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ATP-binding Motifs

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Advanced article

Article Contents

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Adenosine 5′-triphosphate (ATP) binds to a great number of proteins to elicit a wide variety of effects, including energy production and molecular signalling. Proteins have evolved different strategies to specifically recognize ATP, utilizing different ways of binding the phosphoryl moieties as well as the adenine base. The most common, conserved sequence and structural motif for binding ATP is the Walker-A motif, or P-loop, found in many different protein structural families. Greater variation in the sequence of the P-loop is being recognized, as more ATP-binding proteins are being structurally and functionally characterized. In contrast to the P-loop, recognition of the adenine base often makes use of conserved structural motifs of main-chain atoms via hydrogen-bonding interactions, or side-chains in stacking interactions, without a definitive amino acid sequence pattern.

s0001

Introduction

p0001 Many proteins of prokaryotic and eukaryotic origin bind the mononucleotides adenosine 5′-phosphate (ATP) or guanosine 5′-phosphate (GTP). P-loop NTPases (nucleoside triphosphatases), as an example, represent ~ 10–18% of proteins in sequenced genomes (Koonin *et al*., 2000). These proteins are associated with numerous functions, including various kinases that fulfil fundamental metabolic functions via a variety of signalling, regulatory and transport molecules through transfer of the γ-phosphoryl moiety from ATP. Other enzymes, nucleotide hydrolases, often utilize ATP or GTP hydrolysis as a source of chemical energy for transport of ions, small molecules and proteins or to elicit conformational changes in proteins. Specific residues of proteins recognize distinct regions of the ATP molecule (adenine base, ribose sugar and triphosphate), and have evolved over time, such that in many cases a fingerprint motif can be discerned, either at the level of a conserved sequence or a conserved structural arrangement. The sequence and structural features of proteins responsible for specific recognition of ATP continues to be an active area of research, as more proteins are identified from large-scale genome sequencing projects. Parallelly, the enormous increase in protein structural information contained within the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do; Berman *et al*., 2000) now allows a more systematic, molecular-level view of ATP–protein interactions, as well as their classification. The breadth of sequence space devoted to proteins that bind ATP is impressive, with over 300 sequence families of ATP-binding proteins annotated within the Protein Families (Pfam) database (http://pfam.sanger.ac.uk/; Finn *et al*., 2008). **See also**: Adenosine Triphosphate; Bacterial Membrane Transport: Superfamilies of Transport Proteins; Ion Motive ATPases: V- and P-type ATPases; Nucleotides: Structure and Properties; Protein Structure

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P-loop or Walker-A Motif

s0002

Sequences and structural features of the P-loop

s0003

p0002 The best-known and earliest motif related to ATP-binding is the Walker-A sequence motif, $\underline{GX_4GK[S/T]}$, originally found as a common nucleotide-recognition sequence in diverse proteins (Walker *et al*., 1982; **Table 1**). Subsequently, the Walker-A sequence and variants thereof have been identified and classified in a great number of ATP-binding proteins (reviewed in Koonin, 1993; Traut, 1994; Leipe *et al*., 2002, 2003, 2004). Subsequent structural analyses, beginning with that of adenylate kinase (Schulz *et al*., 1974), and subsequently of numerous other proteins revealed that this sequence forms a Gly-rich loop often within a core α/β/α fold (Milner-White *et al*., 1991). Several classes of proteins that bind and act on ATP or GTP have such Gly-rich loops (Bossemeyer, 1994) or the P-loop (Saraste *et al*., 1990). The loop region, found between a β- AU:1

strand and an α helix, contains a pair of sequence- and structure-conserved Gly residues important for main-chain hydrogen-bonding interactions with the phosphoryl moieties of the nucleotide, a partially conserved basic residue near the *N*-terminus of the α helix that has been implicated in phosphoryl transfer, as well as a conserved Ser or Thr residue that directly or indirectly coordinates a catalytic Mg^{2+} ion. The conserved Gly residues are key structural elements in this sequence, imparting the flexibility needed for adopting the loop structure, and have torsion angles that would, for the most part, be incompatible with residues that have side-chains (Schulz, 1992). The conserved basic residue, either a Lys or Arg, has been postulated to have a catalytic role via neutralization of the negative charge on the β-γ bridge oxygen atom during phosphoryl transfer. This Lys can also contribute in determining the relative orientation of the β- and γ-phosphoryl groups, such that they can adopt a higher-energy eclipsed conformation, as in phosphoenolpyruvate carboxykinase (PCK) (Delbaere *et al*., 2004), as opposed to a lower-energy staggered conformation. An example of a P-loop motif, from yeast adenylate kinase, is depicted in **Figure 1**.

p0003 A second sequence, the Walker-B motif, contains a conserved Asp or Glu residue at the *C*-terminal end of a β-strand, often preceded by a stretch of four hydrophobic residues, with this acidic residue coordinating a Mg^{2+} ion (or to lesser extent Mn^{2+} or Ca^{2+}) essential for catalysis, either directly or indirectly via a water molecule. In some cases, such as the nucleotide-binding domains (NBD) of ABC (ATP-binding cassette) transporters, the acidic residue acts as a base, abstracting a proton from water to effect ATP hydrolysis (Rai *et al*., 2006; Carrier and Gros, 2008). The P-loop kinases and NTPases can be differentiated by the presence of an additional core β-strand in the later, as well as a conserved glutamate residue, which functions by activating a water to attack and hydrolyse the terminal (γ) phosphate (Leipe *et al*., 2003). Leipe *et al*. (2003) differentiate two differing groups of P-loop NTPases, based on the relative positions of the Walker-A and Walker-B motifs. In the 'KG-group', the Walker-A and Walker-B motifs are located on adjacent β-strands. A second group, including several other P-loop ATPases, is found to have an additional β-strand within the β-sheet that separates the two motifs from one another. In some cases, notably in shikimate and gluconate kinases, the Walker-B motif is absent and is instead replaced by a DXD motif. Not all P-loop-containing proteins possess a consensus Walker-B motif, one of the most noteworthy examples being the PCK/HprK kinases, in which this region is completely absent (Leipe *et al*., 2003). In PCK, the coordination sphere of the Mg^{2+} ion is completed by three water molecules that in turn form H-bonds to Asp268 and Asp269 (Tari *et al*., 1996).

Themes and variations on the Walker-A and Walker-B Motifs

s0004

p0004 With the large number of ATP-binding sequences and structures now available, it is not surprising that deviations and variations on the P-loop/Walker-A motif have been discovered. The Ser/Thr residue can be found replaced by Asp or Gly in some kinases, such as those that act on nucleosides (Leipe *et al*., 2003). Further, examples are coming to light where sequences that are not part of the canonical Walker-A or Walker-B motifs alone are important for high-affinity ATP-binding. An example of this is the role of a sequence-conserved Asp residue, located within the sensor 1 motif, which is important for high-affinity ATP-binding by *Escherichia coli* DnaA, a member of the AAA+ superfamily (Kawakami *et al*., 2006). An AU:2 example containing a deviant motif is the family of proteins typified by MinD, a protein involved in bacterial cell division, which also includes the nickel-binding ATPase COOCl (Jeoung *et al*., 2009). This family of proteins con- AU:3 tains a deviant Walker-A sequence, GKGGhGK[S/T], where h is a hydrophobic residue (Koonin, 1993). Both Lys residues are found to be necessary for function, with both necessary for ATPase activity. Moreover, the first conserved Lys residue plays a key role in stabilizing the dimeric form of the enzyme in the presence of bound ATP, via interactions between subunits. The nucleotide-binding domain of fungal ABC transporters from *Candida albicans* and related organisms contain a deviant Walker-A sequence, $GX_4GK/CT/S$, with the Cys residue important in ATPase activity but not in ATP-binding (Jha *et al*., 2003). In some proteins, such as a subset of AAA+ ATPases, the conserved Thr/Ser of the Walker-A motif is replaced by Asn, yielding GX_4GKN. Substitution of the conserved Thr in another AAA+ ATPase, ClpB, with Asn, resulted in an enzyme in which nucleotide-binding and activity were effectively uncoupled, demonstrating the functional importance of the conserved Thr in most AAA+ ATPases (Nagy *et al*., 2009).

G-loop of protein kinases

s0005

p0005 Protein kinases exhibit a conserved core structure of an *N*- and *C*-terminal domain, separated by a cleft. Nucleotide-binding involves primarily the *N*-terminal domain, involving interactions with residues from β-strands within this segment. A loop (Gly-rich loop or G-loop) between the β1–β2 strands within this domain adopts the consensus sequence GXGXXG and folds down and clamps over the α- and β-phosphoryl moieties of ATP, precisely orienting the γ-phosphate for phosphoryl transfer to the acceptor protein (Bossemeyer, 1994). The structural context of the G-loop is distinctly different from that of the P-loop, where the loop occurs between a β-strand and an α helix. The functional importance of the conserved Gly residues has been substantiated by structural studies of protein kinases bound to ATP (Zheng *et al*., 1993), as well as site-directed mutagenesis, showing diminished ATP-binding and

catalysis, to varying extents, depending on the particular protein kinase (Hirai *et al*., 2000 and references therein). The inherent flexibility of the loop, due to the Gly-rich sequence, represents an important aspect of its functional AU:4 importance (Bártová *et al*., 2004). In a recent study, an extended consensus sequence has been identified, [K/R$_{-4}$LGXG$_0$XF[G/A][E/D]$_{+4}$V, (Barouch-Bentov *et al*., 2009) that encompasses the original G-loop sequence. The conserved Lys/Arg forms a salt bridge with the corresponding Glu/Asp, limiting the flexibility of the G-loop and thereby influencing ATP-binding and catalysis. Mutations that disrupt this salt bridge are found to impair *in vivo* function. This sequence motif is found in phylogenetically diverse kinases, including the tyrosine kinases belonging to the Src (sarcoma) family and Abl (Abelson murine leukaemia viral oncogene), as well as the Ser/Thr protein kinases CK2A1 and SLK (Barouch-Bentov *et al*., 2009). **See also**: AMP-Activated Protein Kinase (AMPK)

s0006 ## Classification of P-loop containing proteins

p0006 P-loop nucleoside hydrolases are very ancient proteins, and probably evolved early due to the use of ATP hydrolysis as an essential way of generating energy for biomolecular reactions. Since the early studies on the identification of the sequence and structural description of this motif, many studies have collectively defined the various protein families containing the sequence and structural signatures of the P-loop. Some (but not all) kinases contain a P-loop, and are one of the larger and important groups of P-loop-containing proteins, and have been classified at the level of structure and sequence. P-loop-containing proteins can be divided into $\sim$40 distinct families within the P-loop NTPase fold (Leipe *et al*., 2003). It has been suggested that a common ancestral enzyme led to the P-loop kinases and GTPase classes, respectively. A similar analysis has been performed on the sequences and structures of P-loop GTPases (Leipe *et al*., 2002). A new class of P-loop NTPases, the signal transduction ATPases with numerous domains (STAND), has also been identified (Leipe *et al*., 2004).

s0007 # Recognition of the Adenine base

p0007 It is not just the phosphoryl moieities of the nucleotide that bind to sequence or structure-conserved elements within proteins. It became evident some time ago that the recognition of the adenine portion of ATP bound to proteins involved various combinations of residues, without revealing a specific motif that could be recognized at the level of protein sequence. Although the importance of hydrophobic 'sandwich' interactions for adenine-binding were recognized, the idea that there could be a conserved pattern of hydrogen-bond donor and acceptor interactions was not initially appreciated, due no doubt, in part, to the limited quantity of structural information available when the first systematic analysis was performed (Moodie *et al*., 1996). The first clues that this would be a structural motif rather than one based on sequence came from the common adenine-binding site identified within D-Ala: D-Ala ligases and cAMP (cyclic adenosine monophosphate)-dependent protein kinases (Kobayashi and Go, 1997). This recognition site involved four residues that formed main-chain donor (amide NH or carbonyl O atoms) and acceptor H-bond interactions, with the N1 and N6 atoms of the adenine base. The concept of a structural motif for adenine-binding was further reinforced in a study involving additional ATP-bound structures, including proteins from 12 different folds, revealing a common mode of adenine-binding in 8 of these folds (Denessiouk and Johnson, 2000). All 12-folds revealed two common H-bond interactions and three conserved aliphatic interactions with the adenine base. Subsequently, this analysis was expanded, showing that the mode of protein-adenine recognition found for ATP was in fact common to adenine found as a part of other cofactors, including coenzyme A (CoA) and NAD/NADP (nicotinamide adenine dinucleotide phosphate) (Denessiouk *et al*., 2001). It appears that the requirements for adenine recognition by proteins should be thought of using the concept of atomic specificity as opposed to residue specificity (Kuttner *et al*., 2003), which helps to understand why no single, specific sequence motif responsible for adenine-binding has been identified. **See also**: Enzymes: Coenzyme A dependent; NAD^+ and $NADP^+$ as Prosthetic Groups for Enzymes AU:5

p0008 In many P-loop-containing proteins, a conserved Arg residue can often be identified that interacts with the adenine base (Leipe *et al*., 2003). This Arg is part of a sequence motif, usually $\underline{R}X_{(2-3)}\underline{R}$, with the first Arg interacting with the nucleotide base. The second Arg residue is frequently found to interact with the γ-phosphoryl group of ATP and is predicted to compensate the negative charge in the transition state. Both residues can be substituted by Lys in some sequences and in the case of the first Arg, other residues (Tyr/His) capable of stacking against the apolar face of the base can be found instead. These residues are located in helix 4.1, a structural element of the LID module of P-loop NTPases. **See also**: Amino Acid Side-chain Hydrophobicity AU:6

p0009 The molecular basis for the recognition of the adenine portion of adenylate molecules, including ATP, has recently been systematically analysed based on 68 crystal structures of at least 2.5 Å resolution available in the Protein Data Bank (Mao *et al*., 2004). This analysis has revealed that protein–adenine interactions are dominated by hydrogen bonds, stacking (π–π interactions) between aromatic residues (Phe, Tyr and Trp) with the adenine base, and interactions between the positively charged side-chains of Lys or Arg with the adenine aromatic ring (cation–π interactions; Mao *et al*., 2003). On average, each adenine–protein interaction involves 2.7 hydrogen bonds, 1 π–π stacking interaction and 0.8 cation–π interactions (Mao *et al*., 2004). In the case of hydrogen-bonding interactions, these most often involve main-chain atoms, rather than side-chains, resulting in adenine-binding structural motifs

that are not easily recognizable at the sequence level alone. Two patterns of these main-chain hydrogen-bonding networks have been identified, involving either three residues (*i* and *i*−2) or one residue. In either case, it is the N1 and N6 atoms of adenine that are involved in forming the hydrogen bonds. The conserved nature of these two structural motifs for adenine recognition, despite their presence in a variety of different protein folds, suggests a common biological solution to the problem of binding this base. The majority of adenine rings in nucleotides are in the *anti*conformation but in some instances, the adenine ring bound to kinases adopts the *syn* conformation (Tari *et al*., 1996).

Q-motif

The Q-motif was discovered during experiments that characterized functionally important regions upstream of the Walker-A sequence region in DEAD-box RNA (ribonucleic acid) helicases (Tanner *et al*., 2003; Cordin *et al*., 2004). This motif is found ~ 17 residues *N*-terminal to the Walker-A motif and contains a conserved region of 9 amino acids, ending with a highly conservedGlx. An aromatic residue, usually Phe and less often Trp, is found a further 17 residues *N*-terminal to the Q-motif. Both *in vivo* and *in vitro* experiments have demonstrated the functional importance of residues within the Q-motif, in particular for ATP-binding and hydrolysis. The conserved Glxwithin the motif is observed to form H-bonds to the adenine base of the nucleotide based on crystallographic data, whereas the conserved aromatic residue is predicted to stack with the adenine base. Since its original discovery, the Q-motif has also been identified to function in viral terminase proteins that package DNA (deoxyribonucleic acid) (Mitchell *et al*., 2002; Tsay *et al*., 2009).

A-loop

Recently, a region containing a conserved aromatic residue found in the nucleotide-binding domain of ABC transporters, designated the A-loop, has been identified. This conserved residue is located ~ 25 residues upstream of the Walker-A motif and appears to function through stacking interactions with the ATP adenine base (Ambudkar *et al*., 2006). This residue is usually a Tyr, but can be substituted by a Trp, or less often, by Phe. Substitution of Tyr by Phe or Trp retained function in human P-glycoprotein, whereas mutations that replaced the aromatic residue by Ala resulted in loss of function (Kim *et al*., 2006). Crystal structures of the NBD domains of ABC transporters with bound nucleotide further demonstrate a stacking interaction between the aromatic residue of the A-loop with the adenine base. There appear to be parallels between the A-loop and Q-motifs, in that they share conserved aromatic residue 25–30 amino acids *N*-terminal to a Walker-A motif.

ATP-grasp Fold

In addition to the P-loop motif, a second class of ATP-binding proteins has been identified having a different fold, designated the palmate or ATP-grasp fold (Galperin and Koonin, 1997; **Figure 2**). Enzymes having the ATP-grasp fold include D-Ala:D-Ala ligase, biotin carboxylase and glutathione synthetase. The ATP-grasp structure motif consists of a pair of β-strands connected by a loop, together creating a cleft in which the ATP binds. Most ATP-grasp sequences contain a pair of conserved Lys or Arg residues involved in binding the α- and β-phosphoryl groups of ATP, as well as conserved Asp or Glu residues associated with Mg^{2+}-binding.

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References

Ambudkar SV, Kim I-W, Xia D and Sauna ZE (2006) The A-loop, a novel conserved aromatic acid subdomain upstream of the Walker A motif in ABC transporters, is critical for ATP binding. *FEBS Letters* **580**: 1049–1055.

Aravind L and Koonin EV (1999) DNA polymerase beta-like nucleotidyltransferase superfamily: identification of three new families, classification and evolutionary history. *Nucleic Acids Research* **27**: 1609–1618.

Barouch-Bentov R, Che J, Lee CC *et al*. (2009) A conserved salt bridge in the G loop of multiple protein kinases is important for catalysis and for *in vivo* Lyn function. *Molecular Cell* **33**: 43–52.

Bártová I, Otyepka M, Kriz Z and Koca J (2004) Activation and inhibition of cyclin-dependent kinase-2 by phosphorylation; a molecular dynamics study reveals the functional importance of the glycine-rich loop. *Protein Science* **13**: 1449–1457.

Berman HM, Westbrook J, Feng Z *et al*. (2000) The Protein Data Bank. *Nucleic Acids Research* **28**: 235–242.

Bossemeyer D (1994) The glycine-rich sequence of protein kinases: a multifunctional element. *Trends in Biochemical Sciences* **19**: 201–205.

Carrier I and Gros P (2008) Investigating the role of the invariant carboxylate residues E552 and E1197 in the catalytic activity of Abcb1a (mouse Mdr3). *FEBS Journal* **275**: 3312–3324.

Cordin O, Tanner NK, Doère M, Linder P and Banroques J (2004) The newly discovered Q motif of DEAD-box RNA helicases regulates RNA-binding and helicase activity. *EMBO Journal* **23**: 2478–2487.

Delbaere LTJ, Sudom AM, Prasad L, Leduc Y and Goldie H (2004) Structure/function studies of phosphoryl transfer by phosphoenolpyruvate carboxykinase. *Biochimica et Biophysica Acta* **1697**: 271–278.

Denessiouk KA and Johnson MS (2000) When fold is not important: a common structural framework for adenine and AMP binding in 12 unrelated protein families. *Proteins: Structure, Function, and Genetics* **38**: 310–326.

Denessiouk KA, Rantanen V-V and Johnson MS (2001) Adenine recognition: a motif present in ATP-, CoA-, NAD-, NADP- and FAD-dependent proteins. *Proteins: Structure, Function, and Genetics* **44**: 282–291.

Finn RD, Tate J, Mistry J *et al*. (2008) The Pfam protein families database. *Nucleic Acids Research* **36**: D281–D288.

Galperin MY and Koonin EV (1997) A diverse family of enzymes with ATP-dependent carboxylate-amine/thiol ligase activity. *Protein Science* **6**: 2639–2643.

Hirai TJ, Tsigelny I and Adams JA (2000) Catalytic assessment of the glycine-rich loop of the v-Fps oncoprotein using site-directed mutagenesis. *Biochemistry* **39**: 13276–13284.

Jeoung J-H, Giese T, Grunwald M and Dobbek H (2009) CooCl from *Carboxydothermus hydrogenformans* is a nickel-binding ATPase. *Biochemistry* (in press).

Jha S, Karmani N, Lynn AM and Prasad R (2003) Purification and characterization of the *N*-terminal nucleotide binding domain of an ABC drug transporter of *Candida albicans*: uncommon cysteine 193 of Walker A is critical for ATP hydrolysis. *Biochemistry* **42**: 10822–10832.

Kawakami H, Ozaki S, Suzuki S *et al*. (2006) The exceptionally tight affinity of DnaA for ATP/ADP requires a unique aspartic acid residue in the AAA+ sensor 1 motif. *Molecular Microbiology* **62**: 1310–1324.

Kim I-W, Peng X-H, Sauna ZE *et al*. (2006) The conserved tyrosine residues 401 and 1044 in ATP sites of human P-glycoprotein are critical for ATP-binding and hydrolysis: evidence for a conserved subdomain, the A-loop in the ATP-binding cassette. *Biochemistry* **45**: 7605–7616.

Kobayashi N and Go N (1997) ATP-binding proteins with different folds share a common ATP-binding structural motif. *Nature Structural & Molecular Biology* **4**: 6–7.

Koonin EV (1993) A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. *Journal of Molecular Biology* **229**: 1165–1174.

Koonin EV, Wolf YI and Aravind L (2000) Protein fold recognition using sequence profiles and its application in structural genomics. *Advances in Protein Chemistry* **54**: 245–275.

Kuttner YY, Sobolev V, Raskind A and Edelman M (2003) A consensus-binding structure for adenine at the atomic level permits searching for the ligand site in a wide spectrum of adenine-containing complexes. *Proteins: Structure, Function, and Genetics* **52**: 400–411.

Leipe DD, Koonin EV and Aravind L (2003) Evolution and classification of P-loop kinases and related proteins. *Journal of Molecular Biology* **333**: 781–815.

Leipe DD, Koonin EV and Aravind L (2004) STAND: a class of P-loop NTPases including animal and plant regulators of programmed cell death: multiple, complex domain architectures, unusual phyletic patterns, and evolution by horizontal gene transfer. *Journal of Molecular Biology* **343**: 1–28.

Leipe DD, Wolf YI, Koonin EV and Aravind L (2002) Classification and evolution of P-loop GTPases and related ATPases. *Journal of Molecular Biology* **317**: 41–72.

Mao L, Wang Y, Liu Y and Hu X (2003) Multiple intermolecular interaction modes of positively charged residues with adenine in ATP-binding proteins. *Journal of the American Chemical Society* **125**: 14216–14217.

Mao L, Wang Y, Liu Y and Hu X (2004) Molecular determinants for ATP-binding in proteins: a data mining and quantum chemical analysis. *Journal of Molecular Biology* **336**: 787–807.

Milner-White EJ, Coggins JR and Anton IA (1991) Evidence for an ancestral core structure in nucleotide-binding proteins with the type A motif. *Journal of Molecular Biology* **221**: 751–754.

Mitchell MS, Matsuzaki S, Imai S and Rao VB (2002) Sequence analysis of bacteriophage T4 DNA packaging/terminase genes 16 and 17 reveals a common ATPase center in the large subunit of viral terminases. *Nucleic Acids Research* **30**: 4009–4021.

Moodie SL, Mitchell BO and Thornton JM (1996) Protein recognition of adenylate: an example of a fuzzy recognition template. *??????????* **263**: 486–500.

Nagy M, Wu H-C, Liu Z, Kedzierska-Mieszkowska S and Zolkiewski M (2009) Walker-A threonine couples nucleotide occupancy with the chaperone activity of the AAA+ ATPase ClpB. *Protein Science* **18**: 287–293.

Rai V, Gaur M, Kumar A *et al*. (2008) A novel catalytic mechanism for ATP hydrolysis employed by the *N*-terminal nucleotide-binding domain of Cdr1p, a multidrug ABC transporter of *Candida albicans*. *Biochimica et Biophysica Acta* **1778**: 2143–2153.

Rai V, Gaur M, Shukla S *et al*. (2006) Conserved Asp327 of Cdr1p of *Candida albicans* has acquired a new role in ATP hydrolysis. *Biochemistry* **45**: 14726–14739.

Saraste M, Sibbald PR and Wittinghofer A (1990) The P-loop – a common motif in ATP- and GTP-binding proteins. *Trends in Biochemical Sciences* **15**: 430–434.

Schulz GE (1992) Binding of nucleotides by proteins. *Current Opinion in Structural Biology* **2**: 61–67.

Schulz GE, Elzinga M, Marx F and Schirmer RH (1974) Three-dimensional structure of adenylate kinase. *Nature* **250**: 120–123.

Tanner NK, Cordin O, Banroques J, Doere M and Lidner P (2003) The Q-motif: a newly identified motif in DEAD box helicases may regulate ATP-binding and hydrolysis. *Molecular Cell* **11**: 127–138.

Tari L, Matte A, Pugazhenthi U, Goldie H and Delbaere LTJ (1996) Snapshot of an enzyme reaction intermediate in the structure of the ATP-oxalate ternary complex of *Escherichia coli* PEP carboxykinase. *Nature Structural & Molecular Biology* **3**: 355–363.

Traut TW (1994) The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide binding sites. *European Journal of Biochemistry* **222**: 9–19.

Tsay JM, Slippy J, Feiss M and Smith DE (2009) The Q motif of a viral packaging motor governs its force generation and communicates ATP recognition to DNA interaction. *Proceedings of the National Academy of Sciences of the USA* **106**: 14335–14360.

Walker JE, Saraste M, Runswick M and Gay NJ (1982) Distantly related sequences in the a- and b-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO Journal* **1**: 945–951.

Zheng J, Knighton DR, ten Eyck LF *et al*. (1993) Crystal structure of the catalytic subunit of cAMP-dependent protein kinase complexed with MgATP and peptide inhibitor. *Biochemistry* **32**: 2154–2161.

Further Reading

Chakrabarti P and Samanta U (1995) CH/π interaction in the packing of the adenine ring in protein structures. *Journal of Molecular Biology* **251**: 9–14.

Fry DC, Kuby SA and Mildvan AS (1986) ATP-binding site of adenylate kinase: mechanistic implications of homology with ras-encoded p21, F_1-ATPase, and other nucleotide-binding proteins. *Proceedings of the National Academy of Sciences of the USA* **83**: 907–911.

Kjeldgaard M, Nyborg J and Clark BF (1996) The GTP binding motif: variations on a theme. *Federation of American Societies for Experimental Biology Journal* **10**: 1347–1368.

Smith CA and Rayment I (1996) Active site comparisons highlight structural similarities between myosin and other P-loop proteins. *Biophysical Journal* **70**: 1590–1602.

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Key concepts:

- A major class of ATP-binding proteins are those that contain a P-loop or Walker-A motif.
- P-loops or glycine-rich loops function by binding the phosphoryl groups of ATP.
- Several sequence variations on the Walker-A motif are now known and have been functionally characterized.
- The Walker-B motif contains a conserved acidic residue (Glu/Asp) that functions to bind directly or indirectly a metal ion important in catalysis.
- Adenine-binding does not occur through specific sequence motifs, but rather uses a conserved pattern of polar and nonpolar interactions within a structural motif.
- Both main-chain hydrogen bonding and aromatic residue stacking contribute to adenine-binding by proteins.

Glossary: None

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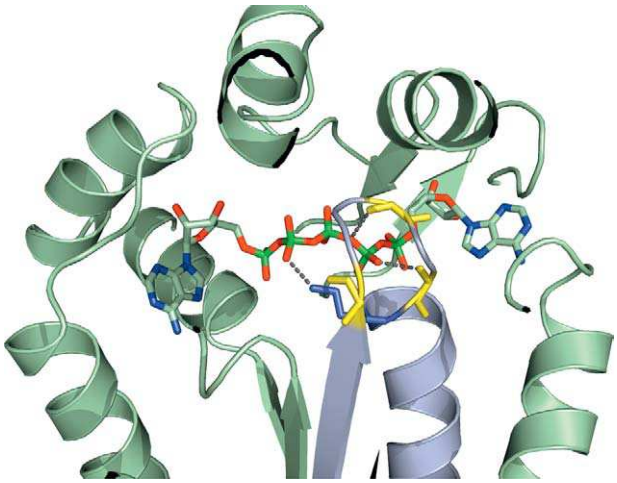
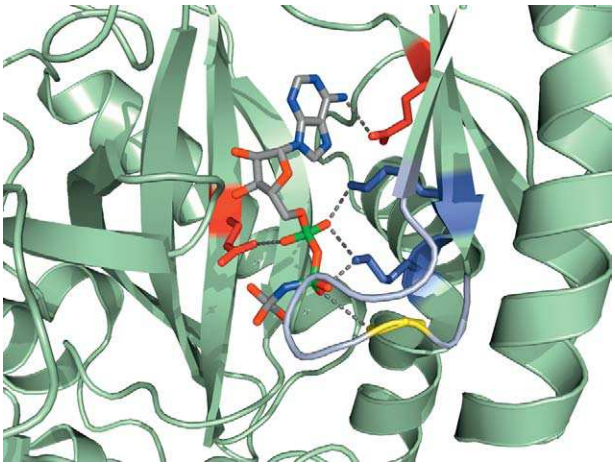
t0001 **Table 1** Representative sequence motifs of ATP-binding proteins

Designation	[a]Consensus	[a]Variant(s)	Function	Reference
P-loop-associated motifs				
Walker-A	GX_4GK[S/T]		Binding and orientation of phosphoryl groups, phosphoryl transfer	Walker *et al.* (1982) and Koonin (1993)
		GX_4GK[G/D]	Found in nucleoside monophosphate kinases	Leipe *et al.* (2003)
		KGGXGK[S/T]T	ATP hydrolysis, dimerization	Koonin (1993) and Jeoung *et al.* (2009)
		RGXPGXGK[S/T]		
		GXGXXG	G-loop or Gly-rich loop – binds α and β phosphates of ATP in protein kinases	Bossemeyer (1994)
		[K/R_{-4}LGXG$_0$XF[G/A][E/D]$_{+4}$V	Modified G-loop containing basic and acidic residues to form salt-bridge stabilizing loop	Barouch-Bentov *et al.* (2009)
		GX_4GK/CT/S	Found in fungal nucleotide-binding domains of ABC transporters	Jha *et al.* (2003) and Rai *et al.* (2008)
		GX_4GKN	Subfamily of AAA+ ATPases	Nagy *et al.* (2009)
Walker-B	[b]hhhhD	hhhhE hhhDXTN	Interaction with metal ion (Mg^{2+}) directly or via water molecule; H-bond to S/T of Walker-A motif	Walker *et al.* (1982)
DxD motif	DXD		Alternative to Walker-B; interaction with metal ion, H-bond to S/T of Walker-A motif (selected kinases and nucleotidases only)	Aravind and Koonin (1999)
Not-specified	R(X_{2-3})R	K(X_{2-3})K	LID module of P-loop kinases; interaction with adenine base, interaction with γ-phosporyl group of transition state	Leipe *et al.* (2003)
Other motifs				
A-loop	X-Y/W/F/-X		Essential for ATP-binding in ABC transporters; located ~ 25 residues upstream of the Walker-A motif	Ambudkar *et al.* (2006) and Kim *et al.* (2006)
Q-motif	F/W/Y-X_{16}-G-X_7-Q		Found in DEAD box RNA helicases, and DNA terminases, regulates ATP-binding and hydrolysis	Mitchell *et al.* (2002); Tanner *et al.* (2003) and Tsay *et al.* (2009)

[a]Underlined residues have specific functional significance as discussed within the text. 'X' is any residue.
[b]Here 'h' designates a hydrophobic residue.



f0001 **Figure 1** Crystal structure of yeast adenylate kinase (pale green) bound to the bi-substrate inhibitor bis(adenosine)-5′-pentaphosphate, (Ap5A) and Mg^{2+} at 1.96 Å resolution (PDB 1AKY). The β-strand-loop-α-helix motif containing the P-loop is coloured light blue, with the catalytic Lys16 coloured blue, and the three Gly residues of the P-loop coloured yellow. Hydrogen-bonding interactions with the Ap5A inhibitor (red = oxygen, blue = nitrogen, grey = carbon and green = phosphorous) are indicated as grey dashed lines. This figure was prepared using PyMol 1.0r2 (http://www.pymol.org).



f0002 **Figure 2** Crystal structure of biotin carboxylase (pale green) from *Staphylococcus aureus* (PDB 2VPQ), highlighting interactions between ATP with proteins belonging to the ATP-grasp fold. The ATP-grasp fold consists of a $\alpha/\beta/\alpha$ unit, including a pair of antiparallel β-strands connected by a loop. This loop (light blue) contains a conserved Gly (Gly161) that forms a hydrogen bond with the β-phosphoryl moiety. Additionally, sequence-conserved hydrogen-bonding interactions involve a polar residue (Glu199) with the N6 atom of the adenine base, as well as a pair of Lys residues (Lys115, Lys157) with the α- and β-phosphoryl moietieis of ATP. This figure was prepared using PyMol 1.0r2 (http://www.pymol.org/).

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