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A common mechanism for ATP hydrolysis in ABC transporter and helicase superfamilies

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ABC (ATP-binding cassette) transporters and helicases are large superfamilies of seemingly unrelated proteins, whose functions depend on the energy provided by ATP hydrolysis. Comparison of the 3D structures of their nucleotide-binding domains reveals that, besides two well-characterized ATP-binding signatures, the folds of their nucleotide-binding sites are similar. Furthermore, there are striking similarities in the positioning of residues thought to be important for ATP binding or hydrolysis. Interestingly, structures have recently been obtained for two ABC proteins that are not involved in transport activities, but that have a function related to DNA modification. These ABC proteins, which contain a nucleotide-binding site akin to those of typical ABC transporters, might constitute the missing link between the two superfamilies.

Many biological events such as vectorial pumping of molecules or ions across membranes, DNA modifications, directed movement or assembly of macromolecules, are energetically unfavourable and proteins involved in these processes need energy to function. Usually, these proteins have evolved to harness the chemical energy provided by the hydrolysis of the β–γ phosphate bond of nucleotides[1], mainly ATP or GTP, to trigger conformational modifications essential for their cellular function.

ATP-utilizing enzymes

ABC (ATP-binding cassette) transporters[2,3] are an example of proteins that obtain the energy required for function from nucleotide hydrolysis. These transporters represent one of the largest families of proteins found in all living organisms[4,5]. Members of this family are involved in the transport of a wide diversity of compounds including sugars, ions, peptides and complex organic molecules[6]. In addition, many clinically relevant transporters belong to this family, such as the chloride channel CFTR (cystic fibrosis transmembrane conductance regulator) and the multidrug resistance (MDR) P-glycoprotein[7,8]. The basic structural organization of ABC transporters comprises two membrane-spanning hydrophobic domains, responsible for translocation of the substrate across the membrane, and two hydrophilic domains, which bind and hydrolyse ATP to power the transporter[3]. These four domains are either found as separate polypeptides, as in the prokaryotic histidine permease $HisQMP_2$ (His Q and His M being the membrane subunits and His P, present as two copies, representing the ATP-binding subunit), or fused together in different combinations to form three, two or even a single polypeptide (e.g. the mammalian P-glycoprotein is a single polypeptide)[6]. The highest similarity between all ABC transporters is found within the nucleotide-binding domain (~30% identity), which notably contains the signature of this family: a stretch of ~12 residues usually starting with LSGGQ (Refs 2,5). Although the role of this signature is so far unknown, it is located only a few residues before the Walker-B motif (motif xxxxD, where x is mostly a hydrophobic residue, see hereafter for a thorough description). Surprisingly, a few proteins that are not involved in transport activities also contain this typical signature and are therefore related to the ABC transporters[3]. These ABC-related proteins are usually involved in nucleic acid modification; for example, the UvrA protein of the bacterial nucleotide excision repair complex, the MutS protein of the DNA mismatch repair complex and the Rad50 protein of the DNA double-strand break repair complex[9].

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Table 1. Structural homology between the ABC proteins, helicases and RecA[a,b]

		HisP	MalK	Rad50	MutS	PcrA	Rep	T7 helicase	RecA	eIF4A	NS3
HisP	*Z* score	–	22.3	12.5	10.8	4.5	6.1	8.3	7.4	3.5	2.3
	rmsd	–	3.2	3.4	2.6	3.2	3.7	3.6	3.3	3.9	3.4
RecA	*Z* score	7.4	7.1	8.2	8.0	6.6	7.6	19.4	–	6.0	6.3
	rmsd	3.3	3.8	3.0	4.3	4.2	11.8	2.6	–	3.5	3.6

[a]Abbreviations: ABC, ATP-binding cassette; eIF4A, eukaryotic translation initiation factor 4A (Ref. 24) (PDB entry: 1qva, chain A); HisP, subunit of the histidine ABC transporter[26] (1b0u, chain A); MalK, subunit of the maltose ABC transporter[29] (1g29, chain 1); MutS, DNA mismatch repair protein[31] (1e3m, chain A); NS3, RNA helicase[22] (1hei, chain A); PcrA, DNA helicase[12] (1pjr); Rad50, DNA double-strand break repair protein[30] (1f2u, both chains A and B have been used here as they belong to the same polypeptide, but were overexpressed as separated fragments for the crystallization); RecA, DNA recombination protein[17] (2reb); Rep, DNA helicase[23] (1uaa, chain A); T7 helicase, helicase domain of the T7 DNA helicase–primase[25] (1cr1, chain A).

[b]The comparison between the whole tertiary structure of a protein (here HisP or RecA) and any known protein structure was performed on the Dali server[27,28]. On this server, the strength of the structural similarity is reported as a *Z* score, which is a statistical significance determined empirically as a function of the chain length of the best structural alignment between the two proteins (see Ref. 28). A *Z* score >2 is considered significant. The rmsd (root mean square deviation of superimposed Cα atoms) of the structural alignment obtained from the Dali server is also shown.

Helicases, a family that encompasses many enzymes involved in DNA or RNA unwinding, are another example of ATP-utilizing enzymes[10,11]. These enzymes play major roles in a variety of processes such as DNA replication, repair and recombination, as well as in transcription, which probably explains their apparent disparate nature with regard to sequence, substrate specificity and oligomeric state[12]. Despite a low overall sequence similarity, conserved motifs have been identified between the helicases, which allows their classification into five different subfamilies[13,14].

Walker A and B nucleotide-binding motifs

Both helicases and ABC proteins contain two well-characterized ATP-binding signatures in their primary structures, known as the Walker A- and B-motifs[15]. Initially, the A-motif, A/Gx_4GKT/S, was found in some ATP-utilizing enzymes; for example, RecA, the α and β subunits of F_1-ATPase, and myosin[15]. This motif was then later discovered in many GTP-utilizing enzymes (or G proteins), such as EF-Tu and rasP21 (Ref. 16). The B-motif was also detected in some ATPases, but its sequence was much less conserved than that of the A-motif. The B-motif is composed of four residues, almost exclusively hydrophobic, that form a putative β strand that is followed by an Asp residue[15]. Elucidation, in the presence of ADP-Mg, of the first crystal structure of an ATPase harbouring both the Walker A- and B-motifs, namely the RecA protein involved in homologous DNA recombination, revealed a new nucleotide-binding architecture distinct from that exhibited by the G proteins as exemplified by RasP21 (Ref. 17). However, both RecA and G proteins exhibited a high structural similarity around the A-motif. This motif invariably follows a β strand, forms a loop that wraps around the polyphosphate moiety of the bound nucleotide and is followed by an α helix[18,19]. Therefore, this motif was also referred to as the P-loop[16]. In addition, the RecA structure showed that the Asp residue of the B-motif was indeed located at the end of a β strand and that its function was to interact with the magnesium ion that chelated the bound nucleotide[17].

A unique ATP fold for proteins containing both Walker A- and B-motifs?

Resolution of the F_1-ATPase structure indicated that both its α and β subunits had a nucleotide-binding fold similar to that of RecA (Ref. 20), which led to the proposal that RecA was a paradigm for all proteins sharing both canonical Walker A- and B-motifs[21]. Consistent with this, 3D structures of several helicases displayed a nucleotide-binding domain related to that of RecA (Refs 12,22–25), although RecA belongs to a different family of DNA-repair proteins with no sequence similarity to the helicases except for the Walker A- and B-motifs[9]. More recently, the structure of HisP, the nucleotide-binding subunit of the histidine ABC transporter, was solved[26]. Surprisingly, Hung *et al*.[26] reported that this protein exhibited only limited similarities with RecA, and with the α and β subunits of F_1-ATPase.

Here, we have investigated this matter by using the Dali algorithm, which allows an objective comparison between the 3D structures of two proteins[27,28]. Apart from the recently crystallized MalK subunit of the maltose ABC transporter[29], the two closest structural neighbours of the HisP protein identified by the search were the DNA double-strand break repair protein Rad50 and the DNA mismatch repair protein MutS (Refs 30–32). In structural comparisons with HisP, these two ABC-related proteins[9], whose 3D structures have recently been determined, gave *Z* score values (statistical significance of the best structural alignment; the higher the value the more significant the structural similarity) of 12.5 and 10.8, with rmsd (root mean square deviation; the lower the value, the closer the two structures) values for superimposed Cα atoms of 3.4 Å and 2.6 Å, respectively (Table 1). Interestingly, the next two structural neighbours identified by the search were the RecA protein and the helicase domain of the bacteriophage T7 helicase–primase with *Z* scores of 7.4 and 8.3, and rmsd values of 3.3 Å and 3.6 Å, respectively. The structure of the bacteriophage T7 helicase–primase has previously been reported to be remarkably similar to that of RecA (Ref. 25 and Table 1). Other

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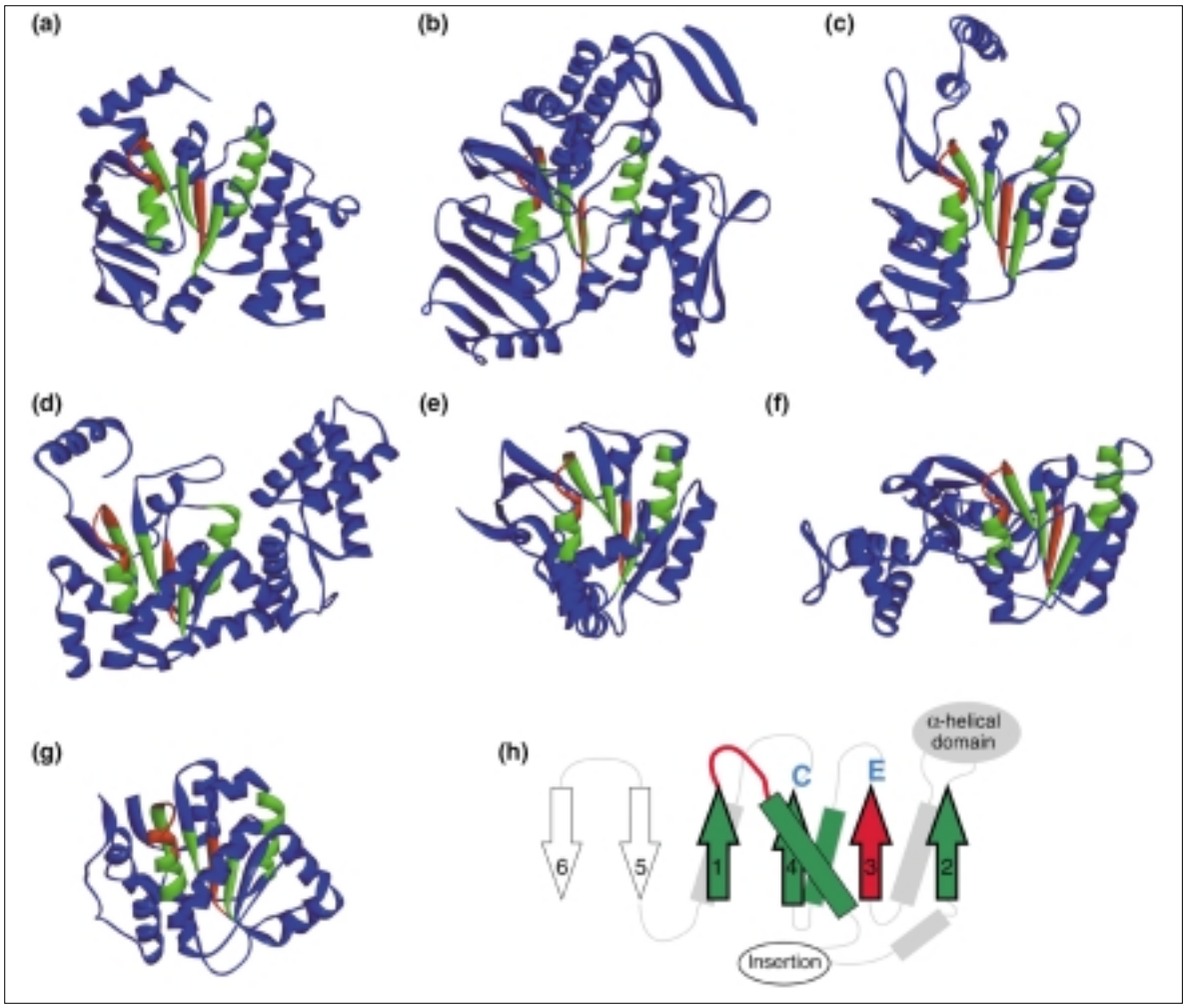

Fig. 1. Topology of the nucleotide-binding fold of ABC (ATP-binding cassette) proteins, helicases and RecA. (a) HisP, (b) Rad50, (c) MutS, (d) PcrA (only the first half of the PcrA structure is shown, truncated after residue 305), (e) helicase domain of the T7 DNA helicase–primase, (f) RecA, (g) eIF4A. Only the HisP structure is used as a representative of the ABC transporter family because the nucleotide-binding site of MalK is highly homologous to that of HisP. Likewise, the structure of the Rep DNA helicase, which is highly homologous to that of PcrA, is not shown. In the structure of the NS3 RNA helicase, the N-terminal part that presumably contributes to the adenine binding subsite was missing and the structure is therefore not included here. The 3D structures were drawn using WebLab Viewer (http://www.msi.com). The common core for the nucleotide-binding fold for both structures is shown in red (Walker A- and B-motifs) and green. The same colour coding is used for the scheme of the HisP structure (h) with the α helices represented as cylinders and the β strands as arrows. E represents a putative catalytic carboxylate residue and C a putative residue involved in triggering the conformational changes that occurred during ATP hydrolysis. For RecA and the T7 DNA helicase domain, the proposed catalytic carboxylate residue is located at the end of β strand number two (corresponding to the fourth strand in the HisP scheme).

members of the helicase family, including the highly homologous Rep (Ref. 23) and PcrA (Ref. 12) proteins, the translation initiation factor eIF4A (Ref. 24) and the NS3 RNA helicase[22], also displayed significant homology with HisP, with *Z* scores ranging from 6.1 for Rep to 2.3 for NS3, and rmsd values from 3.2 Å for PcrA to 3.9 Å for eIF4A. Using RecA as a query, these four helicases, which were shown to contain at least one RecA-like domain[12,33], gave *Z* scores between 6.6 and 6.0, with a rmsd between 3.5 Å and 4.2 Å (except for Rep, which had a *Z* score and rmsd of 7.6 and 11.8 Å, respectively). Overall, these results indicate that there is a significant structural homology between all these proteins that was previously overlooked between the ABC proteins, the RecA and the helicase proteins.

As anticipated, the homology between all these proteins occurs mainly at their ATP-binding site (Fig. 1). The common core comprises a central pleated β sheet formed by four parallel β strands flanked by two α helices. If the β strand that precedes the A-motif is defined as number one in the β sheet, the B-motif is invariably located at the third β strand position in the sheet, regardless of its order of appearance in the protein sequence (see subsequent text). Also, the second β strand in the primary structure is always located at the fourth position in the β sheet (Fig. 1h). Otherwise, the order of the β strands in each β sheet is variable. For instance, this order is 65**14<u>3</u>2** in HisP (the common core of the β sheet is shown in bold and the position of the B-motif is underlined), 6**15<u>4</u>2**3 in PcrA and 07**16<u>5</u>2**43 in eIF4A (the β strand numbered 0 indicates that, in the protein sequence, this β strand is located before that preceding the A-motif). The orientation of each

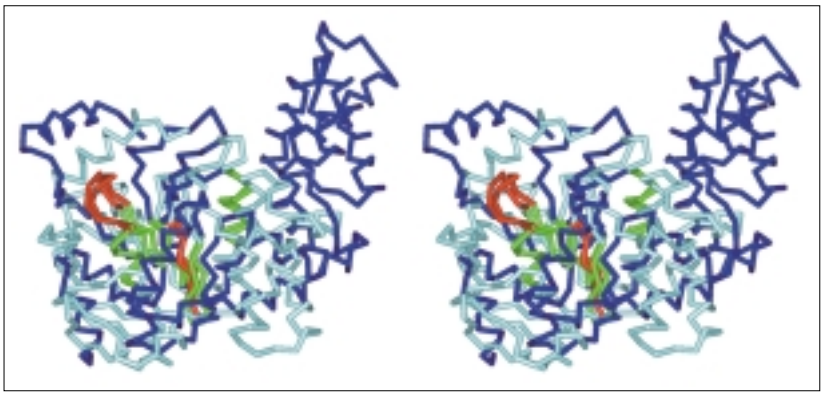

Fig. 2. Stereo-view of the superimposed nucleotide-binding fold of HisP and PcrA. The HisP and PcrA structures are shown in light and dark blue, respectively. The red and green colours represent the common core for the nucleotide-binding fold, with the Walker A- and B-motifs being depicted in red. These two structures were superimposed on the most conserved elements (red and green) by using the tools of the ANTHEPROT program[49]. Only the first half of the structure of PcrA is shown (truncated after residue 305).

additional β strand is either parallel or anti-parallel to the common core of the β sheet. The spacing between the Walker A- and B-motifs in the primary structure is also variable, even within each family. The shortest spacing is found for RecA and MutS (74 and 75 residues, respectively) whereas the largest is seen with Rad50 (788 residues). This variability is a consequence of the insertion of additional domain(s) comprising either a huge coiled-coil region in Rad50 (Refs 9,30), an α-helical domain in the PcrA helicase[12], or both an α-helical domain and an insertion in HisP (Fig. 1h). This insertion of ~40 residues in HisP contributes to a different domain of the protein that was proposed to be involved in dimerization[26], but whether or not this additional domain is indeed responsible for the dimerization of ABC proteins in general is still a matter of debate[29,30,32]. Superposition of the structures of the ABC protein HisP and the helicase PcrA (Fig. 2) gave an rmsd value for the ATP-binding core of 2.6 Å for 49 Cα atoms, showing that there is indeed a good conservation for the fold of the ATP-binding site.

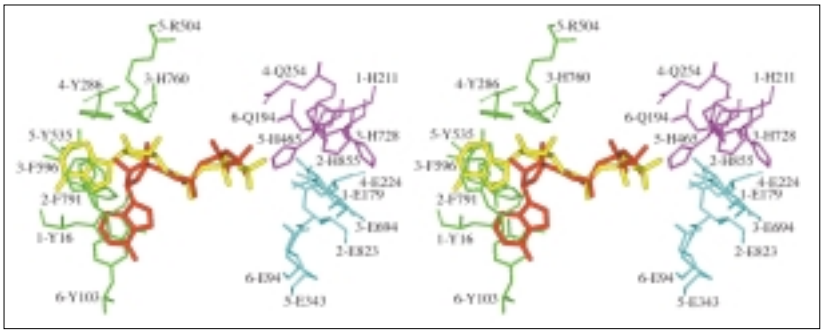

Fig. 3. Stereo-view of some residues of the nucleotide-binding fold of ABC (ATP-binding cassette) proteins, helicases and RecA. The first number refers to the protein structure as follows: 1, HisP; 2, Rad50; 3, MutS; 4, PcrA, 5, T7 DNA helicase domain; 6, RecA. These structures have been superimposed as in Fig. 2 and only a few crucial residues involved in base stacking (green), or putatively either in catalysis (cyan) or transmission of conformational modification (purple) are shown. In the Rad50 structure, F791 is provided by the second subunit of the dimer. The Walker A- and B-motifs and the residues from the eIF4A structure have been omitted. The two ATP molecules have been added subsequently from the HisP (red) or Rad50 (yellow) structures.

Crucial residues occupy a similar spatial position

Interestingly, superposition of all 3D structures from Fig. 1 reveals that, in addition to the well-characterized Walker A- and B-motifs, additional residues that might be crucial for ATP binding and/or hydrolysis occupy a similar spatial position (Fig. 3). Notably, a Glu residue located next to the Asp residue of the Walker B-motif in ABC proteins and in most helicases (Figs 3,4; cyan residue) points towards the γ-phosphate of the bound nucleotide. This residue is conserved in the majority of helicases, in particular in some RNA helicases in which it is part of a modified version of the Walker B-motif characteristic of these proteins, known as the DEAD, DEAH or DExH box[14,34]. The role of this residue in helicase function has been emphasized in several studies in which its mutation drastically reduced, or totally abolished, the ATPase activity without affecting the nucleotide-binding ability of the enzyme[35–37]. In ABC proteins, this Glu is conserved[38], except in a few eukaryotic transporters in which it is replaced by either a Gln, Asn or Ser residue in one of the two nucleotide-binding domains[4]. Based on the HisP structure, it was proposed that either this Glu (E179) or a Gln (Q100) residue activates a catalytic H_2O molecule involved in hydrolysis of the γ-phosphate[26]. Although recent mutational studies of the ABC-transporter Mdr1 definitely ruled out this possibility for the Gln residue[39], these results left some uncertainties about the role of the Glu. Replacement of this residue with a Gln still allowed the formation of a transition state, apparently subsequent to the hydrolysis of the γ-phosphate, although the overall ATPase activity of the protein was completely abolished[38]. Therefore, Urbatsch *et al.*[38] suggested that this Glu residue might be involved in the release of ADP-Mg rather than in the activation of a catalytic H_2O molecule. However, it can be argued that a Gln instead of a Glu residue would still allow, albeit less efficiently, the activation of an H_2O molecule as was proposed, for instance, in the case of RasP21 (Refs 40,41). In RecA, T7 DNA helicase and the β subunit of F1-ATPase (as in ABC proteins and the majority of helicases), the proposed catalytic Glu residue originates from the end of the fourth, rather than the third, β strand[17,20,25] (Fig. 1). In F1-ATPase, site-directed mutagenesis combined with chemical modifications have shown unambiguously that this Glu is indeed the catalytic residue[42], but this is the only extensive characterization of the role of this residue reported so far. Based on our comparison, we propose that, regardless of its position in each protein sequence, the Glu shown in Fig. 3 is the catalytic residue. During the evolution of nucleotide-binding sites, this residue would have occurred at the end of either the third or the fourth β strand in the β sheet.

The specific Gln residue in RecA (Q194 in Fig. 3) has been proposed to act as a γ-phosphate sensor to

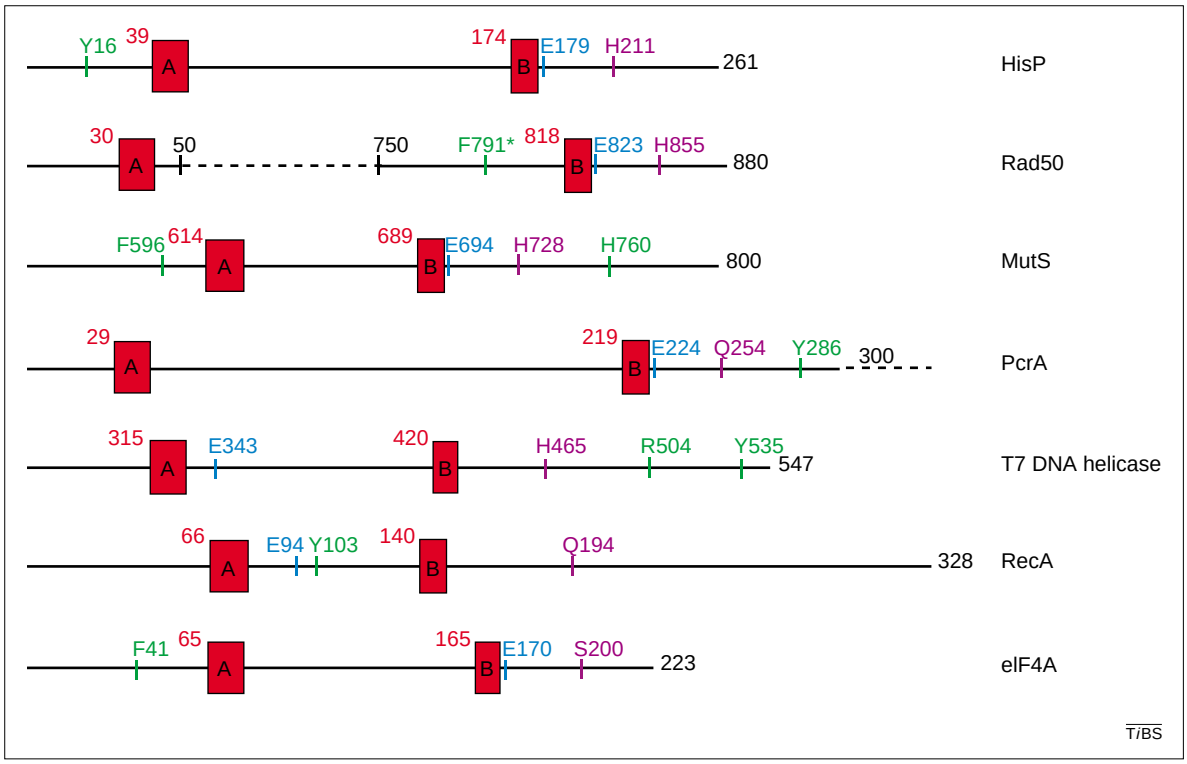

Fig. 4. The structures of ABC (ATP-binding cassette) proteins, helicases and RecA. Residues highlighted in Fig. 3 are shown here by using the same colour coding: residues involved in base stacking (green), or putatively either in catalysis (cyan) or transmission of conformational modification (purple). The red boxes represent the Walker A- and B-motifs. The asterisk in the Rad50 structure indicates that this residue is provided by the second subunit of the dimer. For eIF4, although an indirect role in transmission of the conformational modification has been proposed for this protein[24], we have also displayed the most likely equivalent residue, namely S200, based on the superimposition of its structure with those shown in Fig. 3.

propagate the conformational changes from the nucleotide-binding site to residues involved in DNA binding[17]. Mutations of this residue in RecA gave strong support to this proposal as the mutant proteins were unable to promote the stimulation of the ATPase activity induced by DNA binding, in contrast to the wild-type enzyme[43]. Also, the basal ATPase activity of the mutant proteins was unaltered compared to that of the wild-type enzyme, showing that Q194 is not a crucial residue for the intrinsic ATPase activity of the protein. Mutational analysis of the equivalent residue in PcrA (Q254) also supported that this Gln residue is the key initiator for transmitting the conformational modification from the ATP-binding site to the DNA-binding site. Indeed, the Q254 PcrA mutants were unable to couple ATPase activity to DNA unwinding[44]. Figure 3 shows that a highly conserved His residue, for instance His211 in HisP, occupies a similar spatial position in ABC proteins. Interestingly, a His residue instead of a Gln is also found at this crucial position in the T7 DNA helicase[25] (Fig. 4, purple residues) and, conversely, a few ABC transporters have a Gln instead of a His residue at this position[4]. It is therefore conceivable that, irrespective of the nature and function of the protein, either a Gln or His residue can act as a γ-phosphate sensor. This is the first committed step for transducing the energy from the ATP-binding site to a site (or domain) specific for each protein function.

Residue(s) interacting with the adenine moiety of the bound nucleotide are more scattered than those interacting with the polyphosphate moiety, in both the tertiary (Fig. 3, green residues) and the primary (Fig. 4) structures. This is related to the flexibility of the nucleotide allowing the adenine ring to adopt different conformations according to the location of interacting residue(s), as illustrated here for the bound ATP in two ABC proteins. A similar observation has been made for the bound nucleotides in RecA and PcrA structures[12,44]. The recent observation that, in the MalK protein, the electron density was only seen for the pyrophosphate moiety of the bound ADP (Ref. 29) might be related to this. Such a conformational adaptability of the adenine ring explains why the interacting residue(s) are so scattered. However, both the nature of the residue (i.e. at least one hydrophobic residue) and the type of interaction (stacking) are conserved among the different proteins.

Conclusions

The architecture of the nucleotide-binding sites and the positioning of crucial residues in the helicase and ABC families exhibit similarities that were previously unsuspected. In particular, a spatially conserved Glu residue is most likely responsible for the activation of a catalytic H_2O molecule involved in hydrolysis of the γ-phosphate in ABC proteins, as previously proposed for RecA and helicases. Also, a His or Gln spatially conserved residue might trigger

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the conformational modification associated with ATP hydrolysis in each protein family. Overall, this suggests that these two families share a common mechanism for 'harvesting' the chemical energy produced by ATP hydrolysis to achieve their specific function. Hence, the most plausible explanation is that the ABC and the helicase families have evolved from a common ancestor. Such a hypothesis[45] has been suggested to explain the structural resemblance between the nucleotide-binding domain of the myosin ATPase and G proteins[46], and also to explain why so many ATP or GTP hydrolysing enzymes bear a Walker A-motif in their sequence[47,48]. In this regard, the ABC transporters and helicase families could be considered as 'close relatives' and the DNA-interacting ABC proteins might constitute the missing link between these two large families.

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