stroke (for information on how an internal combustion engine works, go to http://www.britannica.com). By analogy, we can imagine that myosin binding to actin creates the spark that triggers the working stroke. Where the analogy breaks down is that in a two-stroke engine, the duration of the working and recovery strokes are the same because of the coupling of the pistons to the crankshaft; the duty ratio is 0.5. However, motor proteins are not so constrained.

It is instructive to continue the analogy to an internal combustion engine. If kinesin’s heads really do alternate between attached and detached phase with a duty ratio of 0.5—a model that we will examine in later chapters—then its motion is analogous to that of a two-cylinder, two-stroke engine in which one piston (analogous to one head) is always in its power stroke phase of the cycle. The analogy for myosin is to an engine with more strokes. A four-stroke engine has an intake, a compression, an expansion, and an exhaust stroke, and therefore spends only 25% of its time in its working stroke (the expansion stroke). Thus, in a loose sense, we can think of unloaded myosin, with a duty ratio of 1%, as being a 100-stroke engine; smooth motion requires 100 cylinders.

Another useful analogy is to walking and running. The gait of a biped is defined as a walk if “the feet move alternatively with one foot not clear of the ground before the other touches” (Merriam Webster’s Collegiate Dictionary, 10th Edition). While walking, each foot must spend ≥50% of the time on the ground. On the other hand, during a run, there are times when neither foot is in contact with the ground. Thus a two-headed motor protein walks if it is always in contact with the filament, and it runs if it spends time completely detached from the filament. Using these definitions, kinesin walks along the microtubule (Cross, 1995) and myosin runs along the actin filament. A walking motor moves one working distance per ATP hydrolyzed. A running motor, by contrast, can move a much greater distance because it is carried by its fel-
low motors while it is detached. In this way the total distance moved can be much larger than the molecular distance. Where the analogy breaks down this time is that whereas the runner relies on momentum to carry her through the air, the motor, which has no inertia (Chapters 2 and 3), must rely on its neighbors to carry it forward while it is detached from its filament.

**Summary: Adaptation to Function**

The high duty ratios of conventional kinesin and myosin V appear to be adaptations for processivity; the low duty ratios of myosin II and outer-arm dynein appear to be adaptations for high speed. With a working distance of 8 nm and an ATPase rate of ~50 s⁻¹, kinesin attains a top speed of only 800 nm/s. By contrast, with a low duty ratio of 0.01, myosin and dynein can attain speeds of 10 μm/s with smaller working distances and ATPase rates. The consequent high speeds of muscle contraction and sperm motility can clearly confer selective advantage to the organism and to the germ cell. Another advantage of a low duty ratio is that a large distance can be moved during a single hydrolysis cycle: An unloaded muscle can shorten from an initial sarcomere length of 2.5 μm down to an almost fully contracted length of 1.9 μm within about the time it takes to complete the hydrolysis of a single molecule of ATP; yet the thin and thick filaments have slid 300 nm, perhaps 30 times the individual working distance.