

Review

Ras GTPases and Myosin: Qualitative Conservation and Quantitative Diversification in Signal and Energy Transduction

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ABSTRACT:

Most GTPases and many ATPases belong to the P-loop class of proteins with significant structural and mechanistic similarities. Here we compare and contrast the basic properties of the Ras family GTPases and myosin, and conclude that there are fundamental similarities but also distinct differences related to their specific roles. © 2016 The Authors.

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INTRODUCTION

It has been argued that proteins referred to as ATPases and GTPases are wrongly named, since in essentially all cases, the purpose of their hydrolytic activity is not the production of inorganic phosphate and the corresponding nucleoside diphosphate (ADP or GDP) but the

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exploitation of the high free energy of triphosphate hydrolysis to drive an energy consuming process.¹ This is most easily understood for ATPases, which provide the energy for such diverse processes as movement, facilitating energetically unfavorable chemical reactions, transporting ions and other entities across membranes or driving protein folding and chromatin remodeling. GTPases are primarily involved in signal transduction and regulatory processes, in which the significance of the free energy of hydrolysis is less obvious but nevertheless essential for the processes involved.² There are many conserved features amongst ATPases, and in particular GTPases, and here we present a comparison of a motor protein, myosin, with GTPases of the Ras superfamily. We refer mainly to data from myosin II and to Ras, but there are quantitative differences in other members of the respective families, with these differences being related to specific functional properties. However, the discussion is mainly limited to fundamental aspects that are likely to be of general importance.

COMPARISON OF MYOSIN AND RASGTPASES

Nucleotide Binding to Myosin and GTPases

Many ATPases, and probably all GTPases involved in signal transduction and regulation, belong to a structurally related class of proteins that can be called the P-loop NTPases.^{3,4} (see Figure 1 for a comparison of the structures illustrating the strong similarity of the NTPase cores of myosin and Ras⁵). This nomenclature arises from a conserved sequence motif (GxxxxGKS/T), the phosphate binding loop or P-loop, involved in backbone and side chain interactions with the phosphate groups of the nucleotide.⁶ Originally, it was identified by Walker et al. in 1982 and is therefore also called the Walker A

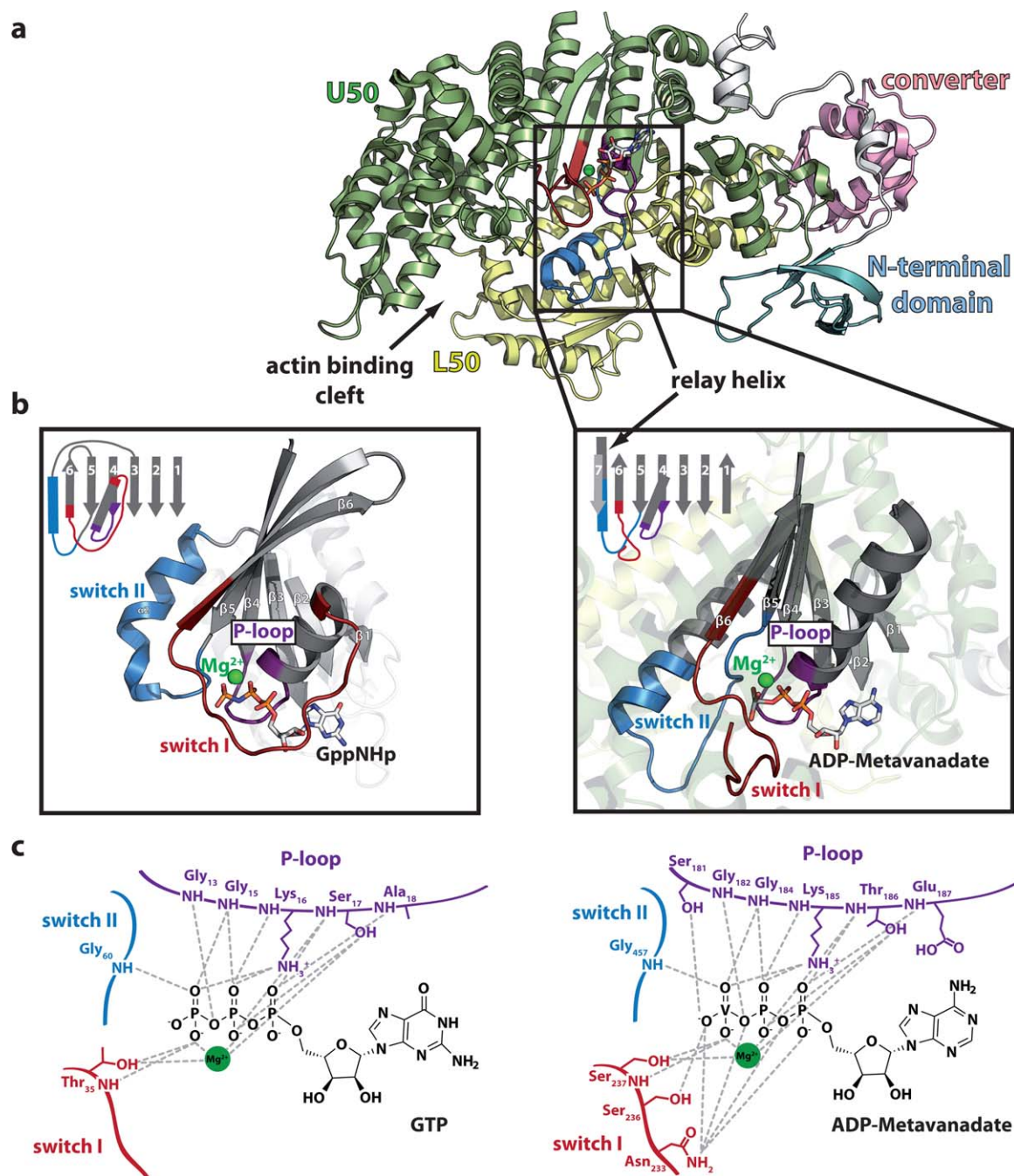


FIGURE 1 Structural comparison of the nucleotide binding pocket of Ras and myosin. (a) The myosin head (pdb 1FMW) is in cartoon representation showing the upper 50 kDa (U50, green), the lower 50 kDa (L50, yellow), the N-terminal (cyan) as well as the converter (magenta) domain.³⁸ The nucleotide binding pocket (P-loop – violet, switch I – red, switch II – blue) constitutes only a minor part of the myosin head and is located between the U50 and the L50 domains. Note the relay helix that transmits the conformational change of the switch II region to the converter domain (and finally the lever arm as indicated in Figure 2). (b) Close-up view of the conserved secondary structure elements surrounding the nucleotide binding pocket of HRas (left, pdb 1CTQ) and myosin II (right, pdb 2X9H) highlighting their notable similarities. The nucleotide-binding region is composed of several β -strands (six in Ras and seven in myosin, the additional 7th helix in myosin is indicated in light grey in the topology diagram, but not in the cartoon representation). The P-loop is located between β -sheet 4 and a conserved α -helix, switch I is located at the N-terminus of β -sheet 6 and switch II connects β -sheet 5 and another α -helix (the “relay” helix in myosin). (c) Schematic presentation of the amino acids within the P-loop and the switch regions contacting the α -, β - and γ -phosphates in the Ras:GTP structure (left) and the myosin:ADP-metavanadate structure (right). Whereas contacts involving the P-loop and switch II are very similar, switch I forms several additional interactions in myosin compared to Ras. Contacts involving additional water molecules are not shown in this scheme, and the reader is referred to the main text for further details.

motif.³ Two other conserved elements are referred to as the switch I and switch II regions.^{7,8} The switch I region was originally identified as the effector binding loop in the Ras protein and contains a threonine or serine residue (Thr35 in Ras, Ser237 in myosin) that provides a hydroxyl group as a ligand for the essential Mg^{2+} ion, which in turn is also coordinated to the β - and γ -phosphate groups of the nucleotide and a serine or threonine in the P-loop (Fig. 1c). The switch II region contains a conserved DxxG motif that is involved in NTPase activity and Mg^{2+} binding. The role of the P-loop appears to be the same in all proteins of this class and can be described as structural, in the sense that together with the Mg^{2+} ion, it holds the β - and γ -phosphates in an appropriate configuration for attack of a water molecule, which in turn is positioned by interactions with the switch I and switch II regions. In addition to these positioning properties, the interactions with the P-loop also serve to withdraw electrons from the β - and γ -phosphorus atoms, thus enhancing both the leaving group properties of the bridging oxygen atom and the electrophilicity of the γ -phosphorus. Among the significant differences between myosin and Ras is the presence of an NKxD motif that is conserved in GTPases, but not in ATPases,⁴ leading to high specificity for binding GTP due to an interaction between the Asp residue of this motif and the NH_2 -group and ring NH group of the guanine base.⁹ A further important difference is that while the active site of myosin is complete in terms of hydrolytic activity, the Ras family GTPases require additional residues from a GAP (GTPase activating protein) to exhibit significant GTPase activity. In the case of myosin, actin is required to release phosphate and ADP from the long-lived myosin:ADP:P_i state in an interaction similar to that of GEFs (guanine nucleotide exchange factor), as discussed in a later section.

Nucleotide Dependence of Conformations

The conserved regions (switch I and switch II) play the dual role of being involved in catalytic activity and, crucially, in interactions with other proteins in the case of the GTPases or (indirectly) with more distal parts of the protein structure in the case of myosin. In the GTPases, changes in the structure of these regions of the proteins between the GTP and GDP states is the essential property leading to changes of affinities and kinetics of their interaction with other proteins, in particular effectors (defined as molecules that bind more strongly to the GTP- than the GDP-form), and these phenomena are well understood in a large number of cases.¹⁰ Stated simply, GTP binding favors a state that approaches that seen when effectors bind to the GTPase. In the case of myosin, the possible role of changes in switch II structure have been apparent for some time,^{11,12} and can be described briefly as follows (see contribu-

tion of Geeves in this issue for a more detailed discussion). Switch II appears to exist mainly in 2 states, a closed state, in which the glycine of the DxxG motif is near to and can indeed interact via its backbone NH group with the γ -phosphate of ATP, if present, and an open state, in which switch II has moved significantly away from the position of the γ -phosphate. Movement of switch II appears to be coupled to a long-range conformational change of the myosin molecule transmitted via the relay helix and the converter domain (Figures 1 and 2),^{13,14} which results in an angle change, relative to the motor domain, of a long C-terminal α -helical region (the "lever arm") of the motor domain. The lever arm is stabilized structurally by interaction with several myosin light chains or their equivalents, depending on the type of myosin molecule. The angle change of this region of the molecule is thought to represent the power stroke in the contractile mechanism.¹²

Analogies Between GEF and Actin Activity

The change of lever arm angle in myosin and its coupling to the loss of the phosphate group of ATP is considered to reflect the crucial relationship between structure and nucleotide state in this system, and is thus an important discovery. However, this is only part of the picture, since another, equally important feature of the cyclical changes occurring in the cross-bridge cycle is the coupling of nucleotide state to actin affinity. Thus, myosin:ATP and myosin:ADP:P_i bind weakly to its polymeric partner protein actin, while myosin:ADP and nucleotide-free myosin bind strongly, and it is the transition between weakly and strongly bound states which is the fundamental thermodynamic event in the cross-bridge cycle leading to production of mechanical work.¹⁵ A direct link between the structural states of the actin and nucleotide binding sites was first observed a little over 10 years ago,¹⁶⁻¹⁸ but before this time there was no structural evidence for a direct influence of the state of switch I on regions of the myosin molecule known to be involved in actin binding, leaving these two significant aspects of the cross-bridge cycle (change of lever arm angle and change of actin affinity) conceptually uncoupled.

In contrast, the role of switch I in GTPases was apparent at a much earlier point in time, starting with mutational analysis leading to the concept of an effector loop. Switch I was seen to be in a well-defined, highly ordered state when GTP or a GTP analog is at the active site.^{7,8,19} On hydrolysis to GDP, this conformation is lost, and switch I becomes disordered and difficult to define crystallographically.¹⁰ The reasons for this can be found at the simplest level in the loss of interactions with the nucleotide. These are interactions occurring with Thr35 in Ras or its equivalent in other GTPases, and include the side chain interaction with Mg^{2+} , a backbone NH interaction with the

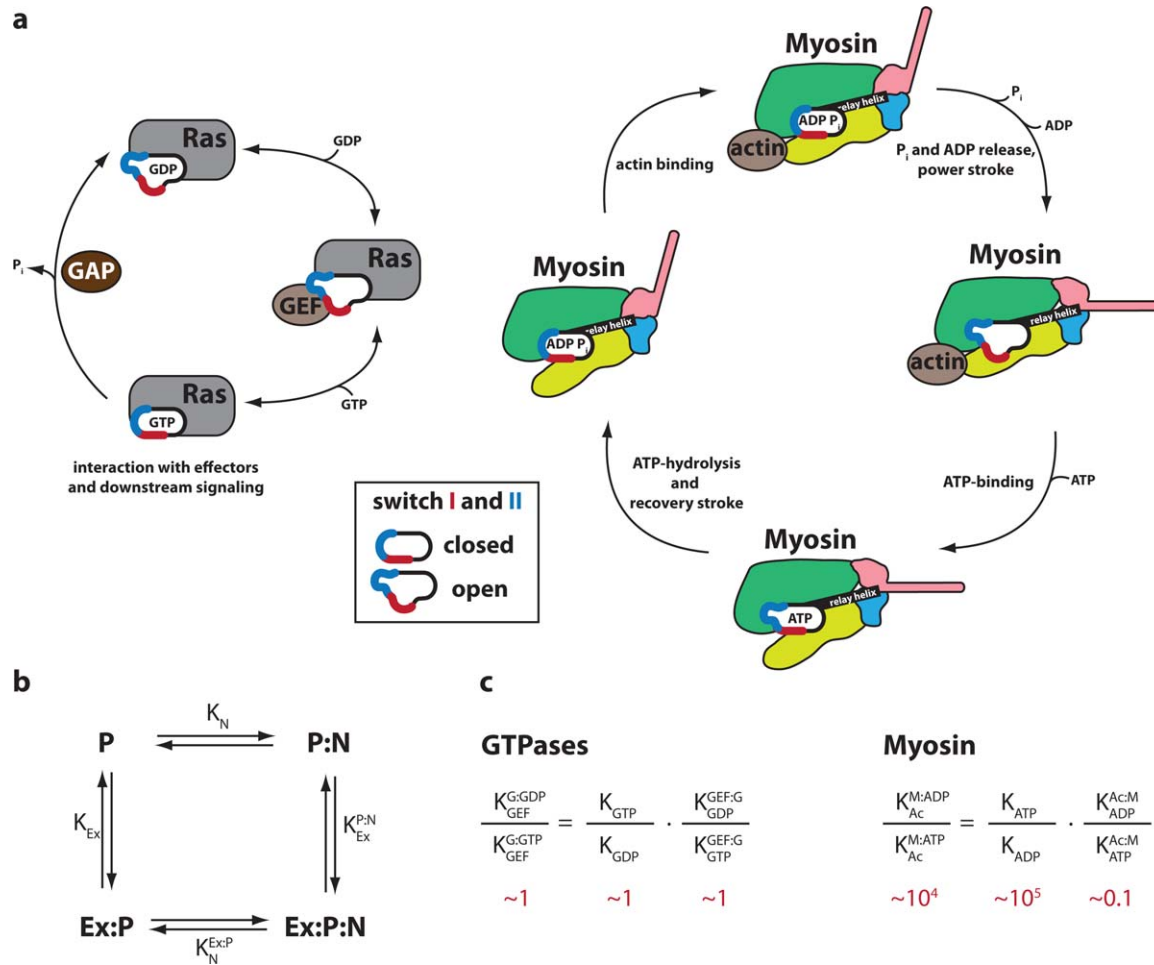


FIGURE 2 Comparison of the NTPase cycle in small GTPases and myosin. (a) Whereas the cycling in Ras provides a controlled switch for signal transduction, the cross bridge cycle in myosin couples the energy of ATP hydrolysis to mechanical work. The role of actin towards myosin is to accelerate the slow rate of phosphate and ADP release from the the myosin:ADP:P_i complex, similarly to GEFs in the GTPase cycle. Whereas hydrolysis of ATP is fast in myosin, GTP hydrolysis is slow in small GTPases and an additional regulatory factor (GAP) is required for their inactivation. Open and closed conformations of the switch I (red) and switch II (blue) regions are indicated for the different states in the NTPase cycle (both open in the nucleotide-free actomyosin state). (b) Equilibria in a system of an NTPase, (P) a nucleotide (N) and an exchange factor (Ex). The equilibrium constants for the individual steps are defined as association constants. The subscripts refer to the species binding in the specific reaction step, while the superscript denotes the presence of absence of N or Ex in complex with P prior to association with the species of the subscript. (c) The thermodynamic dependencies of the equilibria for GTPase, their GEFs and GTP/GDP (left) or myosin, actin and ATP/ADP (right). Whereas GTPases have similar affinities for GDP and GTP, respectively and consequently GEFs act similarly on GTPases bound to GDP or GTP, myosin binds much more strongly to ATP than ADP and actin acts as an ATP-specific exchange factor (K_{GTP} and K_{GDP} are the association constants of the GTPase to GTP and GDP respectively, $K_{GEF:G}^{G:GTP}$ and $K_{GEF:G}^{G:GDP}$ are the association constants between the GEF and the GTPase:GTP and GTPase:GDP complexes, respectively, and $K_{GTP}^{G:GEF}$ and $K_{GDP}^{G:GEF}$ are the association constants between GTPase:GEF complexes and GTP and GDP, respectively. Similarly, K_{ATP} and K_{ADP} are the association constants of myosin to ATP and ADP, $K_{Ac}^{M:ATP}$ and $K_{Ac}^{M:ADP}$ are the association constants between actin and the myosin:ATP and myosin:ADP complexes, and $K_{ATP}^{M:Ac}$ and $K_{ADP}^{M:Ac}$ are the association constants between actomyosin and ATP and ADP).

γ -phosphate group and a backbone C=O interaction with the attacking water molecule. Loss of these interactions results in loss of the well-defined structure of switch I needed for interaction with effector molecules. Thus, the ordered structure of switch I appears to be unstable in the absence of the interactions mentioned above, and arguments can be made which suggest that this destabilization is by a factor of approximately 100-1000 (equilibrium constant between the open and closed (GTP-like) conformation in the GDP state²). The energetic cost of the transition of switch I into the ordered state is presumably the reason for the fact that GTP and GDP bind to many GTPases with similar affinity, in spite of the fact that there are more interactions between GTP and switch I than for GDP. This is in stark contrast to myosin, which shows a loss of nucleotide affinity of ca. 5 orders of magnitude on losing the γ -phosphate group.

It has been pointed out that the properties of actin towards myosin are equivalent to those of GDP/GTP exchange factors (GEFs) towards GTPases.²⁰ The principle of the mechanism of action of these factors is that they bind with high affinity to the nucleotide-free form of GTPases, but with low affinity to nucleotide-bound forms. Since they do not compete directly with nucleotide binding, a ternary complex between GTPase, GEF and nucleotide can be formed, in which the lowered affinity of the nucleotide is expressed as an acceleration of its rate of release. The same effect is seen in the interaction of myosin with actin, since the effect of actin is to bind with high affinity to myosin at a site distal to the nucleotide binding site and in doing so reduces the affinity of this site for nucleotides and in particular accelerates the release of inorganic phosphate from the myosin:ADP:P_i complex. The thermodynamic and kinetic principles of this effect are the same for GTPases and myosin, with the important quantitative difference that the effect is relatively specific for ATP (or ADP:P_i) in myosin, whereas exchange activity is essentially unspecific (i.e. with respect to GDP or GTP) for the GTPases. Thus, GEFs typically reduce the affinity of both GTP and GDP to GTPases dramatically (typically 5–7 orders of magnitude), while actin has a similar effect on ATP affinity to myosin, but only reduces ADP affinity by about 2 orders of magnitude. As argued (reference²⁰ and below), the transition from the situation seen in GTPases to the situation seen in myosin is fundamentally related to the roles of these NTPases in signal and energy transduction, respectively. Whereas GTPases display similar affinities towards GTP and GDP, and correspondingly similar affinity of the GTPase:GTP and GTPase:GDP complexes to GEFs, there is a dramatic difference in the nucleotide affinity to myosin and a corresponding dramatic increase in affinity of myosin to actin on hydrolysis of ATP to ADP.

Because of the similarities between the action of GEFs and actin, it is illustrative to examine the structural mechanism of

action of the GEF proteins. As an early example, the GTPase Ras was crystallized as a complex with the exchange factor SOS in the nucleotide free form and the structure of this complex was solved.²¹ A major effect of binding of SOS appears to be the opening of the nucleotide binding site of Ras by movement of switch I away from the nucleotide position, which together with a distortion of switch II leads to loss of interactions with the nucleotide and Mg²⁺ ion. As already discussed, changes in switch II had been seen to be important for myosin at a relatively early point in time, but initially, there was no evidence for changes of position or conformation of switch I, for example between the ATP and ADP-bound states. However, it seemed likely that a structural change of the type seen in the Ras:SOS complex would, if it occurred, lead to a lowering of the affinity to nucleotides. The details of such a mechanism would have to be different in myosin, since the actin binding site is distal to the nucleotide binding site, whereas the effect in Ras:SOS occurs by penetration of an α -helix of SOS into the region of the Ras active site, but it appeared highly likely that the end effect in terms of removing interactions with the respective nucleotide would be similar.

In myosin, the situation of switch I appears to be different, in spite of the remarkable overall structural similarity of the active site to those of GTPases. Thus, regardless of whether ATP or ADP was bound, and even in the absence of nucleotide in earlier structures, the switch I region was seen in an essentially closed state. Interpreting this in the same manner as for GTPases, this suggests that the closed state of switch I in myosin is an inherently stable, or at least easily reached conformation, even when no nucleotide is at the active site. This was surprising in view of the major changes seen in the structurally similar GTPases and the high degree of structural and mechanistic similarity between the two classes of proteins. However, the idea that switch I in myosin is relatively stable in the closed form leads to significant understanding of some important features of the mechanism of energy transduction. In contrast to the GTPases, where GTP and GDP bind (in most cases) with similar affinity, ATP binds ca. 10⁵ fold more strongly than ADP to myosin.²² As argued above, the additional interactions between GTP and GTPases with switch I appear to be used to stabilize an otherwise unstable configuration of this region, and are thus not expressed as increased affinity. In myosin, switch I appears to be much more stable in the closed state than in GTPases, so that the additional interactions of ATP compared with ADP with this region contribute dramatically to its higher affinity. Moreover, there are more interactions of the switch I region of myosin with both ATP and ADP than there are of switch I of the GTPases with GTP (and none with GDP; Figure 1, c). In Ras:GTP, these interactions are restricted to interaction of the backbone NH of Thr35 (in Ras) with the

γ -phosphate group and the interaction of the side chain of the same protein residue with the Mg^{2+} ion. Both of these are lost on GTP hydrolysis, leading to the disordered state of switch I referred to previously. In myosin, equivalent interactions of Ser237 (numbering of residues according to the Dictyostelium sequence) with the γ -phosphate and the Mg^{2+} ion are present in the ATP state (and presumably in the ADP: P_i state, an assumption that is supported by structures of putative transition or intermediate state analogs such as ADP-vanadate²³). There are, however, several additional interactions of switch I with ATP (Figure 1c), including a side chain interaction of Ser236 with the γ -phosphate and a possibly highly significant interaction of the side chain NH_2 group of Asn233 with an oxygen of the α - and the γ -phosphate as well as with the α , β - and β , γ -bridging oxygens. Several water-mediated interactions can also be seen, for example with the side chain and the backbone carbonyl of Asn235. After ATP hydrolysis and loss of phosphate from the active site, several of these interactions are lost, in particular those with the γ -phosphate group. However, in contrast to what is seen in GTPases, the interaction of the Ser237 side chain with Mg^{2+} is retained, as is the side chain interaction of Asn233 with the β -phosphate of ADP. This is only possible because switch I stays in the closed state, and does not take up either an open configuration as seen in the Ras:SOS and other GTPase:GEF complexes or the disordered state seen in GTPase:GDP complexes.

The first indications that an “open” form of switch I in myosin can occur came from the structure of myosin V in the nucleotide-free state.¹⁷ Two striking features of this structure distinguish it from those previously determined. First, in the active site region, switch I moves away from the position normally occupied by the nucleotide and thus from the P-loop. If nucleotide and/or P_i were still bound to the active site, all the above mentioned interactions to switch I would be lost (also those to switch II, since this is also in an open configuration), with a resulting loss of affinity. Coupled to this change in switch I is a significant closure of a major groove between the upper and lower domains of the so-called 50K region of the motor domain and the adoption of the “down” position of the lever arm, which is equated with the end of the contractile power stroke in the actomyosin complex. Kinetic evidence indicates that nucleotide-free myosin V can bind to actin without undergoing a conformational change, in contrast to other myosins.¹⁷ The conclusion was reached that a state of myosin V had been characterized which is identical, or very similar, to that in the strongly bound actomyosin state. This interpretation was confirmed by EM structures of actomyosin in the nucleotide-free state, showing a closed constellation of the 50K cleft,¹⁸ and this has been confirmed and elaborated on in more recent higher resolution EM structures.²⁴ For a more detailed

account of the significance of these results for understanding the cross-bridge cycle, see the article by Geeves in this issue.

These results with myosin confirm the analogy with GTPases, since binding of myosin to actin requires closure of the so called 50 kD cleft, which is coupled to opening of switch I, as seen in GTPase:GEF complexes. Conversely, binding of ATP leads to switch I closure, opening of the 50kD cleft and dissociation of myosin from actin, in an analogous manner to GTP binding to GTPase:GEF complexes (a comparative schematic presentation of the NTPase cycle for Ras and myosin is shown in Figure 2a).

The Significance of the Relative Affinities of NTP and NDP in Myosin and GTPases and of Specific Versus Non-Specific Nucleotide Exchange Activity

We can describe the equilibria involved in generation of GTPases or myosin with nucleotides and with GEFs or actin as shown in Figure 2b. Since the overall change in free energy to generate the ternary complex (Ex:P:N) is the same regardless of which route is taken from nucleotide-free protein (P), we can derive the following relationship:

$$\frac{K_N^{Ex:P}}{K_N} = \frac{K_{Ex}^{P:N}}{K_{Ex}} \quad (1)$$

with the equilibrium constants defined as in the legend to Figure 2. This means that the reduction of affinity of nucleotide to NTPase in the ternary- with respect to the binary-complex is equal to the reduction of affinity of the exchange factor in the ternary complex. This is the thermodynamic basis for the expectation and observation that for GTPases, the effect of GEFs is not specific for GDP, as is often assumed. Thus, if a GEF could weaken the binding of GDP but not GTP to a GTPase, the affinity of the GTPase for the exchange factor would be reduced in the GDP containing ternary complex, but not in the GTP-containing complex, making the GEF:GTPase:GTP complex essentially a dead-end. This would mean that the activated form of the GTPase, the GTPase:GTP complex, would not be produced in significant amounts, and in addition to this would sequester the GEF in this complex so that it could not act catalytically on further GTPase molecules. Several studies have confirmed the similar effects of GEFs on GDP and GTP complexes with GTPases.^{20,25,26} Thus, GEFs simply act to establish the equilibrium between the GDP and GTP forms of GTPases, which is dictated by the relative prevailing GDP and GTP concentrations in the cell and the affinities to the respective GTPase, which tend to be similar for GDP and GTP, rather than catalyzing exchange in a particular direction.

That the situation with myosin would be different was, in retrospect, already apparent from the very large difference in

affinities between myosin and ADP on the one hand ($K_D \approx 1 \mu\text{M}$) and ATP on the other ($K_D \approx 10\text{pM}$).²² Extensive work on actomyosin ATPase led to the conclusion that the effect of ATP on the affinity between myosin and actin is dramatic (reduction of affinity by ca. 5 orders of magnitude, meaning that the ATP affinity in the ternary complex is also reduced by this factor), but is much less in the case of ADP (ca. 2 orders of magnitude).²⁷ Hydrolysis of ATP to ADP:P_i does not have a major effect on the affinity of myosin for actin (both are defined as weakly bound states), and also occurs with a small change in free energy, but after loss of the phosphate group, the strongly bound state actomyosin:ADP is produced. The free energy change associated with this step is the energy source which drives the conformational change in the cross-bridge leading to relative movement of the myosin-containing thick filaments and the actin-containing thin filaments in contractile actomyosin systems. To estimate the amount of energy potentially available in this step, we can imagine 2 schemes of the type shown in Figure 2b, one for ADP and one for ATP. There are then 2 versions of equation (1) (i.e. for ATP and for ADP, and similarly for GDP and GTP in the case of small GTPases), from which we can derive the expression shown in Figure 2c. The very large value of K_{ATP}/K_{ADP} (ca. 10^5) keeps the value of $K_{Ac}^{M:ADP}/K_{Ac}^{M:ATP}$ high, even though $K_{ADP}^{M:Ac}/K_{ATP}^{M:Ac}$ has a value of less than 1 (ca. 0.1, see reference²⁸). The value of $K_{Ac}^{M:ADP}/K_{Ac}^{M:ATP}$ is then calculated to be ca. 10^4 , corresponding to a ΔG° value of ca. -23 kJ mol^{-1} for the step leading to relative translation of the myosin and actin filaments, compared to the overall free energy of ATP hydrolysis of ca. -31 kJ mol^{-1} , suggesting that the energy made available for mechanical work from this step is a significant fraction of the available free energy. Thus, the combination of the large difference in affinity of ATP and ADP for myosin with the strong coupling with actin binding in the case of ATP/ADP:P_i, but weak coupling for ADP, leads to a thermodynamically realistic model of the cross-bridge cycle.

In GTPases, the basic functional mechanism requires strong binding of GDP in order to prevent spontaneous replacement by GTP and therefore spontaneous signal activation of a signaling event. If GTP is to compete well with GDP at the thermodynamic level, its affinity must also be high. There is therefore a good reason to expect a not too drastic difference in GTP and GDP affinity (i.e. $K_{GTP}/K_{GDP} \approx 1$, Figure 2c). At the level of GEF catalyzed GDP/GTP exchange, we also expect a roughly symmetrical situation, since specific activity towards the GTPase:GDP state would lead to a thermodynamic trap, as discussed above.

It should be noted that the interaction constants can be manipulated in a quite different manner to produce an energy transducing system, as is the case for kinesin, which is also a

P-loop protein belonging to the TRAFAC (translation factor) family⁴ (see article by Cross in this issue). Here, ADP release from kinesin is very slow, and is accelerated by microtubules, leading to formation of a stably bound microtubule:kinesin:ATP complex and rapid hydrolysis to ADP and P_i. After similarly rapid release of P_i, the weakly bound ADP state is released from microtubules.²⁹ Thus the properties of this system have evolved away from a putative generic GTPase precursor that is essentially symmetric in terms of the interactions of a GEF with the GDP and GTP forms of the GTPase towards one in which there is a specific exchange of the microtubule GEF analog towards the kinesin:ADP state, allowing transformation to the strongly bound nucleotide-free and ATP states in a transition that can occur with a favorable ΔG change. This is actually equivalent to the postulated situation that would occur with a GTPase and a GEF with GDP-specific exchange activity alluded to above, and leading to a “dead-end” ternary complex. In the case of kinesin and microtubules, this thermodynamic trap is exited via rapid ATPase activity under the appropriate conditions, generating the weakly microtubule bound ADP state and leading to dissociation and completion of the cycle.

The Hydrolysis Competent States in Ras and Myosin

As stated earlier, Ras has a very low intrinsic rate of GTP hydrolysis ($k_{\text{hydr}} = 2 \cdot 10^{-4} \text{ s}^{-1}$)³⁰ and the rate is dramatically increased in the presence of a GTPase activating protein (GAP), the first one of which was already identified in 1987.³¹ The structural basis of GAP stimulated hydrolysis for Ras was resolved in 1997 by Scheffzek et al., showing the importance of an Arg finger that completes the active site of Ras by interacting with the phosphate groups and also stabilizing switch II and especially the important Gln61 residue in a hydrolysis competent conformation (Figure 3).^{32,33} Although some other small GTPases have a different mechanism of GAP stimulated hydrolysis (see article by Lambright in this issue), the active site needs additional contributions from other amino acids in all cases.

The active site of myosin also contains an Arg residue (Arg238 in switch I following the Thr35_{Ras} equivalent Ser237), but this is quite differently positioned to the Arg residue in Ras:RasGAP and other GTPase:GAP complexes. Arg238 forms a salt bridge with the essential Glu459, and this salt bridge appears to be important for several reasons, one of which is possibly the stabilization of the hydrolysis competent configuration around the γ -phosphate.^{12,34} This effect alone is probably not enough to explain why the rate of hydrolysis of ATP by myosin is approximately seven orders of magnitude faster than that of Ras (k_{hydr} ca. 100 s^{-1}).^{35–37} The Arg from RasGAP

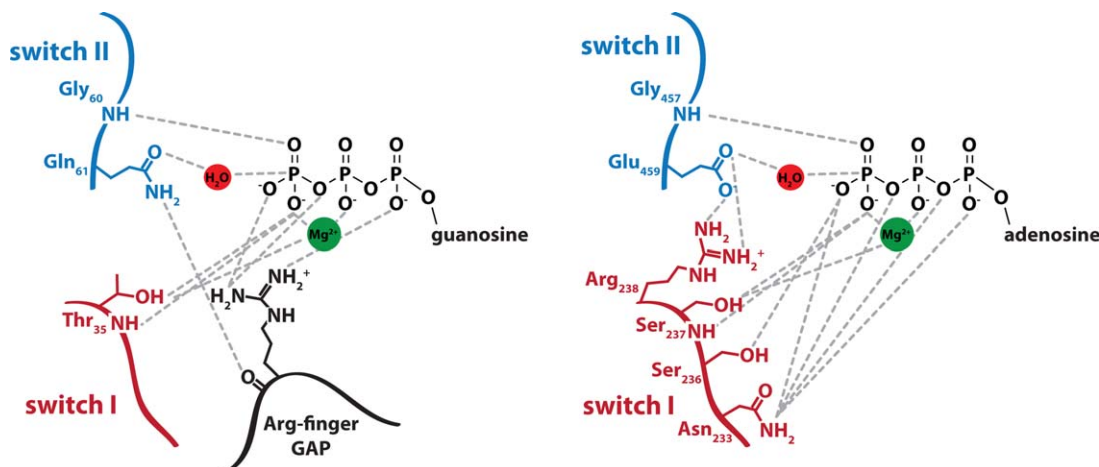


FIGURE 3 Comparison of the putative hydrolysis competent states in Ras and myosin. As discussed in the text, whereas Ras (left) needs an additional factor (GAP) providing an essential Arg-residue to complete the active site for hydrolysis, the active site of myosin (right) contains an Arg residue (Arg238) within the switch I region. In both cases, the Arg residues appear to stabilize the switch II regions and Gln61 in Ras/Glu459 in myosin in a hydrolysis competent conformation.^{32–34,39} In the Ras:RasGAP transition state analog structure, the arginine is also involved in important interactions with the phosphates/phosphate structure, but similar interactions of the switch I Arg residue are not observed in myosin. Instead, Asn233 appears to play a similar role in myosin.

stabilizes the position of Gln61 in Ras via an interaction with its backbone carbonyl group but importantly also interacts with the α - and γ -phosphates via its side chain guanidinium group, interactions that are not observed in myosin. However, in myosin several interactions between the phosphate groups and additional residues in switch I (Ser236 and Asn233) are seen, as discussed above. Notably, switch I Asn233 in myosin occupies a position very similar to that of the catalytic Arg residue provided by RasGAP in the Ras:RasGAP structure and presumably plays a similar role in stabilization of the transition state in myosin (Figure 3). Thus, the role of the essential arginine in RasGAP and many other GAPs appears to be shared between an arginine and other residues, including an asparagine from switch I in myosin. Here we see that also at this level, there are certain striking basic similarities between myosin and GTPases, but with differences directly related to their roles and modes of action.

CONCLUSION

The comparison of myosin and GTPase structures and mechanisms illustrates important conserved features of these P-loop proteins, but also shows how quantitative modifications to the interaction affinities and kinetics can lead from a protein together with its cognate GEF with the required properties of a signal transducing system to one in which the interaction of the NTPase with a GEF-like factor (actin) is capable of trans-

ducing the free energy of hydrolysis of ATP into mechanical work. At the structural level, the main differences are in the switch I region, with its higher stability in the closed state and significantly more interactions in the ATP than the ADP state, leading to a 10^5 fold higher affinity of myosin for ATP than ADP, an important factor in the energy conversion mechanism. Additionally, the nucleotide binding site in myosin contains all residues necessary for efficient catalysis of ATP hydrolysis. In contrast, small GTPases have similar affinities for GDP and GTP and need an additional factor (GAP) for efficient hydrolysis of GTP, two important properties of this class of proteins that provide the basis for a controllable switch in signal transduction pathways.

Intriguingly, the kinesin-microtubuli system has evolved into one with ADP-specific exchange properties, in exactly the opposite direction to myosin, which has ATP-specific exchange properties. In both systems that generate movement, the transition in which movement and mechanical work are generated can be identified, and is essentially a tightening of the interaction of motor domains with their partners actin or microtubuli, in the case of myosin on loss of P_i and in kinesin on loss of ADP and binding of ATP.

The structural and mechanistic basis of the GTPase and ATPase reactions of the compared protein classes also show pronounced similarities, with the functionally significant difference that the hydrolysis machinery is complete in myosin, but needs to be completed by additional residues from GTPase

activating proteins in the case of GTPases, thus introducing an additional level of control in the signal transduction cascades regulated by small GTPases.

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