# Switches, Latches, and Amplifiers: Common Themes of G Proteins and Molecular Motors

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In a universe where entropy is increasing, living organisms are a curious anomaly. The organization that distinguishes living organisms from their inanimate surroundings relies upon their ability to execute vectoral processes such as directed movements, ion pumping across membranes, and the assembly of macromolecules and organelle systems. Such phenomena are executed by protein machines that harness chemical energy to drive processes that would be otherwise energetically unfavorable.

Two of the best studied protein machines are the ATP-hydrolyzing motor proteins and the GTP-hydrolyzing G proteins. Motor proteins (kinesins, myosins, and dyneins) move along cytoskeletal filaments and are involved in generating most of the cellular and intracellular motions that are associated with living organisms. G proteins are a diverse group of molecular switches that regulate information flow in signal transduction pathways and orchestrate an orderly sequence of interactions in protein synthesis, protein translocation across membranes, and trafficking of vesicles in the endocytotic and secretory pathways. Essential to the mechanism of both classes of proteins, the NTP and NDP bound forms of the enzymes exhibit distinct conformations, which enables them to change affinity for target proteins (cytoskeletal polymers for motors and various target proteins for G proteins).

Historically, the scientific investigations of motor proteins and G proteins have proceeded along separate paths. However, many of the key questions regarding the role of nucleotide hydrolysis are the same: What is the nature of the conformational switch between NTP and NDP states? How do changes in the nucleotide-binding site affect the binding interaction with target proteins? How are the rate constants governing transitions in the nucleotidase cycle set and controlled? Recent structural and kinetic studies have uncovered intriguing parallels between molecular motors (kinesin and myosin) and G proteins which may be important for understanding these questions. The premise of this article is to compare and contrast these enzymes with the purpose of understanding common and distinct themes in the mechanism of chemical energy transduction.

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## *Biological Activities and Enzymatic Cycles of G Proteins and Molecular Motors*

### *G Proteins*

Although the roles of G proteins are enormously broad, these proteins all operate by a fundamentally similar mechanism (8, 9). With GTP bound in its active site, the G protein binds to and changes the activity of a downstream target protein (Fig. 1). As examples, Ras-GTP activates its target kinase Raf in mitogenic signaling pathways, EF-Tu-GTP delivers an aminoacyl-tRNA to the A site of the ribosome-mRNA complex during protein synthesis, and transducin-α-GTP activates cGMP phosphodiesterase during the light-mediated signal transduction cascade in the visual system. Phosphate release following hydrolysis of GTP causes the dissociation of the G protein–target complex and thus terminates the "active state" of the G protein. As a result, activation of downstream targets ceases in signal transduction pathways, and G proteins disengage from their targets in synthetic or membrane trafficking pathways so that they can be used for subsequent rounds of docking events.

Nucleotide hydrolysis and product dissociation are both regulated steps in the GTPase cycle, since these parameters determine when and for how long the G protein is "switched on" (Fig. 1; Table I). There are two limiting steps in the cycle: cleavage of the β–γ phosphate bond and the dissociation of GDP from the active site (51). The bond cleavage step limits the transition rate from the active to inactive state and therefore serves as a molecular clock. In signal transduction cascades, this clock governs the lifetime of the activated G protein–target enzyme complex and thereby determines the amplification gain of the production or destruction of soluble second messengers by the target enzyme (8). In synthetic reactions, the time lag of the GTPase reaction acts as a proofreading mechanism to enhance the fidelity of proper docking

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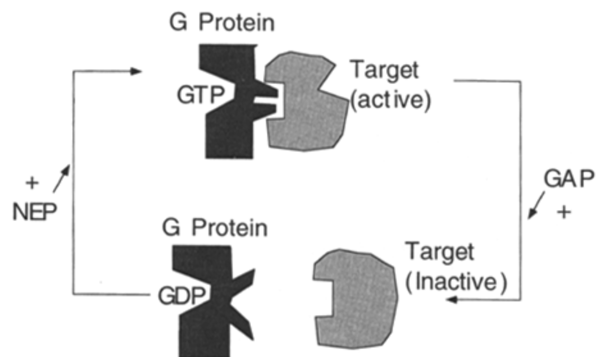
**G Proteins**




**Motor Proteins**

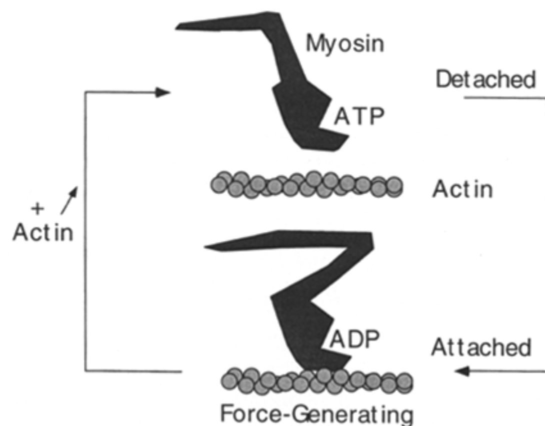



*Figure 1.* Enzymatic cycles of G proteins and the myosin motor protein. See the text for details. For kinesin, the enzymatic cycle is less well defined. However, current work indicates that the ATP state is a tight microtubule-binding state and an ADP state might be weak binding, which would be more akin to G proteins. In the kinesin cycle, microtubules act both as an NAP (nucleotidase accelerating protein) and an NEP (nucleotide exchange protein) (see Table I).

events (80). For the majority of G proteins, the bond cleavage reaction is accelerated by structural changes that occur when the G protein binds to a "GTPase-activating protein" (GAP)[1] (Table I). The GAP can be the target protein itself (ribosome in the case of EF-Tu [71] or phospholipase C-β1 for $G_q$/11 [6]), although in other cases (e.g., Ras), the GAP is a regulatory protein that is distinct from the target (10).

A second slow step in the cycle returns the G protein from an inactive to an active conformation. The rate of this transition is limited by the dissociation of GDP from the active site, since phosphate release is fast (53) and GTP (at concentrations present in the cytoplasm) rebinds rapidly (<1 ms) to an empty site. The rate of spontaneous GDP dissociation is very low for most G proteins, but it can be accelerated several orders of magnitude by regulatory proteins termed "nucleotide exchange proteins" (NEPs) (Table I and Fig. 1). In the case of many signal transduction pathways, the receptor-ligand complex acts as the NEP. In conclusion, the two kinetically slow steps in the GTPase cycle correspond to functional transitions between active and inactive conformations and both of these steps are accelerated by specific protein–protein interactions.

1. *Abbreviations used in this paper:* GAP, GTPase-activating protein; NEP, nucleotide exchange protein.

*Table I. Transition Rate Constants*

| Transition | 1 | 2 | 3 |
|---|---|---|---|
| Protein-NTP → | Protein-NDP-Pi → | Protein-NDP → | Protein + NDP |

| Transition | Rate ($sec^{-1}$) Ras | Myosin | Kinesin |
|---|---|---|---|
| 1. Bond cleavage | | | |
| − GAP/Polymer | 0.0003 | 50–100 | 9 |
| + GAP/Polymer | 15 | 30 | 100 |
| 2. Phosphate release | | | |
| − GAP/Polymer | – | 0.1 | >9 |
| + GAP/Polymer | <100 | 20–80 | 13 |
| 3. NDP release | | | |
| − NEP/Polymer | 0.0004 | 1 | 0.01 |
| + NEP/Polymer | 0.8 | 300–500 | 40 |

Values shown here for Ras alone were obtained from Neal et al. (51), for Ras with GAP were from Nixon et al. (53), and for Ras with NEP (mammalian C-CDC25) were from Jacquet et al. (31). Myosin rate constants were obtained from a review by Taylor (79) and references to original work can be found within. For dimeric kinesin, the value of phosphate release was taken from (19), and other values are from Ma and Taylor (40, 41). Rate constants shown include rate-limiting protein isomerizations that precede the transition. Buffer conditions and temperatures differ in these studies, so comparison of values can only be interpreted as approximations. More information on the nucleotidase cycles of motors and G proteins can be found in recent reviews (23, 79, 88).

## *Motor Proteins*

The majority of transport and force-generating activities executed by eukaryotic cells involve motor proteins that use chemical energy provided by ATP hydrolysis to move unidirectionally along protein polymers. Cells are populated with large numbers of molecular motors. Mammals, for instance, probably contain >100 motor genes, each of which may have a specialized force-generating function within the cell. Well-known roles of motor proteins include muscle contraction, ciliary beating, cytokinesis, mitosis, and organelle movement, and the repertoire of biological activities of motors is continuing to expand as new motors are being discovered.

Three superfamilies of motor proteins have been identified: myosin, which moves along actin filaments, and kinesin and dynein, which travel along microtubules. Members within each of the superfamilies share a similar motor domain (30–50% amino acid identity) that can function autonomously as a force-generating element. The motor domains of myosin and kinesin are better understood than that of dynein, and are the primary focus of this review. Although the motor domains are specialized for particular types of force-generating activities (discussed later), many of the unique self-assembly (e.g., filament formation by muscle myosin) or binding interactions (e.g., attachment of motors to membranes) that govern biological function are conferred by the nonmotor or "tail" domains, which can differ greatly among motors within a given superfamily (20, 49).

Like G proteins, the binding affinity of motors for their protein partners, the cytoskeletal filaments, depends upon the nucleotide that occupies the active site (Fig. 1). In the myosin enzymatic cycle, myosin-ATP and myosin-ADP-Pi are both weakly bound to actin. The cleavage of the β–γ phosphate bond in the active site does not produce a large change in free energy ($\Delta G = -1.4$ kcal/mol) (90), in contrast with the nonenzymatic reaction in solution ($\Delta G = \sim -7.5$ kcal/mol). This remarkable shift in the equilibrium constant indicates that energy must be stored in the enzyme-nucleotide complex. It is after the phosphate is released that a large free energy change and concomitant protein structural rearrangement most likely occurs which enables myosin-ADP to bind 10,000-fold tighter to the actin filament (79). The transition from the weak to the strong binding state of the motor-filament complex is thought to elicit the conformational change that enables the motor to produce force and move unidirectionally along the filament (15, 79) (discussed later). Thus, in contrast to G proteins, the ATP to ADP transition is considered to convert the motor to its "active" state. Subsequent ADP-ATP exchange dissociates the myosin-filament complex which enables the motor to rebind to a new actin subunit.

Transitions in the nucleotidase cycle determine when motors enter into and exit from the force-generating state. Therefore, the enzymatic cycle can be considered to be a partnership between the motor and the filament. Analogous to the situation described above for G proteins, critical rate constants are modulated by polymer binding. The overall ATPase rate of motors is generally low, but it is stimulated by several orders of magnitude by the polymer binding partner. In the case of kinesin, microtubules stimulate the bond cleavage step by 10-fold (Table I), and hence the polymer can be considered to act as a nucleotidase accelerating protein (NAP). However, polymer stimulation of the ATPase rate occurs primarily by a large increase in the rate of product release (both phosphate and ADP release in the case of myosin, and ADP release for microtubule stimulation of kinesin ATPase activity). Thus, polymers act in a fashion analogous to the nucleotide exchange factors that interact with G proteins (88). The rate of ADP release can also be modulated by applied tension and becomes slower under high load (14, 16). This important adaptation allows motors to prolong their force-generating state when working against an opposing force.

While many attributes of the nucleotidase cycles of motors and G proteins are similar, the intrinsic hydrolytic rates of motor proteins are faster than G proteins, which reflects the different biological settings in which these proteins operate. After conversion to their active GTP state, G proteins must diffuse to find protein targets in the cytoplasm or membrane bilayer. Hence, low hydrolytic rates are essential for target association and activation. In contrast, motor proteins and filaments are generally held in close proximity, either by highly structured filaments (e.g., muscle or cilia), motor-associated proteins (e.g., CLIP proteins [61]), or by the intrinsic mechanism of the motor itself (e.g., kinesin, which manages to move between microtubule-binding sites without letting go of the filament [7, 28, 84]). Furthermore, the rate of chemomechanical cycles of motors must be high in order to execute mechanical steps at a sufficient frequency to produce velocities of movement in the μm/s range.

## Structural Features

### G Proteins

The crystal structures of several G proteins (Ras [45, 55, 81], Ran [64], ARF1 [2], transducin-α [37, 54], $G_{i\alpha 1}$ [11, 47], EF-Tu [3, 35], and EF-G [1, 13]) reveal a common core structure (~21 kD) that consists of five helices packed against a hydrophobic 6-stranded β-sheet (five parallel and one antiparallel) (Figs. 2 and 3). GTP is bound in a cleft with the purine located near the cleft entrance and the phosphates buried within the protein. This core or "G domain" is virtually superimposable for all G proteins (Fig. 3).

Four distinct regions in the G domain contain absolutely conserved residues involved in nucleotide binding (Fig. 2; Table I). The first (G-1) consists of a loop emerging from a β-strand that cradles the α,β phosphates (phosphate binding or P-loop). This motif is not unique to G proteins, but is shared by many nucleotide-binding proteins including motor proteins, recA, the mitochondrial F1-ATPase, and adenylate kinase (68, 85). Two other conserved residues/motifs (G-2 and G-3) are part of loops that emerge from β-strands adjacent to the P-loop (Fig. 2). These loops, which lie at the very rear of the nucleotide cleft, interact with the γ-phosphate and act as critical "switch regions" that undergo conformational changes between GDP and GTP states (discussed below). The G-4 motif, which resides in a loop that contacts the guanine ring, plays an important role in determining nucleotide specificity (Fig. 2).

Members of the G protein superfamily have acquired unique domains, which contribute to their diversity of function (Fig. 3). These acquisitions, which are topological extensions of loops or chain elongations of the $NH_2$ and COOH terminus of the G domain, are involved in binding interactions with target proteins or modulating GTPase activity. In the case of EF-Tu, its >200 amino acid COOH-terminal extension (red in Fig. 3) undergoes large movements in response to GTP-GDP transitions in the G domain which may serve to reversibly dock aa-tRNA (3, 35, 52). Within the G domain itself, a highly variable region is located between the G-1 and G-2 motifs. In the Gα subunits of heterotrimeric G proteins, this insertion is large and folds into a predominantly helical domain (green portion of transducin-α in Fig. 3). This domain is thought to function, at least in part, as an intrinsic GAP that accounts for the higher GTPase rates of the heterotrimeric G proteins compared to smaller G proteins of the Ras family (9).

### Motor Proteins

Crystal structures have now been obtained for the motor domains of chicken skeletal muscle myosin and cytoplasmic myosin II from *Dictyostelium* (17, 60, 67, 69). Despite the species differences and their different biological roles (muscle contraction vs cytokinesis), the structures of these motor domains are nearly identical. The motor's catalytic region is composed of an ellipsoidal domain (~45 × 60 × 90 Å dimensions) that contains an ATP-binding pocket

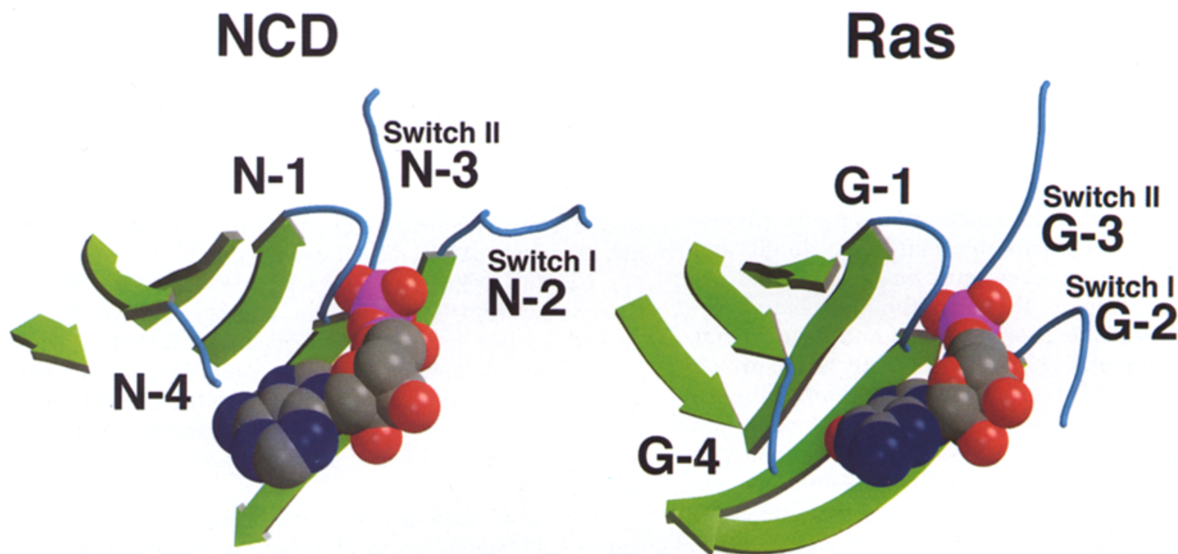



*Figure 2.* The β-strands and nucleotide-contacting loops that are shared in common with motors and G proteins. A similar set of β-strands with identical orientation (five parallel strands and one anti-parallel strand on the right) and similar position can be identified in kinesins (NCD shown here) and G proteins (Ras shown here). The region shown represents the entire β-sheet of the G domain, whereas the kinesin and myosin cores have two and one additional β-strands added onto the right side, respectively. Myosins also differ from kinesins and G proteins in that the first β-strand on the left runs anti-parallel. The location of the loops containing the N1-N4 and G1-G4 motifs indicated in Table II are shown. The nucleotide (GDP for Ras and ADP for NCD) is shown. Although the spatial placement of the nucleotide motifs is the same, the order of the β-strands in the primary sequence is different for the two β-strands on the left (*left to right*, G protein β-strand order is 6, 5, 4, 1, 3, 2; kinesin β-strand order is 0, −1, 4, 1, 3, 2).

and a prominent cleft that separates two actin-binding regions. A striking feature of this motor is an 85 Å helix that emerges from the COOH terminus of the catalytic domain and is stabilized by interactions with two light chains of 16 and 21 kD (helix without light chains is shown in Fig. 3).

For microtubule-based motors, atomic structures have been determined for the ~40-kD force-generating domains of human kinesin (36) and the *Drosophila* kinesin–related protein NCD (63). Microtubules are polar structures, and these two members of the kinesin superfamily move in opposite directions (towards the "plus-end" for kinesin and towards the "minus-end" for NCD). Despite the fact that these two motors move with opposite polarity, their three-dimensional structures are very similar. The motor domain of the kinesin superfamily consists of a single, arrowhead-shaped domain (70 × 45 × 45 Å) dominated by a central 8-stranded β-sheet with three helices on each side (Fig. 3). The nucleotide sits in an exposed binding cleft.

The motor domains of kinesin and myosin differ considerably in size, and computer alignments reveal no significant amino acid sequence identity. Surprisingly, however, these motor superfamilies share a common fold surrounding the nucleotide consisting of six helices and seven β-strands (which constitutes the majority of the secondary structural elements of the kinesin motor) (36, 63). The size difference of the two motors is explained by the fact that two "insertions" that project from the core (shown in green and orange for the motors in Fig. 3) and the $NH_2$- and COOH-terminal segments adjoining the core are all substantially larger in myosin compared to kinesin. In myosin, residues within the two large core insertions are involved in actin binding (59). Residues in the corresponding insertions in kinesin have also been found to be important for microtubule binding as determined by alanine scanning mutagenesis (Woehlke, G., A. Ruby, C. Hom-Booher and R. Vale, unpublished observations). Therefore, the two unique core insertions may be largely responsible for determining the distinct polymer binding interactions of these two motor superfamilies.

The nearly identical topology of the phosphate-binding loop can be used as a common reference to superimpose the motor structures with those of the G proteins. When this is done, six of the β-strands and four of the helices are in similar locations, but the spatial alignment is not as strong as between kinesin and myosin. Nonetheless, several features of the myosin/kinesin core have intriguing similarities to the G domain. Notably, kinesin and myosin contain a group of conserved residues (termed N1-N4) that contact the nucleotide and which show sequence similarity to the G1-G4 motifs of G proteins (63) (Table II). Moreover, after alignment of the P-loops (N1/G1 regions) of motors and G proteins, the N2-N4 motifs fall in approximately the same location as the G2-G4 motifs, suggesting that they serve similar functions (Fig. 2).

The six-stranded β-sheet of G proteins also corresponds in position and orientation to six of the eight strands of kinesin's β-sheet (Fig. 2). Furthermore, the position of the nucleotide and the N1-N4/G1-G4 loops relative to these common β-strands is identical in motors and G proteins (Fig. 2 [left to right]- strand 2 is followed by N4/G4, strand 4 is followed by N1/G1, strand 5 is followed by N3/G3, and the antiparallel strand 6 is preceded by N2/G2) (63, 68). This relationship is not a general feature of all P-loop–containing proteins, since recA and adenylate kinase have different strand orientations and do not have the conserved

# Table II

Nucleotide Binding Motifs

| | α,β-PO₄ N-1 | γ-PO₄ N-2 | γ-PO₄ N-3 | Purine N-4 |
|---|---|---|---|---|
| G Proteins | GxxGxGKS/T | T | DxxG | NKxD |
| Kinesins | GQTxxGKS/T | NxxSSR | DxxGxE | RxRP |
| Myosins | GESxxGKS/T | NxxSSR | DxxGxE | NP |

G proteins motifs shown are taken from a review by Bourne et al. (9), and the motor protein motifs were described in Sablin et al. (63). The residues shown are universally conserved in the G protein, kinesin, and myosin superfamilies. The kinesin and myosin motifs shown in green are found in a similar location in the structures. The red residues align spatially in kinesin, myosin, and G proteins and very likely serve similar functions. The first N-2 serine in myosin was assigned a similar role to the N-2 threonine of G proteins, since it forms hydrogen bonds with the $AlF_4$ or $VO_4$ in the myosin-ADP-$AlF_4$ and myosin-ADP-$VO_4$ structures. The assignment of the corresponding hydrogen bonding serine in kinesins is less clear, since only the kinesin-ADP and NCD-ADP structures have been solved. The locations of the N1-N4 and G1-G4 motifs are shown in Fig. 2.

Ser/Thr and AspxxGly motifs in the loops corresponding to N2/G2 and N3/G3, respectively (68, 74). Therefore, motors and G proteins appear to be more similar to one another than they are to other nucleotide-binding proteins. Although the relative strand order in the primary structure differs between G proteins and motors (Fig. 2 legend), these two superfamilies may have arisen by divergent evolution from an ancient nucleotidase precursor (Kull, J., R. Vale, and R. Fletterick, manuscript in preparation).

## *The Switch between NTP and NDP Conformations*

### *G Proteins*

The common structural features of motors and G proteins suggest that they share similar strategies for undergoing conformational change. A common property uniting motor proteins and G proteins is their ability to switch conformation between NTP and NDP states. To accomplish this feat, these proteins must be able to sense a relatively small change in the active site—the presence or absence of a γ-phosphate. The "γ-phosphate sensor" is best understood for G proteins, since several G protein structures have been solved in both GDP and GTP conformations (3, 11, 35, 37, 45, 47, 54, 55, 81). The sensor relies primarily upon two key residues: the G-2 threonine and the G-3 glycine. Both residues, which reside on mobile loops located at the rear of the nucleotide binding cleft, form hydrogen bonds with the γ-phosphate when GTP is in the cleft. In several G proteins, this interaction is accompanied by a significant movement (~4 Å) of the G-2 and G-3 loops towards the nucleotide in comparison with their positions in the GDP state. Therefore, residues in the G-2 and G-3 regions, which are also called switch I and switch II, respectively, act like gates that "swing in" when GTP is in the site and "swing out" when the hydrolyzed phosphate is released.

In addition to the direct interactions with the γ-phosphate described above, contacts between the switch I and II regions help to stabilize the protein's GTP conformational state. In transducin-α, the β-strands following G-2 and preceding G-3 move closer to one another and form additional hydrogen bonds in the GTP state (37). In EF-Tu, the β-strands do not change position in different nucleotide states, but the switch I and II loops move closer together and enclasp via a salt bridge (Arg59 in switch I and Asp87 in switch II) (3). Collectively, these contacts, together with additional interactions between switch II and adjacent regions of the protein which are discussed below, enhance the cooperativity in the γ-phosphate–dependent conformational switch.

### *Motor Proteins*

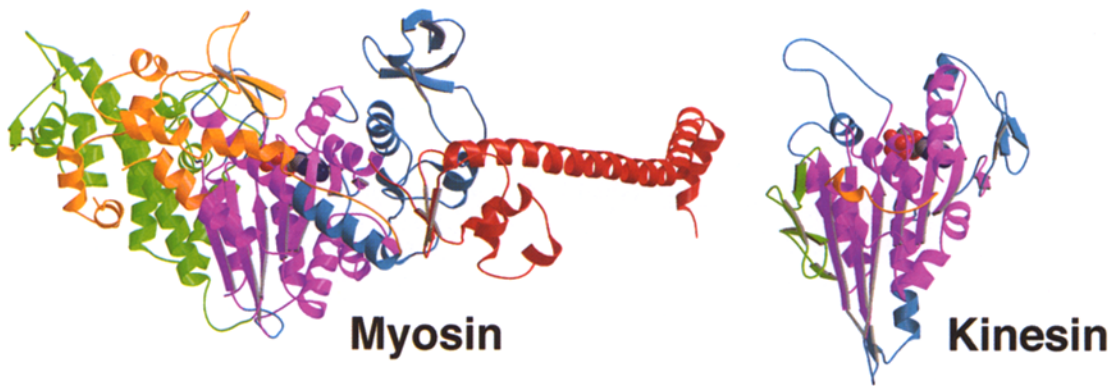
The N-2 and N-3 residues in motor proteins also appear to function as γ-phosphate sensors. Analogous to the observations with G proteins, the first serine residue in N-2 and the glycine in N-3 form hydrogen bonds with $AlF_4$ or $VO_4$ in the myosin-ADP-$AlF_4$ and myosin-ADP-$VO_4$ crystals, respectively (both of these structures may mimic a myosin-ADP-Pi state) (17, 69). Mutations of the N-3 glycine to alanine in myosin (62) and kinesin (Ruby, A., G. Woehkle and R. Vale, unpublished results) yield completely inactive motors, suggesting a critical role for this residue in the mechanochemical cycle. Comparable results have been obtained with switch II Gly to Ala mutations in several G proteins (39, 46, 75). Moreover, the arginine in N-2 and glutamate in the N-3 loops form a salt bridge in the myosin-ADP-$AlF_4$ and myosin-ADP-$VO_4$ structures, which is similar to the switch I-switch II salt bridge described above for EF-Tu. Interestingly, myosin-ADP-$BeF_x$ does not exhibit these changes (17). Since the beryllium-fluoride bond length is slightly shorter than that of aluminum-fluoride, N-2 and N-3 may be out of range for hydrogen bonding.

## *Nucleotide-dependent Interactions with Target Proteins*

### *G Proteins*

While the γ-phosphate sensing mechanism appears to be very similar in G proteins and motors, it can be used to control a myriad of different protein–protein interfaces. How is the coupling between the nucleotide site and various protein–protein interfaces achieved?

Insight into this question comes from examining the solvent-exposed regions of G proteins that change conformation in response to trigger movements from the γ-phosphate sensor. While the precise changes differ for each G protein, two general themes emerge. First, movement of the G-3 glycine towards the γ-phosphate changes the angle and/or partially melts portions of the subsequent helix (referred to here as the switch II helix) (3, 37, 45, 47). A second common theme is that the switch II loop/helix behaves like a "latch" that interacts with a nearby loop or domain (a "catch") which results in these two regions



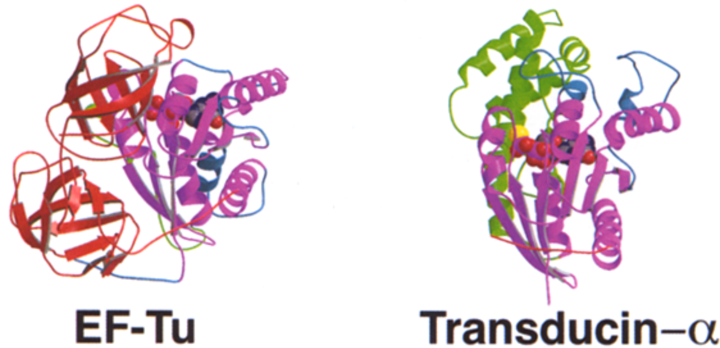




*Figure 3.* The common core domains and unique insertions of the motors and the G proteins. The structural elements that superimpose between kinesin and myosin are shown in magenta. A distinct core that defines G proteins is also shown in magenta in EF-Tu and transducin-α. Unique loops emerging from the core are indicated in blue. In the motors, two distinct but corresponding insertions that extend from the core are shown in green (insertion 1: human kinesin residues 138-173; chicken skeletal myosin residues 270-455) and orange (insertion 2: human kinesin residues 272-280; chicken skeletal myosin residues 506-656) (36). These insertions may be involved in binding polymers (actin in the case of myosin and microtubules in the case of kinesin). The unique COOH-terminal region of myosin (including the long helix) is shown in red. Kinesin may also contain a helical region (see Fig. 5) which is disordered and thus not visible in the present crystal structures. In the G protein family, two distinct types of insertions are illustrated in the examples of EF-Tu and transducin-α. Transducin-α, and other members of the heterotrimeric G protein family, contain a large helical domain (aa 54-175; shown in green) inserted between the G1 and G2 motifs (Table II). The corresponding region of EF-Tu consists of a smaller loop (aa 37-60). EF-Tu, on the other hand, has added two domains to the COOH terminus of the G domain (aa 200-405; shown in red). All proteins in this figure were aligned by their P-loops and are displayed in the same orientation. ADP is shown in the active sites of both myosin and kinesin, and GppNHp and GTPγS are shown in the active sites of EF-Tu and transducin-α, respectively.

moving closer together (Fig. 4). In the case of the Gα subunit of heterotrimeric G proteins, the catch is a nearby loop/helix (termed switch III; magenta in transducin-α in Fig. 4) that forms contacts with the mobile switch II region in the GTP state (37). In the case of EF-Tu in the GTP state, domains II/III (red in Fig. 3) serve as the catch that interacts with the switch II helix (3). Thus, the latch-catch interaction, under the control of the γ-phosphate sensor, helps to amplify small conformational changes that occur in the nucleotide-binding site.

The switch regions and the latch-catch interaction play an important role in reversibly assembling and disassembling protein interfaces. Recent atomic resolution structures of G protein–target complexes (EF-Tu-EF-Ts [34], Rap-1-Raf [50], and the Giαβγ [87] and transducin-αβγ [38] complexes) have confirmed that the nucleotide sensitive switch I and II regions participate directly in the binding of G proteins to their targets. The Raf protein, for example, forms an anti-parallel β-strand interaction with the switch I region of Rap-1 (a close Ras relative) in the GTP state, and the βγ subunits of heterotrimeric G proteins form extensive interactions with the switch II loop/helix of the α subunit in the GDP state. Remarkably, the nucleotide-dependent changes that enable these specific interactions to occur involve only a small portion of the protein surface. In the case of transducin-α, ~14% of the surface changes between the GDP and GTP states, which is nevertheless sufficient to confer nucleotide dependence to several binding interactions (meta-rhodopsin [the NEP], the βγ-subunits [which bind transducin-α-GDP], and cGMP phosphodiesterase [the target enzyme which is activated by transducin-α-GTP]) (37).

### *Motor Proteins*

Motor-polymer crystal structures have not yet been ob-

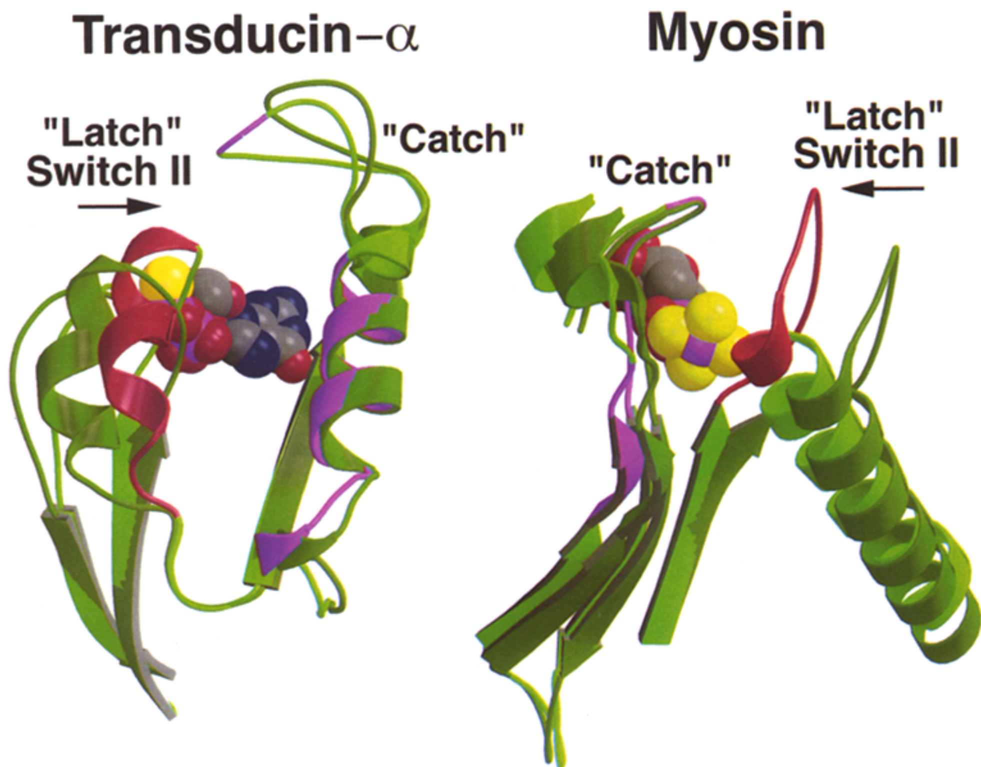



*Figure 4.* Conformational changes of motors and G proteins between NDP and NTP states. Shown here are myosin-$BeF_x$ (dark green, switch residues in N-2 and N-3 are not hydrogen bonded to the phosphate analogue), myosin-$AlF_4$ (light green, switch residues are hydrogen bonded), transducin-α-GDP (dark green, switch residues in G-2 and G-3 are not hydrogen bonded to the nucleotide), and transducin-α-GTPγS (light green, switch residues are hydrogen bonded). This figure illustrates the conformational change of the switch II loop and helix between these two nucleotide states. With a γ-phosphate or equivalent in the active site, the switch II region acts like a "latch" (*red*) which forms interactions with an adjacent region of the protein (a "catch" [*magenta*]). The general direction of movement of the switch II loop/helix, however, is opposite in myosin and transducin-α. The locations of residues that form NTP-specific interactions are shown in red (transducin-α- aa 201-211 [part of switch II]; chicken skeletal muscle myosin- aa 456-468) and magenta (transducin-α- aa 232-255 [part of switch "III"]; chicken skeletal muscle myosin- aa 230-238 and aa 260-264) (details of the side chain interactions that cause the latch and catch to interact are described by Fisher et al. [17] for myosin and Lambright et al. [37] for transducin-α). A GTPγS and an ADP-$AlF_4$ are shown in the active sites of transducin-α and myosin, respectively.

tained. However, the present structural data suggests that motor proteins may modulate polymer binding interfaces using conformational changes that are somewhat similar to those used by G proteins. Similar to the switch II helix of G proteins, both myosins and kinesins have a conserved helix following the N-3 motif that is tilted at an ~45° to the β-sheet (63). In the case of myosin, this helix rotates in response to movement of the N-3 glycine towards the $AlF_4$ in the ADP-$AlF_4$ crystal structure (17) (Fig. 4). Furthermore, the myosin motor domain also employs a latch and catch mechanism whereby the switch II loop on the lower portion of the cleft (red in Fig. 4) moves to form bonds with the side chains of residues in the upper cleft (magenta in Fig. 4). This is accompanied by a rigid body motion of the lower half of the catalytic domain, which may be important for modulating actin-binding affinity during the nucleotidase cycle.

Motors differ from G proteins, however, in that their switch regions are largely buried and very likely do not participate directly in the binding interface with the polymer (an exception being the solvent exposed switch II loop of kinesins which could participate in binding). How then is the binding interaction coupled to the γ-phosphate sensor? In kinesin, the switch II helix forms extensive contacts with the putative microtubule binding loop of insertion 2 (amino acids 270-280 in human kinesin) (36, 63). Similarly in myosin, the actin-binding region between Pro529 and Lys553 (59) is located only ~15 Å away from the switch II loop shown in Fig. 4. Thus, transitions in the enzymatic cycle can be relayed by direct associations between the polymer interface and the γ-phosphate sensing switch regions.

## *Timing of Transitions in the Nucleotidase Cycle*

### *NTP Hydrolysis*

The cleavage of the β–γ phosphate bond is required for the transition from the "active" to the "inactive" state in G proteins and from the nonforce generating to the force-generating state in motor proteins. In both classes of proteins, the hydrolysis reaction involves an attack of an ordered water molecule on the γ-phosphate. However, bond cleavage rates differ greatly among G proteins and motors (Table I), indicating that this rate constant can be tuned by the nucleotide-binding environment. It has been speculated that a residue(s) serving as a catalytic base may activate the water molecule (e.g., 56), although this hypothesis has been questioned for both G proteins (11, 42, 65) and myosin (17, 68). Other work has indicated that the hydrolytic reaction can be accelerated by stabilizing the increasing negative charge that develops on the β–γ bridging oxygen during bond cleavage. Hydrogen bonds contributed by residues in the P-loop (42), as well as an arginine residue in the switch I region of heterotrimeric G proteins (11, 42, 70) or an arginine supplied to the Ras active site by Ras

GAP (10), might contribute to such charge stabilization. However, a critical role of the active site environment of all motors and G proteins is the precise positioning of the catalytic water for an in-line attack on the γ-phosphate (11, 17, 69, 70). Reorientation of this water molecule may represent another mechanism by which GAPs (or microtubules in the case of kinesin) can accelerate the bond cleavage step.

### *NDP Release*

It has been speculated that nucleotide exchange proteins release NDP by prying open the binding pocket (9). However, the recent atomic resolution structure of the complex between EF-Tu and its NEP, EF-Ts, suggests an alternative mechanism (34). Formation of the EF-Tu-EF-Ts complex alters the conformation of the switch II region which changes the position of the universally conserved aspartate in the G-3 motif. This aspartate, whose position does not change between GTP and GDP states, plays an essential role in stabilizing the active site $Mg^{2+}$ ion, contacting it via a bridging water molecule. $Mg^{2+}$ is required for high affinity binding of the nucleotide, since chelation by EDTA (51) or mutations of the G-3 aspartate (32) substantially increase the dissociation rate of the nucleotide. Hence, these results raise the intriguing possibility that NEPs expel GDP through subtle conformational changes that first release the $Mg^{2+}$ ion, which in turn causes the release of the nucleotide.

Kinesin and myosin also have a universally conserved aspartate in N-3 that coordinates the nucleotide-bound $Mg^{2+}$ via a bridging water. Strikingly, when the G proteins and motor proteins are superimposed by alignment of their P-loops, the positions of these aspartate side chains are virtually identical to one another in these two classes of molecular machines (63, 68). Since chelation of $Mg^{2+}$ by EDTA efficiently strips the otherwise tightly bound nucleotide from motors of the kinesin superfamily (66), it is tempting to speculate that rearrangements of this switch II aspartate may play a role in polymer-induced ADP release.

### *Building a Motor from a Molecular Switch*

What properties distinguish a molecular switch from a molecular motor? Both motors and G proteins undergo cyclic interactions with partner proteins. Even the magnitude of nucleotide-dependent conformational change can be similar, as EF-Tu can undergo large interdomain rearrangements comparable to those described for myosin (~40 Å in both cases [3, 91]). A unique attribute of motors, however, is that each binding and release cycle must result in movement to a new subunit in one direction along a filament. To undergo such unidirectional motion, molecular motors must contain a structural element(s) that can store the potential energy derived from ATP binding to the enzyme and convert this stored energy into force and directional movement as the enzyme proceeds through its cycle. To accomplish this feat, this structural element, which in effect acts like a spring, must be coupled to and be capable of amplifying small conformational changes that occur in the γ-phosphate sensor and polymer binding regions of the motor.

Determining the structural basis of myosin motility has represented a major quest for the past 25 years. The most widely accepted theory proposes that the motor docks in a fixed orientation on the filament and a distal portion of the motor undergoes a large lever arm–like motion in the direction of movement. Support for the lever arm model of motility has come from recent structural work on myosin (59, 60). The proposed myosin lever arm is the 85 Å α helix that emerges from the catalytic domain (Figs. 3 and 5), and recent electron microscopic studies revealed that the end of the long helix can move by 35–50 Å in different nucleotide states (33, 91). The magnitude of this change agrees with some measurements of the myosin step size per ATPase cycle using high resolution microscopes (16, 48), although other studies have measured steps sizes that are too great to be accounted for by this lever arm motion (Ishijima, A., and T. Yanagida, personal communication). Additional support for the lever arm model comes from recent molecular genetic experiments in which shortening or lengthening of the lever arm helix, respectively, decreased or increased the velocity of movement in a linear manner (83).

How are conformational changes in the nucleotide-binding site communicated to the amplifier helix? In myosin, several highly conserved structural elements converge at the base of the long helix (Fig. 5): (*1*) the switch II helix (red, Fig. 5), (*2*) a smaller helix that undergoes conformational changes during the nucleotidase cycle (17, 89) (the reactive thiol helix; purple, Fig. 5), (*3*) the lower actin-binding region (orange, Fig. 5), and (*4*) a three-stranded β-sheet near the COOH terminus (blue, Fig. 5) that forms a distinct subdomain and that could act as a flange to support the movement of the lever-arm helix. These four regions, which have been hot spots for myosin mutations that interfere with function (57), may act in concert to give rise to the nucleotide- and polymer-dependent movement of the long helix.

Strikingly, the kinesin/NCD structures reveal an intriguing similarity to myosin with regard to a potential communication pathway from the nucleotide-binding site to a mechanical amplifier. Kinesin has structural elements corresponding in location to the switch II, polymer binding, and reactive thiol regions of myosin (same color scheme in Fig. 5). In addition, kinesin has a three-stranded β-sheet subdomain that could potentially serve a similar function to the three-stranded β-sheet of the myosin. While this subdomain is positioned near the $NH_2$- rather than COOH-terminal portion of the polypeptide chain (in contrast to myosin), it is nevertheless located close to the switch II (red) and the adjacent COOH-terminal helix (purple). Thus, kinesin and myosin may use a similar set of structural elements to transmit and amplify conformational changes from the γ-phosphate sensor.

Kinesin also has a ~50–amino acid segment that extends from the COOH terminus of the catalytic core which may serve as a mechanical amplifier (termed the "neck" region; indicated between arrows in Fig. 5). Approximately half of the neck region is present in the kinesin protein that was crystallized, but it is mobile and hence not visible in the crystal structure. Circular dichroism studies of synthetic peptides show that the COOH-terminal 25–30 aa of the neck region forms an α-helical coiled-coil (Hodges, R., B.

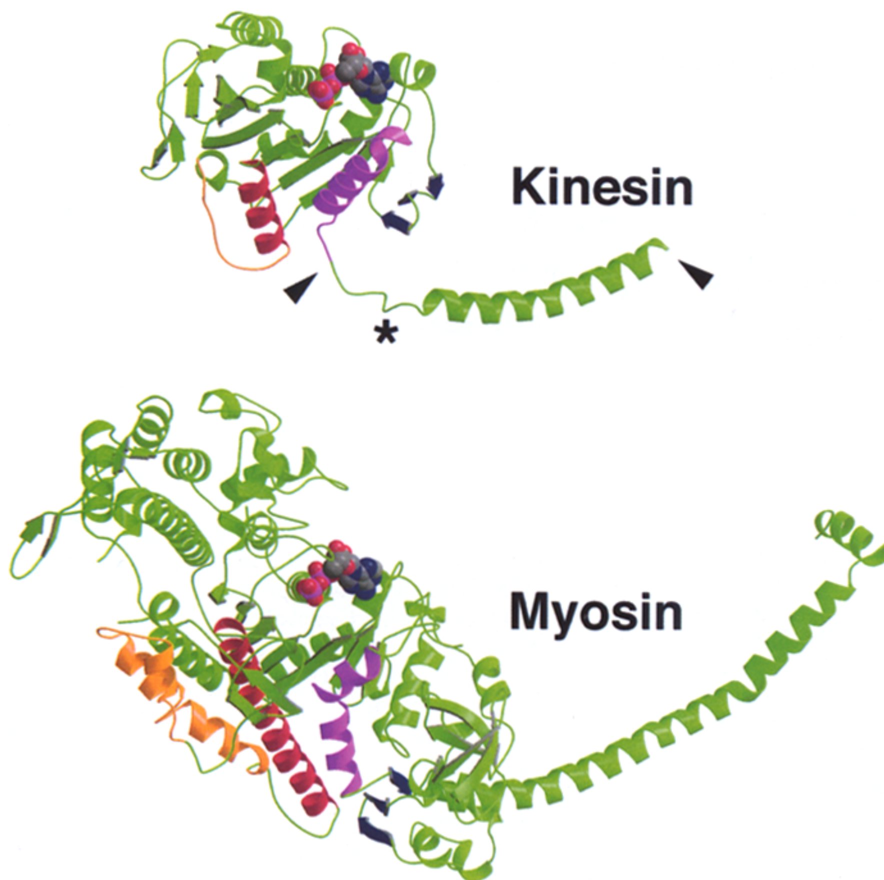



*Figure 5.* Structural elements of kinesin and myosin that may be involved in conformational change amplification and force-generation. Highlighted here are (*1*) the switch II helix (*red*; chicken skeletal muscle myosin- aa 475-506; human kinesin- aa 257-270), an actin-binding region of myosin (aa 517-552) and a proposed microtubule-binding region of kinesin (aa 270-280) (*brown*), the reactive thiol region of myosin (aa 688-707) and the corresponding region of kinesin (aa 308-323) (magenta), and a three-stranded β-sheet of myosin (aa 714-717, 758-761, 764-767) and kinesin (aa 30-34, 38-41, 44-47) (*blue*). All of these elements converge at the base of a COOH-terminal helix proposed to be involved in conformational change amplification and force-generation. (Note: the region between the two arrowheads in the kinesin "neck" region is not visible in the present crystal structure. The assignment of random coil and helix is based upon secondary structure prediction and CD spectral studies; the precise boundary of the neck helix and location of the neck region relative to the core is not known.) The asterisk denotes a kinesin truncation (aa 340 in *Drosophila* kinesin and aa 332 in human kinesin) that is monomeric and functions as a motor, albeit at slow velocities (7 nm/s) (84). A motor with 10 additional COOH-terminal amino acids (which is still monomeric and predicted to contain little, if any, of the helix) produces velocities of movement (120 nm/s [84]) that are more similar to full-length kinesin (500–600 nm/s). This illustrates that the COOH-terminal helix is not absolutely essential for force-generation, although it may be required for processive movement (4, 84).

Tripet, and R. Vale; H. Morii, T. Takenawa, F. Arisaka and T. Shimizu, unpublished results). Therefore, kinesin and myosin appear to contain long helices extending from the core catalytic domains, although the means by which these helices are stabilized are distinct (light chains maintain the helical structure for myosin and a coiled-coil interaction with a partner polypeptide stabilizes the helix in the case of the kinesin dimer).

A role for the neck region in motility has been confirmed by mutagenesis studies. Deletion of the neck region helix (truncation indicated by the asterisk in Fig. 5) yields a monomeric kinesin motor which fails to move for long distances along a microtubule without detaching (termed processive movement) (5, 84). This finding suggests that the coiled-coil dimer interface in the neck region could be involved in coordinating the actions of two motor domains in the native kinesin dimer during processive movement. One way in which this could occur is if relatively small conformational changes in one motor head could be communicated to the partner head through the linkage between the two polypeptide chains. The strain generated by the force-generating head could serve to dissociate a partner head from a rearward microtubule-binding site and/or bias the detached head to rebind to a forward tubulin-binding site in the direction of movement (12, 21, 27, 58). This type of hand-over-hand mechanism, which also must involve coordination of the enzymatic cycles of the two heads (19, 21, 22), could also explain how the relatively small kinesin core motor domain (~75 Å in its longest dimension) could travel between tubulin-binding sites spaced 80 Å apart on the microtubule (76).

Does the kinesin neck region execute a lever arm motion as proposed for myosin? This remains an open question. Monomeric motors lacking the neck region helix, while not processive, still produce gliding movement in an assay where multiple monomeric motors attached to a glass slide can interact simultaneously with a single microtubule ([4, 5, 73, 84, 92], see Fig. 5 legend for details). Thus, a lever arm motion of the α helix in the neck region cannot be essential for force-generation. If truncated, monomeric kinesin does not have a lever arm helix, then how do they work? One possibility is that the region of the neck before the helix (whose structure is not known) acts like chain that can pull or push. For example, while this segment is mobile in the kinesin-ADP crystal structure (36), it could interact with the core in another part of the cycle. Such a transition between disordered and ordered states could function as a spring, as suggested by Harrington (24). An atomic resolution structure of the neck region in kinesin may provide insight into these issues. Al-

ternatively, changes at the polymer binding interface may be important for motility (26, 29). Thus, with several models still in contention, these are still early days in understanding the precise conformational changes that accompany motor mechanics.

### *Adaptations of Motors: Velocity, Direction, and Processivity*

Despite the fact that motor proteins in the myosin and kinesin superfamilies share a common core, they exhibit a remarkable diversity of motile properties. Motors such as kinesin are designed to move for a hundred ATPase cycles along a microtubule without dissociating (19, 22, 84), and this processivity enables single or relatively few motors to transport organelles efficiently. Muscle myosin, on the other hand, is weakly bound for the majority of its ATPase cycle so that nonforce-generating motors do not interfere with other myosins generating force on the same actin filament in the sarcomere (72). Velocities and even the direction of movement are additional tunable variables. Myosin I of the intestinal epithelium, for example, produces movement at $\sim$0.025 μm/s, whereas myosin from the algae *Nitella* transports organelles at 2,000-fold greater rates. Even more remarkably, some members of the kinesin superfamily move to the microtubule plus-end (e.g., kinesin), whereas others move in the opposite direction (e.g., NCD) (43, 86). Understanding how motors achieve these diverse activities will provide important clues into the motility mechanism itself.

Several variable loops in the myosin motor have received a great deal of attention as key modulators of kinetic parameters in the ATPase cycle (for review see references 72, 77). The modular nature of these loops has been clearly demonstrated by loop swap experiments between myosins with different properties. For instance, when an important actin-binding loop (termed loop 2) from various myosins (rabbit skeletal, chicken smooth, rat cardiac α and rat cardiac β) was substituted in place of the corresponding loop of *Dictyostelium* myosin II, the relative magnitudes of the ATPase activities of the chimeric myosins were similar to the myosin source from which the loop was derived (82).

The amplifier region adjacent to the motor core (neck region of kinesin and long helix of myosin) may also confer unique types of motility to kinesin and myosin motors (e.g., processivity of the kinesin motor described previously). This region only exhibits strong sequence conservation within motor subclasses with related functions, suggesting that it might also confer unique motile properties to motors within the kinesin and myosin superfamilies. In the case of myosins, for example, amplifier helices differ in length and can bind different light chains that confer unique forms of regulation (e.g., $Ca^{2+}$-based regulation for scallop myosin and phosphorylation-based regulation for smooth muscle myosin). In kinesin motors, the putative amplifier or neck region may even be placed at either the $NH_2$ or COOH terminus of the core motor domain (conventional kinesin has its motor domain at the $NH_2$ terminus of the polypeptide chain, while the NCD motor domain is found at the COOH terminus). Since the $NH_2$ and the COOH termini of the motor core are located close to one another in space, both $NH_2$- and COOH-terminal amplifiers would both be positioned near the switch II helix and could respond to changes in the γ-phosphate sensor (63). How these different amplifier regions could generate opposite polarities of movement, however, remains a major unresolved question.

### *Perspective*

In conclusion, kinesin and myosin are more similar to one another and to G proteins than one could have dared imagine a few years ago. All of these proteins are most similar in the environment of the γ-phosphate, but have created distinct strategies for coupling the nucleotide switch to different protein–protein interfaces or to mechanical activity. The discovery of similarities between these proteins also hint that functional overlaps between G proteins and motors may emerge as well. For example, dynamin, a GTPase that is thought to contain a G domain, may generate mechanical activity in constricting membranes during endocytosis (25, 78), and motor protein homologues that function as switches or mechanical gates (rather than generating movement) could also be uncovered. It will also be interesting to explore whether the shared design features of G protein and kinesin/myosin are found in other proteins, such as dyneins or enzymes that translocate along nucleic acid polymers (e.g., helicases).

In the past, researchers studying G proteins and motor proteins have rarely crossed paths. However, each field potentially can learn a great deal from the other. The structural changes that occur during the enzymatic cycle are presently better understood for G proteins than molecular motors, particularly, since several atomic resolution structures of G proteins with interacting proteins have been determined. On the other hand, the kinetics and thermodynamics of the enzymatic cycle have received far more attention in the motor protein field. Moreover, it is now possible to follow the motility of individual protein motors and measure nanometer-sized displacement events, piconewton scale forces, and single molecule ATP turnovers from individual motor proteins (12, 16, 18, 30, 44, 48, 76), thereby providing a dynamic picture of the enzymatic cycle that has not yet been achieved with G proteins. Through the combined efforts and the interchange of ideas in both fields, a detailed understanding of how molecular machines work is within sight. With such knowledge, practical applications such as engineering new types of protein switches or designing molecular motors that operate along artificial polymers may be possible.

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