Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1659 (2004) 1–18

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

Review

RecA-like motor ATPases—lessons from structures

Jiqing Ye[a,1], Andrew R. Osborne[a,1], Michael Groll[b], Tom A. Rapoport[a,*]

[a] *Department of Cell Biology, Harvard Medical School, HHMI, 240 Longwood Ave., LHRRB 613, Boston, MA 02115, USA*
[b] *Institut fuer Physiologische Chemie, Ludwig Maximilians Universitaet Muenchen, Butenandtstr. 5, Haus B, 81377 Muenchen, Germany*

Received 5 March 2004; received in revised form 15 June 2004; accepted 16 June 2004
Available online 6 July 2004

Abstract

A large class of ATPases contains a RecA-like structural domain and uses the energy of nucleotide binding and hydrolysis to perform mechanical work, for example, to move polypeptides or nucleic acids. These ATPases include helicases, ABC transporters, clamp loaders, and proteases. The functional units of the ATPases contain different numbers of RecA-like domains, but the nucleotide is always bound at the interface between two adjacent RecA-like folds and the two domains move relative to one another during the ATPase cycle. The structures determined for different RecA-like motor ATPases begin to reveal how they move macromolecules.

Keywords: ATPase; RecA; Structure; Motor protein; Oligomerization

1. Introduction

Many ATPases that use the energy of nucleotide binding and hydrolysis for mechanical work have a common structural domain first seen in RecA, an *E. coli* protein involved in DNA recombination [1]. A large number of these enzymes move macromolecules or move along macromolecules; in both situations, the ATPase is moving relative to the macromolecule with either the former or the latter being stationary. Classic molecular motors, such as myosin or kinesin, move along a polymeric filament formed from actin or tubulin, but—with the exception of dynein—the RecA-like motor ATPases use nonfilamentous polypeptide chains or nucleic acid strands as substrates. Some RecA-like ATPases perform just a single round of mechanical work, e.g., they ''pull'' on a protein or ''pry'' apart an oligomeric assembly of proteins.

Several prominent members of the RecA-like motor ATPases are listed in Table 1. The list contains several proteases with associated ATPases that move a polypeptide substrate into a proteolytic chamber for degradation. For example, a hexameric ring of ATPases is located at the base of the 19S regulatory subunit of the proteosome and moves polypeptides into the 20S proteolytic subunit. Similarly, in bacteria, the ATPase rings of ClpA or HslU (ClpY) move polypeptides into the proteolytic chambers formed by ClpP or HslV (ClpQ), respectively. The list includes SecA, an ATPase that transports polypeptides across the bacterial membrane, the ABC transporter PrtD that exports metalloproteases from *Erwinia chrysanthemi*, and TrwB, an ATPase that moves DNA across the bacterial membrane during conjugation. We have also listed several examples for which the movement of macromolecules is more speculative. For example, the disassembly of protein aggregates by Hsp104 might occur by moving a polypeptide chain through the central pore of the hexameric ring of the ATPase and releasing it on the other side of the ring in an unfolded conformation. The ATPase p97 may ''pull'' proteins out of the endoplasmic reticulum membrane and move them into the cytosol. The list contains F1 ATPase, which differs from all other examples by being a rotary motor; the γ-subunit rotates in the central pore of the hexameric arrangement, either generating ATP from a trans-membrane proton gradient, or vice versa, generating a proton gradient from ATP hydrolysis. Another special case is dynein, a molecular motor that moves cargo along microtubules. We also include the clamp loader, an ATPase that opens a dimeric protein

* Corresponding author. Tel.: +1-617-432-0676; fax: +1-617-432-1190.
E-mail address: tom_rapoport@hms.harvard.edu (T.A. Rapoport).
[1] These authors contributed equally to this work.

0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2004.06.003

Table 1
RecA-like motor ATPase

ATPase	Subfamily	Function	
Dimer[a]			
PcrA	Helicase superfamily I	DNA helicase	[6]
Rep	Helicase superfamily I	DNA helicase	[84]
SecA	Helicase superfamily II	protein translocation	[8,9]
HCV NS3	Helicase superfamily II	RNA helicase	[7,85,86]
RecG	Helicase superfamily II	DNA helicase	[87]
UvrB	Helicase superfamily II	DNA helicase	[88–90]
ABC dimer			
Rad50	ABC family	involved in double strand break repair	[10]
MalK	ABC family	Maltose importer	[38,91]
MutS	ABC family	mismatch repair	[92–95]
MJ1267	ABC family	branched-chain amino acid importer	[48]
MJ0796	ABC family	Unknown	[11]
SMC	ABC family	sister chromatid cohesion, chromosome condensation	[96]
MsbA	ABC family	multiple drug exporter, lipid flippase	[56,57]
BtuCD	ABC family	Vitamin B importer	[45]
TAP	ABC family	antigen peptide presentation	[47]
HlyB	ABC family	RTX-toxin translocation	[46]
PrtD[b]	ABC family	metalloprotease translocation	
Pentamer			
Clamp loader	AAA+	Loads clamp onto DNA	[12,97]
Hexamer			
F1-ATPase	F- and V-type ATPase/Rho	proton gradient formation	[13,31,61]
Rho	F- and V-type ATPase/Rho	Transcriptional termination, RNA helicase	[17]
19S regulatory subunit[b]	AAA	translocation of protein into the proteosome chamber	
TrwB	AAA	translocation of DNA across membranes	[14]
HP0525	AAA	translocation of DNA across membranes	[98,99]
FtsH	AAA	protease, retrotranslocation of membrane proteins	[100,101]
HslU(ClpY)	AAA+	protein unfolding and translocation	[18,19]
Dynein[b]	AAA+	microtubule associated motor	[72]
Cdc6/Cdc18[c]	AAA+	assembly of prereplication complex	[102]
SV40 Large T antigen	Helicase superfamily III	DNA helicase	[50]
T7 gp4	Helicase superfamily III	DNA helicase	[15,16]
RepA	Helicase superfamily III	DNA helicase	[49]
RuvB	Helicase superfamily III	DNA branch migration	[103]
Dodecamer			
P97	AAA	membrane fusion, ER protein degradation	[25,26]
NSF	AAA	membrane fusion	[54]
ClpA	AAA+	protein unfolding and translocation into the ClpP proteolytic chamber	[28]
ClpB	AAA+	protein unfolding	[30]
Hsp104[b]	AAA+	protein chaperone	

[a] Two RecA domains in one polypeptide chain.
[b] X-ray structure unknown.
[c] Oligomerization state unclear.

ring, the clamp, for DNA to enter; the clamp subsequently slides along the DNA, allowing the bound replicative polymerase to be processive over long distances. The table also contains several helicases that move along nucleic acid strands and displace a second strand, thereby unwinding base-paired duplexes. A special case is Rho transcription terminator, an RNA/DNA helicase that loads onto mRNA, translocates to the site of transcription, and disengages the polymerase. The list of RecA-like motor ATPases is much longer than shown in Table 1, and in many cases the functions of the ATPases remain unclear.

2. The RecA-like core structure

At the primary sequence level, the RecA-like motor ATPases belong to different classes of P-loop ATPases. These include the AAA+ family (which contains the subfamily AAA), the ABC family, and helicase superfamilies I, II, and III (Table 1) [2,3]. The overall sequence similarity between proteins with the same RecA-like fold is low, confined to short sequence motifs that make up the ATPase site.

The RecA-like fold is characterized by a central β-sheet (the strand order in RecA is 51432) flanked by α-helices (Fig. 1A) [1,4]. A highly conserved sequence motif (A/GxxxxGKT/S, where x is any amino acid) is located at the tip of the β1-strand, between β1 and the following α-helix (shown in red), and is called the P-loop (phosphate loop) or Walker A motif. It is responsible for coordination of the γ-phosphate of ATP during the hydrolysis reaction (see below). The less conserved Walker B motif (ϕϕϕϕDE, where ϕ is a hydrophobic residue) is located in the β3-strand (in blue) [5]. It coordinates the magnesium ion and activates the attacking water molecule (see below). Other conserved motifs are discussed in the following sections.

Fig. 1B shows an alignment of the structures of several members of the RecA-like motor ATPases with the core structure of RecA (the left figures show the alignment, the right ones the localization of the RecA-like domains in the respective complete molecules). The comparison shows that the β-sheet and the loops in the active site are highly conserved, whereas the α-helices on the outside show larger deviations. Invariably, the nucleotide is bound at the interface between two RecA-like domains, but its precise orientation differs among the ATPases.

The ATPases contain different numbers of RecA-like domains (Table 1). Two RecA-like domains in a single polypeptide chain are found in many superfamily 1 and 2 helicases, such as PcrA [6] and NS3 [7], as well as in the protein translocase SecA [8,9]. The ABC transporters contain two RecA-like domains, with roughly twofold symmetry, and two ATP molecules bound at the interface [10,11]. Five RecA-like folds, only a subset of which bind and hydrolyse ATP, are found in the clamp loaders [12]. A hexameric arrangement of RecA folds is found in many proteins, including the F1 ATPase [13], TrwB [14], T7 gp4 helicase [15,16], the Rho transcriptional terminator [17], and the HslU protease [18,19]. Some ATPases, for example, the T7 primase–helicase, thermoautotropicum MCM protein, and RuvB, can form both hexameric and heptameric rings [20–22]. Finally, there is a hexameric arrangement in which two RecA domains are covalently linked. These are stacked on top of one another and form two hexameric rings, resulting in a total of 12 RecA-like domains. Examples include NSF [23,24], p97 [25,26], Hsp104 [27], ClpA [28,29], and ClpB [30].

3. Coupling ATP binding and hydrolysis to conformational changes

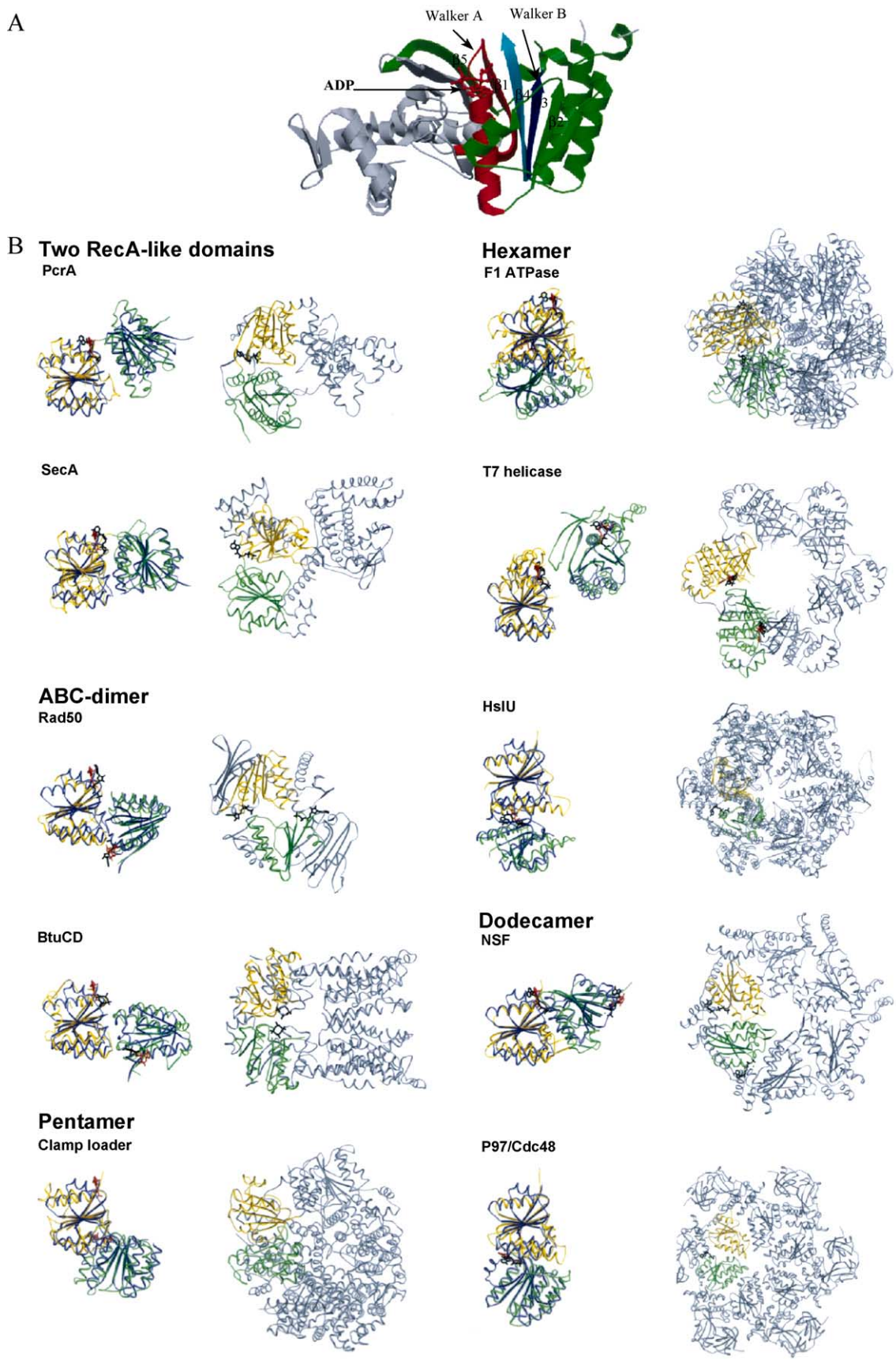
The common structure of the RecA-like motor ATPases indicates that they hydrolyze ATP by the same mechanism. In each case, hydrolysis is catalyzed by stabilization of a pentacovalent intermediate at the γ-phosphate (Fig. 2A). One or more general acids, arginines or lysines, coordinate the γ-phosphate, and a general base, usually a glutamate or aspartate, polarizes the attacking water molecule. The position of important residues can be seen in the structure of F1 ATPase bound to ADP:AlF4 which may represent the transition state or a state shortly thereafter (Fig. 2B and C) [31]. The conserved lysine in the Walker A motif (β Lys162) and an arginine from a neighboring subunit (α Arg373) interact with the β- and γ-phosphate groups. The attacking water is polarized by a glutamic acid residue (β Glu188) which, in contrast to most other ATPases, is not located in the Walker B motif but at the tip of a neighboring β-strand. An Mg^{2+} ion also participates in the stabilization of the transition state. It is coordinated by negatively charged residues at the end of the Walker B strand (β3), and by the side chain hydroxyl group of the Thr/Ser residue in the Walker A motif. Ala158 has also been implicated in stabilizing the transition state [32].

In all RecA-like motor ATPases, the nucleotide is bound primarily to one RecA-like domain, with the phosphates always positioned in a similar manner by the Walker A motif. However, hydrolysis requires residues from a neighboring RecA-like domain, explaining why the ATPase activity in the oligomeric state is often much higher than in the monomer. It is likely that the energy of nucleotide binding or hydrolysis is harnessed for mechanical work, inducing conformational changes of the protein [33].

The simplest possibility is that two RecA-like domains, between which a nucleotide is bound, move relative to one another as rigid bodies. A common mechanism of communication between RecA-like domains is through an "arginine finger", a residue provided by one domain that pokes into the active site of the other. For example, in F1 ATPase, Arg373 of an α-subunit interacts with the nucleotide bound to the neighboring β-subunit (Fig. 2C) [31]. ATP binding will result in the movement of the arginine finger and thus of the entire RecA-like domain to which it is attached.

Members of the AAA family have their arginine finger in the "second region of homology (SRH)" motif, which emerges from the β-strand between the Walker A and B motifs and is in close proximity to the catalytic center of a neighboring RecA-like domain (Fig. 3 shows the SRH motif and arginine finger for the ATPase p97) [34,35]. Although superfamily I and II helicases share only little sequence similarity with AAA family ATPases, they also have an arginine finger (located in motif 6) that serves the same function [36]. In the case of ABC transporters, the signature motif LSGGQ may replace the arginine finger; the backbone amide groups of the glycines could coordinate the γ-phosphate and communicate changes in the active site to the neighboring RecA-like domain [10,37,38] (Fig. 4A).

Although it is attractive to assume that communication between neighboring RecA-like domains is mediated by a single arginine residue, things may be more complicated. The arginine finger concept was initially proposed for Ras, a


A
Walker A
Walker B
ADP
β5
β1
β4
β3
β2
B
Two RecA-like domains
PcrA
SecA
ABC-dimer
Rad50
BtuCD
Pentamer
Clamp loader
Hexamer
F1 ATPase
T7 helicase
HslU
Dodecamer
NSF
P97/Cdc48

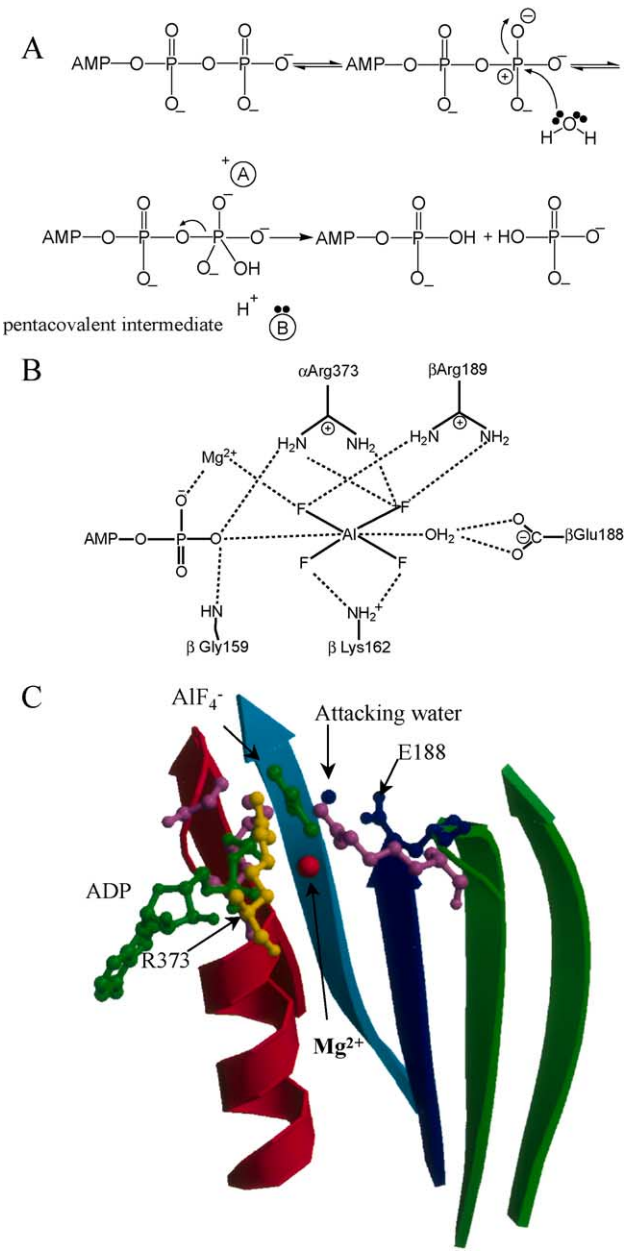


Fig. 2. Mechanism of ATP hydrolysis. (A) Scheme showing how the γ-phosphate is attacked by a water molecule, leading to a pentacovalent transition state that is stabilized by a general acid (A) and a general base (B). (B) Scheme of the transition state in the F1 ATPase in a structure containing ADP and AlF_4^- [31]. The Al atom mimics the phosphorus and the F atoms the oxygens. The transition state is stabilized by a magnesium ion, residues in β-subunit, and the "arginine finger" (Arg373) from a neighboring α-subunit. (C) Structure of the transition state of the F1 ATPase. Some of the residues shown in B are indicated.

small GTPase, whose GTP hydrolysis rate is greatly stimulated by Ras-GAP. The structure of the complex showed that Ras-GAP pokes an arginine finger into the active site of Ras [39] (Fig. 4B). This arrangement is similar to the arginine finger in the RecA-like ATPases (Fig. 3) and the signature motif in ABC transporters (Fig. 4A). Surprisingly, the structure of another small GTPase and its GAP (Ran and Ran-GAP) indicates that no arginine is involved in catalysis; rather the catalytic glutamine is correctly positioned by the GAP [40]. The contacts between the subunits may position other catalytic residues in the RecA-like motor ATPases as well, perhaps in addition to the arginine finger.

An alternative to the rigid body movement of RecA-like domains is that certain residues transmit changes at the nucleotide binding site to other regions of the molecule. This concept is based on the analysis of Ras in its GTP- and GDP-bound forms, which identified two regions (switch I and II) that undergo large conformational changes [41]. In both switch regions, there are one or two "sensor" residues close to the γ-phosphate that may move upon nucleotide hydrolysis and initiate larger conformational changes at the periphery. Similar sensor residues have been proposed for AAA family ATPases and ABC transporters [3]. A potential sensor I residue, usually a serine, threonine, asparagine, glutamine, or histidine, is found at the C-terminus of the β-strand between the Walker A and B strands, close to the γ-phosphate of ATP (Fig. 3). This residue is immediately adjacent to the SRH region in AAA proteins (Fig. 3), which could be the equivalent of the switch II region in GTPases. Some members of the RecA-like motor ATPases have a putative second sensor residue, sensor II, which is located in the C-terminal helical domain (for example Arg205 in RuvB) [42]. Although sensor residues are supposed to passively sense the presence or absence of the γ-phosphate, they may actually participate in ATP hydrolysis and/or binding [43,44]. In addition, from the few structures determined in both ATP- and ADP-bound states, it appears that RecA-like domains undergo only small conformational changes compared to GTPases [41]. It is therefore not yet possible to say whether and how the conformational changes of the active site are propagated to other regions of the ATPases.

4. How is the oligomeric state maintained during domain movement?

Although the RecA-like fold itself is highly conserved among the ATPases, the interfaces between neighboring

Fig. 1. The RecA-like fold. (A) Structure of RecA with bound ADP [1]. The β-strands (51432) are numbered according to their appearance in the primary sequence. The core of the RecA domain is colored and the remainder is gray. The Walker A motif, the preceding β1-strand, and the following α helix are colored red. The Walker B motif is colored blue. The β4-strand between the Walker A and B motifs is colored cyan. (B) Left panels: Alignment of the core of the RecA fold with various ATPases. One RecA-like domain of the indicated ATPases is always shown in the same orientation (in yellow). A neighboring RecA-like domain is shown in green. The core of RecA (in blue), comprising residues 61 to 155 and 167 to 228, was docked into each of these domains individually. Right panels: The position of the two RecA-like domains in the full-length protein is shown (the additional regions are in grey). Nucleotide bound to RecA and to the aligned ATPase is shown in red and black, respectively.

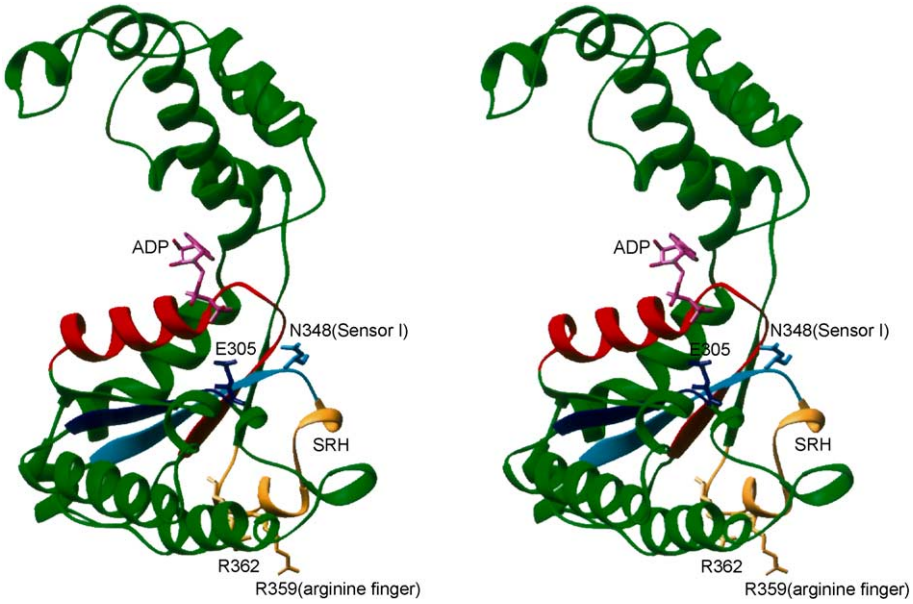


Fig. 3. Residues in proximity of the γ-phosphate of ATP in p97 ATPase. A stereo view of the structure of the D1 domain of p97 with bound ATP is shown, together with residues close to the γ-phosphate [82]. The sensor I region may sense the presence of the γ-phosphate and transmit changes upon ATP hydrolysis to the SRH domain, to which the arginine finger Arg359 is attached. This could communicate the change to the active site in the neighboring subunit (not shown). Changes could also be transmitted through Arg362, which interacts with the water-polarizing Glu305 of the neighboring subunit.

domains are variable. How neighboring RecA-like domains interact may dictate the kind of relative movement between them. Since RecA-like domains generally move relative to one another during the ATPase cycle, the interface cannot be rigid. In some cases, such as for PcrA, the two RecA-like domains may even move apart in one of the nucleotide states. These considerations therefore suggest that regions outside the RecA-like fold maintain the oligomeric state of the ATPases. In the case of proteins with two RecA-like folds, both are in the same polypeptide chain and domains emerging from them interact with one another and keep the RecA-like folds in proximity (e.g. PcrA [6], SecA [8,9]; see Fig. 1B). For many ABC transporters (e.g. BtuCD [45]; see Fig. 1B), the membrane-embedded domains are essential for maintaining the oligomeric state of the ATPase. Indeed, several isolated ATPase domains of ABC transporters are monomeric in the absence of the trans-membrane region [46–48].

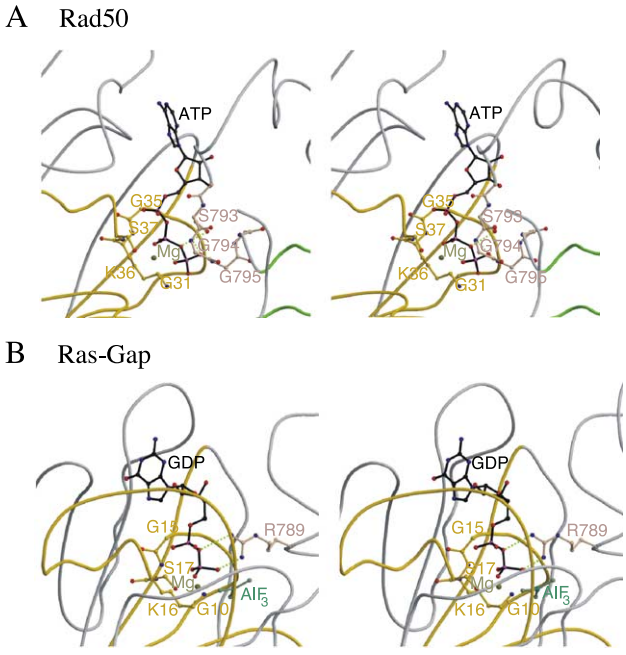


Fig. 4. Comparison of the signature motif in ABC proteins with the "arginine" finger in the Ras/Ras-GAP complex. (A) Stereo view of the nucleotide binding site in the ABC protein Rad50. The signature motif from the neighboring RecA-like fold contains the residues Ser793, Gly794, and Gly795. (B) Stereo view of the nucleotide binding site in the Ras/Ras-GAP complex. Arg789 is the "arginine finger" contributed by Ras-GAP.

We understand best how hexameric arrangements of RecA-like domains are kept together during the ATPase cycle. Although several hexameric ATPases crystallized as spirals or "lock-washer"-like open rings [1,15,17,30], some X-ray structures show closed rings. The hexamerization interface is reasonably conserved within a subfamily. There seem to be two different ways how a hexamer is kept together: either through interactions at the periphery of the hexameric ring or through interactions in an additional ring stacked on top of the hexamer. An example for the first principle is provided by HslU [18,19]. There are two major contact regions between neighboring subunits: one between a loop N-terminal to the β1-strand and the C-terminal helical domain, the other between two helices at the edge of the central β-sheet (Fig. 5A). The first interaction is at the outer rim, contributes most of the

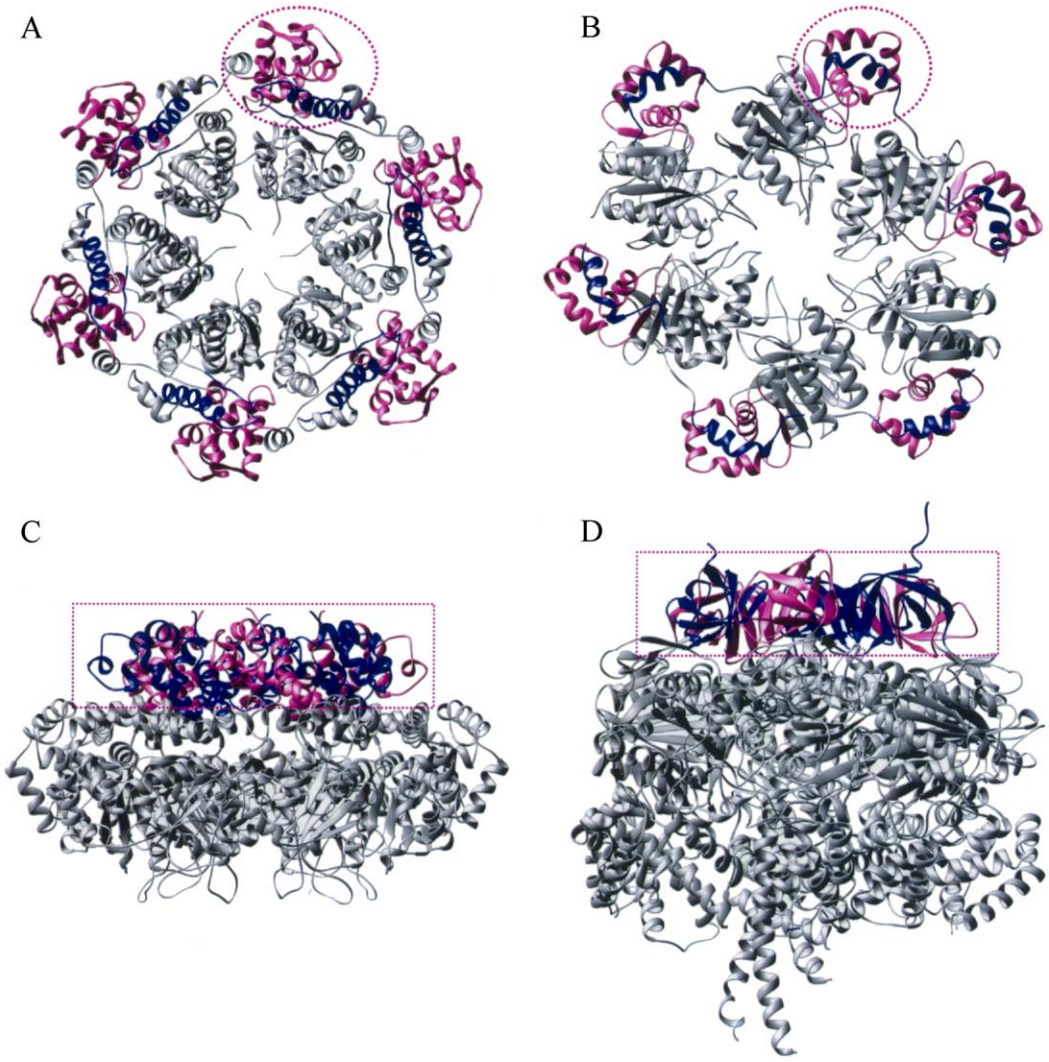


Fig. 5. Two principles of maintaining the oligomeric state of hexameric RecA-like ATPases. (A, B) The main interface between subunits is at the periphery of the HslU [19] (A) and T7 gp4 ATPases [16] (B). The circled regions are mostly responsible for maintaining the oligomeric state. (C, D) The main interface between subunits of the SV40 large T antigen [50] (C) and the F1 ATPase [83] (D) is located in a second ring (boxed area), stacked on top of the ring formed by the RecA-like folds. Regions from neighboring subunits that interact with each other are colored in blue and pink.

buried interface, and is probably largely responsible for maintaining the oligomeric state of the flat ring. During the ATPase cycle, the outer rim remains relatively stationary but provides enough flexibility for the inner RecA-like cores to move up and down.

A similar principle applies to the T7 gp4 helicase [16] and RepA [49]. Here, a C-terminal helical domain is lacking, and the hexamer is kept together by an interaction between a helical region near the edge of the RecA-like fold and an N-terminal helix (shown for T7 gp4 in Fig. 5B). Again, the ring is flat and the peripheral elements probably help to maintain the oligomeric state, allowing the internal parts to move (Fig. 5B).

The second type of hexamerization principle is seen in the helicase domain of the large T antigen of SV40 (Fig. 5C) [50]. The protein forms two rings stacked on top of each other, a smaller one formed by the N-terminal domains (D1), and a larger one formed by interactions of the RecA-like D2 domains. The Zn^{2+}-binding D1 domain is required for hexamer formation. A central hole through which DNA is moved extends through both rings, but different crystal forms show that the diameter of the D2 ring is significantly more variable than that of the D1 ring. Thus, the D1 ring appears to keep the hexamer together, while the D2 ring undergoes larger conformational changes that distort and move DNA.

A similar hexamerization mechanism is seen in the rotary motor F1 ATPase [31]. Again, the hexamer is held together above the ring by a "crown" formed from the N-terminal domains of the subunits, here a β-barrel structure, and the "crown" remains stationary during the ATPase cycle while the C-terminal domains move (Fig. 5D).

ATPases with two hexameric rings stacked on top of each other appear to combine the principles discussed for single-ring ATPases. One of the two rings is responsible for most of the stability of the oligomeric assembly. For example, the D1 ring of the p97 ATPase alone forms hexamers, while the D2 ring alone does not [51,52]. Within the D1 hexamer of p97, the interface between the subunits is similar to that in the T7 gp4 helicase, mostly located at the periphery of the ring. In NSF, the D2 domain is essential for oligomerization, while the D1 domain is not [53], and again, within the D2 ring, it is a peripheral region that provides most of the interface between the subunits [54]. ClpB is similar to NSF, although here the D1 domain also affects hexamerization [30,55].

5. Performing mechanical work

How exactly is motion generated by ATP binding and hydrolysis? Surprisingly, the movements generated in the various RecA-like ATPases are quite different. In the following, we discuss several examples for which a mechanism has been proposed, starting with ATPases that contain two RecA-like folds and moving to those with more. In most cases, the conformational changes during the nucleotide cycle remain largely speculative.

6. Motion in the dimeric RecA-like ATPase PcrA

Among the ATPases with two RecA-like domains, the 3′ to 5′ helicase PcrA is best understood [6]. PcrA is composed of four domains, two RecA-like domains 1A and 2A, with the nucleotide binding site in the cleft between them, and two other domains, called 1B and 2B. These interact with double-stranded DNA, destabilize the duplex, and initiate strand separation. The single-stranded region of the nucleic acid interacts with both RecA-like domains, bridging the cleft between them.

PcrA appears to utilize an inchworm mechanism to move along a nucleic acid strand. Binding of ATP initiates a relative movement of domains 1A and 2A, which leads to closure of the interdomain cleft and movement of domains 1B and 2B (Fig. 6). The movement is mediated by the formation of new contacts between the γ-phosphate of ATP and residues in the two RecA-like domains including the arginine finger (Arg610) in domain 2A. ATP hydrolysis leads to movement of the two RecA domains and to the coordinated binding and release of nucleic acid by each RecA-like domain, resulting in translocation of PcrA along the nucleic acid. Although the motion of the two RecA-like domains is largely a rigid body movement, there are also some small, but important conformational changes within each domain. For example, in the ATP-bound state, domain 1A is thought to release the single-stranded region of the DNA by a small motion of the peptide backbone and a rotation of a phenylalanine side chain into one of the DNA binding pockets.

7. Motion in ABC transporters

The structures of the bacterial multidrug exporter MsbA [56,57] and of the vitamin B12 importer BtuCD [45] have led to models of how the ATPase cycle allows

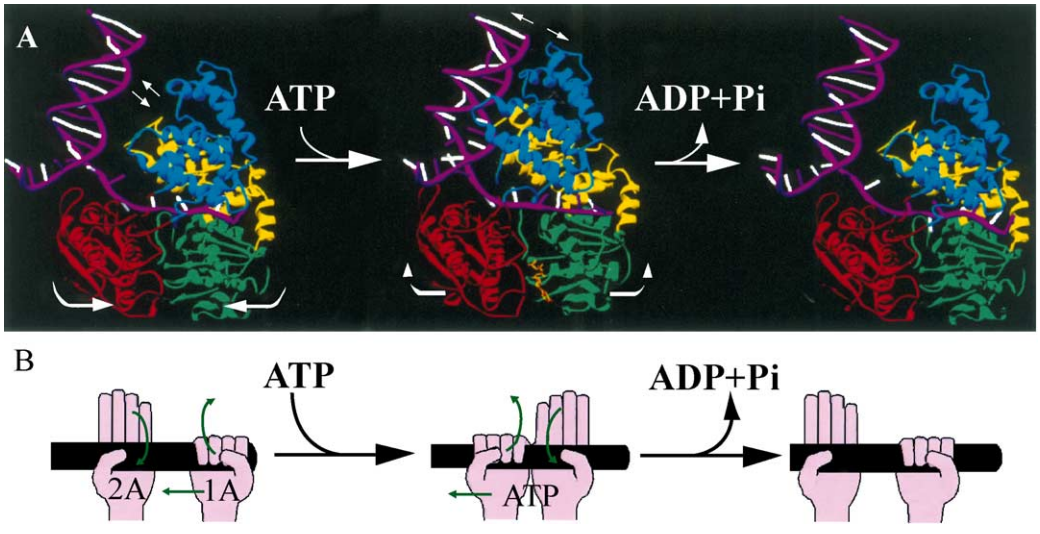


Fig. 6. Inchworm mechanism proposed for the PcrA helicase. (A) Structures of the different states of the PcrA ATPase with bound DNA substrate [6]. The two RecA-like folds (red, domain 2A; green, domain 1A) move towards and away from one another upon ATP binding and hydrolysis, respectively. (B) Scheme illustrating the relative movement of the RecA-like domains and the alternating binding and release of the single-stranded DNA segment. With permission from Velankar et al. [6].

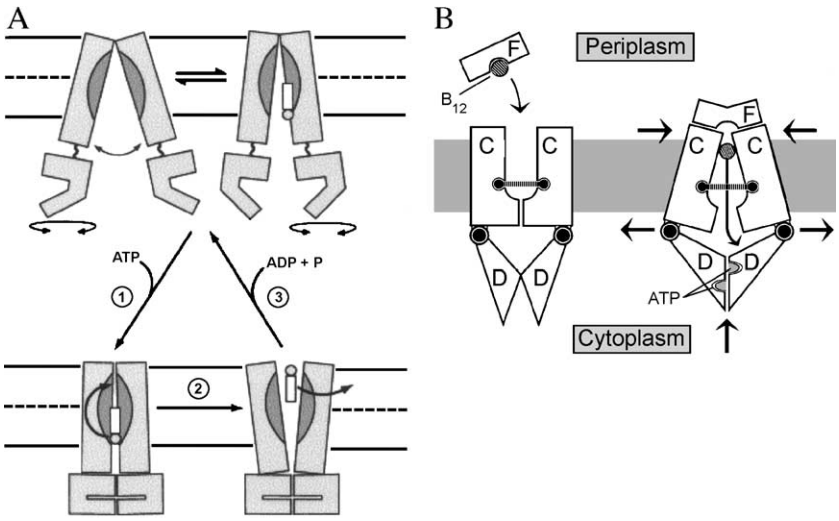


Fig. 7. Proposed mechanisms for the transport of substrates by ABC transporters. (A) The multidrug transporter MsbA binds substrates dissolved in the cytoplasmic leaflet of the membrane in an internal cavity. Upon ATP binding, the two RecA-like folds come into proximity with the active sites at their interface. Following ATP hydrolysis, a conformational change occurs and the substrate is released into the outer leaflet of the membrane. The RecA-like domains may now be free to rotate relative to the membrane-embedded domain [56,57]. (B) The vitamin B12 transporter BtuCD (labeled C and D) first binds the complex of BtuF (labeled F) and B12 from the periplasmic space and releases the substrate into a periplasmic cavity of the membrane protein [45]. A conformational change is induced in BtuCD on the other side of the membrane, allowing ATP binding. This opens the gate towards the cytosol, releasing the substrate into the cytosol. ATP hydrolysis resets the system (not shown). With permission from Chang [57] and Locher et al. [45].

molecules to cross the membrane. In both cases, the functional unit is a dimer, consisting of membrane-embedded domains and cytosolic domains containing RecA-like folds. Although the details of the membrane-embedded segments are different for these proteins, they might both undergo conformational changes that create alternating openings towards the inside or outside of the cell (Fig. 7). In the case of the multidrug transporter MsbA, a hydrophobic compound dissolved in the inner leaflet of the lipid bilayer may enter a cavity in the protein and, following a conformational change, be released into the outer leaflet (Fig. 7A). The conformational change may be caused by the binding of ATP, resulting in the association of the two RecA-like folds (Fig. 7A). Following hydrolysis of ATP, the RecA-like folds may dissociate and would be free to rotate relative to the membrane-embedded domains. The latter is based on the crystal structure of *E. coli* MsbA, in which the ATP binding sites of the two RecA-like folds are some 50 Å apart from each other and point in opposite directions. However, the structure may be nonphysiological, since the interface of the monomers in the observed dimer is small and may be a crystal contact. Indeed, in the structures of *V. cholera* MsbA [57] and of BtuCD [45] the ATP binding sites of the two RecA-like folds are close to and face one another in the nucleotide-free state, similarly to the situation in the Rad50 protein [10]. In the model proposed for BtuCD [45], the first step is the binding of BtuF with a bound vitamin B12 molecule (Fig. 7B). The hydrophilic substrate would enter a cavity at the external side of the membrane-embedded domain. Following a conformational change on the cytoplasmic side of the membrane, ATP binding would occur between the two RecA-like folds, moving them closer to each other as has been seen in MalK [38]. The change would be propagated through an L-shaped loop in the membrane-embedded subunits, spreading them apart and opening the gate towards the cytosol so that the substrate can be released (Fig. 7B). In contrast to the MsbA model, the RecA-like folds and the membrane-embedded domains would remain in contact during the transport cycle.

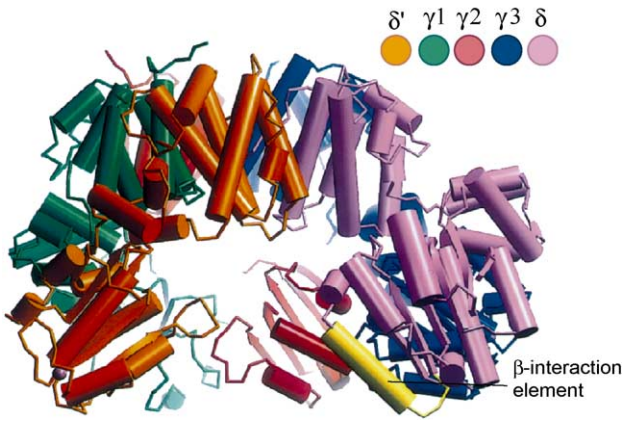


Fig. 8. Structure of the clamp loader. Changes in the relative orientation of the subunits are thought to be induced by ATP binding or hydrolysis [12]. These would open the associated clamp for DNA loading (not shown). With permission from Jeruzalmi et al. [12].

8. Motion in the clamp loader

The *E. coli* clamp loader contains five RecA-like domains, with the subunits arranged in the order δ′, γ1, γ2, γ3, δ (Fig. 8) [12]. δ′ is called the stator, and δ the wrench. In the *E. coli* clamp loader ATP is only hydrolyzed by the γ-subunits. [58]. The structure of the *E. coli* clamp loader showed a partially opened circle (Fig. 8). Initially, it was thought that the δ and δ′ subunits move apart, prying open the clamp for DNA loading. However, recent fluorescence energy transfer experiments [59], and a structure of the yeast clamp loader in a complex with the clamp (J. Kuriyan, personal communication), suggest that the conformational changes are relatively small. Nevertheless, ATP binding and hydrolysis must lead to changes in the relative orientations of the subunits, allowing clamp opening and DNA loading. The clamp loader is an example in which an ATPase performs a single step of mechanical work.

9. Rotary motion in the hexameric F1 ATPase

We understand best how the F1 ATPase uses the energy of ATP binding and hydrolysis to perform mechanical work.

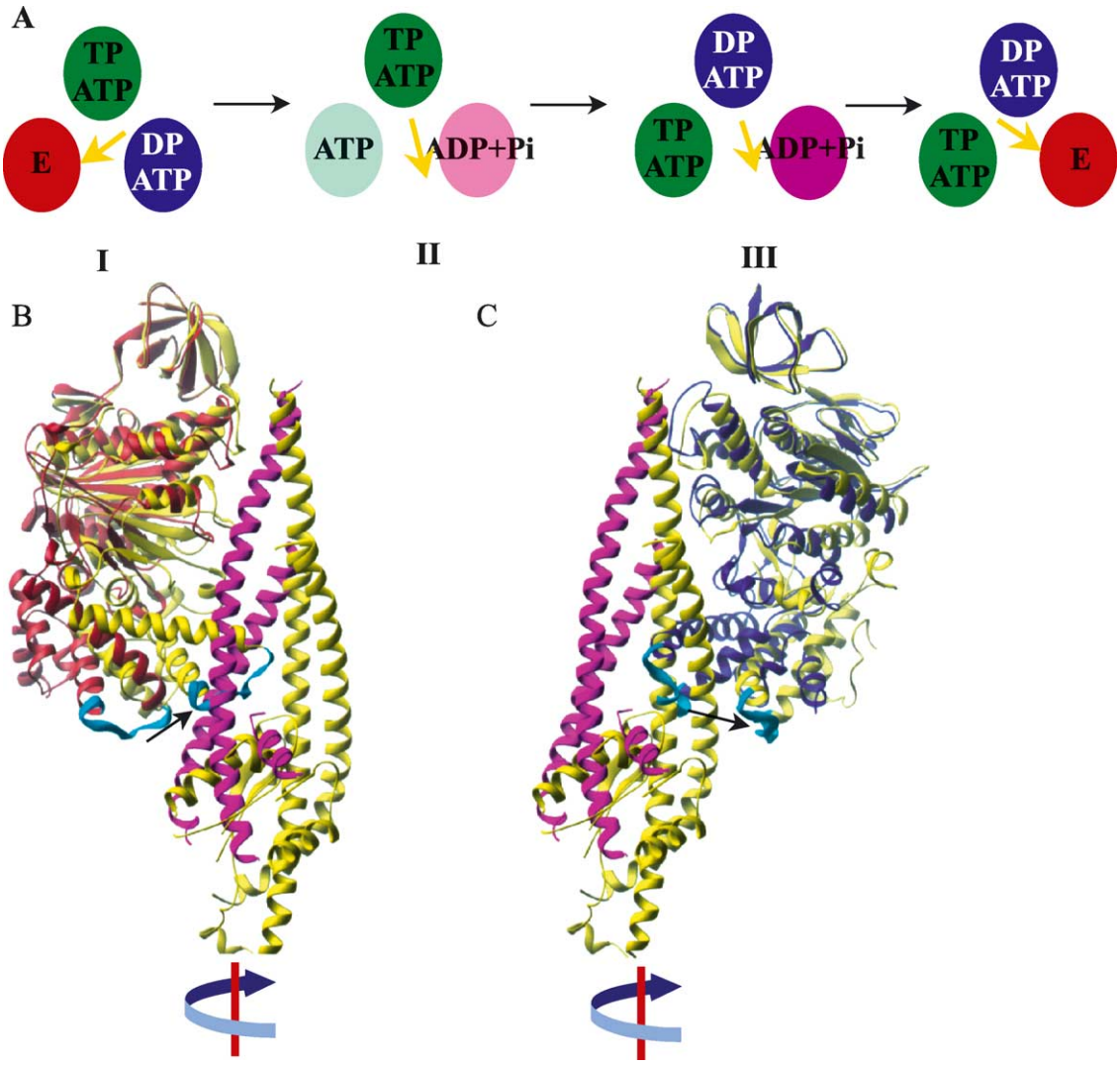


Fig. 9. ATP-dependent movements in the F1 ATPase. (A) Scheme showing the three β-subunits in the hexameric ring during one step of ATP hydrolysis. Scheme I shows the starting point, with the subunit in green with ATP bound (TP), the one in blue in a transition state of ATP hydrolysis (DP), and the one in red with an empty binding site (E). The γ-subunit (yellow) points towards subunit E. The end state, in which previous subunit TP converts into DP, previous E into TP, and previous DP into E is reached through two intermediate states (II and III). (B–C) The β-barrel domains of the E, TP and DP subunits of stage I (represented by PDB 1E1R) were superimposed to the TP, DP and ADP + Pi subunits, respectively, of stage III (represented by PDB 1H8E). The γ-subunit is colored pink and yellow in stages I and III, respectively. (B) The conformational change in the transition from E, stage I (red) to TP (yellow), stage III. The pushing loop (cyan) would clash with the γ-subunit if it did not move. (C) The conformational change in the transition from DP (blue), stage I, to ADP + Pi, stage III (yellow). The β-subunit moves out of the way to accept the incoming γ-subunit [31,83].

The γ-subunit rotates inside the hexameric ring of alternating α- and β-subunits, which both have RecA-like folds and Walker A motifs. However, only the β-subunits hydrolyze ATP, because the α-subunits lack a water-polarizing residue in the ATP binding site. Each step of ATP-binding and-hydrolysis of a β-subunit causes a 90° rotation of the γ-subunit; product release causes an additional 30° rotation [60].

ATP hydrolysis in each of the β-subunits of the hexameric ring occurs sequentially in three steps: an ATP-bound state (TP), a transition state (DP), and a state in which the nucleotide binding site is empty (E) [31]. When ATP binds to the E state of one β-subunit, the γ-subunit rotates and E is converted to TP, the DP state of the next β-subunit is converted to E, and the TP state of the third β-subunit to DP. Each ATP hydrolysis step can be further subdivided into three different conformational intermediates, I to III (Fig. 9A). Intermediates I and III are represented by X-ray structures [31,61], while the structure for intermediate II is not yet available. Nevertheless, the available information allows the reconstruction of the conformational changes that drive the movement of the γ-subunit.

To visualize the conformational changes during the transition from intermediate I to III, we have superimposed the stationary N-terminal β-barrel domains (r.m.s.d. of 0.74 Å for the 216 residues of this region). When ATP binds to state E, the N-terminal half of the RecA-like domain does not change much, while the C-terminal half undergoes a rigid body movement (Fig. 9B). The loop between residues 394 and 400 (colored cyan) appears to push the γ-subunit away, so that it moves anti-clockwise around the sixfold axis (viewed from the C-terminus). The energy for the changes comes from interactions of ATP in its binding site and from hydrogen bonds formed between two β-strands in the RecA-fold [62]. Although the sequence of the pushing loop is conserved, its mutagenesis does not have strong effects, supporting the idea that it simply pushes against the γ-subunit without making specific contacts [63].

Movement of the γ-subunit to its new position in intermediate III would not be possible if the β-subunit in the DP state did not hydrolyze ATP. This induces a conformational change so that the C-terminal half moves outwards, making room for the incoming γ-subunit (Fig. 9C). This movement could even exert some "pulling force".

The transition from intermediate III back to state I involves a further rotation of the C-terminal domain of the β-subunit that was originally in the E state, and an additional small rotation of the γ-subunit [60]. This transition is favored by the reformation of interactions between residues in the pushing loop of the new TP state (Asp394, Glu398) with residue Lys90 of the γ-subunit, as well as between residues of the new E state β-subunit (Asp316, Thr318, and Asp319) with residues Arg254 and Gln255 of the γ-subunit [13]. These "catch" interactions provide resistance required for generating torque force.

The overall picture is that ATP binding induces a conformational change in the E state that induces a C-terminal loop to push the γ-subunit, while at the same time, ATP hydrolysis in the neighboring DP state β-subunit causes a change that makes room for the incoming γ-subunit and may pull on it.

10. Movement of DNA through the central pore in T7 gp4 helicase

The bacteriophage T7 DNA replication system consists of an N-terminal primase and a C-terminal helicase. The helicase functions as a hexamer to unwind duplex DNA at replication forks, moving a single-stranded DNA though the central pore. The primase–helicase complex can also form heptamers with a larger central hole that could accommodate duplex DNA [20], and this may be required for its movement along double-stranded DNA [64]. Nucleotide-dependent conformational changes have only been described for the hexameric state of the helicase.

The closed hexameric ring seen in the X-ray structure of a helicase fragment is a dimer of trimers with subunits in different conformations, called A, B, and C [16]. In each trimer, subunit B is rotated relative to subunit A by 15° and subunit C relative to subunit B by another 15° (Fig. 10A). AMP-PNP is bound to both interfaces. Between the C subunit of one trimer and the A subunit of the other there is a 30° rotation in the other direction, and no nucleotide is bound. Superposition of subunits A, B, and C shows that only the N-terminal helix is different (Fig. 10B), indicating that the RecA-like folds undergo rotations as rigid bodies with a hinge between the N-terminal α-helix and the RecA-like fold. This is distinct from the intra-subunit motion seen with F1 ATPase (see above).

Motion of T7 gp4 helicase is proposed to be triggered by the interaction of an arginine finger from one subunit with the γ-phosphate of ATP bound to another subunit. Upon ATP hydrolysis, the interaction with the arginine finger is broken allowing the neighboring subunit to rotate. This would move the two DNA binding loops inside the pore downwards along the sixfold axis and drag the DNA strand with them (loop 1 in pink, residues 424–439, and loop 2 in blue, residues 464–475; Fig. 10A). Both loops are close to the catalytic center, which explains why DNA stimulates ATPase activity. In the proposed mechanism, two empty subunits in the hexameric ring bind ATP, while two hydrolyze the nucleotide (Fig. 10C). ATP binding would cause a 45° relative rotation between subunit C of one trimer and subunit A of the other, which would place the arginine finger in the correct position for ATP hydrolysis. The movement would

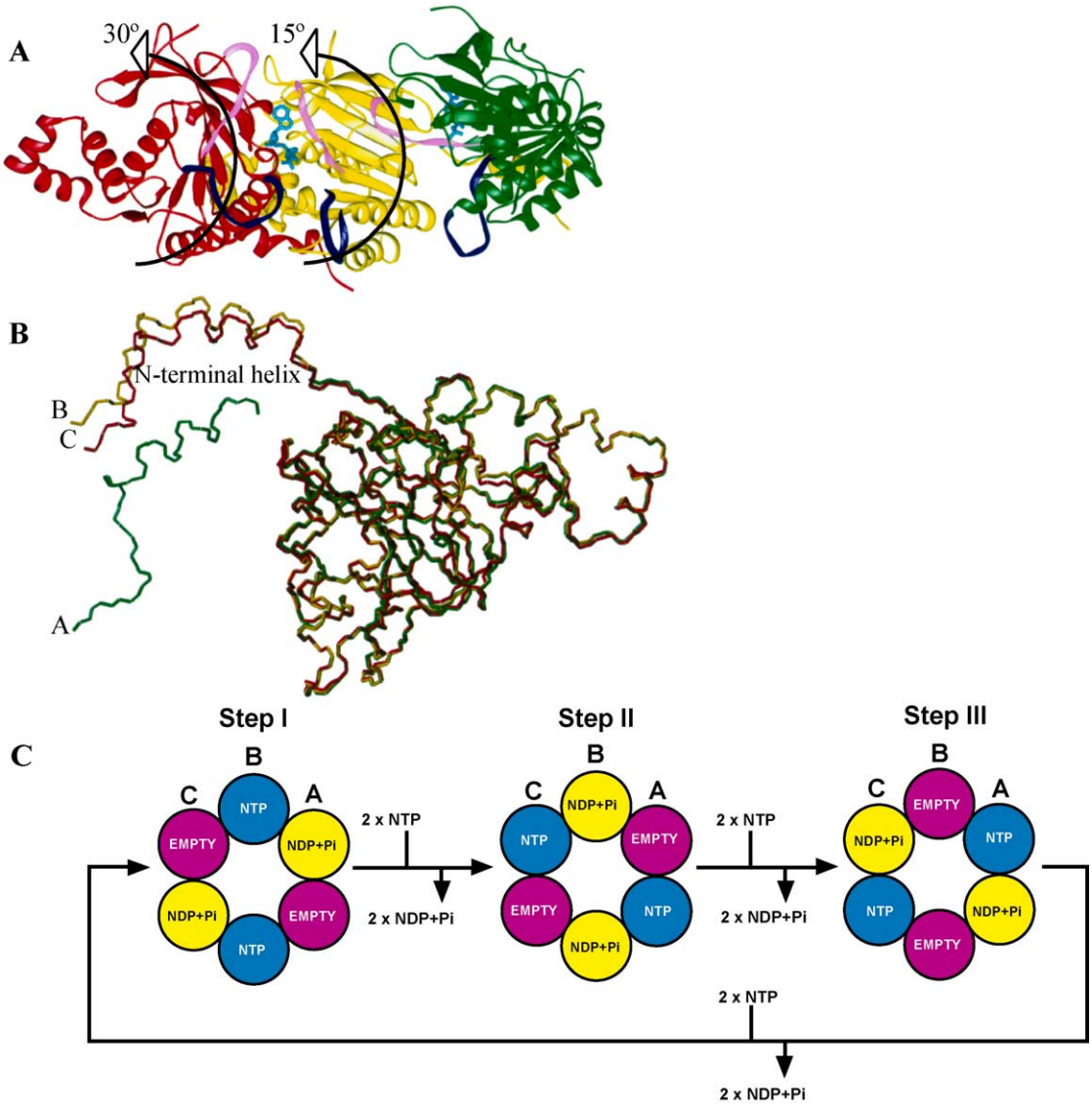


Fig. 10. Proposed nucleotide-dependent movements in T7 gp4 helicase. (A) View of the three subunits, A, B, and C, colored green, yellow and red, respectively, in the hexameric ring of the ATPase. The loops highlighted in blue and pink bind to and move DNA through the central pore. Subunit B is rotated relative to A by 15°, and subunit C relative to B by another 15°. (B) Superposition of the RecA-like folds of the three subunits, showing that only the N-terminal helices differ. (C) Model of sequential ATP hydrolysis in the subunits. The active site of subunit A contains ADP and Pi, that of subunit B contains ATP, and that of C is empty. This is repeated in the second half of the hexameric ring. ATP binding and hydrolysis change the states of the three subunits sequentially [16]. With permission from Singleton et al. [16].

be brought about by a 30° rotation of subunit A in the second trimer and a 15° rotation of subunit C in the first trimer in the opposite direction. The latter would be triggered by hydrolysis in subunit A in the first trimer, causing subunits B and C to move together. At the same time, hydrolysis in A breaks the arginine interaction with B, so that B can rotate back. Despite the elegance of the proposed mechanism of inter-subunit rotation, it should be pointed out that it is based on a single structure, which may not be an intermediate in the catalytic cycle. While the relative orientation of the neighboring subunits changes when a nucleotide is present or absent, the local conformation of the catalytic centre remains the same (Fig. 10B).

The T7 gp4 helicase may provide a paradigm for the large class of hexameric RecA-like ATPase with a central pore through which a macromolecule is transported. In all these cases, the subunits may hydrolyze the nucleotide sequentially and rotate inwards. This, together with alternating binding affinities for the substrate, would drag the polypeptide chain or nucleic acid strand through the central pore. Sequential ATP hydrolysis by the subunits in the ring, similar to what has been proposed for the T7 gp4 helicase, is suggested by the asymmetry of the hexamers

formed by DnaB helicase [65–67], bacteriophage SPP1 gp40 helicase [68], and papilloma E1 helicase [69]. Biochemical studies also show that ATP hydrolysis does not occur synchronously within the ring, and that ATP binds with different affinity to the different subunits (reviewed in Ref. [70]). Since the macromolecular substrate in the pore is not expected to make identical contacts with all subunits in the ring, this could also induce non-synchronous ATP hydrolysis.

Synchronized ATP hydrolysis has, however, been suggested for several RecA-like motor ATPases with hexameric rings, such as p97 and NSF [3]. For example, the electron microscopy structure of p97 [71] is symmetrical and the ATPase sites behave identically. However, F1 ATPase is also symmetrical without Mg^{2+} or the γ-subunit, raising the possibility that symmetry is an artifact of nonphysiological conditions, such as the absence of the macromolecular substrate and/or Mg^{2+}-ATP. On the other hand, it is also possible that, unlike T7 gp4, these ATPases do not move the macromolecule through the central pore, or that ATPases with two stacked RecA rings, such as p97 and NSF, behave differently.

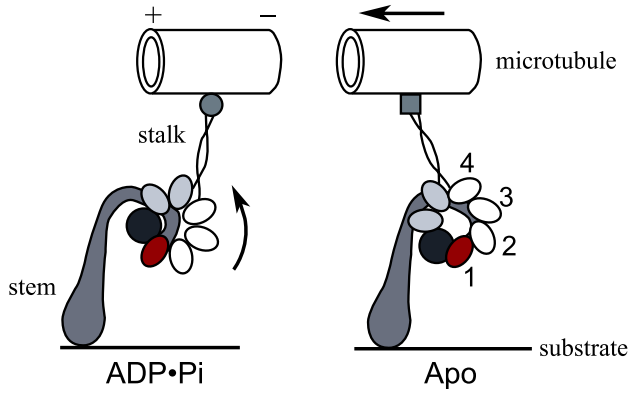


Fig. 11. Proposed mechanism for movement of axonemal dynein along microtubules. Electron microscopy shows that dynein forms a ring with seven domains, probably six contributed by the RecA-like folds and one by the C-terminal region. Emerging from the ring are a stem that binds cargo and a stalk that binds to microtubules. Nucleotide hydrolysis in domain 1 causes a conformational change that is propagated to domain 4, which may change the relative orientation of stem and stalk, allowing movement along the microtubule [72]. With permission from Burgess et al. [72].

11. Movement of dynein along microtubules

Dynein is a motor protein that in axonemes produces the propagating bending motions of cilia and flagella, and in all cells, as cytoplasmic dynein, moves cargo along microtubules. It contains six predicted RecA-like folds in one polypeptide chain, but only one of them hydrolyzes ATP at an appreciable rate. Both axonemal and cytoplasmic dynein form ring structures visible in the electron microscope [72] (Samso and Koonce, personal communication). The 25-Å resolution structure of cytoplasmic dynein shows a seven-member ring with probably six domains contributed by the RecA-like folds, and one by the C-terminal region. Emerging from the ring on opposite sides are two arms, called stem and stalk, which bind cargo and microtubules, respectively. Structures of axonemal dynein determined in two different nucleotide states indicate that the stem and stalk can change their relative orientation, possibly providing the power stroke for movement along the microtubule (Fig. 11). In this case, a conformational change in one RecA-like fold is propagated through the ring, resulting in movement of the arms attached to them. This is a dramatically different mechanism than proposed for other hexameric RecA-like ATPases.

12. Iris-like contraction in SV40 large T antigen

SV40 large T antigen (LTag) melts viral DNA at the replication origin (which it does as a double hexamer) and unwinds duplex DNA at replication forks (which it does as a single hexamer) [73]. A single LTag hexamer consists of a small ring, formed by the D1 domain, and a large ring containing the RecA-like D2 domains, as well as the D3 domains protruding from the D2 domains [50] (Fig. 5C). Although only the nucleotide-free conformation of LTag has been crystallized, a comparison of different crystal forms indicates that the molecule is flexible and that the top and bottom rings can twist relative to one another. This results in the constriction or opening of the channel. DNA passes through the central pore of LTag, and a nucleotide-dependent iris-movement may distort/melt the replication origin. During the subsequent unwinding process, two hexamers assembled in a back-to-back manner could move a single-stranded DNA loop through lateral holes in the hexameric ring. Two loops of single-stranded DNA emerging from the barrel are indeed seen by electron microscopy [73]. LTag may thus undergo both an iris-like motion and a motion similar to that seen in hexameric helicases.

13. Movement of a coiled-coil domain at the periphery of ClpB

ClpB and its eukaryotic homolog Hsp104 are chaperones that can disassemble aggregated proteins. They have two hexameric rings of RecA-like domains stacked on top of each other (Fig. 12A) [27,30]. At the outside of the first ring (D1) are six mobile and extended coiled-coil domains, which contain segments called motifs 1 and 2 at opposite ends. Aggregated proteins may bind to motif 1 and motif 2 of adjacent subunits. A small nucleotide-dependent conformational change in the D1 domain could be propagated into

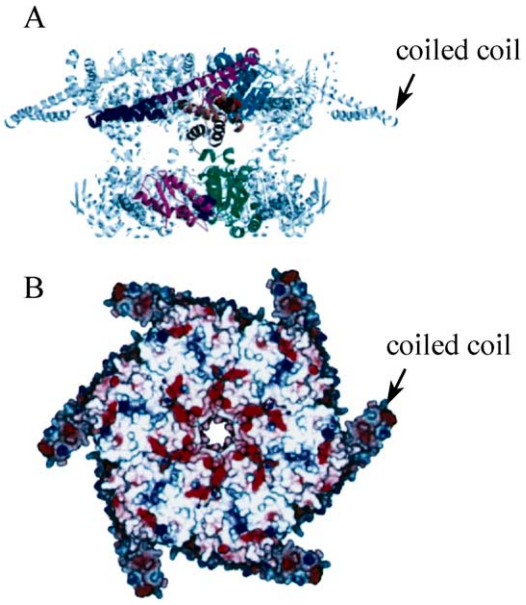


Fig. 12. Coiled-coil domains in the ClpB ATPase. (A, B) Side and top views of the double barrel structure of ClpB. At the outside of the first ring (D1) are six extended coiled-coil domains, which may serve as "crow bars" to pry apart aggregated proteins [30]. With permission from Lee et al. [30].

a large motion of the coiled-coil, which could pry apart the bound aggregate. It has also been proposed that, once the aggregates are smaller, ClpB may also use a translocation mechanism, similar to that discussed for T7 gp4, or a "capture-and-release" mechanism, to further unfold the proteins.

14. Conclusions and open questions

We are beginning to understand how RecA-like ATPases perform mechanical work. Invariably, the nucleotide is bound at the interface between two RecA-like domains and hydrolysis requires residues from both domains. Thus, the relative orientation of the two RecA-like folds is sensitive to the state of nucleotide binding and/or hydrolysis and this can be employed for large-scale conformational changes. However, the details of how ATP binding and hydrolysis is coupled to conformational changes are still largely unclear. As discussed, there are a number of proposals for different ATPases on how motion is generated, but most of these ideas are based on single structures, often without a substrate present. Experience has shown that such speculations often do not stand the test of time. One major direction of future work is therefore to obtain more structures in different nucleotide states, similarly to what has been done in pioneering work on the F1 ATPase. In addition, structures with the substrate present are needed, which is a particular challenge in the case of polypeptide substrates.

The hexameric arrangement of RecA-like domains appears to be common and it may be the one that is closest to a complete picture. For some cases it is clear that a macromolecule—either a nucleic acid or a polypeptide—is moved through the central pore. These ATPases might all use a mechanism similar to that proposed for the T7 gp4 helicase, in which the subunits hydrolyze ATP sequentially and rotate relative to one another, dragging the macromolecule with it [15,16]. However, as discussed before, even for T7 gp4 there are many open questions about the mechanism of converting chemical energy into movement. For the proteosome 19S subunit, for which a structure is not yet available, there is evidence that the polypeptide substrate inserts into the hexameric ATPase ring as a loop [74]. The diameter of the central pore in the associated 20S proteosome is about 13 Å [75], which would be sufficient to accommodate two extended polypeptide strands. In some hexameric ATPases, such as p97, the central opening is narrower and significant conformational changes need to be invoked to allow a polypeptide substrate to move through it [26].

ATPases with two hexameric rings stacked on top of one another are much less understood than those with a single ring. Why does one need two rings in the first place? For example, ClpA contains two stacked RecA rings, while ClpX is a single hexameric ring, but they both perform the same function, i.e., they push a polypeptide substrate into the proteolytic chamber of ClpP [76]. In double-barrel ATPases the two rings might communicate with one another, so that the entire structure essentially acts as a single ring. One may also imagine that the two rings allow the ATPase to switch directions of movement, or that one ring simply serves to keep the other in a hexameric arrangement, similar to the function of the "crown" domains of SV40 LTag or F1 ATPase. Finally, one ring may trigger the opening and closure of the other, thus allowing the loading and unloading of a macromolecule. The ATPase cycle within the rings and the communication between the rings are also unresolved issues. In contrast to single-ring ATPases, there is little evidence that ATP is hydrolyzed sequentially within a ring. In the case of NSF, the second ring (D2) does not hydrolyze ATP at all, although it does bind ATP [53,77]. In the related p97 protein, both rings may hydrolyze ATP, and mutagenesis studies suggest that the D1 and D2 rings alternate in ATP hydrolysis [52]. Surprisingly, it has been reported that the hexameric rings are stacked in opposite orientations in the two related proteins, NSF [23] and p97 [25,26]. For some double-ring ATPases, such as ClpA, it is likely that they move a substrate through the central pore [78], but in other cases, the situation is less clear. For example, hydrolysis in the D1 ring of NSF could move the three molecules of α-SNAP that are bound at the outside of the double barrel, which in turn could pry open the four-helix bundle of the SNARE complex [79]. This

would, of course, be drastically different from a translocation mechanism.

How is a macromolecule loaded into the central pore of a ring structure? Nucleic acids with a free end may simply be loaded head-on, and polypeptide chains might be threaded into the pore either head-on or as loops. In other cases, loading may require opening/closure or disassembly/reassembly of the ring structure. Many helicases, such as DnaB [80] and papilloma virus E1 [81], use specialized loading proteins to gain entry into the interior of the rings. Others, such as the Rho transcription terminator, appear to load without accessory factors. The determined structure of Rho resembles a ''lock-washer'' with a gap at the opening that is wide enough to accommodate single-stranded RNA. This conformation has been proposed to be required for loading [17].

RecA-like ATPases are found in essentially all cellular processes. For many members of this class we do not yet even understand what function they have. However, given that all better understood members perform mechanical work, it seems likely that many of them will also move macromolecules or move along macromolecules. In fact, given the enormous numbers of RecA-like ATPases, it is possible that most mechanical work in cells is performed by RecA-like ATPases. A detailed understanding of their functions, using a combination of structural and biochemical techniques, is thus an important research direction for the future.

Acknowledgements

We thank Tom Ellenberger, John Kuriyan, Briana Burton, Yihong Ye, and Kent Matlack for critical reading of the manuscript. The work was supported by a grant from the NIH to T.A.R., who is a Howard Hughes Medical Institute Investigator. J.Y. is supported by a fellowship from Jane Coffin Childs memorial fund for medical research.

References

[1] R.M. Story, I.T. Weber, T.A. Steitz, The structure of the *E. coli* recA protein monomer and polymer, Nature 355 (1992) 318–325.
[2] A.N. Lupas, J. Martin, AAA proteins, Curr. Opin. Struct. Biol. 12 (2002) 746–753.
[3] T. Ogura, A.J. Wilkinson, AAA+ superfamily ATPases: common structure—diverse function, Genes Cells 6 (2001) 575–597.
[4] L. Pellegrini, D.S. Yu, T. Lo, S. Anand, M. Lee, T.L. Blundell, A.R. Venkitaraman, Insights into DNA recombination from the structure of a RAD51–BRCA2 complex, Nature 420 (2002) 287–293.
[5] E.V. Koonin, A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif, J. Mol. Biol. 229 (1993) 1165–1174.
[6] S.S. Velankar, P. Soultanas, M.S. Dillingham, H.S. Subramanya, D.B. Wigley, Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism, Cell 97 (1999) 75–84.
[7] N. Yao, T. Hesson, M. Cable, Z. Hong, A.D. Kwong, H.V. Le, P.C. Weber, Structure of the hepatitis C virus RNA helicase domain, Nat. Struct. Biol. 4 (1997) 463–467.
[8] J.F. Hunt, S. Weinkauf, L. Henry, J.J. Fak, P. McNicholas, D.B. Oliver, J. Deisenhofer, Nucleotide control of interdomain interactions in the conformational reaction cycle of SecA, Science 297 (2002) 2018–2026.
[9] V. Sharma, A. Arockiasamy, D.R. Ronning, C.G. Savva, A. Holzenburg, M. Braunstein, W.R. Jacobs Jr., J.C. Sacchettini, Crystal structure of Mycobacterium tuberculosis SecA, a preprotein translocating ATPase, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 2243–2248.
[10] K.P. Hopfner, A. Karcher, D.S. Shin, L. Craig, L.M. Arthur, J.P. Carney, J.A. Tainer, Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily, Cell 101 (2000) 789–800.
[11] P.C. Smith, N. Karpowich, L. Millen, J.E. Moody, J. Rosen, P.J. Thomas, J.F. Hunt, ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer, Mol. Cell 10 (2002) 139–149.
[12] D. Jeruzalmi, M. O'Donnell, J. Kuriyan, Crystal structure of the processivity clamp loader gamma (gamma) complex of *E. coli* DNA polymerase III, Cell 106 (2001) 429–441.
[13] J.P. Abrahams, A.G. Leslie, R. Lutter, J.E. Walker, Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria, Nature 370 (1994) 621–628.
[14] F.X. Gomis-Ruth, G. Moncalian, R. Perez-Luque, A. Gonzalez, E. Cabezon, F. de la Cruz, M. Coll, The bacterial conjugation protein TrwB resembles ring helicases and F1-ATPase, Nature 409 (2001) 637–641.
[15] M.R. Sawaya, S. Guo, S. Tabor, C.C. Richardson, T. Ellenberger, Crystal structure of the helicase domain from the replicative helicase-primase of bacteriophage T7, Cell 99 (1999) 167–177.
[16] M.R. Singleton, M.R. Sawaya, T. Ellenberger, D.B. Wigley, Crystal structure of T7 gene 4 ring helicase indicates a mechanism for sequential hydrolysis of nucleotides, Cell 101 (2000) 589–600.
[17] E. Skordalakes, J.M. Berger, Structure of the Rho transcription terminator: mechanism of mRNA recognition and helicase loading, Cell 114 (2003) 135–146.
[18] M. Bochtler, C. Hartmann, H.K. Song, G.P. Bourenkov, H.D. Bartunik, R. Huber, The structures of HsIU and the ATP-dependent protease HsIU–HsIV, Nature 403 (2000) 800–805.
[19] M.C. Sousa, C.B. Trame, H. Tsuruta, S.M. Wilbanks, V.S. Reddy, D.B. McKay, Crystal and solution structures of an HslUV protease–chaperone complex, Cell 103 (2000) 633–643.
[20] E.A. Toth, Y. Li, M.R. Sawaya, Y. Cheng, T. Ellenberger, The crystal structure of the bifunctional primase–helicase of bacteriophage T7, Mol. Cell 12 (2003) 1113–1123.
[21] X. Yu, M.S. VanLoock, A. Poplawski, Z. Kelman, T. Xiang, B.K. Tye, E.H. Egelman, The *Methanobacterium thermoautotrophicum* MCM protein can form heptameric rings, EMBO Rep. 3 (2002) 792–797.
[22] T. Miyata, K. Yamada, H. Iwasaki, H. Shinagawa, K. Morikawa, K. Mayanagi, Two different oligomeric states of the RuvB branch migration motor protein as revealed by electron microscopy, J. Struct. Biol. 131 (2000) 83–89.
[23] J. Furst, R.B. Sutton, J. Chen, A.T. Brunger, N. Grigorieff, Electron cryomicroscopy structure of *N*-ethylmaleimide sensitive factor at 11 A resolution, EMBO J. 22 (2003) 4365–4374.
[24] P.I. Hanson, R. Roth, H. Morisaki, R. Jahn, J.E. Heuser, Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy, Cell 90 (1997) 523–535.

[25] T. Huyton, V.E. Pye, L.C. Briggs, T.C. Flynn, F. Beuron, H. Kondo, J. Ma, X. Zhang, P.S. Freemont, The crystal structure of murine p97/VCP at 3.6A, J. Struct. Biol. 144 (2003) 337–348.
[26] B. DeLaBarre, A.T. Brunger, Complete structure of p97/valosin-containing protein reveals communication between nucleotide domains, Nat. Struct. Biol. 10 (2003) 856–863.
[27] D.A. Parsell, A.S. Kowal, S. Lindquist, *Saccharomyces cerevisiae* Hsp104 protein. Purification and characterization of ATP-induced structural changes, J. Biol. Chem. 269 (1994) 4480–4487.
[28] F. Guo, M.R. Maurizi, L. Esser, D. Xia, Crystal structure of ClpA, an Hsp100 chaperone and regulator of ClpAP protease, J. Biol. Chem. 277 (2002) 46743–46752.
[29] M. Kessel, M.R. Maurizi, B. Kim, E. Kocsis, B.L. Trus, S.K. Singh, A.C. Steven, Homology in structural organization between *E. coli* ClpAP protease and the eukaryotic 26 S proteasome, J. Mol. Biol. 250 (1995) 587–594.
[30] S. Lee, M.E. Sowa, Y.H. Watanabe, P.B. Sigler, W. Chiu, M. Yoshida, F.T. Tsai, The structure of ClpB: a molecular chaperone that rescues proteins from an aggregated state, Cell 115 (2003) 229–240.
[31] R.I. Menz, J.E. Walker, A.G. Leslie, Structure of bovine mitochondrial F(1)-ATPase with nucleotide bound to all three catalytic sites: implications for the mechanism of rotary catalysis, Cell 106 (2001) 331–341.
[32] Y.H. Ko, M. Bianchet, L.M. Amzel, P.L. Pedersen, Novel insights into the chemical mechanism of ATP synthase. Evidence that in the transition state the gamma-phosphate of ATP is near the conserved alanine within the P-loop of the beta-subunit, J. Biol. Chem. 272 (1997) 18875–18881.
[33] M.R. Singleton, D.B. Wigley, Multiple roles for ATP hydrolysis in nucleic acid modifying enzymes, EMBO J. 22 (2003) 4579–4583.
[34] A.F. Neuwald, L. Aravind, J.L. Spouge, E.V. Koonin, AAA+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes, Genome Res. 9 (1999) 27–43.
[35] A. Beyer, Sequence analysis of the AAA protein family, Protein Sci. 6 (1997) 2043–2058.
[36] M.C. Hall, S.W. Matson, Helicase motifs: the engine that powers DNA unwinding, Mol. Microbiol. 34 (1999) 867–877.
[37] P.M. Jones, A.M. George, Subunit interactions in ABC transporters: towards a functional architecture, FEMS Microbiol. Lett. 179 (1999) 187–202.
[38] J. Chen, G. Lu, J. Lin, A.L. Davidson, F.A. Quiocho, A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle, Mol. Cell 12 (2003) 651–661.
[39] K. Scheffzek, M.R. Ahmadian, W. Kabsch, L. Wiesmuller, A. Lautwein, F. Schmitz, A. Wittinghofer, The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants, Science 277 (1997) 333–338.
[40] M.J. Seewald, C. Korner, A. Wittinghofer, I.R. Vetter, RanGAP mediates GTP hydrolysis without an arginine finger, Nature 415 (2002) 662–666.
[41] M.V. Milburn, L. Tong, A.M. deVos, A. Brunger, Z. Yamaizumi, S. Nishimura, S.H. Kim, Molecular switch for signal transduction: structural differences between active and inactive forms of protooncogenic ras proteins, Science 247 (1990) 939–945.
[42] K. Yamada, N. Kunishima, K. Mayanagi, T. Ohnishi, T. Nishino, H. Iwasaki, H. Shinagawa, K. Morikawa, Crystal structure of the Holliday junction migration motor protein RuvB from *Thermus thermophilus* HB8, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1442–1447.
[43] D.A. Hattendorf, S.L. Lindquist, Analysis of the AAA sensor-2 motif in the C-terminal ATPase domain of Hsp104 with a site-specific fluorescent probe of nucleotide binding, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2732–2737.
[44] D.A. Hattendorf, S.L. Lindquist, Cooperative kinetics of both Hsp104 ATPase domains and interdomain communication revealed by AAA sensor-1 mutants, EMBO J. 21 (2002) 12–21.
[45] K.P. Locher, A.T. Lee, D.C. Rees, The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism, Science 296 (2002) 1091–1098.
[46] L. Schmitt, H. Benabdelhak, M.A. Blight, I.B. Holland, M.T. Stubbs, Crystal structure of the nucleotide-binding domain of the ABC-transporter haemolysin B: identification of a variable region within ABC helical domains, J. Mol. Biol. 330 (2003) 333–342.
[47] R. Gaudet, D.C. Wiley, Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing, EMBO J. 20 (2001) 4964–4972.
[48] N. Karpowich, O. Martsinkevich, L. Millen, Y.R. Yuan, P.L. Dai, K. MacVey, P.J. Thomas, J.F. Hunt, Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter, Structure (Camb) 9 (2001) 571–586.
[49] T. Niedenzu, D. Roleke, G. Bains, E. Scherzinger, W. Saenger, Crystal structure of the hexameric replicative helicase RepA of plasmid RSF1010, J. Mol. Biol. 306 (2001) 479–487.
[50] D. Li, R. Zhao, W. Lilyestrom, D. Gai, R. Zhang, J.A. DeCaprio, E. Fanning, A. Jochimiak, G. Szakonyi, X.S. Chen, Structure of the replicative helicase of the oncoprotein SV40 large tumour antigen, Nature 423 (2003) 512–518.
[51] Q. Wang, C. Song, C.C. Li, Hexamerization of p97-VCP is promoted by ATP binding to the D1 domain and required for ATPase and biological activities, Biochem. Biophys. Res. Commun. 300 (2003) 253–260.
[52] Y. Ye, H.H. Meyer, T.A. Rapoport, Function of the p97–Ufd1–Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains, J. Cell Biol. 162 (2003) 71–84.
[53] S.W. Whiteheart, K. Rossnagel, S.A. Buhrow, M. Brunner, R. Jaenicke, J.E. Rothman, *N*-Ethylmaleimide-sensitive fusion protein: a trimeric ATPase whose hydrolysis of ATP is required for membrane fusion, J. Cell Biol. 126 (1994) 945–954.
[54] C.U. Lenzen, D. Steinmann, S.W. Whiteheart, W.I. Weis, Crystal structure of the hexamerization domain of *N*-ethylmaleimide-sensitive fusion protein, Cell 94 (1998) 525–536.
[55] A. Mogk, C. Schlieker, C. Strub, W. Rist, J. Weibezahn, B. Bukau, Roles of individual domains and conserved motifs of the AAA+ chaperone ClpB in oligomerization, ATP hydrolysis, and chaperone activity, J. Biol. Chem. 278 (2003) 17615–17624.
[56] G. Chang, C.B. Roth, Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters, Science 293 (2001) 1793–1800.
[57] G. Chang, Structure of MsbA from *Vibrio cholera*: a multidrug resistance ABC transporter homolog in a closed conformation, J. Mol. Biol. 330 (2003) 419–430.
[58] M.J. Davey, D. Jeruzalmi, J. Kuriyan, M. O'Donnell, Motors and switches: AAA+ machines within the replisome, Nat. Rev., Mol. Cell. Biol. 3 (2002) 826–835.
[59] E.R. Goedken, M. Levitus, A. Johnson, C. Bustamante, M. O'Donnell, J. Kuriyan, Fluorescence measurements on the *E. coli* DNA polymerase clamp loader: implications for conformational changes during ATP and clamp binding, J. Mol. Biol. 336 (2004) 1047–1059.
[60] R. Yasuda, H. Noji, M. Yoshida, K. Kinosita Jr., H. Itoh, Resolution of distinct rotational substeps by submillisecond kinetic analysis of F1-ATPase, Nature 410 (2001) 898–904.
[61] M.A. Bianchet, J. Hullihen, P.L. Pedersen, L.M. Amzel, The 2.8-A structure of rat liver F1-ATPase: configuration of a critical intermediate in ATP synthesis/hydrolysis, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 11065–11070.
[62] A.G. Leslie, J.E. Walker, Structural model of F1-ATPase and the implications for rotary catalysis, Philos. Trans. R. Soc. Lond., B Biol. Sci. 355 (2000) 465–471.
[63] K.Y. Hara, H. Noji, D. Bald, R. Yasuda, K. Kinosita Jr., M. Yoshida, The role of the DELSEED motif of the beta subunit in rotation of F1-ATPase, J. Biol. Chem. 275 (2000) 14260–14263.

[64] D.L. Kaplan, M. O'Donnell, DnaB drives DNA branch migration and dislodges proteins while encircling two DNA strands, Mol. Cell 10 (2002) 647–657.
[65] M.C. San Martin, N.P. Stamford, N. Dammerova, N.E. Dixon, J.M. Carazo, A structural model for the *Escherichia coli* DnaB helicase based on electron microscopy data, J. Struct. Biol. 114 (1995) 167–176.
[66] X. Yu, M.J. Jezewska, W. Bujalowski, E.H. Egelman, The hexameric *E. coli* DnaB helicase can exist in different Quaternary states, J. Mol. Biol. 259 (1996) 7–14.
[67] S. Yang, X. Yu, M.S. VanLoock, M.J. Jezewska, W. Bujalowski, E.H. Egelman, Flexibility of the rings: structural asymmetry in the DnaB hexameric helicase, J. Mol. Biol. 321 (2002) 839–849.
[68] M. Barcena, C.S. Martin, F. Weise, S. Ayora, J.C. Alonso, J.M. Carazo, Polymorphic quaternary organization of the *Bacillus subtilis* bacteriophage SPP1 replicative helicase (G40 P), J. Mol. Biol. 283 (1998) 809–819.
[69] E.T. Fouts, X. Yu, E.H. Egelman, M.R. Botchan, Biochemical and electron microscopic image analysis of the hexameric E1 helicase, J. Biol. Chem. 274 (1999) 4447–4458.
[70] J.P. Richardson, Rho-dependent termination and ATPases in transcript termination, Biochim. Biophys. Acta 1577 (2002) 251–260.
[71] I. Rouiller, V.M. Butel, M. Latterich, R.A. Milligan, E.M. Wilson-Kubalek, A major conformational change in p97 AAA ATPase upon ATP binding, Mol. Cell 6 (2000) 1485–1490.
[72] S.A. Burgess, M.L. Walker, H. Sakakibara, P.J. Knight, K. Oiwa, Dynein structure and power stroke, Nature 421 (2003) 715–718.
[73] R. Wessel, J. Schweizer, H. Stahl, Simian virus 40 T-antigen DNA helicase is a hexamer which forms a binary complex during bidirectional unwinding from the viral origin of DNA replication, J. Virol. 66 (1992) 804–815.
[74] C.W. Liu, M.J. Corboy, G.N. DeMartino, P.J. Thomas, Endoproteolytic activity of the proteasome, Science 299 (2003) 408–411.
[75] M. Groll, T. Clausen, Molecular shredders: how proteasomes fulfill their role, Curr. Opin. Struct. Biol. 13 (2003) 665–673.
[76] R. Grimaud, M. Kessel, F. Beuron, A.C. Steven, M.R. Maurizi, Enzymatic and structural similarities between the *Escherichia coli* ATP-dependent proteases, ClpXP and ClpAP, J. Biol. Chem. 273 (1998) 12476–12481.
[77] M. Sumida, R.M. Hong, M. Tagaya, Role of two nucleotide-binding regions in an *N*-ethylmaleimide-sensitive factor involved in vesicle-mediated protein transport, J. Biol. Chem. 269 (1994) 20636–20641.
[78] T. Ishikawa, F. Beuron, M. Kessel, S. Wickner, M.R. Maurizi, A.C. Steven, Translocation pathway of protein substrates in ClpAP protease, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4328–4333.
[79] R.C. Yu, R. Jahn, A.T. Brunger, NSF N-terminal domain crystal structure: models of NSF function, Mol. Cell 4 (1999) 97–107.
[80] M. Barcena, T. Ruiz, L.E. Donate, S.E. Brown, N.E. Dixon, M. Radermacher, J.M. Carazo, The DnaB.DnaC complex: a structure based on dimers assembled around an occluded channel, EMBO J. 20 (2001) 1462–1468.
[81] Y.S. Seo, F. Muller, M. Lusky, E. Gibbs, H.Y. Kim, B. Phillips, J. Hurwitz, Bovine papilloma virus (BPV)-encoded E2 protein enhances binding of E1 protein to the BPV replication origin, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 2865–2869.
[82] X. Zhang, A. Shaw, P.A. Bates, R.H. Newman, B. Gowen, E. Orlova, M.A. Gorman, H. Kondo, P. Dokurno, J. Lally, G. Meyer, H. Meyer, M. van Heel, P.S. Freemont, Structure of the AAA ATPase p97, Mol. Cell 6 (2000) 1473–1484.
[83] K. Braig, R.I. Menz, M.G. Montgomery, A.G. Leslie, J.E. Walker, Structure of bovine mitochondrial F(1)-ATPase inhibited by Mg(2+) ADP and aluminium fluoride, Struct. Fold. Des. 8 (2000) 567–573.
[84] S. Korolev, J. Hsieh, G.H. Gauss, T.M. Lohman, G. Waksman, Major domain swiveling revealed by the crystal structures of complexes of *E. coli* Rep helicase bound to single-stranded DNA and ADP, Cell 90 (1997) 635–647.
[85] H.S. Cho, N.C. Ha, L.W. Kang, K.M. Chung, S.H. Back, S.K. Jang, B.H. Oh, Crystal structure of RNA helicase from genotype 1b hepatitis C virus. A feasible mechanism of unwinding duplex RNA, J. Biol. Chem. 273 (1998) 15045–15052.
[86] J.L. Kim, K.A. Morgenstern, J.P. Griffith, M.D. Dwyer, J.A. Thomson, M.A. Murcko, C. Lin, P.R. Caron, Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding, Structure 6 (1998) 89–100.
[87] M.R. Singleton, S. Scaife, D.B. Wigley, Structural analysis of DNA replication fork reversal by RecG, Cell 107 (2001) 79–89.
[88] N. Nakagawa, M. Sugahara, R. Masui, R. Kato, K. Fukuyama, S. Kuramitsu, Crystal structure of *Thermus thermophilus* HB8 UvrB protein, a key enzyme of nucleotide excision repair, J. Biochem. (Tokyo) 126 (1999) 986–990.
[89] K. Theis, P.J. Chen, M. Skorvaga, B. Van Houten, C. Kisker, Crystal structure of UvrB, a DNA helicase adapted for nucleotide excision repair, EMBO J. 18 (1999) 6899–6907.
[90] M. Machius, L. Henry, M. Palnitkar, J. Deisenhofer, Crystal structure of the DNA nucleotide excision repair enzyme UvrB from *Thermus thermophilus*, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 11717–11722.
[91] K. Diederichs, J. Diez, G. Greller, C. Muller, J. Breed, C. Schnell, C. Vonrhein, W. Boos, W. Welte, Crystal structure of MalK, the ATPase subunit of the trehalose/maltose ABC transporter of the archaeon *Thermococcus litoralis*, EMBO J. 19 (2000) 5951–5961.
[92] M.S. Junop, G. Obmolova, K. Rausch, P. Hsieh, W. Yang, Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair, Mol. Cell 7 (2001) 1–12.
[93] E. Alani, J.Y. Lee, M.J. Schofield, A.W. Kijas, P. Hsieh, W. Yang, Crystal structure and biochemical analysis of the MutS.ADP.beryllium fluoride complex suggests a conserved mechanism for ATP interactions in mismatch repair, J. Biol. Chem. 278 (2003) 16088–16094.
[94] M.H. Lamers, A. Perrakis, J.H. Enzlin, H.H. Winterwerp, N. de Wind, T.K. Sixma, The crystal structure of DNA mismatch repair protein MutS binding to a G × T mismatch, Nature 407 (2000) 711–717.
[95] G. Obmolova, C. Ban, P. Hsieh, W. Yang, Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA, Nature 407 (2000) 703–710.
[96] J. Lowe, S.C. Cordell, F. van den Ent, Crystal structure of the SMC head domain: an ABC ATPase with 900 residues antiparallel coiled-coil inserted, J. Mol. Biol. 306 (2001) 25–35.
[97] M. Podobnik, T.F. Weitze, M. O'Donnell, J. Kuriyan, Nucleotide-induced conformational changes in an isolated *Escherichia coli* DNA polymerase III clamp loader subunit, Structure (Camb) 11 (2003) 253–263.
[98] S.N. Savvides, H.J. Yeo, M.R. Beck, F. Blaesing, R. Lurz, E. Lanka, R. Buhrdorf, W. Fischer, R. Haas, G. Waksman, VirB11 ATPases are dynamic hexameric assemblies: new insights into bacterial type IV secretion, EMBO J. 22 (2003) 1969–1980.
[99] H.J. Yeo, S.N. Savvides, A.B. Herr, E. Lanka, G. Waksman, Crystal structure of the hexameric traffic ATPase of the *Helicobacter pylori* type IV secretion system, Mol. Cell 6 (2000) 1461–1472.
[100] H. Niwa, D. Tsuchiya, H. Makyio, M. Yoshida, K. Morikawa, Hexameric ring structure of the ATPase domain of the membrane-integrated metalloprotease FtsH from *Thermus thermophilus* HB8, Structure (Camb) 10 (2002) 1415–1423.
[101] S. Krzywda, A.M. Brzozowski, C. Verma, K. Karata, T. Ogura, A.J. Wilkinson, The crystal structure of the AAA domain of the ATP-

dependent protease FtsH of *Escherichia coli* at 1.5 A resolution, Structure (Camb) 10 (2002) 1073–1083.

[102] J. Liu, C.L. Smith, D. DeRyckere, K. DeAngelis, G.S. Martin, J.M. Berger, Structure and function of Cdc6/Cdc18: implications for origin recognition and checkpoint control, Mol. Cell 6 (2000) 637–648.

[103] K. Yamada, T. Miyata, D. Tsuchiya, T. Oyama, Y. Fujiwara, T. Ohnishi, H. Iwasaki, H. Shinagawa, M. Ariyoshi, K. Mayanagi, K. Morikawa, Crystal structure of the RuvA–RuvB complex: a structural basis for the Holliday junction migrating motor machinery, Mol. Cell 10 (2002) 671–681.