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Structural frameworks for considering microbial protein and nucleic-acid dependent motor ATPases

Nathan D. Thomsen and James M. Berger

Quantitative Biology Institute and Dept. of Molecular and Cell Biology, 374D Stanley Hall #3220, University of California at Berkeley, Berkeley, CA 94720

James M. Berger: jmberger@berkeley.edu

Abstract

Many fundamental cellular processes depend on enzymes that utilize chemical energy to catalyze unfavourable reactions. Certain classes of ATPases provide a particularly vivid example of the process of energy conversion, employing cycles of nucleotide turnover to move and/or rearrange biological polymers such as proteins and nucleic acids. Four well characterized classes of ATP-dependent protein/nucleic acid translocases and remodelling factors are found in all three domains of life (bacteria, archaea, and eukarya): ASCE P-loop NTPases, GHL proteins, actin-fold enzymes, and chaperonins. These unrelated protein superfamilies have each evolved the ability to couple ATP binding and hydrolysis to the generation of motion and force along or within their substrates. The past several years have witnessed the emergence of a wealth of structural data that help explain how such molecular engines couple nucleotide turnover to conformational change. In this review, we highlight several recent advances to illustrate some of the mechanisms by which each family of ATP-dependent motors facilitates the rearrangement and movement of proteins, protein complexes and nucleic acids.

Introduction

Numerous protein families have evolved the ability to catalyze molecular transactions using ATP, the universal energy currency of the cell. ATPases can function as nucleotide-dependent switches, ion pumps, and polymer-dependent molecular motors, to name a few (Vetter and Wittinghofer, 1999). While many ATPases undergo significant conformational changes as they hydrolyze nucleotide, the polymer-dependent motors further couple these conformational changes to the often-dramatic rearrangement and mechanical manipulation of polypeptides, protein complexes and nucleic acids. The vast majority of these motors are built from one of four basic folds – ASCE P-loop, GHL, actin, and chaperonin – that have evolved the ability to push, pull and wind or unwind target substrates. The cellular roles of these motors beautifully illustrate the adaptive powers of evolution, both through the divergence of function for closely related motors, and the convergence of function between unrelated ATPase superfamilies.

The microbial ATPase folds covered here each illustrate a unique mechanism by which ATPase activity is coupled to motion or force generation. However, four main generalizations can be drawn about the requirements for an ATP dependent motor, summarized here and in Table 1. First, to generate the large conformational changes required for motor function, ATP and a divalent cation are generally bound at an interdomain or intersubunit interface, with conserved amino acids being contributed by both

ATP binding subunits or subdomains. At a minimum, this configuration leads to instances in which two adjacent domains within a single polypeptide create a monomeric motor; however, this arrangement often leads to large multimeric motor assemblies that respond to complex allosteric interactions between multiple ATP and target-substrate binding sites. Second, binding of adenosine nucleotides often relies on a flexible glycine rich loop (generally termed a P-loop), in which backbone amides help hydrogen bond to the phosphate groups of ATP. In most motor ATPases, the P-loop is accompanied by adenine binding residues, which in some protein classes are highly conserved. Third, motors typically utilize a conserved acidic residue to position and activate a water molecule for attack on the γ -phosphate of ATP. Finally, in order to physically respond to different nucleotide states, the motor must be able to sense the difference between ATP and its hydrolysis products. This action is accomplished either by direct interactions between a positively-charged γ -phosphate sensor and ATP, or indirectly via a negatively-charged residue that binds a divalent cation (typically Mg^{2+}) in contact with the γ -phosphate. Although these conserved features are present in all four microbial motor ATPase families, it is their unique positioning within distinct folds that is primarily responsible for generating the remarkable diversity of ATP dependent motor mechanisms.

Given the broad nature of the topic, this review will focus primarily on structural studies that have begun to reveal the nature and scope of ATP-dependent conformational changes in microbial protein/nucleic-acid motor ATPases, and how these rearrangements drive function. We will first discuss the conserved modules for ATP binding and hydrolysis and how these elements differ among families, and then describe the ATP-dependent conformational changes in the context of quaternary motor assemblies. GTP-hydrolyzing tubulin family members, the Walker-A Cytoskeletal ATPases (WACA) (which are phylogenetically classified as P-loop GTPase family members) (Leipe *et al.*, 2002), and the nucleotide-dependent RNA and DNA polymerizing machineries, though motor proteins in their own right, are too broad to be contained in this survey; the reader is directed to a number of recent reviews for coverage of these NTPase families, (Michie and Lowe, 2006; Rothwell and Waksman, 2005; Steitz, 2006; Thanbichler and Shapiro, 2008). We will focus instead on those motor proteins whose members almost exclusively use ATP to translocate or remodel their target substrates.

ASCE P-loop Motors

The Additional Strand Catalytic “E” (ASCE) P-loop family (Leipe *et al.*, 2003) is one of the largest and most functionally diverse classes of ATP-dependent motors. Although ASCE P-loop members are sometimes referred to as “RecA-like” proteins, due to the similarity of their fold to the RecA recombination factor (Story *et al.*, 1992), there are critical differences between this subgroup and other ASCE proteins. For example, the core nucleotide-binding folds of RecA-like and AAA+ (ATPases Associated with various cellular Activities) proteins are related, but differ in the details of their topology, conserved ATP-binding residues, and the orientation of individual motor domains in higher-order quaternary states (Caruthers and McKay, 2002; Erzberger and Berger, 2006; Iyer *et al.*, 2004b; Wang, 2004). Despite these differences, RecA and AAA+ proteins both rely on similar mechanisms for the generation of movement, in which ATP binding induces internal conformational changes within a single motor subunit, which are then amplified by the remodelling of inter-subunit interfaces via *trans*-acting γ -phosphate sensor motifs.

RecA-like Motors

The RecA-like family can be divided into four major evolutionarily related groups: the ABC ATPases, superfamily 1/superfamily 2 (SF1/SF2) helicases, the PilT/FtsK secretory ATPases, and the classic RecA/F₁ family (Iyer *et al.*, 2004b). RecA-like ATPases are

defined by a six- to seven-strand β -sheet sandwiched between a series of α -helices (Fig. 1A) (Story and Steitz, 1992; Story *et al.*, 1992). Residues responsible for ATPase function are located almost exclusively on loops connecting the C-termini of central β -strands to downstream helical elements. In almost all cases, non-conserved accessory domains are attached to or inserted within the RecA-like fold, such as the C-terminal helical bundles commonly found in RecA/F₁ family members, or domains Ib and IIb found in SF1/SF2 helicases.

Conserved elements known as Walker-A and Walker-B motifs, so termed for their identification in the sequence of the bovine mitochondrial F₁-ATPase and other ATP binding enzymes (Walker *et al.*, 1982), contain the only catalytic residues that are strictly conserved among all RecA family members. The Walker-A motif (W-A), also referred to as the phosphate-binding, or P-loop, is located between strand β -1 and helix α -A, and generally bears the consensus sequence [G/A]xxxxGK[T/S] (Fig. 1A) (Walker *et al.*, 1982). The backbone amide groups of the glycines coordinate the phosphate groups of ATP, the conserved lysine forms ion pairs with the β - and γ -phosphates, and the threonine/serine residue ligands a critical Mg²⁺ ion (Fig. 1B). The Walker-B motif (W-B), located between strand β -4 and helix α -D, contains the consensus sequence $\phi\phi\phi\phi$ [D/E] (in which ϕ is a hydrophobic residue) (Walker *et al.*, 1982), where the conserved acidic group further coordinates the Mg²⁺ ion associated with the β - and γ -phosphates of ATP (Abrahams *et al.*, 1994; Story and Steitz, 1992). The spatial position of the catalytic glutamate that gives its name to the ASCE superfamily is conserved in the active site of different RecA-like family members, but is located at different points in the primary amino acid sequence. For example, in the F₁/RecA and PilT/FtsK families, the catalytic residue is located on a loop between strand β -2 and helix α -B (Fig. 1A) (Story and Steitz, 1992; Yeo *et al.*, 2000). By contrast, in SF1/SF2 and the ABC ATPase families, the catalytic glutamate is located immediately after the aspartate of the W-B motif (Fig. 1A) (Geourjon *et al.*, 2001; Hung *et al.*, 1998; Subramanya *et al.*, 1996). In both instances, this residue is postulated to activate a water molecule for nucleophilic attack on the γ -phosphate (Geourjon *et al.*, 2001). Another component of the active site conserved in many RecA-like ATPases is a polar residue at the C-terminal tip of strand β -5 that might function as a γ -phosphate sensor in order to transmit conformational changes to other parts of the protein upon ATP binding (Iyer *et al.*, 2004b; Story and Steitz, 1992; Yoshida and Amano, 1995). Such a function might parallel the analogous switch-II region of G-proteins (Milburn *et al.*, 1990), which are distantly related to ASCE P-loop ATPases within the broader P-loop NTPase superfamily (Leipe *et al.*, 2002). Finally, nearly all RecA-like ATPases, apart from the ABC class (see below and Fig. 1 and S1), contain an arginine (or lysine) finger similar to those first observed in GTPase activating proteins (Wittinghofer *et al.*, 1997). Since this amino acid typically contributes to the active site of a partner subunit *in-trans*, the arginine finger helps couple ATP binding and hydrolysis to large-scale conformational changes between adjacent RecA folds. The positive charge on the guanidinium group of the arginine is critical for ATPase activity, and helps to stabilize the pentavalent phosphoanhydride transition state that forms during hydrolysis (Braig *et al.*, 2000; Nadanaciva *et al.*, 1999; Ogura *et al.*, 2004).

RecA-like ATPases typically exhibit nucleotide-dependent movement of substrate-binding loops that are adjacent to the ATP binding site. In some cases, this movement appears to be coupled to twisting of the central β -sheet of the RecA-fold. This β -sheet deformation has been proposed to store energy elastically (Sun *et al.*, 2003) and can lead to large motions in accessory domains. The F₁-ATPase, which has served as a model system for studies of oligomeric RecA-type proteins, illustrates both of these effects clearly (Fig. 1C) (Abrahams *et al.*, 1994; Kabaleeswaran *et al.*, 2006). F₁ is a hetero-hexamers containing three active β -subunits and three inactive α -subunits in an alternating arrangement (Abrahams *et al.*, 1994). Two loops, designated here as L1 and L2 in accordance with the nomenclature for RecA

(Story *et al.*, 1992), contact the internal γ -shaft subunit and undergo small but distinct conformational changes between the ATP-bound and empty subunits. Similar ATP-dependent loop movements have also been observed in other well-studied hexameric or filamentous RecA-like motors whose nucleic acid binding sites reside in the central channel formed by the L1/L2 loops. These proteins include RecA itself (Krishna *et al.*, 2007b), the phage T7-gp4 helicase (Singleton *et al.*, 2000), the bacteriophage ϕ 12 P4 RNA packaging motor (Mancini *et al.*, 2004), and the *E. coli* Rho transcription termination factor (Skordalakes and Berger, 2006). Interestingly, in the SF1 helicases PcrA and UvrD that bind target nucleic acid substrates in a very different fashion, loops topologically equivalent to L1/L2 also undergo ATP-dependent conformational shifts and contain residues that base stack with bound nucleic acid (Fig. 1D) (Lee and Yang, 2006; Velankar *et al.*, 1999). No other RecA-like proteins to date have illustrated the drastic flexing of the β -sheet observed in F₁, which might reflect a unique characteristic of this motor but could also be due to the fact that structures of RecA-like hexameric translocases bound to both ATP and their protein or nucleic acid substrates have not been obtained.

Although the intra-domain conformational changes due to ATP binding are relatively small, the oligomeric nature of RecA-like motors allows these transitions to be amplified through inter-subunit or inter-domain (in the case of SF1/SF2 family members) interfaces. Structural analyses of the SF1 helicases PcrA and UvrD have illustrated this principle particularly well, since they remain the only RecA motors imaged in the presence of nucleic-acid substrates in multiple nucleotide-bound states (Fig. 1D) (Lee and Yang, 2006; Velankar *et al.*, 1999). In the inactive or empty conformation, the two RecA folds of the helicases splay apart, removing the arginine finger in domain IIa from the ATP-binding site in domain Ia. Upon binding ATP, domain IIa rotates inward to insert its arginine finger into the catalytic pocket of domain Ia, thereby promoting ATP hydrolysis. This movement is thought to be coupled to the translocation of single-stranded nucleic acid across the tandem RecA domain surface, using base stacking and nucleic acid backbone-binding residues located on substrate-binding loops to grip and pull DNA (Velankar *et al.*, 1999). Unwinding of duplex DNA occurs concomitantly with this motion by splitting the duplex across a wedge element in domain IIa (Lee and Yang, 2006; Velankar *et al.*, 1999). Significant inter-domain movements have been observed in the conserved RecA domains of other nucleic-acid bound SF1 helicases such as *E. coli* Rep (Korolev *et al.*, 1997), as well as in SF2 helicases such as HCV-NS3 (Kim *et al.*, 1998), archaeal Rad54 (Durr *et al.*, 2005), and Hel308 (Buttner *et al.*, 2007). It is notable that only nucleic-acid bound SF1/SF2 helicases have illustrated both the open and closed states clearly. A possible explanation was provided by recent single-molecule fluorescence resonance energy transfer (FRET) studies of substrate dependent domain movements in the SF2 RNA helicase YxiN from *Bacillus subtilis* (Theissen *et al.*, 2008). This study revealed that cooperative binding of both ATP and nucleic acid substrate were required for the helicase to adopt the closed conformation. Although the substrate binding modes and quaternary organization of the hexameric motors are quite different, the movements observed in SF1/SF2 helicases currently serve as the most complete model of inter-domain communication in the RecA-like polymer-dependent ATPases.

The catalytic motifs and inter-subunit/domain conformational changes described thus far can be generalized to most RecA-like proteins with the exception of the ABC ATPases. Although the small molecule transporters of the ABC class are outside the scope of this review, a number of ABC proteins have been identified as DNA-dependent remodelling or repair factors (Hopfner and Tainer, 2003). Structures of these ATPases, including structural maintenance of chromosomes (SMC) proteins (Lammens *et al.*, 2004), the mismatch repair enzyme MutS (Obmolova *et al.*, 2000), and the double-strand break repair enzyme Rad50 (Hopfner *et al.*, 2000), have all revealed that ABC ATPases form head-to-head dimers with conserved ABC motifs forming two bipartite ATP binding sites along a dyad symmetry axis

(Fig. S1) (Hopfner *et al.*, 2000). A notable exception to this organization is illustrated by UvrA, a protein involved in nucleotide excision repair, in which the ATP binding sites are formed at the interface between tandem ABC folds contained in a single polypeptide chain (Pakotiprapha *et al.*, 2008). Both arrangements differ markedly from the head-to-tail dimers that generate a single ATP binding site in classical RecA-like proteins. As a result, the unique ABC dimers lack the arginine fingers found in other RecA family members, but have gained several new motifs (Table I and Fig. S1). Another distinction is that ABC ATPase multimers do not form the ring-shaped assemblies characteristic of many RecA ATPases, but rather use cycles of ATP-dependent dimerization events to capture and/or bind to DNA (Lamers *et al.*, 2000; Obmolova *et al.*, 2000). In this way, ABC transporters share some functional parallels with GHL enzymes (see below), highlighting an intriguing functional convergence between two unrelated ATPase folds.

While SF1/SF2 and DNA-dependent ABC ATPases typically couple the ATP-dependent rearrangement of two RecA folds to movement along or association with nucleic acid substrates (Hopfner and Tainer, 2003; Singleton *et al.*, 2007), how nucleotide turnover is linked among the multiple active sites of ring-shaped hexameric RecA-like motors is less well understood (Singleton *et al.*, 2007). In this regard, the F_1 -ATPase has served as a valuable reference system for ring shaped RecA-like motors. This heterohexameric assembly, which participates in ATP synthesis *in-vivo*, displays structural and catalytic asymmetry among its three active β -subunits, which alternate between high, medium and low affinity for ATP+Mg²⁺ (Abrahams *et al.*, 1994). The asymmetry of F_1 is directly coupled to the position of the γ -shaft subunit in the centre of the ring, and ensures that only the high affinity site can hydrolyze ATP at any one time. ATP turnover proceeds around the F_1 ring, directly driving rotation of the internal γ -shaft (Mao and Weber, 2007; Noji *et al.*, 1997; Weber and Senior, 2003). In contrast to F_1 , most toroidal RecA-type ATPases assemble and function as homohexamers, and retain the potential to accommodate six catalytically competent ATP binding sites. To date, structures of these motors have revealed a variety of ring symmetries. These structural states are often observed to have rotational three-fold, or “trimer of dimers” symmetry, as has been observed in an archaeal secretion ATPase of the GspE family (Yamagata and Tainer, 2007), the Rho transcription termination factor (Skordalakes and Berger, 2006), and the bacterial replicative helicases RepA of plasmid RSF1010 (Ziegelin *et al.*, 2003) and DnaB (Bailey *et al.*, 2007). By contrast, other oligomeric RecA motors have been imaged as dimers of trimers (Satyshur *et al.*, 2007; Singleton *et al.*, 2000; Xu *et al.*, 2003), as six-fold symmetric particles (Gomis-Ruth *et al.*, 2001; Mancini *et al.*, 2004; Niedenzu *et al.*, 2001; Yeo *et al.*, 2000), or even as heptamers (Toth *et al.*, 2003). Based on these structures and on F_1 , multiple mechanisms have been proposed for how inter-subunit conformational changes drive substrate through the motor ring, the most predominant of which rely on a concerted wave of L1/L2 loop movements that progress around the helicase in a rotary fashion. Nonetheless, given that a ring shaped RecA-type ATPase has not yet been visualized in the presence of both nucleotide and a nucleic acid or protein substrate, the detailed mechanism by which this family of motors uses ATP turnover to power the movement of target molecules remains a point of vigorous debate (Singleton *et al.*, 2007).

AAA+ Motors

The AAA+ family of ASCE P-loop ATPases represents a second way in which nature has utilized conserved Walker-A and Walker-B motifs, and a mixed α/β fold, to perform mechanical work. The structures of domain II of eukaryotic N-ethylmaleimide Sensitive Factor (NSF) (Lenzen *et al.*, 1998; Yu *et al.*, 1998) and the δ' subunit of the *E. coli* DNA polymerase III clamp-loader complex (Guenther *et al.*, 1997), along with subsequent phylogenetic analyses, have formed the basis for classifying this family separately from

other ASCE P-loop ATPases (Iyer *et al.*, 2004a; Neuwald *et al.*, 1999). The AAA+ family has been divided further into seven evolutionarily-related subgroups, or clades: clamp loaders, initiators/helicase loaders, classic AAAs, superfamily 3 (SF3) helicases, HCLR (HslU, ClpABC-CTD, LonAB, RuvB), helix 2 insert (H2I), and pre-sensor 2 insert (PS2I) (Iyer *et al.*, 2004a). The structural core of the AAA+ ATPases consists of a five-stranded, parallel β -sheet sandwiched between α -helices that is similar to, but slightly more compact than, the RecA-like fold (Fig. 2A). This core is typically appended with a three-helix bundle at its C-terminus, sometimes referred to as the “lid”, which is conserved in six of the seven AAA+ clades (Erzberger and Berger, 2006; Iyer *et al.*, 2004a).

The AAA+ family possesses a greater number of conserved catalytic residues than does the RecA-like family (Fig. 2B). The putative water-activating acidic residue is almost always located in the Walker-B motif (similar to SF1/SF2 helicases), while the arginine finger invariably lies on the C-terminus of helix α -D (Erzberger and Berger, 2006; Iyer *et al.*, 2004a). The AAA+ family is also characterized by two additional, highly conserved catalytic residues, the sensor-I (SI) and sensor-II (SII) motifs first identified in the δ' subunit of the *E. coli* clamp-loader complex (Guenther *et al.*, 1997). The sensor-I motif is typified by a polar residue located at the C-terminus of strand β -4, a position structurally analogous to the switch-II or sensor residue found at the C-terminus of strand β -5 in many P-loop GTPases and RecA-like ATPases (Milburn *et al.*, 1990; Story and Steitz, 1992). The sensor-I motif might play a role in orienting the catalytic water molecule for attack on the γ -phosphate (Guenther *et al.*, 1997). The sensor-II motif is a positively-charged residue at the N-terminus of helix α -G of the AAA+ lid and is thought to couple ATP binding and hydrolysis to intra-subunit conformational changes between this region and the primary nucleotide-binding fold (Erzberger and Berger, 2006; Wang *et al.*, 2001b). Due to the absence of a canonical AAA+ lid, SF3 helicases lack this element.

Among microbial AAA+ motor proteins, the ATPases of proteolytic degradosomes have been the most thoroughly characterized structurally. For example, the HslU engine of the HslU/V protease has been imaged at high resolution in numerous conformational states and bound to a variety of nucleotides (Bochtler *et al.*, 2000; Song *et al.*, 2000; Sousa *et al.*, 2000; Wang *et al.*, 2001a; Wang *et al.*, 2001b). These different states have revealed intra-subunit conformational changes that are characteristic of most members of the AAA+ family (Fig. 2C). The most obvious conformational changes occur in the AAA+ lid domain that, in addition to the sensor-II arginine, contributes a number of residues directly to the active site. The lid can undergo a rigid body rotation of nearly 20° upon entering an ATP-bound state, allowing productive interactions to form between the sensor-II region and the γ -phosphate of ATP. Structures bound to SO₄ (presumably mimicking a post hydrolysis PO₄) and ADP populate conformations along this same rotation vector, suggesting that the observed configurations represent a functionally significant set of transitions (Wang *et al.*, 2001b). Studies of numerous AAA+ motor proteins, including the σ -factor remodelling proteins NtrC (Lee *et al.*, 2003b) and PspF (Rappas *et al.*, 2005), and the chaperone ClpB (Lee *et al.*, 2003a), have revealed marked variability in the positioning of the AAA+ lid domain. Distinct nucleotide-dependent conformational transitions have also been observed in the AAA+ domains of the DnaA initiator (Erzberger *et al.*, 2002; Erzberger *et al.*, 2006), and archaeal Replication Factor C (RFC) clamp loader (Seybert *et al.*, 2006), as well as in the protein translocase motor of the protease FtsH (Niwa *et al.*, 2002; Suno *et al.*, 2006). Thus, movement of the AAA+ lid appears to be a general feature of most motor proteins of this family and is linked directly to the formation of a competent ATP-binding site.

The structure of the superfamily 3 (SF3) helicase E1 from papillomavirus bound to DNA represents one of the most complete structures of an AAA+ motor/substrate complex visualized to date. Although the complex is a homohexamer, E1 adopts a markedly

asymmetric conformation in which the spatial displacement of each subunit varies sequentially around the ring and is coupled directly to the position of DNA-binding loops that protrude into the centre of the helicase (Enemark and Joshua-Tor, 2006). This configuration strongly suggests that the ATPase sites of E1 fire in a sequential manner similar to that seen for F₁-ATPase, although six catalytic sites are utilized instead of three (Abrahams *et al.*, 1994; Enemark and Joshua-Tor, 2006). Comparison of the differences between empty and nucleotide-bound active sites indicates that ATP-binding triggers inter-subunit clamping movements similar to those observed in RecA-type proteins (Fig. 2D). These movements, in turn, appear coupled to the shifting of substrate-binding loops emanating from the AAA+ fold and the translocation of single-stranded DNA through the central pore of the hexameric E1 motor (Enemark and Joshua-Tor, 2006). Structures of HslU (Bochtler *et al.*, 2000; Song *et al.*, 2000; Sousa *et al.*, 2000; Wang *et al.*, 2001a; Wang *et al.*, 2001b), NtrC (Lee *et al.*, 2003b), PspF (Rappas *et al.*, 2005; Rappas *et al.*, 2006), SV40 large T-antigen (Gai *et al.*, 2004), and FtsH (Niwa *et al.*, 2002; Suno *et al.*, 2006) likewise exhibit ATP-dependent clamping movements and/or the displacement of internal substrate-binding loops, suggesting that a binding-ratchet motion might be a conserved feature of these oligomeric motors.

Despite relying on a common set of ATP-induced conformational changes, the relative relationship between inactive and active catalytic centres and their firing order might be variable. For example, the asymmetric structure of the E1 helicase bound to DNA strongly supports a rotary mechanism for ATP turnover (Enemark and Joshua-Tor, 2006) in which all six subunits sequentially take their turns binding and hydrolyzing nucleotide. By contrast, the SV40 large T-antigen has been imaged in a number of symmetric, nucleotide-dependent conformational states that have been interpreted as evidence for a concerted, all-or-none DNA translocation mechanism (Gai *et al.*, 2004). Biochemical analysis of a covalently-linked ClpX hexamer, which allowed exquisite control over the number and placement of mutant subunits, has shown that activity is related to the number of active subunits within the hexamer, and that even a single catalytic subunit can support peptide translocation (Martin *et al.*, 2005); these latter findings have been used to invoke a stochastic firing mechanism for the enzyme. As with the RecA-like ring-shaped motors, the coupling of ATP hydrolysis within the ring assembly to substrate movement through the motor might depend on whether the ATPase motor acts on protein or nucleic acid targets. In addition, it should be noted that E1 is the only substrate-bound hexameric AAA+ ATPase to be imaged at high resolution, raising the possibility that many of the symmetric structures observed in hexameric ATPases might not reflect fully functional states.

With respect to ASCE ATPases as a group, it is interesting that members of two different subgroups, E1 (AAA+) and F₁ (RecA) have markedly different functions in the cell, yet both appear to use a sequential approach for coupling ATP turnover to the movement of target macromolecules. Despite this similarity, both proteins orient their ATPase folds in a distinct manner with respect to the central axis of the ring, and both use different secondary-structural elements to engage substrate (Wang, 2004). Given these parallels and differences, it will be interesting to see if other members of these two ASCE families have evolved similar motor mechanisms.

GHL Proteins

GHL proteins comprise a subset of molecular motors and switches within the GHKL ATPases, a diverse group of proteins named for the principal nucleotide-binding fold found in type II topoisomerases (DNA Gyrase), the chaperone Hsp90, the histidine Kinases and MutL repair factors (Dutta and Inouye, 2000), reviewed in (Corbett and Berger, 2004; Pearl and Prodromou, 2006; Wolanin *et al.*, 2002; Yang, 2000). Like ASCE-class motor proteins,

structural studies of GHL enzymes have revealed a common set of physical mechanisms that underlie a rich functional diversity. In particular, all microbial GHL proteins use ATP to trigger the dimerization of their ATPase domains and regulate the engagement of client macromolecular substrates.

GHL ATPases are composed of two structural domains, the ATP-binding GHKL core, and an α/β domain, sometimes referred to as the transducer element (Fig. 3A). The GHKL domain is a two layered α/β sandwich consisting of an eight-stranded, predominantly anti-parallel β -sheet with four to six α -helices situated on one face (Wigley *et al.*, 1991). The transducer domain consists of a four- or five-stranded mixed β -sheet sandwiched between three to four interconnecting α -helices, and constitutes a fold found also in certain RNA binding proteins (Murzin, 1995). The GHL ATPase architecture was first observed in the structure of the *E. coli* gyrase B N-terminal domain (Wigley *et al.*, 1991); however the family was defined only after comprehensive sequence analyses (Bergerat *et al.*, 1997), along with structural studies of MutL (Ban and Yang, 1998) and Hsp90 (Meyer *et al.*, 2003; Prodromou *et al.*, 1997), revealed the conservation of the nucleotide-binding fold (Dutta and Inouye, 2000). Unlike ASCE P-loop motors, which situate the ATP-binding site on loops protruding from the C-terminal tips of a central β -sheet, the ATP-binding site of GHL proteins is located on the α -helical face of the GHKL domain within a large cleft partially bounded by the transducer domain (Wigley *et al.*, 1991). Another distinct feature of GHL proteins is the presence of an N-terminal segment of polypeptide chain that is used by each subunit to engage the ATPase site of a partner protomer. This region, sometimes termed the “strap,” often contributes amino acids that directly coordinate bound nucleotide and help seal off the active site cleft (Ali *et al.*, 2006; Ban and Yang, 1998; Brino *et al.*, 2000; Wigley *et al.*, 1991).

Four conserved sequence motifs, all of which contribute residues to the ATP-binding site, are found in GHKL family members (Dutta and Inouye, 2000), along with a fifth motif in the transducer that defines the GHL subclass (Fig. 3B). Motif I, located at the C-terminus of helix α -B contains a conserved glutamate that activates a water molecule for attack on the γ -phosphate (Ban and Yang, 1998; Jackson and Maxwell, 1993). Motif I also includes a conserved asparagine residue that ligands a Mg^{2+} ion required for nucleotide binding and hydrolysis (Wigley *et al.*, 1991). Motif II contains an aspartate and glycine at the C-terminus of strand β -3 that make specific contacts to the adenine ring and strictly enforce the use of ATP over other nucleoside triphosphate cofactors (Dutta and Inouye, 2000; Wigley *et al.*, 1991). Motif III, located between helix α -C and helix α -D, is composed of a glycine-rich, phosphate-binding region that is part of a large loop containing one or more α -helices known as the “ATP-lid” (Dutta and Inouye, 2000; Wigley *et al.*, 1991). Motif IV, which lies adjacent to motif II at the N-terminus of strand β -7, contains a conserved threonine that makes further base-specific contacts with the adenine ring. Finally, motif V consists of a conserved basic residue located on a loop emanating from the transducer domain between strand β -13 and helix α -H. This positively-charged residue acts as a γ -phosphate sensor and is thought to be linked to the propagation of nucleotide-dependent conformational changes between the GHKL and transducer folds (Ali *et al.*, 2006; Ban *et al.*, 1999; Corbett and Berger, 2005; Wigley *et al.*, 1991). In this regard, motif V is a functional analogue of the arginine finger found in ASCE P-loop motors.

Two distinct types of intra-subunit conformational changes occur during ATP turnover in GHL proteins. One is movement of the transducer domain with respect to the N-terminal GHKL region, while the other is the opening and closing of the ATP binding lid. The effects of nucleotide binding on these structural transitions have been seen in MutL (Ban *et al.*, 1999), Hsp90 (Ali *et al.*, 2006) and type II topoisomerases (Fig. 3C) (Bellon *et al.*, 2004; Corbett and Berger, 2005). In the presence of ATP mimetics or transition-state analogues,

the transducer domain rotates up and into the GHKL fold, inserting the motif V lysine into the active site where it contacts the γ -phosphate. In the absence of nucleotide, the transducer rotates away from the GHKL region, flipping the lysine into solution. Interestingly, in type II topoisomerases, ADP can support the adoption of both ATP-bound “restrained” and apo “relaxed” states, suggesting that nucleotide hydrolysis might not “power” conformational changes directly, but instead likely serves to facilitate the transition between the different structural states (Corbett and Berger, 2005; Wei *et al.*, 2005). Multiple conformations of the transducer domain in relation to the GHKL domain also have been observed in the *E. coli* Hsp90 homologue HtpG in the presence of ADP, highlighting the importance of the γ -phosphate in stabilizing the restrained state (Huai *et al.*, 2005; Shiau *et al.*, 2006). The conformation of the lid region, which varies in size among different GHKL family members, is also altered in an ATP-dependent manner. In type II topoisomerases and MutL, ATP binding orders the flexible lid, further stabilizing GHKL dimer contacts (Ban and Yang, 1998; Ban *et al.*, 1999; Corbett and Berger, 2003; Lamour *et al.*, 2002). A variety of conformations likewise have been observed for this region in different nucleotide-bound states of *E. coli* HtpG (Huai *et al.*, 2005; Shiau *et al.*, 2006) and yeast Hsp90 (Ali *et al.*, 2006). The conformational variability in the particularly large Hsp90-family lids appears to alternatively expose or hide a number of hydrophobic residues, including some that are important for inter-subunit contacts, and others that might play a role in client protein binding (Shiau *et al.*, 2006).

Structural studies of full-length Hsp90 chaperones (Ali *et al.*, 2006; Shiau *et al.*, 2006) and type IIB topoisomerases (Corbett *et al.*, 2007; Graille *et al.*, 2008) have at last begun to reveal the scope and nature of the large scale inter-subunit conformational changes that take place in intact GHKL assemblies. Most notably, ATP-induced dimerization of the GHKL domains leads to a large, *en-bloc* movement akin to the closing of pincers (Fig. 3D). Although Hsp90 is thought to utilize this movement to pull on or remodel proteins and protein complexes, type II topoisomerases and MutL use it to trap DNA substrates in a central cavity between the GHKL and C-terminal dimerization domains (Fig. 3C inset). It remains to be seen precisely how GHKL proteins engage target protein/nucleic acid substrates and how these binding events are linked to the timing of ATP hydrolysis.

Actin fold

The actin-fold superfamily includes two types of motor/remodelling proteins in bacteria: the filament forming actin-related proteins and the Hsp70 class of ATP-dependent chaperones (Kabsch and Holmes, 1995). Both actin and Hsp70 function as ATP-dependent molecular switches that couple conformational changes to directed action upon a protein substrate (Genevaux *et al.*, 2007; Goloubinoff and De Los Rios, 2007; Mogilner and Oster, 2003; Sousa and Lafer, 2006), however, the cellular functions of these two motors are very different. The Hsp70 monomer assists with protein folding, intracellular transport, and the remodelling of protein complexes by undergoing physical rearrangements that move a substrate-binding domain in relation to the actin fold (Sousa and Lafer, 2006). By contrast, filamentous actin polymerizes into extended multi-subunit assemblies that are able to exert force on objects at the filament ends via a Brownian ratchet mechanism (Mogilner and Oster, 2003). Although force generation for prokaryotic actins has yet to be measured directly, numerous lines of evidence suggest that these proteins play a motor function in the cell (Carballido-Lopez, 2006; Garner *et al.*, 2004; Garner *et al.*, 2007; Graumann and Defeu Soufo, 2004; Michie and Lowe, 2006). Despite their significant oligomeric and functional differences, actin-fold motors utilize a common nucleotide-dependent clamping mechanism, in which the differential movement of a pair of catalytic modules either directly generates movement of client-substrate binding domains (Hsp70), or generates binding interfaces that facilitate polymerization (actin).

The actin fold was first observed in the structure of hexokinase (Fletterick *et al.*, 1975). It was not until 15 years later, however, that structures of eukaryotic actin and Hsp70 proteins became available to define the family (Bork *et al.*, 1992; Flaherty *et al.*, 1990; Flaherty *et al.*, 1991; Kabsch *et al.*, 1990); the discovery of prokaryotic actin filaments came later still (Jones *et al.*, 2001; van den Ent *et al.*, 2001). All structures of microbial actin homologues determined to date have revealed a two-domain architecture, with each domain divided into one large and one variable small subdomain. The two large subdomains (Ia and IIa) each consist of a repeat of a five-stranded, mixed β -sheet sandwiched between three α -helices. These two elements form the conserved core of the actin fold (Fig. 4A), with the base of the ATP binding cleft formed by helices α -C and α -F on the rear face of each β -sheet (Bork *et al.*, 1992).

Five sequence motifs, all lining the ATP-binding site, are highly conserved among the actin fold family, with a sixth motif found specifically in the actin and Hsp70 motors (Fig. 4B) (Bork *et al.*, 1992). Motifs I and III form glycine-rich, phosphate-binding loops between strands β -1/ β -2 and β -6/ β -7, respectively. Both loops contribute to the binding of a single molecule of ATP (Flaherty *et al.*, 1990; Kabsch *et al.*, 1990). Each of these loops also contains a single acidic residue used in coordinating a divalent cation, typically Mg^{2+} , which contacts the β - and γ -phosphates of bound ATP (Flaherty *et al.*, 1990; Kabsch *et al.*, 1990). Motif IV is rich in hydrophobic residues that stack against the hydrophobic face of the adenine ring, and contains an invariant glycine that is in close proximity to the α -phosphate (Flaherty *et al.*, 1991). Motifs II and V lie on helices α -C and α -F, respectively, and define the closest contact points between the two large subdomains (Flaherty *et al.*, 1990; Kabsch *et al.*, 1990). The presence of a conserved alanine in motif II and a glycine in motif V is likely favoured by the fact that these amino acids define a major hinge point for ATP-dependent structural transitions in the actin fold (Bork *et al.*, 1992). A negatively-charged or polar residue in motif II at the N-terminus of helix α -C additionally is involved in coordinating the divalent cation located in the active site (Flaherty *et al.*, 1990; Kabsch *et al.*, 1990). Motif VI consists of a single conserved acidic residue located in strand β -7 that is thought to activate a water molecule for in-line attack on the γ -phosphate of ATP (Flaherty *et al.*, 1991). Finally, the two smaller subdomains (Ib and IIb) further contribute residues to the ATP binding centre. The structures and placement of these elements can vary among different actin fold members. For example, in the prokaryotic actin homologue FtsA, subdomain Ib is located between helix α -B and strand β -4, whereas in other actin and Hsp70 homologues, this module is positioned between helix α -A and strand β -3 (Fig. 4A) (van den Ent and Lowe, 2000).

Early structures of hexokinase provided the first glimpse into the conformational plasticity of the actin fold (Steitz *et al.*, 1981). A common inter-domain “propeller rotation” mechanism has since been suggested to unify the action of the family (Galkin *et al.*, 2002). This movement is somewhat akin to the ATP-clamping action of SF1/SF2 helicases. However, the bipartite ATP-binding site in SF1/SF2 proteins is formed via a front-to-back arrangement of two homologous RecA domains, whereas the bipartite ATP-binding site of the actin fold is formed via a back-to-back arrangement of two homologous actin-fold subdomains (Kabsch *et al.*, 1990; Subramanya *et al.*, 1996). Structural studies of the bacterial actin homologue ParM, which is believed to use its polymerizing capabilities for segregating low copy number plasmids (Garner *et al.*, 2007), clearly illustrate the nucleotide-dependent conformational changes that take place in a microbial actin (Fig. 4C) (van den Ent *et al.*, 2002), revealing that the two large subdomains of the protein undergo a 25° rigid body rotation upon binding nucleotide. Interestingly, examination of the catalytic centre of actin-fold family members (Fig. 4B) shows that these proteins do not contain conserved arginine/lysine γ -phosphate sensors like GHL and ASCE P-loop motors. Instead, these proteins contain Mg^{2+} -binding residues in the Ia and IIa subdomains that appear to

undergo significant conformational changes upon adopting a nucleotide-bound conformation (Figs. 4A, 4C). Thus, these Mg^{2+} sensors might serve as signal relays for ATP-induced domain movements.

Hsp70-class chaperones differ from actin-based polymerization motors by virtue of an additional, C-terminal substrate-binding domain, the movement of which might be coupled to ATP-dependent conformational changes in the N-terminal actin fold. Most structures of Hsp70 homologues have exhibited an open or inactive orientation between the two large subdomains of the actin fold, but a recent structure of the yeast Hsp70 paralogue Sse1 has successfully captured an ATP-bound state for the first time (Liu and Hendrickson, 2007). Together with structurally-informed mutational analyses, comparison of this intermediate with other structures of prokaryotic and eukaryotic Hsp70 family members suggests that an actin-like inter-domain conformational rearrangement serves as the basis of function in this chaperone family (Liu and Hendrickson, 2007).

The relative orientation between subunits in different actin-fold families appears to vary significantly. The first high-resolution experimental structure of a filamentous actin (F-actin) was the bacterial actin homologue MreB bound to the ATP analogue AMP-PNP (van den Ent *et al.*, 2001). This structure revealed a subunit interface similar to a theoretical model of eukaryotic F-actin obtained by fitting the crystal structure to X-ray fibre diffraction data (Fig. 4D) (Holmes *et al.*, 1990; van den Ent *et al.*, 2001). Remarkably, the subunit spacing captured in MreB crystals proved nearly identical to that seen in electron micrographs of MreB filaments formed *in vitro* (van den Ent *et al.*, 2001), and is similar to the inter-subunit interactions within crystallographic filaments of the nucleotide-bound archaeal actin homologue Ta0583, from *Thermoplasma acidophilum* (Roeben *et al.*, 2006). By contrast, ParM exhibits large structural differences in its inter-subunit interface compared to other prokaryotic and eukaryotic actins (Fig. 4D), primarily due to differences in the structure of its Ib and IIb subdomains (van den Ent *et al.*, 2002). Two recent models of the ParM filament based on three-dimensional electron microscopic (EM) reconstructions reveal a unique set of subunit interfaces and an opposite helical handedness to that of F-actin, providing further evidence of the differences between ParM and other actin homologues. However, there is still some disagreement as to the relative orientations of actin monomers within the filament (Orlova *et al.*, 2007; Popp *et al.*, 2008). Based on the observation of both right and left handed filaments, it has been suggested that polymerization of actin proteins might have arisen more than once in evolution (Egelman, 2003; Orlova *et al.*, 2007).

Hsp70 chaperones do not typically form higher-order oligomers, but are regulated by and require nucleotide exchange factors (NEFs) for function (Genevaux *et al.*, 2007). Yeast Sse1 is thought to act as an NEF for cytosolic Hsp70s, and a recent Sse1 structure exhibits a dimeric arrangement of protomers that might recapitulate the Sse1-Hsp70 interface (Liu and Hendrickson, 2007). Interestingly, this dimer interface arises primarily from contacts between the small Ib and IIb domains as well as the Hsp70-specific substrate binding domain (SBD α) of both subunits (Liu and Hendrickson, 2007), forming a head-to-head orientation that differs from the head-to-tail configuration observed between protomers in actin filaments (Fig 4D). Thus, unlike other ATP-dependent molecular motors, members of the actin-fold family appear to have linked a common internal conformational change to unique forms of inter-subunit associations, giving rise to significantly different motor mechanisms.

Chaperonins

Chaperonins are among the most specialized and best-understood class of ATP-dependent molecular motors. Two families of chaperonins exist and, although their quaternary

organizations are unique, both are based on a structurally homologous three-domain fold. The type I chaperonins, found in bacteria, mitochondria and chloroplasts, consist of 14 identical subunits arrayed into two heptameric rings stacked back-to-back against each other. By comparison, type II chaperonins are found in archaea and eukaryotes, and consist of two eight- or nine-subunit hetero-oligomeric rings stacked back-to-back (Horovitz and Willison, 2005). During the reaction cycle, the rings of type I chaperonins can be capped by a co-chaperonin lid (Horovitz and Willison, 2005), creating an interior cavity for protein substrate folding. Typically, only one of the rings is capped by the lid at any given time, leading to an enclosed “cis-ring” and an open “trans-ring” (Chen *et al.*, 1994; Ishii *et al.*, 1992; Langer *et al.*, 1992; Saibil *et al.*, 1993; Xu *et al.*, 1997), although doubly capped complexes might also play a role in the chaperonin cycle (Azem *et al.*, 1994; Beissinger *et al.*, 1999; Inobe *et al.*, 2008; Llorca *et al.*, 1994, 1997a, b; Schmidt *et al.*, 1994; Sparrer *et al.*, 1997). The *cis*- and *trans*-rings are thought to alternate back and forth, utilizing positive cooperativity for ATP binding within a single ring, and negative cooperativity for ATP binding between rings to drive the process (Bochkareva *et al.*, 1992; Bochkareva and Girshovich, 1994; Gray and Fersht, 1991; Horovitz and Willison, 2005; Rye *et al.*, 1997; Rye *et al.*, 1999). During this reaction, chaperonin subunits undergo dramatic conformational changes that are believed to promote active unfolding of mis-folded substrates (Krishna *et al.*, 2007a; Lin and Rye, 2006; Motojima *et al.*, 2004; Roseman *et al.*, 1996; Shtilerman *et al.*, 1999; Xu *et al.*, 1997).

GroEL, a type I bacterial chaperonin, has the most complex topology of the microbial protein/nucleic-acid dependent motor ATPases. The chaperonin protomer consists of three distinct domains that are each composed of N- and C-terminal regions of the polypeptide chain (Fig. 5A) (Braig *et al.*, 1994). The equatorial domain, which forms the inter-ring contacts and contains most of the ATP-binding residues, is composed of a two-layered α -helical sandwich and a series of small β -hairpins (Braig *et al.*, 1994). Like other motor families, the phosphate groups of ATP are bound by a series of flexible loops, but the chaperonins represent the only class whose ATP-binding fold is not predicated on a central β -sheet element. The elongated intermediate domain, which contains other motifs involved in ATP binding, comprises a three-stranded β -sheet and a three-helix bundle, and serves to transmit allosteric signals between the equatorial and apical domains (Braig *et al.*, 1994; Xu *et al.*, 1997). The apical domain is composed of two stacked β -sheets and a series of five α -helices, and is involved in binding both substrate polypeptides and the GroES lid (Lin and Rye, 2006).

The conserved ATP-binding residues, as observed in structures of GroEL bound to nucleotide, can be divided into five motifs (Fig. 5B). Motifs I and II lie at the base of the β -2/ β -3 hairpin and contain residues involved in binding both the α -phosphate of ATP and a K^+ ion that is critical for hydrolysis (Boisvert *et al.*, 1996; Wang and Boisvert, 2003). Motif II further includes an aspartate that is thought to activate a water molecule for nucleophilic attack on the γ -phosphate (Chaudhry *et al.*, 2003; Ditzel *et al.*, 1998). Motif III resides on a loop between helices α -C and α -D and contains a highly conserved GDGTTT sequence that acts as the primary β - and γ -phosphate binding loop (Boisvert *et al.*, 1996). The conserved aspartate in this region also helps to coordinate a Mg^{2+} ion, which contacts all three phosphates of ATP (Boisvert *et al.*, 1996). Motif IV consists of a conserved aspartate on helix α -M of the intermediate domain and coordinates Mg^{2+} when GroES is bound (Chaudhry *et al.*, 2003). Motif V is located on the hairpin loop between strands β -15 and β -16 of the equatorial domain and is primarily involved in making specific contacts to the adenine ring (Boisvert *et al.*, 1996).

All structures of GroEL illustrate some degree of conformational variability within their 14 subunits; however, structures bound to ATP, protein substrate, and the co-chaperonin lid

GroES (alone or in combination) have revealed the wide scope of conformational changes that take place within the chaperonin during catalysis. ATP binding causes three distinct but subtle conformational changes per protomer: movement of the β -2/ β -3 hairpin, a lateral translation of helix α -C, and a subtle rigid-body rotation of the apical and intermediate domains with respect to the equatorial domain (Boisvert *et al.*, 1996; Ranson *et al.*, 2001; Wang and Boisvert, 2003). Substrate binds to the apical domain surface formed by helices H and I (Chen and Sigler, 1999), and induces modest conformational shifts in this domain with respect to the rest of the protein (Wang and Chen, 2003). In light of a recent apo structure of GroEL (Bartolucci *et al.*, 2005), as well as a cryo-EM study of a GroEL substrate complex (Falke *et al.*, 2005), both substrate and ATP binding appear to cause a counter clockwise rotation of the apical domains.

Structural studies of GroEL-GroES bound to ADP have revealed that the binding of GroES creates much more drastic conformational changes in GroEL than nucleotide alone, giving rise to a downward motion of the intermediate domain to close off the nucleotide-binding site, and a dramatic upward movement and twist of the apical domain to engage the co-chaperonin lid (Fig. 5C and D) (Xu *et al.*, 1997). This conformational change moves helices α -I and α -H from an exposed position in the centre of the ring to a buried position in the GroEL-GroES interface, creating a large, hydrophilic interior cavity. Since these two helices form the primary interaction between GroEL and hydrophobic peptides (Chen and Sigler, 1999; Wang and Chen, 2003), this GroES-induced rearrangement might constitute one mechanism by which substrate is released into the central cavity. Structures of the GroEL-GroES complex bound to the transition-state mimic ADP·AlF₃, as well as a subsequent computational analysis of this and previous structures, have suggested that the γ -phosphate of ATP stabilizes the GroES-bound state by interacting with the motif IV aspartate through the active-site Mg²⁺ ion (Chaudhry *et al.*, 2003; Chaudhry *et al.*, 2004). A nearly identical interaction was observed in an ADP·AlF₃ bound structure of the archaeal thermosome, a type II chaperonin (Ditzel *et al.*, 1998). As with the actin fold family, chaperonins appear to lack a conserved arginine or lysine γ -phosphate sensor. The motif IV aspartate might serve instead as a Mg²⁺ sensor responsible for transmitting ATP binding signals between the equatorial and intermediate domains.

As might be expected from such large conformational transitions, the inter-subunit contacts among the 14 GroEL subunits also change significantly during the ATPase cycle. Consideration of the range and nature of observed conformations helps to explain the positive and negative cooperativity present in the allosteric transitions of this enzyme. Crystallographic models of the GroEL-GroES-ADP and GroEL-ATP complexes, as well as recent cryo EM studies of GroEL-GroES- and GroEL-nucleotide complexes, have all provided support for direct communication between the ATP binding sites of neighbouring subunits within a contiguous ring (Boisvert *et al.*, 1996; Ranson *et al.*, 2001; Ranson *et al.*, 2006; Xu *et al.*, 1997). Specifically, ATP-dependent movement of the β -2/ β -3 hairpin disrupts a four-stranded β -sheet formed between this hairpin and the β -1/ β -18 elements of an adjacent subunit. The large conformational change induced by GroES binding further alters many inter-subunit interactions (Fig. 5D). The most notable of these is a new contact formed between helix α -M of one subunit and helix α -C of an adjacent protomer. The fact that both of these helices are involved in intra- and inter-subunit conformational changes provides a possible explanation for the all-or-none cooperativity observed to arise from ATP-binding to a single GroEL ring (Bochkareva *et al.*, 1992; Gray and Fersht, 1991). Finally, the inter-ring interface contains a series of structurally important salt bridges that provide direct communication between ATP-binding equatorial domains in either half of the particle (Bartolucci *et al.*, 2005; Cabo-Bilbao *et al.*, 2006; Xu *et al.*, 1997). Cryo-EM studies of GroEL and GroEL-GroES complexes in the presence or absence of ATP have supported this idea by revealing conformational changes in the inter-ring interface (Ranson *et al.*, 2001;

Ranson *et al.*, 2006). These findings provide a physical explanation for the negative cooperativity of ATP binding between the back-to-back GroEL heptamers (Bochkareva and Girshovich, 1994; Horovitz and Willison, 2005; Rye *et al.*, 1997; Rye *et al.*, 1999), and indicate that ATP binding to the *trans*-ring may provide the energy to eject Mg^{2+} -ADP, GroES and substrate allosterically from the *cis*-ring to restart the cycle (Fig. 5D inset). Although the story is far from complete, these studies beautifully illustrate how a combination of low- and high-resolution structural analyses on distinct catalytic intermediates can reveal the dynamic architectural transitions in a complex molecular machine.

Conclusion

In microbes, ATP-dependent protein/nucleic-acid motors and remodelling factors range from the relatively specialized chaperonins to the remarkably diverse ASCE P-loop ATPases. Although each of these families utilizes a unique motor mechanism, certain common principles appear to underlie their functions. The phosphate groups of ATP are generally bound using flexible, often glycine-rich, loops that connect secondary structural elements. Acidic or polar residues are typically used to orient and/or activate a water molecule for nucleophilic attack on the γ -phosphate of ATP, promoting hydrolysis. Active sites are always located near an inter-domain or inter-subunit interface, allowing ATP binding to create or rearrange global protein-protein contacts. Finally, adjacent γ -phosphate (ASCE P-loop, GHL) or Mg^{2+} (actin fold, chaperonin) sensors provided by neighbouring domains or subunits help propagate conformational changes within quaternary motor assemblies. These distinct sensor mechanisms constitute an intriguing difference between the four motor families. In all instances, when linked to disparate substrate binding sites or accessory domains, the conformational changes resulting from nucleotide turnover are capable of propelling a motor along a substrate or forcibly remodelling biological polymers.

Despite the large number of conformational states imaged by high-resolution structural studies over the past few years, much remains to be learned. For the majority of motors, it is still unclear how the conformational changes observed thus far are linked to the directed movement of protein and nucleic acid polymers. Moreover, there is often little direct evidence to explain how ATP binding and hydrolysis are coupled between different subunits in oligomeric motor complexes. These questions still exist, in part, because most structures imaged to date have been obtained in the absence of client substrates. Definitive insights into motor protein activity and the role of ATP await more high-resolution structures examining the effects of nucleotide turnover on protein- or nucleic-acid bound complexes. Such studies will help connect structural models to biochemical and single-molecule efforts to examine motor dynamics in solution. Given that most microbial motor ATPases and their eukaryotic homologues are critical for many fundamental cellular processes, mechanistic understanding of their function is likely to have important consequences for basic science and human health alike.

Supplementary Material

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Fig. 1. Nucleotide binding and conformational changes in RecA-like ATPases

A. Topology diagram of the RecA fold. Conserved motifs and catalytic residues are shown as solid coloured circles (see key). Motifs and catalytic residues conserved spatially in the structure, but not in primary amino acid sequence are shown as open circles. β -sheets and α -helices are coloured yellow and blue respectively. Secondary structure elements shown in grey are not conserved in all members of the family. Abbreviations: RF = arginine finger, CE = catalytic glutamate, W-A = Walker A, W-B = Walker B.

B. The active site of the *Saccharomyces cerevisiae* F₁-ATPase β -subunit (PDB entry 2HLD) (Kabaleeswaran *et al.*, 2006), highlighting the location of catalytic residues common to all RecA-like ATPases. The adjacent α -subunit, which contributes the arginine finger, is shown in dark grey. Magnesium is shown as a yellow sphere. Residues are coloured as in A.

C. Structural superposition of the conserved RecA-fold of the ATP bound (β TP) subunit (dark blue) and the empty (β E) subunit (light blue) of *S. cerevisiae* F₁-ATPase (PDB entry 2HLD) (Kabaleeswaran *et al.*, 2006), with most loops removed for clarity. Arrows highlight ATP-dependent conformational changes discussed in the text. Inset: the location of the superposed subunits with respect to the quaternary structure of F₁-ATPase.

D. ATP-dependent conformational changes between two RecA-like folds illustrated in the structure of *E. coli* UvrD bound to DNA (PDB entry 2IS1 and 2IS4) (Lee and Yang, 2006). ATP is shown as coloured spheres. Domains 1A and 2A comprising the RecA folds are shown in yellow and cyan respectively, while domains 1B and 2B are shown in light and dark grey. DNA illustrated in stick representation is coloured orange.

Fig. 2. Nucleotide binding and conformational changes in AAA+ ATPases

A. Topology diagram of the typical two-domain AAA+ fold. Conserved motifs, catalytic residues and secondary structural elements are coloured as in Figure 1. Abbreviations: RF = arginine finger, CE = catalytic glutamate, W-A = Walker A, W-B = Walker B, SI = sensor I, SII = sensor II.

B. The active site of *Aquifex aeolicus* DnaA (PDB entry 2HCB) (Erzberger *et al.*, 2006) highlighting the location of catalytic residues common to all AAA+ ATPases. Active site elements are coloured as in Figure 1.

C. Structural superposition of the conserved AAA+ fold of ATP-bound (dark blue) and empty (light blue) subunits of *E. coli* HslU (PDB entry 1DO2) (Bochtler *et al.*, 2000), with most loops removed for clarity. Arrows highlight ATP-dependent conformational changes discussed in the text. Inset: the location of the superposed subunits with respect to the quaternary structure of HslU. Note that the β -sheet domain stays relatively immobile while the lid moves, compared to oligomeric RecA-type proteins where the β -sheet can flex considerably (Fig. 1C).

D. ATP-dependent conformational changes between two AAA+ folds illustrated in the structure of the human papillomavirus E1 helicase bound to DNA (PDB entry 2GXA) (Enmark and Joshua-Tor, 2006). ADP is shown as coloured spheres, and the bound chloride ion (which mimics the γ -phosphate) is shown as a green sphere. The cyan and purple subunits are coloured according to the inset. Inset: views of E1 from the bottom up relative to the main figure, illustrating the position of empty and ATP bound subunits within the quaternary structure.

Fig. 3. Nucleotide binding and conformational changes in GHL ATPases

A. Topology diagram of the two-domain GHL fold. The GHKL and transducer domain are labelled. Conserved motifs, catalytic residues and secondary structural elements are coloured as in Figure 1. Roman numerals define conserved motifs of GHL motors.

B. The active site of *A. aeolicus* topoisomerase VI (PDB entry 1MX0) (Corbett and Berger, 2003) highlighting the location of catalytic residues common to all GHL ATPases. Active site elements are coloured as in Figure 1.

C. Structural superposition of the GHL fold of ATP-bound (PDB entry 1MX0) (dark blue) and empty (PDB entry 1MU5) (light blue) B-subunits of *Sulfolobus shibatae* topoisomerase VI (Corbett and Berger, 2003), with most loops removed for clarity. Arrows highlight ATP-dependent conformational changes discussed in the text. Inset: the location of the ATP-bound subunit (shown in dark blue) with respect to the quaternary structure of the topoisomerase VI heterotetramer (with subunits shown in light and dark grey).

D. ATP-dependent conformational changes in a GHL homodimer illustrated by the structures of apo *E. coli* HtpG (PDB entry 2IOQ) (Shiau *et al.*, 2006) and ATP-bound *S. cerevisiae* Hsp90 (PDB entry 2CGE) (Ali *et al.*, 2006). ATP is shown as coloured spheres. Subunits of the homodimer are coloured cyan and dark grey, or yellow and light grey, with the cyan and yellow portion of each subunit corresponding to the conserved GHL fold.

Fig. 4. Nucleotide binding and conformational changes in actin-fold ATPases

A. Topology diagram of the two-domain actin fold. Conserved motifs, catalytic residues and secondary structural elements are coloured as in Figure 1. Roman numerals define conserved motifs of the actin fold. Triangles represent common insertion sites for the Ia and Ib subdomains.

B. The active site of *Thermotoga maritima* MreB (PDB entry 1JCG) (van den Ent *et al.*, 2001) highlighting the location of catalytic residues common to all actin fold ATPases. Active site elements are coloured as in Figure 1.

C. Structural superposition of the actin fold of ADP-bound (PDB entry 1MWM) (dark blue) and apo (PDB entry 1MWK) (light blue) subunits of *E. coli* ParM (van den Ent *et al.*, 2002), with most loops removed for clarity. Arrows highlight ATP-dependent conformational changes discussed in the text.

D. A variety of higher order oligomers observed in the actin-fold family. Structures correspond to *T. maritima* MreB (PDB entry 1JCE) (van den Ent *et al.*, 2001), *E. coli* ParM (PDB entry 2QU4) (Orlova *et al.*, 2007) and *S. cerevisiae* Sse1 (PDB entry 2QXL) (Liu *et al.*, 2007). Subunits of the oligomers are coloured cyan and dark grey, or yellow and light grey, with the cyan and yellow portion of each subunit corresponding to the conserved actin fold. Bound nucleotide is shown as coloured spheres. The actin fold refers specifically to subdomains Ia and IIa, while subdomains Ib and IIb can be more variable. Regions coloured in grey correspond either to the variable Ib and IIb subdomains, or to the substrate binding domains found only in Hsp70-class chaperones.

Fig. 5. Nucleotide binding and conformational changes in chaperonins

A. Topology diagram of the three-domain chaperonin fold. Conserved motifs, catalytic residues and secondary structural elements are coloured as in Figure 1. Roman numerals define conserved motifs of the chaperonin fold.

B. The active site of *E. coli* GroEL (PDB entry 1PCQ) (Chaudhry *et al.*, 2003) highlighting the location of catalytic residues common to type I and type II chaperonins. Active site elements are coloured as in Figure 1. The active-site potassium ion is shown as a purple sphere.

C. Structural superposition of the equatorial and intermediate domains of ADP- and GroES-bound (dark blue) and apo (light blue) subunits of *E. coli* GroEL (PDB entry 1AON) (Xu *et al.*, 1997), with most loops removed for clarity. Arrows highlight ATP dependent conformational changes discussed in the text.

D. ATP-dependent conformational changes within the GroEL-GroES complex (PDB entry 1AON) (Xu *et al.*, 1997). The equatorial (pink and green), intermediate (light orange and cyan) and apical (yellow and light blue) domains of two adjacent protomers are coloured to reveal the range of conformational changes and altered inter-subunit contacts. GroES is coloured orange. The left structure illustrates a dimer interface in the context of the apo *cis*-ring, while the right structure illustrates the dimer interface bound to ADP and GroES in the context of the *trans*-ring. ADP bound to the *cis*-ring is shown as coloured spheres. Inset: Effect of conformational changes on the GroEL₁₄-GroES₇ complex in which the two GroEL rings alternately bind and release GroES and substrate polypeptides. The two GroEL rings are illustrated in light and dark grey, while the subunits shown in the main panel are highlighted in colour.

Table 1

Functional roles of conserved catalytic motifs among motor ATPase families. Abbreviations: W-A = Walker A, W-B = Walker B, RF = arginine finger, CE = catalytic glutamate, H = H loop, D = D loop, Q = Q loop, C = C region or ABC signature motif, SI = sensor I, SII = sensor II. All other motifs are listed as roman numerals.

	P-loop	Metal binding	γ -phosphate sensor	Hydrolysis	Adenine binding
RecA	W-A	W-A	RF	CE	-
		W-B			
ABC	W-A	W-A	C	CE	-
		W-B		D	
				H	
				Q	
AAA+	W-A	W-A	RF	CE	-
		W-B	SII	SI	
GHL	III	I	V	I	II
					IV
Actin	III	I	-	VI	IV
		II			
		III			
Chaperonin	III	I	-	II	V
		II			
		III			
		IV			