

The PRT protein family

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Members of the homologous PRT family are catalytic and regulatory proteins involved in nucleotide synthesis and salvage. New crystal structures have revealed key elements of PRT protein function, as well as glimpses of how the fold has evolved to perform both catalytic and regulatory functions.

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Abbreviations

GPATase	glutamine PRPP amidotransferase
PDB	Protein Data Bank
PP_i	pyrophosphate
PRPP	5-phosphoribosyl- α 1-pyrophosphate
PRTase	phosphoribosyltransferase

Introduction

PRT proteins comprise a homologous family of enzymes and regulatory proteins of the nucleotide synthesis and salvage pathways. Members of the family are defined by the protein fold and by a short sequence motif. The name PRT comes from the phosphoribosyltransferase (PRTase) enzymes, which carry out phosphoryl transfer reactions on 5-phosphoribosyl- α 1-pyrophosphate (PRPP), an activated form of ribose-5-phosphate. PRPP is a central metabolite connecting the nucleotide synthesis and salvage pathways. A broad understanding of the structure and function of the PRT family comes from the analysis of nearly 40 PRT crystal structures of 16 different PRT proteins, including representatives from all known PRT subfamilies [1–22,23•,24,25••,26•].

Enzymes catalyzing PRTase reactions are the most numerous members of the PRT family. PRTases catalyze the displacement of pyrophosphate (PP_i) from PRPP by a nitrogen-containing nucleophile, such as ammonia, adenine, guanine, hypoxanthine, xanthine, orotate or uracil, producing a β -substituted ribose-5-phosphate and PP_i. Not all PRT proteins are enzymes. For example, in some bacteria, PRT proteins regulate the expression of purine and pyrimidine synthetic genes. Conversely, not all enzymes with PRTase activity are members of the PRT family. The PRT sequence motif is only found in PRTases from the nucleotide synthesis and salvage pathways. Other PRTases, from the tryptophan, histidine and nicotinamide synthetic and salvage pathways, lack the PRT sequence motif. One of these enzymes, quinolinate PRTase, was classified as a ‘type II PRTase’ because its structure is

unrelated to the PRT family (‘type I PRTases’) [27,28]. The sequences of the other tryptophan, histidine and nicotinamide enzymes appear to be unrelated to each other and unrelated to the PRT family (see also Update). Here we review only members of the homologous PRT family.

Like many protein families, the PRT family was identified at the sequence level and the family relationship was confirmed later by three-dimensional structures. Nearly all PRT proteins include a 13-residue sequence motif easily recognized in database searches. The motif consists of four hydrophobic amino acids, two acidic amino acids and seven amino acids of variable character, usually including glycine and threonine. The motif was correctly predicted to be a PRPP-binding site in advance of structural information [29,30]. Apart from the PRT sequence motif, different PRT proteins have a low level of sequence identity, generally <15%.

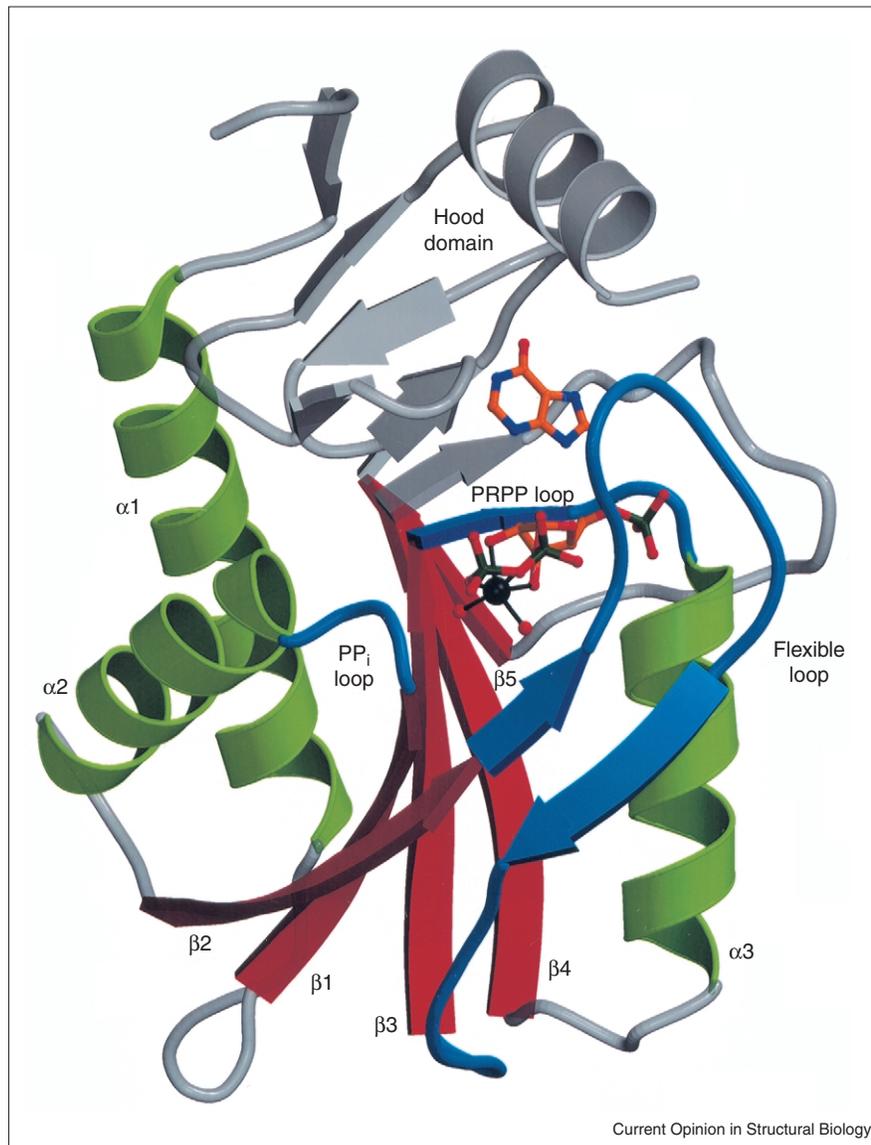
The PRT fold binds PRPP

PRT proteins have a common core consisting of a five-stranded, parallel β sheet (β 2– β 1– β 3– β 4– β 5) sandwiched between layers of α helices, α 1 and α 2 on one face of the β sheet and α 3 on the other face (Figure 1). The core fold is expanded by between two and five additional secondary structures, which vary among PRT family members. At the C-terminal edge of the core β sheet, a second domain or subdomain, known as the ‘hood’, is formed predominantly by residues at the N terminus of the protein.

PRT proteins have a conserved PRPP-binding site, as expected for a homologous protein family with a common ligand. PRPP binds in association with a divalent metal ion, usually Mg²⁺. The PRPP-binding site comprises three loops: a ‘PP_i loop’ between β 1 and α 2; a ‘flexible loop’ between β 2 and β 3; and a ‘PRPP loop’ between β 3 and α 3 (Figure 1). PRPP binds to the C-terminal edge of the central β sheet between the PP_i and PRPP loops. The sequences of the three loops of the PRPP-binding site are highly conserved for each PRT protein, although there are no sites of strict conservation across the PRT family.

The PRPP loop constitutes the last nine residues of the 13-residue PRT sequence motif and binds the ribose 5-phosphate group of PRPP (Figure 2). The length, sequence and conformation of the PRPP loop are highly conserved in PRPP-binding sites throughout the PRT family. Two acidic residues at the start of the loop are conserved in the PRPP-binding sites of all PRTs, except uracil PRTase, which has only one acidic residue. These characteristic acidic sidechains form hydrogen bonds with the ribose hydroxyl groups. The ribose 5-phosphate group binds in a positively charged pocket that is formed by backbone amides, polar sidechains and the dipole of helix α 3, and

Figure 1



The PRT fold. The common core is highlighted as green α helices and red β strands in this representation of *T. cruzi* hypoxanthine/guanine PRTase [16] (PDB code 1TC2). The Mg•PRPP-binding loops (PRPP loop, PP_i loop and flexible loop) are highlighted in blue. The remainder of the protein is shaded gray. Full atomic details are shown for the substrates Mg•PRPP and hypoxanthine (a purine base), colored according to atom type (carbon, orange; oxygen, red; nitrogen, blue; phosphorus, green; Mg, black; water, red spheres). This and other figures were made using MOLSCRIPT [43] and Raster3D [44].

includes at least one glycine and one threonine. This interaction is apparently essential to ligand binding. The ribose ring is disordered in nucleoside complexes that lack 5-phosphate [15,24]. Anions from the crystallization solution, such as sulfate, phosphate or sulfonate, nearly always fill the 5-phosphate site of PRT structures lacking specific ligands [5,11–13,15,19,23•,26•].

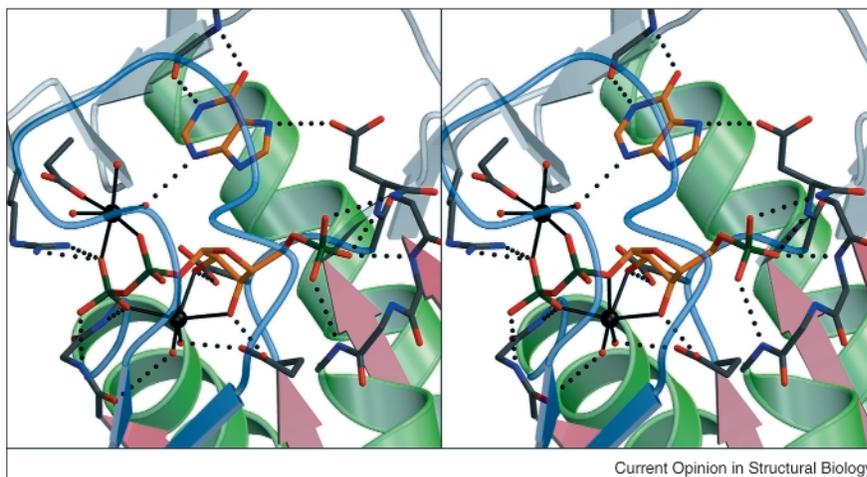
The four-residue PP_i loop binds the PP_i group of PRPP. The conformation of the PP_i loop is conserved across the PRT family, but sequence similarity is restricted to the fourth residue (generally glycine, alanine or serine), which contacts the adjacent PRPP loop. A key element of Mg•PRPP recognition is a rare, nonproline *cis* peptide between the first and second residues of the PP_i loop (Figure 2). The N–H and C=O groups of the *cis* peptide point into the PRPP-binding site and form hydrogen

bonds with the metal•PP_i group of bound PRPP. The *cis* peptide may occur only in PRT proteins for which PRPP recognition is essential to *in vivo* function. For example, the *cis* peptide is not present in PRPP synthetase [23•], for which PRPP is the product of the reaction, or in the regulatory protein PyrR [12], whose coeffector is UMP. Anions from the crystallization solution bind in the β -phosphate position in a few PRT crystal structures lacking PRPP or PP_i [8,11,19,26•].

The flexible loop closes over bound Mg•PRPP in the PRT enzymes (Figure 3a,b). This long crossover connection between strands β 2 and β 3 has strikingly different lengths in different PRT proteins. The flexible loop is ordered and closed only in PRPP-bound forms of PRT proteins [10,16–18,20,25••] and, generally, is unfolded or highly mobile in the absence of PRPP (Figure 3a,b). Closed

Figure 2

Stereo view of Mg•PRPP interactions in the PRPP-binding site. The protein backbone is rendered as in Figure 1, with muted colors. Atoms are rendered for selected parts of the PRPP and PP_i loops, and for the substrates Mg•PRPP and hypoxanthine. Atoms are colored according to atomic type, with dark gray protein carbons and orange substrate carbons. Hydrogen bonds are indicated by dotted lines and Mg²⁺ coordination by thin black lines. The figure is based on a ternary complex of *T. cruzi* hypoxanthine/guanine PRTase [16]. In this view, the primary Mg²⁺ site common to all PRTases is below the PP_i group and the secondary site in purine PRTases is above.



flexible loops contact PRPP, usually involving a few basic sidechains. The sequences of flexible loops are highly conserved for each PRT family member but, similar to the PP_i loop sequences, are poorly conserved among different PRT proteins. The closed state of the flexible loop appears to be transient, as not all structures containing appropriate ligands have closed flexible loops [4,14,22,24].

Metal binding

Mg²⁺ is associated with the PP_i of PRPP and appears to be essential to PP_i binding in the PRPP site. In all closed-loop PRT structures, Mg²⁺ in a primary site is coordinated by a β-phosphate oxygen, by both PRPP ribose hydroxyls, by the ribose–PP_i bridging oxygen and by two water molecules. One of the water molecules is hydrogen bonded to the C=O of the *cis* peptide in the PP_i loop (Figure 2). Remarkably, the Mg²⁺ ion in this site has no direct protein ligands.

In some open-loop PRT structures, Mg²⁺ occupies an alternative position within the primary site. The main and alternative positions are closer than 2 Å and thus cannot be occupied simultaneously. In the alternative position, Mg²⁺ is coordinated by the two ribose hydroxyls and by the two conserved carboxyl sidechains in the PRPP loop, with polar sidechains or water molecules completing the octahedral coordination sphere. Nucleotide feedback inhibitor complexes of glutamine PRPP amidotransferase (GPATase) bind Mg²⁺ in the alternative position [9,10] (Figure 3a). Mg²⁺ occupies a similar position in the ternary complex of a guanine PRTase with a nucleotide product analog and PP_i [24], and in a xanthine PRTase structure with sulfate in the β-phosphate site [8]. These structures may represent the pre-catalytic or post-catalytic arrangement of substrates [24].

Purine PRTases might all have a second metal site, fully compatible with the primary site. In the second site, Mg²⁺ is coordinated by α and β PP_i oxygens, three water molecules and a conserved aspartate sidechain from the hood subdomain ([16,18,20,22,24,25••]; Figure 2). The second

Mg²⁺ is linked to the purine substrate through a water ligand and might orient the purine base for catalysis. The hydrogen bond between water and purine N3 is independent of the protonation state of N3. Thus, the second Mg²⁺ also contributes to purine binding and recognition [31].

PRT quaternary structure

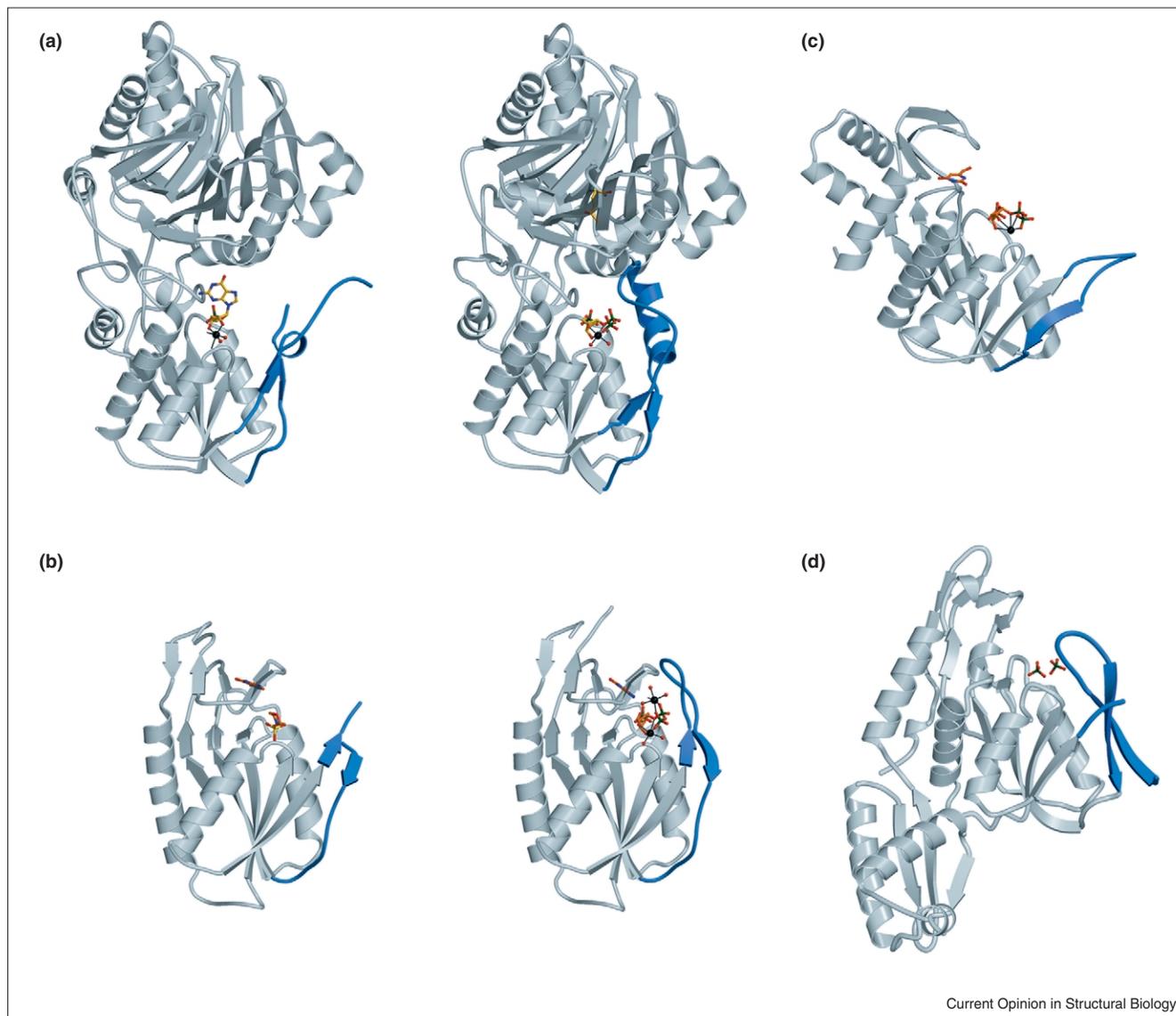
No monomeric PRT proteins are known. In most cases, the PRT core fold is part of a dimer interface in which a basic residue from each monomer contacts Mg•PRPP in the neighboring subunit. Regions of the structure involved in cross-subunit Mg•PRPP interactions differ among PRT proteins and include the flexible loop, the hood, the PP_i loop and the bottom of the core domain. Despite these cross-subunit interactions, most PRT proteins do not display cooperative Mg•PRPP binding.

For a few PRT proteins, allostery is important to function. GPATase catalyzes the first step of purine biosynthesis and is feedback-inhibited by the end products of the pathway. Two different feedback inhibitor-binding sites overlap the Mg•PRPP site [2,9–11]. PRPP synthetase connects the nucleotide synthesis and salvage pathways, and is activated by inorganic phosphate (P_i) and inhibited by ADP. These effector molecules bind to overlapping sites between subunits of the hexameric enzyme, remote from the active site [23•]. Uracil PRTase, which is part of the cellular scheme for balancing uracil and cytidine nucleotides, is regulated by GTP [32], although the structural basis of this allosteric behavior is unknown. Allostery is expected to be an element of function for the PRT regulatory proteins PurR and PyrR, which bind to nucleic acid in response to a small-molecule effector.

The amazing, multifunctional flexible loop

The PRT flexible loop is disordered in most crystal structures, confounding structural biologists who seek an intact PRT active center. In PRTases, the loop orders only when Mg•PRPP binds, completing the active site and

Figure 3



Variable conformations of the PRTase flexible loop. The proteins are oriented identically, with flexible loops highlighted in blue.

(a) Group 1: the *E. coli* GPATase open form (left) with Mg•GMP bound (PDB code 1ECB) and closed form (right) with Mg•cPRPP bound (PDB code 1ECC) [10]. **(b)** Group 2: *T. cruzi* hypoxanthine/guanine PRTase, open form (left), with the purine

nucleoside formycin B and the buffer *N*-morpholinoethanesulfonic acid bound (PDB code 1TC1) [15] and closed form (right) with Mg•PRPP and 3H-pyrazolo[4,3-d]pyrimidin-7-ol, a hypoxanthine analog, bound (PDB code 1TC2) [16]. **(c)** Group 3: open form of *S. typhimurium* orotate PRTase with Mg•PRPP and orotate bound (PDB code 1OPR) [4]. **(d)** Group 4: open form of *B. subtilis* PurR with bound sulphate [26*].

sequestering the reaction from bulk solvent. Nature has also adapted the large conformational changes of the flexible loop to a variety of functions associated with Mg•PRPP binding, including catalysis, signaling and cooperativity.

The flexible loops of several PRT proteins occur in subunit interfaces and contribute to Mg•PRPP binding in two subunits. For example, arginine sidechains interact with PP_i in the active sites of adjacent subunits of both GPATase [9,10] and orotate PRTase [4]. Similar functions are inferred for the flexible loops of adenine PRTase [19] and PurR [26*], in which an arginine sidechain from the

flexible loop is hydrogen bonded to an anion in the β-phosphate site of an adjacent subunit. In orotate PRTase, a lysine sidechain at the tip of the flexible loop is essential to catalysis in the neighboring subunit of the dimeric protein [33,34], raising the possibility that the flexible loop may close over Mg•PRPP in the neighboring subunit.

The GPATase flexible loop is remarkable in its multiple functions. When it closes over Mg•PRPP, the flexible loop signals PRPP binding by contacting a second catalytic domain and activating production of the nucleophile NH₃ by glutamine hydrolysis. The closed flexible loop also

forms a tunnel for NH_3 to travel between the glutaminase and PRTase active sites. Finally, the flexible loop contacts feedback inhibitors in two subunits, contributing to the cooperative allosteric transition [2,9,10,35–38].

The PRTs can be classified into four groups based on flexible loop conformations (Figure 3). All of the flexible loops form additional β strands, which protrude from or add to the PRT core.

Group 1 includes the GPATases. The flexible loop consists of two short antiparallel β strands (Figure 3a). The first β strand is a continuation of β_2 in the PRT core [2,9,11]. The long, 20-residue connection between these β strands is disordered in the open-loop structures and undergoes a coil→helix transition as it folds and closes over bound $\text{Mg}\bullet\text{PRPP}$ [10].

The open flexible loops of hypoxanthine/guanine/(xanthine) PRTase [3,6,7,15,21,22], xanthine PRTase [8,14], guanine PRTase [24] and uracil PRTase [13], which comprise group 2, form a β hairpin that is nearly perpendicular to the core β sheet (Figure 3b). The hairpin loop, of ten or fewer residues, is disordered in open-loop structures. In closed-loop structures, the β hairpin is closer to the PRT core and the connecting loop is ordered and kinked over bound $\text{Mg}\bullet\text{PRPP}$ [16–18,20,25••].

The flexible loops of group 3 members, including orotate PRTase [1,4,5], PyrR [12] and PRPP synthetase [23•], contribute an additional antiparallel β strand to the PRT core (Figure 3c). The 10-residue loop connecting the antiparallel β strand to β_2 is disordered in open-loop structures of group 3 proteins.

The open flexible loops of PurR [26•] and adenine PRTase [19], comprising group 4, include a two-stranded, antiparallel β ribbon (Figure 3d). The least ordered part of the flexible loop is the distant end of the β ribbon and, in PurR, the connection between β_2 and the β ribbon. There are no structures representing closed forms of proteins from groups 3 and 4, but the flexible loops in neither group are long enough to refold in a helical conformation.

The PRT hood

Above the core fold, PRT proteins have a structure known as the hood. The hood includes peptides both N- and C-terminal to the core, and can be as small as a few loops, as in PurR and xanthine PRTase, or as large as a full domain, as in GPATase and PRPP synthetase.

The structure of the hood is highly variable across the PRT family. In PRT enzymes, the hood binds or generates the nucleophilic substrate. In purine or pyrimidine PRTases and in PyrR, the hood recognizes the appropriate base through specific hydrogen bonds and ligates the second Mg^{2+} in purine PRTases. In GPATase, the hood is a catalytic domain that generates the nucleophilic substrate NH_3 by

glutamine hydrolysis [39]. In PRPP synthetase, the hood produces PP_i from ATP [23•]. No base is known to bind to the hood of PurR, perhaps through the occlusion of potential binding space by two aromatic residues [26•].

Catalysis by PRTases

The available structures provide a wealth of information on states of PRTases relevant to catalysis, effectively providing snapshots along the reaction pathway [31,40]. The closed-loop structures, in particular, provide a remarkably consistent picture of $\text{Mg}\bullet\text{PRPP}$ binding. $\text{Mg}\bullet\text{PRPP}$ binds in a virtually identical manner in these structures, despite the absence of invariant residues among the enzyme members of the PRT family. The structural snapshots are consistent with a substrate-assisted catalytic mechanism, in which the enzyme provides a solvent-excluded, suitably charged environment and binds $\text{Mg}\bullet\text{PRPP}$ in a conformation that promotes transition-state formation. A number of details of $\text{Mg}\bullet\text{PRPP}$ binding support this hypothesis. The PP_i tail is oriented to accept a proton from the incoming nucleophile and to stabilize the planar (trivalent or pentavalent) transition state through a CH–O interaction between the ribose C1 proton and an α -phosphate oxygen [10]. Mg^{2+} ligation by all three exocyclic oxygens of the ribose ring provides electrostatic assistance to catalysis and influences the pucker of the ribose ring. The ribose pucker, which should assist in the formation of a partial double bond at O4–C1 in the transition state, differs among closed-loop structures, but all are within the C3-*exo*/C2-*endo* pseudorotation domain. Puckering at C3 would be optimal for double bond formation at O4–C1. The high-resolution (1.05 Å) structure of hypoxanthine/guanine PRTase has a C2-*endo* ribose pucker in the ternary complex with $\text{Mg}\bullet\text{PRPP}$ and 9-deazaguanine [25••].

The structural snapshots also help answer the question of whether catalysis proceeds by a more dissociative ($\text{S}_{\text{N}}1$) or associative ($\text{S}_{\text{N}}2$) mechanism. The positions of 5-phosphate, PP_i and Mg^{2+} are fixed in the active site. The ribose position varies, however, depending on whether $\text{Mg}\bullet\text{PP}_i$ is present and whether ribose C1 is bonded to PP_i or to the nucleophilic base. In this respect, the PRTase structures, taken as a group, are consistent with a dissociative ($\text{S}_{\text{N}}1$) mechanism.

Regulatory PRT proteins

Two members of the PRT family from *Bacillus subtilis* regulate the expression of purine and pyrimidine biosynthetic genes. In these proteins, the ability of a PRTase to bind its substrate PRPP or its product nucleotide has been co-opted for a regulatory function.

PurR represses transcription initiation of genes of the *de novo* purine pathway by binding to specific operator DNA sequences. PRPP induces the expression of the purine genes by reducing the affinity of PurR for operator DNA [41]. PRPP is presumed to bind to the PRT core in

the same manner as it binds other PRT proteins. The PRT domain of PurR is augmented by a helix-turn-helix domain to bind DNA, thus exploiting the PRPP-binding capability to control transcription initiation [26•].

PyrR regulates the expression of pyrimidine genes by attenuation. PyrR binds a specific stem-loop structure in the regulatory region of RNA transcripts of pyrimidine genes, allowing formation of a downstream terminator [42]. UMP is a co-attenuator, enhancing the RNA affinity of PyrR, and is presumed to bind in the PRPP loop of the PRT core. A basic surface of the PyrR dimer is the proposed binding site for the RNA stem-loop [12]. PyrR also has uracil PRTase activity, although catalytic activity is unlikely to be relevant to its regulatory function. In PyrR, the PRT fold is adapted to bind RNA, thus exploiting the binding of the PRTase product to attenuate gene expression.

Sequence homologs of PurR and PyrR are not widely distributed. They are found in a few bacteria, primarily close relatives of *B. subtilis*. On the other hand, PRT enzymes are ubiquitous. We therefore presume that the PRT regulatory proteins arose from PRT enzymes by relatively recent gene duplications. The closest structural and sequence relative of PurR among the PRTases is adenine PRTase. Despite its uracil PRTase catalytic activity, the closest relative of PyrR is hypoxanthine/guanine PRTase.

A distant relative – PRPP synthetase N-terminal domain

So far, we have discussed PRT proteins that bind Mg•PRPP and are recognized at the sequence level through the PRT motif, which includes the PRPP loop. However, distant relatives in many protein families are not recognizable at the sequence level and are discovered only through three-dimensional structures. To date, only one such distant relative has been identified in the PRT family. PRPP synthetase catalyzes the formation of PRPP from ribose-5-phosphate and ATP. The C-terminal domain of PRPP synthetase includes the PRT sequence motif and, as expected, has the PRT fold, which binds the substrate, ribose-5-phosphate, and the product, PRPP [23•].

Unexpectedly, the N-terminal domain of PRPP synthetase also belongs to the PRT family. The N-terminal domain binds the substrate, ATP, and the allosteric effectors, ADP and P_i, in unique sites that are not topological analogs of the Mg•PRPP site. The topological analog of the PRT flexible loop in the N-terminal domain is found in a subunit interface of the hexameric protein and participates in base-specific recognition of ATP in another subunit. The two PRT domains of PRPP synthetase face one another as if each were the other's 'hood' domain. The N-terminal domain is presumed to have arisen by duplication of the gene encoding the C-terminal domain. Other distant relatives of the PRT family remain to be identified.

Conclusions

Three-dimensional structures have fleshed out hints about a common structure and function among the homologous family of PRT proteins. Part of the characteristic 13-residue sequence motif forms the PRPP loop for binding Mg•PRPP, assisted by the PP_i loop and the flexible loop. The flexible loop has an essential role in the specific biological function of each PRT, such as catalysis, allostery, interdomain signaling and channel formation. The mode of Mg•PRPP binding is virtually identical among PRTases, strongly suggestive of a common catalytic mechanism. The lack of invariant amino acids in the active site leads to obvious proposals for substrate-assisted catalysis. PRTases of only the nucleotide synthesis and salvage pathways possess the characteristic sequence motif; other proteins with PRTase activity are unlikely to belong to the homologous PRT family. The mechanism of regulatory PRT proteins that bind DNA or RNA remains to be determined. Only one distant family member has been identified.

Update

The structure of the tryptophan biosynthetic enzyme anthranilate phosphoribosyltransferase was solved recently (O Mayans, A Ivens, K Kirschner, M Wilmanns, personal communication). As expected, this protein fits the pattern of other PRTases: it does not catalyze a reaction of nucleotide synthesis or salvage, it does not possess the PRT sequence motif, it has a fold different from the PRT proteins discussed in this review and it is not a member of the homologous PRT family. In addition, it has a different structure when compared to quinolinate PRTase, the 'type II PRTase' mentioned in the text.

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