Phosphoribosyl Diphosphate (PRPP): Biosynthesis, Enzymology, Utilization, and Metabolic Significance

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Published in: Microbiology and Molecular Biology Reviews

Link to article, DOI: 10.1128/MMBR.00040-16

Publication date: 2017

Document Version Peer reviewed version

Link back to DTU Orbit

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Phosphoribosyldiphosphate (PRPP): Biosynthesis, Enzymology, Utilization, and Metabolic Significance

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Running title: PRPP metabolism

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SUMMARY

Phosphoribosyl diphosphate (PRPP) is an important intermediate in cellular metabolism. PRPP, synthesized by PRPP synthase: ribose 5-phosphate + ATP → PRPP + AMP, is ubiquitously found in living organisms, and is used in substitution reactions with the formation of glycosidic bonds. PRPP is utilized in the biosynthesis of purine and pyrimidine nucleotides, the amino acids histidine and tryptophan, the co-factors NAD and tetrahydromethanopterin, arabinosyl monophosphodecaprenol and certain aminoglycoside antibiotics. The participation of PRPP in each of these metabolic pathways is reviewed. Central to the metabolism of PRPP is PRPP synthase, which has been studied from all kingdoms of life by classical mechanistic procedures. The results of these analyses are unified with recent progress in molecular enzymology and the elucidation of the three-dimensional structure of PRPP synthases from eubacteria, archaea, and humans. Structure and mechanism of catalysis of the five diphosphoryltransferases are compared, as are those of selected enzymes of diphosphoryltransfer, phosphoryltransfer and nucleotidyltransfer reactions. PRPP is used as a substrate by a large number phosphoribosyltransferases. The protein structure and reaction mechanism of these phosphoribosyltransferases vary and demonstrate the versatility of PRPP as an intermediate in cellular physiology. PRPP synthases appears to originate from a phosphoribosyltransferase during evolution as demonstrated by phylogenetic analysis. PRPP furthermore is an effector molecule of purine and pyrimidine nucleotide biosynthesis either by binding to PurR or PyrR regulatory proteins or as allosteric activator of carbamoylphosphate synthetase. Genetic analysis have disclosed a number of mutants altered in the PRPP synthase-specifying-genes in humans as well as bacterial species.
INTRODUCTION

The compound 5-phospho-D-ribosyl-α-1-diphosphate (PRPP) is an important metabolite required in the biosynthesis of purine and pyrimidine nucleotides, the amino acids histidine and tryptophan, and the co-factors NAD and NADP (1-3). Furthermore, PRPP is utilized in the biosynthesis of methanopterin in certain archaeal species (4), and in the biosynthesis of polyprenylphosphate-pentoses in Mycobacterium tuberculosis (5). By far, the most abundant class of reactions using PRPP as a substrate results in the formation of N-glycosidic bonds, whereas a few reactions result in the formation of O- and C-glycosidic bonds. Kornberg and co-workers discovered PRPP in the mid 1950s, while searching for a reaction that converted orotate to uridylate as well as the enzymes catalyzing these reactions (6, 7).

The enzyme catalyzing the synthesis of PRPP, PRPP synthase (ATP:D-ribose 5-phosphate diphosphotransferase, EC 2.7.6.1) is ubiquitous among free living organisms. Thus, only certain obligate intracellular parasites such as some Chlamydia and Rickettsia species lack a gene encoding PRPP synthase. Therefore, in general an organism contains at least one gene specifying PRPP synthase. A few bacterial species contain more than one prs gene. In contrast, many eukaryotic organisms contain more than one PRPP synthase-specifying gene. The yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe appear to contain five and three PRPP synthase orthologs, respectively (8-10), mammals (humans and rat) contain three PRPP synthase-encoding genes (11-13), and the plants Spinacea oleracea and Arabidopsis thaliana contain four and five PRPP synthase genes, respectively (14-16).

Although prs is believed to be an essential gene (17), a number of prs mutants have been isolated, primarily in Escherichia coli and Salmonella enterica serovar
Typhimurium, that are conditional or specify mutant variants of PRPP synthase with altered enzymatic properties. Additionally, a few knockout mutations (Δprs) have been constructed in vitro. Insertion of these Δprs alleles into strains with a specific genetic background revealed that an organism can be viable in the absence of PRPP synthase activity. Also, a number of naturally occurring variant PRPP synthases have been identified among patients with gout or uric acid overproduction (18).

The present review deals with all aspects of PRPP metabolism with emphasis on biochemical, genetic and physiological aspects of PRPP synthase and the utilization of PRPP in biosynthesis, primarily in microorganisms. The elucidation of high-resolution structures of PRPP synthase from a number of organisms makes this review timely. Previously, a few reviews have been published on PRPP synthase of microbial (1, 19), and of mammalian origin (18, 20). These reviews are complemented by the present.

Sequence analysis was performed with BLAST (21), the program packages of the Integrated Microbial Genomes web-site (http://img.jgi.doe.gov/) (22), and the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) (23), whereas amino acid sequence analysis and alignments were performed with Multalin (http://multalin.toulouse.inra.fr/multalin/) and DNA Strider (24, 25). Figures of three-dimensional structures were prepared with the Pymol Molecular Viewer (http://www.pymol.org) (26).

CHEMISTRY OF PRPP

PRPP is synthesized by transfer, in a single step, of the β,γ-diphosphoryl group of ATP to the C-1 hydroxyl of α-d-ribosyl 5-phosphate with the simultaneous formation of AMP by the following deceptively simple reaction: ribose 5-phosphate + ATP →...
PRPP + AMP (Fig. 1). The reaction is catalyzed by PRPP synthase (6, 7), encoded by a \( prs, prsA \) or \( PRPS \) gene (13, 27, 28). PRPP may be regarded as an activated form of ribose 5-phosphate. Essentially all of the reactions utilizing PRPP as a substrate are substitutions, where usually nitrogen-containing aromatic bases replace the diphosphoryl group with simultaneous inversion of configuration at C1 of the ribosyl moiety (Fig. 1). In the majority of these reactions a ribonucleoside 5’-monophosphate is formed. Thus, ”all” that has happened in reactions such as those shown in Fig. 1 is an attachment of the nitrogenous compound to the ribosyl moiety and the formation of diphosphate (PP\(_i\)). PP\(_i\), i.e. phosphoric acid anhydride with a high negative free energy of hydrolysis, in turn is hydrolyzed to phosphate (P\(_i\)), which makes the phosphoribosyl transfer reaction thermodynamically irreversible.

Immediately after its discovery, it was noted that PRPP was a stable compound as long as it was stored in the cold at neutral pH, whereas acidic or basic conditions and heating readily decomposed the compound. Divalent ions such as Mg\(^{2+}\), Mn\(^{2+}\) and Ba\(^{2+}\) encouraged decomposition. The degradation of PRPP, presumably by hydrolysis, appears to follow either of three pathways: (a) PRPP + H\(_2\)O \(\rightarrow\) ribose 5-phosphate + PP\(_i\), (b) PRPP + H\(_2\)O \(\rightarrow\) 5-phosphoribosyl 1,2-cyclic phosphate + P\(_i\) (6, 7, 29), or (c) PRPP + H\(_2\)O \(\rightarrow\) ribosyl 1,5-cyclic phosphate + PP\(_i\), with (a) and (b) representing the major pathways (30, 31). Degradation through pathway (b) may furthermore continue according to the scheme 5-phosphoribosyl 1,2-cyclic phosphate \(\rightarrow\) ribosyl 1-
phosphate \(\rightarrow\) ribose (32). The binding of divalent ions to PRPP is conveniently measured by \(^{31}\)P nuclear magnetic resonance, and revealed that Mg\(^{2+}\) binds to both the 5-phosphate and the 1-diphosphate moieties of PRPP (33).

The standard free energy (\(\Delta G^\circ\)) for the hydrolysis of PRPP (PRPP + H\(_2\)O \(\rightarrow\) ribose 5-phosphate + PP\(_i\)) has been calculated as approximately -35 kJ mol\(^{-1}\). This
value should be compared to -47 kJ mol\(^{-1}\) for the hydrolysis of the α,β-
phosphoanhydride bond of ATP (34). Consistently, the equilibrium constant for the
diphosphoryltransfer reaction of PRPP synthase was determined as 29 (35).

In spite of the lability of PRPP, procedures have been developed for the
quantitative isolation of the compound. These include extraction of PRPP from cells
with cold formic acid (36-38), cold perchloric acid (39), or boiling followed by
immediate cooling (40), followed by chromatography or phosphoribosyltransferase-
catalyzed conversion of PRPP to ribonucleoside 5’-monophosphate.

A chemically stable analog of PRPP has been synthesized for use as ligand to
various PRPP-binding proteins: 1-α-diphosphoryl-2,3-α-dihydroxy-4-β-cyclopentane-
methanol 5-phosphate (cPRPP) (41, 42). Although an inhibitor of PRPP synthase
activity, the compound has proven valuable as a PRPP analog in X-ray analyses of
PRPP synthase.

BIOSYNTHESIS OF PRPP

PRPP synthase and Phosphoribosyl Bisphosphate Phosphokinase

The synthesis of PRPP is catalyzed by two enzymes, PRPP synthase and
phosphoribosyl bisphosphate phosphokinase. On a quantitative basis, PRPP synthase
is by far the most important. Phosphoribosyl bisphosphate phosphokinase is a
component of the phosphonate catabolic pathway, and is present only in cells that
thrive on phosphonate as Pi source. We shall deal first with PRPP synthase, and with
phosphoribosyl bisphosphate phosphokinase in a later section.

CLASSIFICATION AND PROPERTIES OF PRPP SYNTHASES
Alignment of the amino acid sequences from a variety of organisms revealed a high similarity among PRPP synthases. For example, the overall identity of PRPP synthase amino acid sequences of *E. coli* and humans is 47% (20), that of PRPP synthases of *E. coli* and the Gram-positive organism *Bacillus subtilis* is 51% (43). In addition, a 15- amino acid sequence is highly conserved among PRPP synthase and adenine phosphoribosyltransferase (44, 45). The latter enzyme catalyzes the reaction adenine $+\text{PRPP} \rightarrow \text{AMP} + \text{PP}_i$. This 15-amino acid sequence of adenine phosphoribosyltransferase was designated the PRPP binding site, and later, the ribose 5-phosphate binding loop in PRPP synthase. Subsequently, studies of cDNA libraries of the plants *S. oleracea* and *A. thaliana* revealed the presence of four genes that specify PRPP synthase. Two of these enzymes (isozyme 1 and 2) had primary structures similar to those of *E. coli* and humans, whereas two (isozyme 3 and 4) had primary structures quite different from those of *E. coli* and humans. The amino acid sequence identity of *S. oleracea* isozyme 1 and 2 is 88% and that of isozyme 3 and 4 is 75%. In contrast, the amino acid sequence identity of isozyme 1 or 2 to isozymes 3 or 4 is modest, 22 to 25%. This deviation of amino acid sequence identity of *S. oleracea* PRPP synthase isozymes 3 and 4 from isozymes 1 and 2 and other “classical” PRPP synthases was also reflected in their physico-chemical properties, as further described below. It was therefore suggested that two different classes of PRPP synthases exist, class I and II (46, 47). Additionally, PRPP synthases of archaeal origin show low amino acid sequence identity with class I or class II PRPP synthases. Thus, the amino acid sequence identity of *B. subtilis* and the thermophilic, methanogenic archaeon *Methanocaldococcus jannaschii*, the thermoacidophilic archaeon *Sulfolobus solfataricus* or the thermoacidophilic, facultatively anaerobic, organotrophic archaeon *Thermoplasma volcanium* PRPP synthases is 26 to 28%. 
Analysis of PRPP synthase of *M. jannaschii* revealed biochemical properties that appeared to be a mixture of the properties of class I and II PRPP synthases, and it was suggested to belong to a novel class III PRPP synthases (48). In the present review we shall instead designate these class III enzymes as “archaeal PRPP synthases”. An alignment of representatives of PRPP synthases of class I (*B. subtilis*), class II (*S. oleracea* isozyme 4), and archaea (*M. jannaschii*) is shown in Fig. 2. Overall, 36 amino acid residues are identical among the three sequences, i.e. an amino acid sequence identity of 13% or less. The inclusion of additional 26 conserved amino acid residues results in a similarity of approximately 21%. As we shall see below, the conservation of amino acid residues identified as responsible for catalysis is very high, whereas amino acid residues involved in allosteric regulation of activity of *B. subtilis* PRPP synthase are non-conserved among the three classes, consistent with the fact that only class I PRPP synthases are allosterically regulated.

**Class I PRPP Synthases**

The classical PRPP synthases, i.e. class I, are by far the most widely phylogenetically distributed PRPP synthases. These enzymes contain approximately 315 amino acid residues. PRPP synthase of the bacterial species *S. enterica*, *E. coli* and *B. subtilis*, as well as those of humans and rat are the best studied PRPP synthases. Among the class I enzymes, *B. subtilis* and *E. coli* PRPP synthase and human PRPP synthase isozyme 1 have been crystallized and high-resolution structures have been determined (49-52). A three-dimensional structure has been determined also of PRPP synthase from the Gram-negative bacterium *Burkholderia* (*Pseudomonas*) *pseudomallei* strain 1710b (PDB code 3das) (53). However, there are no biochemical data available for the latter enzyme. The crystal forms of PRPP synthase from the various organisms are...
summarized in Table 1. Some kinetic properties of PRPP synthases of various
organisms are listed in Table 2.

**Eubacterial PRPP synthases.** In this section we shall treat the properties of
PRPP synthase of the bacterial species *B. subtilis, S. enterica, E. coli,* and *M. tuberculosis.* Among these, a three-dimensional structure has been determined of *B. subtilis* and *E. coli* PRPP synthase. The biochemical properties of *B. subtilis* PRPP synthase is much less studied compared to those of *S. enterica* and *E. coli* PRPP synthases. The latter two enzymes are identical except for two conservative amino acid substitutions (*E. coli* PRPP synthase serines 278 and 283 are threonine and alanine, respectively, in the *S. enterica* enzyme). Furthermore, the *S. enterica* and *E. coli* PRPP synthase amino acid sequences are 51% identical to that of the *B. subtilis* enzyme. It is therefore very likely that these three enzymes share identical three-dimensional structures, biochemical properties, and catalytic and regulatory properties. Below, we describe the three-dimensional structure of *B. subtilis* PRPP synthase and, when applicable, the properties of the enterobacterial PRPP synthases with reference to the structure of the *B. subtilis* enzyme.

**(i) Three-dimensional structure of *B. subtilis* PRPP synthase.** A number of
crystal forms, summarized in Table 1, are utilized for the description of the structure
and catalysis of *B. subtilis* PRPP synthase. (a) *SO_4^{2-}-PRPP synthase* with one *SO_4^{2-}* bound in the active site at the position corresponding to the phosphate moiety of
ribose 5-phosphate and a second *SO_4^{2-}* bound at the position corresponding to the the
α-phosphate of ADP at the regulatory site. No divalent metal ion was present, and the
structure was determined to 2.3 Å resolution (PDB code 1dkr); (b) *mADP-PRPP synthase* with methylene ADP bound at the active and regulatory sites. As before no
divalent metal ion was present, and the structure was determined to 2.2 Å resolution.
(PDB code 1dku) (49); (c) \textit{Cd}^{2+}-\text{PRPP synthase} with two \textit{Cd}^{2+} bound in each monomer presumably at the two \textit{Mg}^{2+} sites, AMP bound at the active site, and \textit{SO}_4^{2-} bound in place of the phosphate moiety at the ribose 5-phosphate site. The structure was resolved to 2.8 Å resolution (PDB code 1ibs) (50); (d) \textit{AlF}_3-\text{PRPP synthase}. This crystal form contained in the active site an analog of ATP pieced together by three molecules: (i) AMP representing the AMP moiety of ATP, (ii) \textit{AlF}_3 with \textit{Al}^{3+} representing the β-phosphorus and the three \textit{F}⁻ representing the three oxygens of ATP, and (iii) a second AMP molecule whose phosphate represented the γ-phosphate of ATP. The remaining adenosyl moiety of the latter AMP molecule protruded into an “empty space” between two subunits. Furthermore, the crystal form contained two \textit{Mg}^{2+} (one ligated to the “triphosphate chain”), one ribose 5-phosphate at the active site and one \textit{SO}_4^{2-} located at the position of the α-phosphate of ADP in the allosteric site. This structure was resolved to 2.8 Å resolution. Altogether this complex is thought to resemble the transition state of PRPP synthase; (e) \textit{mADP/R5P-PRPP synthase} with one methylene ADP representing the ADP moiety of ATP as well one ribose 5-phosphate in the active site, two \textit{Mg}^{2+} and one \textit{SO}_4^{2-} representing the α-phosphate of ADP at the allosteric site in each monomer. This structure was resolved to 2.1 Å (54). The structure of the active site is described in more detail including stereo views in the forthcoming section “Mechanism of Catalysis”. Two additional crystal forms (GDP-PRPP synthase and mGDP-PRPP synthase) have been determined and have been particularly useful in elucidating the mechanism of allosteric regulation and will be further described below.

(a) \textit{Tertiary structure}. The PRPP synthase monomer is composed of two domains in a head to tail arrangement. The tertiary structures of the two domains are remarkably similar, although the amino acid sequence identity of the two domains
(amino acid residues 1 to 150 and 152 to 292) is only 11% with additional 10% similar amino acid residues. Thus, the similarity in tertiary structure of the two domains is not at all predictable from their primary structure. Each domain possesses an α/β structure with a five-stranded parallel β-sheet at the center surrounded by four α-helices as well as one 3_{10}-helix in the N-terminal domain (Fig. 3A). Additionally, short antiparallel β-sheets, designated flag regions, flank both domains. This structure resembles that of type I phosphoribosyltransferases. It is likely, therefore, that type I phosphoribosyltransferases and PRPP synthase originate from the same ancestral gene, and that “modern” PRPP synthases may have evolved from duplication of that ancestral gene of half the size of the contemporary prs gene (54).

A number of regions along the amino acid sequence have been highlighted on the basis of their functions. These are called the regulatory flexible loop (Tyr97-Thr113), the diphosphate (PP) loop (Asp174-Gly177), the catalytic flexible loop (Lys197-Met208) the ribose 5-phosphate binding loop (previously called the PRPP binding site) (Gly216-Thr231) (49, 50, 54) (Fig. 2).

There are two types of subunit interactions in PRPP synthase. First, interactions of the α3N and α4N helices of the N-terminal domain result in the formation of a bent head to head arrangement of two subunits, referred to as the bent dimer (Fig. 3B). The relevent amino acid residues involved in this interaction are Asn69, Glu70, Ile 72, Met73, Leu76, Ile77 (α3N) and Leu116 and Leu120 (α4N), many of which are highly conserved and listed in Table 3 (left-hand column). Second, a subunit is aligned in a parallel manner with a neighboring subunit in an arrangement involving residues from both the N- and the C-terminal domains (Fig. 3B). These interactions include hydrophobic interactions and salt bridges of the α1C helix and the flag region of the C-terminal domain as well as interactions of the 3_{10} helices and salt bridges of the N-
terminal domain. Important residues in the formation of this type of dimer, referred to hereafter as the parallel dimer, are Lys115, Gln138, Ile139, Phe142, Asp144, Val178, Asp186, Ile192, Ala193, Ile194, Arg198 and Val211 and are listed in Table 3 (left-hand column) (49). The two types of subunit interactions are shown in Fig. 3B, and it is easy to see how this “trimer” may be formally assembled to the hexameric, propeller like structure shown in Fig. 3C. The enzyme, thus, consist of a trimer of dimers with all of the N-terminal domains forming an inner circle and the C-terminal domains forming the propeller blades at the outside resulting in a three-fold symmetry axis with perpendicular two-fold axes (49).

(b) The active site. The active site must accommodate the substrates ribose 5-phosphate and MgATP. In addition, an overwhelming volume of research data has shown that an additional so-called free Mg\(^{2+}\) is required for activity, because maximal activity is only obtained when Mg\(^{2+}\) is added to the reaction in excess of the MgATP concentration with both bacterial (35, 45, 55-58), and mammalian PRPP synthases (59, 60). Furthermore, most PRPP synthases may accept other divalent metal ions in place of Mg\(^{2+}\), and, thus, Mg\(^{2+}\) may be regarded as a pseudosubstrate (45, 56, 58, 61). In contrast, Ca\(^{2+}\) is an inhibitor of PRPP synthase activity, even in the presence of Mg\(^{2+}\) (45). In the crystal structure one Mg\(^{2+}\) (the MG1 site) coordinates to Asp174, a highly conserved residue of the C-terminal domain, to the oxygens of the hydroxyls of C1, C2 and C3 of ribose 5-phosphate, to an oxygen of the \(\gamma\)-phosphate of ATP and to a water molecule, which forms hydrogen bonds to Asp174 and Asp223. This Mg\(^{2+}\) thus ligates to both of the substrates. A second Mg\(^{2+}\) (MG2) coordinates to His135 of the N-terminal domain, to oxygens of the \(\alpha\)-, \(\beta\)- and \(\gamma\)-phosphates of ATP and to two water molecules, which form hydrogen bonds to the side chains of Asp103 and Arg104 and the carbonyl oxygen of Arg101. The effect of this intricate binding is a
perfect alignment of ribose 5-phosphate and ATP for an in-line attack of the hydroxyl of C1 of ribose 5-phosphate at the β-phosphorus of ATP (54), as described in detail in the section “Mechanism of Catalysis”.

As described above, the two active site Mg$_2^+$ ligate to Asp174 of the C-terminal domain and His135 of the N-terminal domain. Similarly, the ATP binding site is located at the interface of the N- and C-terminal domains of each subunit, but with contributions of amino acid residues of the N-terminal domain of a neighbor subunit of the parallel dimer, for example, subunit A and subunit D (Fig. 3B). The specific amino acid residues that are important in the binding of ATP in *B. subtilis* PRPP synthase are listed in Table S1 (left-hand column). In contrast, the ribose 5-phosphate binding site is located within the C-terminal domain and is formed exclusively by amino acid residues of this domain. A subset of amino acid residues of the ribose 5-phosphate binding site, Gly216-Thr231, have been shown to directly interact with the hydroxyls 2 and 3 (Asp223 and Asp224), or the phosphate moiety (Asp227, Thr228, Ala229, Thr2321 and Ile232) (50, 54), also listed in Table S1 (left-hand column).

Chemical modification studies have confirmed the importance of some of the amino acid residues mentioned above. Thus, chemical modification of *S. enterica* PRPP synthase with 5’-(4-fluorosulfonylbenzoyl)adenosine completely inactivated the enzyme in a one to one molar ratio. ATP protected the enzyme against inactivation, and the site of modification was His130, which corresponds to His135 in *B. subtilis* PRPP synthase (62), providing evidence for the importance of this residue.

Similarly, affinity labelling of *E. coli* PRPP synthase has been performed with the ATP analog 2’,3’-dialdehyde ATP. Three lysine residues were labeled, Lys181, Lys193 and Lys230. Only Lys193 is conserved and corresponds to Lys197 of *B. subtilis* PRPP synthase (63). As we shall see, this Lys197 residue plays a very
important role in the catalysis of PRPP synthase. Chemical modification with
sulphhydryl reagents has been reported to cause inactivation of PRPP synthase that is
protected by the presence of ATP and P$_i$ (64, 65), but cysteine residues have not been
identified from the structural studies to be important in catalysis, which suggests that
these treatments resulted in nonspecific inactivation of the enzyme.

The geometry of the two Mg$^{2+}$ binding sites confirms a wealth of information on
the binding properties of divalent cations to PRPP synthases of particularly S. enterica
and E. coli. Firstly, kinetic analysis of the S. enterica enzyme revealed the binding of
both MgATP and free, i.e. enzyme-bound, Mg$^{2+}$ (56). It was proposed that the
enzyme-bound Mg$^{2+}$ ligates to the α-phosphate of ATP, provided to the enzyme as
β,γ-MgATP (58, 66). However, according to the crystal structure of B. subtilis PRPP
synthase, the true substrate of the enzyme is the α,β,γ-tridentate complex of MgATP,
which is consistent with the MG2 site described above. Additionally, analysis of E.
coli PRPP synthase altered in the ribose 5-phosphate binding site (Asp220Glu,
Asp220Phe and Asp221Ala) revealed that the effects on the values for $V_{app}$ and $K_M$ for
ribose 5-phosphate were dependent on the divalent cation present suggesting that the
binding of ribose 5-phosphate also occurs by interaction with Mg$^{2+}$ (61), which is
consistent with the MG1 site described above. The crystal structure furthermore
completes nuclear magnetic resonance analyses that attempted to elucidate the
conformation of ATP at the active site of S. enterica PRPP synthase. Thus,
paramagnetic line broadening of the C-1 proton or of $^{31}$P of ribose 5-phosphate by
Cr(III) bound to ATP as the exchange-stable αβγ-tridentate complex estimated the
distances from the Cr atom (and presumably Mg$^{2+}$) to the two atoms as 6.7 to 8.0 Å.
This is consistent with proximity of the ribose 5-phosphate C1 hydroxyl and the β-
phosphorus of ATP to the enzyme-bound divalent cation (67).
Steady state kinetic analysis of the inhibition of *S. enterica* or *E. coli* PRPP synthase by substrate analogs revealed an ordered Bi-Bi mechanism with binding of Mg$^{2+}$ first followed by MgATP and then ribose 5-phosphate (41, 56, 57). The ordered kinetic mechanism was further confirmed by equilibrium dialysis. Thus, radioactive ATP and the inactive analog α,β-methylene ATP bound well to the free enzyme with dissociation constants in the micromolar range, whereas ribose 5-phosphate binding could not be detected unless α,β-methylene ATP was also included (68). Kinetic parameters of PRPP synthases from various bacilli and enteric organisms are listed in Table 2.

*(c) The allosteric site.* In addition to the crystal forms SO$_4^{2-}$-PRPP synthase and mADP-PRPP synthase described above, two additional crystal forms have been useful in elucidating the structure of the allosteric site. (f) *GDP-PRPP synthase* (with four Mg$^{2+}$, one Ca$^{2+}$, one GDP, one GTP, two α,β-methylene ATP and two ribose 5-phosphates bound per assymetric unit) resolved to 1.8 Å resolution; (g) *mGDP-PRPP synthase* (with five Mg$^{2+}$, one methylene GDP, three α,β-methylene ATP and two ribose 5-phosphate bound per assymmetric unit) resolved to 1.9 Å resolution (54). The allosteric site of *B. subtilis* PRPP synthase is composed of amino acid residues contributed by three subunits such as A, B and D (Fig. 3B) (49). The amino acid residues involved in the binding of the β-phosphate and the adenyl moiety of ADP are provided by subunit D (Ser52, Arg54, Ala85 and Ser86) and subunit B (Ser310, Val311, Ser312 and Phe315). Subunit B also provides amino acids for the so-called hydrophobic pocket (Leu134, Ile139, Gln140, Asp148 and His149). Finally, subunit A contains the regulatory flexible loop (Lys105, Ala106, Arg107, Ser108 and Arg109) (49, 54). These amino acid residues are listed in Table S2. A stereo view of
the allosteric site is shown in Fig. 4. We shall return to the mechanism of allosteric regulation below in the section “Regulation of PRPP Synthase Activity”.

In general, the activity of class I PRPP synthases is regulated by the presence of ribonucleoside diphosphates, primarily ADP, which inhibits the enzyme in competition with ATP as well as by binding to a second, allosteric site. In some cases GDP is also an inhibitor, although GDP only binds to the allosteric site. Thus, a dual mechanism, competitive and allosteric inhibition, appears to control the activity of class I PRPP synthases. The complex inhibition pattern by ADP of the enzyme from \textit{S. enterica} has been studied in particular detail. Kinetic analysis revealed that ADP competes with ATP at subsaturating ribose 5-phosphate concentrations, and that ADP causes substrate inhibition by ribose 5-phosphate due to the binding at a site different from the active site (69). Direct binding was demonstrated by equilibrium dialysis.

Thus, in the absence of ribose 5-phosphate ADP binds to the same site as ATP, whereas in the presence of ribose 5-phosphate, ADP binds to two sites per monomer (68). Presumably, these are the active and allosteric sites, respectively. Similarly, PRPP synthases from \textit{E. coli} (45), \textit{B. subtilis}, and mammalian sources have been shown to be subject to allosteric regulation by ADP, the enzymes from \textit{B. subtilis}, \textit{Bacillus caldolyticus}, and mammals are also inhibited by GDP (55, 70-72).

As mentioned above, the activity of class I PRPP synthases requires the presence of P\(_i\). In some cases sulfate may substitute for P\(_i\) although a ten-fold higher concentration of sulfate is required (49, 71, 73). Removal of P\(_i\) from \textit{E. coli} and \textit{S. enterica} PRPP synthase causes irreversible inactivation or aggregation (35, 45, 74), although the presence of MgATP or MgPRPP stabilizes the enzyme from \textit{E. coli} or \textit{S. enterica}, respectively (35, 75). \textit{B. subtilis} and mammalian PRPP synthases are stable but inactive upon P\(_i\)-removal (49, 71, 73). Indeed, P\(_i\) and ADP have been shown to
compete for the same binding site in *B. subtilis* and human PRPP synthases, with Pi acting as an allosteric activator and ADP an allosteric inhibitor. Thus, certain mutant variants of PRPP synthase desensitized in inhibition by ribonucleoside diphosphates are reciprocally activated at lower concentrations of Pi (72), and the antitumor agent aminopyrimidopyrimidine ribonucleoside 5'-phosphate binds to the allosteric site of human PRPP synthase isozyme 1 and 2. The concentration needed for half-maximal binding increases with increasing Pi concentrations (76). Finally, a sulfate ion was found in the *B. subtilis* SO\(_4^{2-}\)-PRPP synthase structure. Superimposition of the SO\(_4^{2-}\)-PRPP synthase and mADP-PRPP structures revealed that the sulfate ion was located similarly to the β-phosphate of the methylene ADP molecule of the regulatory or allosteric ADP binding site (49). Altogether, the data demonstrate that Pi and ADP compete for binding to the same site. Steady state kinetic analysis revealed that Pi binds randomly to *E. coli* PRPP synthase, i.e. either before or after the ordered binding of Mg\(^{2+}\), MgATP and ribose 5-phosphate. Similarly, ADP may bind to PRPP synthase in a random fashion, however, with significantly different rate constants constituting the equilibria, so that the pathway where Mg\(^{2+}\), MgATP and ribose 5-phosphate bind in the absence of Pi is much slower than when Pi is bound first. This phenomenon provokes what is termed kinetic cooperativity and thus the sigmoid saturation curves frequently obtained for Pi activation with class I PRPP synthases (75).

As expected from the 51% identity of the amino acid sequences of *B. subtilis* and *E. coli* PRPP synthases, the amino acid residues involved in the formation of dimers as well as the the active site and the allosteric site (Tables 3, S1 and S2) are highly conserved. The identity of this subset of amino acids is 61%. These data are
furthermore confirmed by analysis of the three-dimensional structure of *E. coli* PRPP synthase (52).

(ii) *M. tuberculosis* PRPP synthase. Some attention has been devoted to studies of PRPP synthase of *M. tuberculosis* in searching for possible targets for drug treatment of tuberculosis. Three research groups have characterized this enzyme (77-79). All three groups agree that the quaternary structure of the active enzyme is a hexamer, and that allosteric inhibition is exhibited by the ribonucleoside diphosphates ADP and GDP, whereas there is some disagreement about the kinetic properties (Table 2). In addition, one research group established GTP, UTP and CTP as diphosphoryl donors with $k_{cat}/K_M$ values of 3.8, 3.0 and 2.5 M$^{-1}$ s$^{-1}$, respectively, compared to the value 26 M$^{-1}$ s$^{-1}$ for ATP (78). Interestingly, analytical ultracentrifugation revealed that the apo-enzyme existed as a trimer as well as a hexamer. The presence of ADP greatly shifted the oligomeric state toward the hexamer, whereas the presence of ATP had no effect on the oligomerization state (79). A comparison of the amino acid residues involved in dimer formation, bent as well as parallel dimers, of *M. tuberculosis* and *B. subtilis* PRPP synthases is shown in Table 3. It is evident that essentially all of the amino acids involved in dimer formation of *B. subtilis* PRPP synthase are retained in the *M. tuberculosis* enzyme. Additionally, a comparison of the amino acid residues involved in the binding of the substrates MgATP, ribose 5-phosphate and Mg$^{2+}$ of the two enzymes reveals a high degree of conservation as does a comparison of the amino acid residues involved in the formation of the allosteric site between the two enzymes. All of these data are consistent with the identification of the *M. tuberculosis* enzyme as a class I PRPP synthase. The amino acids involved in the binding of GTP and the pyrimidine ribonucleoside 5’-triphosphates CTP and UTP as substrates for PRPP synthase have
not been mapped, so the propensity of *M. tuberculosis* PRPP synthase to utilize these latter ribonucleoside 5’-triphosphates remains unexplained. Other class I PRPP synthases do not utilize GTP, CTP or UTP.

(iii) Other bacterial PRPP synthases. Although most prokaryotic species contain a single gene encoding PRPP synthase, the genomes of almost 100 species have been annotated as containing two PRPP synthase orthologs. Biochemical data for PRPP synthase are available for only a few of these organisms. One of these is *Lactococcus lactis*, which contains two PRPP synthase-encoding orthologs, *prsA* and *prsB*, and for which some physiologic data exist. The two genes are separated by approximately 300 kBp. The sequence identities of the *prsA*- and *prsB*-specified amino acid sequences with that of *B. subtilis* PRPP synthase are 65% (*L. lactis* PrsA), and 52% (*L. lactis* PrsB), whereas the amino acid sequence identity between the two *L. lactis* Prs sequences is 49%. Comparison of amino acid sequence of *L. lactis* prsA- and prsB-specified gene products and the established active site amino acid residues of *B. subtilis* PRPP synthase reveals that PrsA and *B. subtilis* PRPP synthase are nearly identical, whereas in PrsB the amino acid residues of the catalytic flexible loop vary considerably from those of *B. subtilis* PRPP synthase. Specifically, the catalytically important Lys197 and Arg199 residues of *B. subtilis* PRPP synthase are replaced by Tyr198 and Asp200 in *L. lactis* PrsB (Table S3). Complementation analysis revealed that *L. lactis* prsA complements an *E. coli* prs allele, whereas prsB does not, which demonstrate that prsA encodes a functional PRPP synthase. The function of the prsB gene product remains to be established (P. Bennedsen and J. M., The Technical University of Denmark, unpublished results).

A few bacterial species have three annotated PRPP synthase orthologs. In *Roseiflexus castenholzii* (DSM 13941) one amino acid sequence has a histidine
residue corresponding to *B. subtilis* Arg199 as well as additional six amino acid residues in the catalytic flexible loop, and 43% amino acid sequence identity with *B. subtilis* PRPP synthase. The other two amino acid sequences have 42 and 51% amino acid sequence identity with *B. subtilis* PRPP synthase. Similarly, in *Sphingobium japonicum* strain UT26S one sequence has an aspartate at the position of the *B. subtilis* Lys197 and there is a nine amino acid-insertion in the catalytic flexible loop. In *Pseudomonas stutzeri* strain A 1501 the amino acid sequence identity with *B. subtilis* PRPP synthase varies between 23 and 49%, in *Clostridium beijerinckii* strain NCIMB 8052 these values are 27 and 62%, whereas in *Rhodoferax ferrireducens* strain T118 these values are 24 and 54%. No biochemical studies of any of these putative PRPP synthases are currently available.

**Yeast PRPP synthases.** Among yeast species PRPP synthase has been analyzed only in *S. cerevisiae* and *S. pombe*, but presumably many of the physiologic properties of PRPP metabolism of these two species are similar to those of other yeast species. Although yeast PRPP synthases share typical class I properties, they also contain properties different from the PRPP synthases described so far.

(i) *S. cerevisiae*. *S. cerevisiae* contains five PRPP synthase-specifying orthologs, *PRS1* to *5* (8, 9). Inspection of the amino acid sequences reveals that *PRS2*, *PRS3* and *PRS4* appear to encode typical class I enzymes. Also, *PRS1* and *PRS5* may encode class I enzymes, but *PRS1* - and *PRS5*-specified amino acid sequences contain so-called non-homologous regions (NHRs), which are insertions relative to the sequences specified by *PRS2*, *PRS3* or *PRS4* (Fig. 5). Prs1 contains a single NHR (NHR1) of 105 amino acids in length, whereas Prs5 contains two NHRs of 110 amino acids (NHR5-1) and 63 amino acids (NHR5-2). With Prs1 immunochemical analysis has confirmed that the NHR1 is actually present in the polypeptide found in yeast cells (8,
The Prs1 NHR1 is inserted after Ser199, whereas the Prs5 NHR5-2 is inserted after Ser306. These two positions, Ser199 and Ser306, correspond to Arg202 of the \textit{B. subtilis} PRPP synthase, and, thus the NHRs are inserted in the catalytic flexible loop. In contrast, the Prs5 NHR5-1 sequence is inserted after Pro100, which corresponds to Asp103 of the \textit{B. subtilis} PRPP synthase, and, thus the NHR5-2 is inserted in the regulatory flexible loop. The identity of the amino acid sequences specified by \textit{PRS2}, \textit{PRS3} and \textit{PRS4} with that of \textit{B. subtilis} PRPP synthase is 42-46\%, whereas these values for the \textit{PRS1}- and \textit{PRS5}-specified sequences are 29 and 24\%, respectively. After removal of the NHR amino acid sequences in silico, the latter values increase to 41 \% (Prs1 \text{ΔNHR1}) and 39 \% (Prs5 \text{ΔNHR5-1 ΔNHR5-2}).

(a) Interaction of \textit{PRS} gene products. Unlike most other cloned \textit{prs} genes, the gene products of each of the five \textit{S. cerevisiae} \textit{PRS} genes have no PRPP synthase activity in vivo, when their genes are harbored and expressed individually in an \textit{E. coli} host strain (82). In contrast, the formation of active PRPP synthase requires the expression of two or more \textit{PRS} genes, as established by deletion analysis of \textit{PRS} genes in \textit{S. cerevisiae} or by expression of \textit{PRS} genes in \textit{E. coli}. A single knockout mutation in \textit{PRS1}, \textit{PRS2}, \textit{PRS3}, \textit{PRS4} or \textit{PRS5} results in viable phenotypes (80, 83). Additionally, all 10 combinations of two \textit{PRS} gene knockouts result in viable phenotypes except the \text{Δprs1 Δprs5} and \text{Δprs3 Δprs5} double knockouts, both of which are lethal (83). The latter double mutant, \text{Δprs3 Δprs5}, is lethal because the Prs1 polypeptide is unstable in a \text{Δprs3} genetic background. Thus, strains with the genotype \text{Δprs3 Δprs5} are phenotypically \text{prs1 prs3 prs5} (84). Among the five possible combinations of deletion of three \textit{PRS} genes only \text{Δprs1 Δprs2 Δprs3}, \text{Δprs1 Δprs3 Δprs4} and \text{Δprs2 Δprs3 Δprs4} are lethal, and, thus, \textit{S. cerevisiae} cannot exist with only a
single PRS gene. These results were confirmed by analysis of complementation by relevant combinations of PRS genes of an *E. coli Δprs* allele. This analysis furthermore showed that the simultaneous expression of *PRS1* and *PRS2* or the simultaneous expression of *PRS1* and *PRS4* resulted in PRPP synthase with very low activity in vivo, presumably too low to promote growth of *S. cerevisiae Δprs3 Δprs4 Δprs5* or *Δprs2 Δprs3 Δprs5* deletants. Although complementation of the *E. coli Δprs* allele by combinations of two PRS genes revealed the production of active PRPP synthase in vivo, in vitro PRPP synthase activity could be detected only in cell extract of the strain that expressed both *PRS1* and *PRS3* (82). The conclusions from this analysis are that active PRPP synthase requires either *PRS1* or *PRS5* in addition to at least one more PRS gene product, but not just any PRS gene product. Thus, an active enzyme consists of the *PRS1* and *PRS3* gene products, or an active enzyme consists of the *PRS5 PRS2*, the *PRS5 PRS4*, or the *PRS5 PRS2 PRS4* gene products (83).

Physical interaction of Prs polypeptides was furthermore demonstrated by yeast two-hybrid analysis. The results of this analysis showed interaction between Prs1 and Prs3, between Prs1 and Prs2 and between Prs1 and Prs4. These interactions were demonstrated in a reciprocal manner, i.e. with each PRS gene fused to the binding domain as well as to the activating domain. Additional, some non-reciprocal interactions were also observed between Prs5 and Prs2 and between Prs5 and Prs4 (83). Studies of synthetic lethality of combinations of *prs*-deletions also demonstrated interaction between Prs1 and Prs3 and between Prs1 and Prs5 (84, 85). These Prs-polypeptide interactions, demonstrated by genetic and physical analysis after manually manipulating the relevant DNA molecules, have been by and large confirmed by high-throughput analyses with two-hybrid analysis (86-88), protein-fragment complementation (89, 90), or affinity-capture mass spectrometry (91, 92).
The protein-protein interaction analyses reveal an extensive interaction between the various PRS gene products. Evidently, all of the Prs-polypeptides interact with one another. Presumably, each Prs-polypeptide may be able to form a hexameric structure like that of *B. subtilis* PRPP synthase, as well as mixed hetero-hexamers and eventually larger molecular structures similar to those described for mammalian PRPP synthases (see below).

**(b) Enzyme structure.** *S. cerevisiae* PRPP synthase appears to be a large enzyme. PRPP synthase activity of wild-type *S. cerevisiae* strain YN94-2 eluted as a single symmetrical peak corresponding to a molecular mass of at least 900 kDa by exclusion chromatography (Fig. S1A). Also, the enzyme eluted as a single peak by ion exchange chromatography (Fig. S1B). These results may indicate the presence of a single enzyme form containing all five PRS gene products, although the existence of multiple forms with nearly identical size and charge properties cannot be excluded. Additionally, PRPP synthase activity of extracts of Δprs2, Δprs3 or Δprs4 strains eluted with molecular mass identical to that of the wild-type, whereas no PRPP synthase activity could be determined in the exclusion chromatography-eluates of either a Δprs1 or a Δprs5 strain, even though activity could be easily determined in the cell extracts before loading them to the column (Fig. S1A). Thus, PRPP synthase lacking either of the Prs1 and Prs5 polypeptides may be unstable to the conditions of exclusion chromatography conditions, and the presence of additional PRS gene product(s) apparently stabilizes the enzyme complex (93).

Amino acid residues important in active site formation, i.e. residues involved in the binding of the adenyl moiety and the triphosphate chain of ATP as well as binding of ribose 5-phosphate, and the catalytic flexible loop of *B. subtilis* PRPP synthase are compared to those of each of the five *S. cerevisiae* PRS specified amino acid
sequences (Table S3). The similarity of these selected *B. subtilis* PRPP synthase amino acid residues with each of those of *S. cerevisiae* is 71% (*PRS1*-specified amino acid residues), 88% (*PRS2-, PRS3- and PRS4*-specified amino acid residues), or 50% (*PRS5*-specified amino acid residues). Little is known of the enzymatic properties of *S. cerevisiae* PRPP synthase activity. Some activity remains following dialysis of an *S. cerevisiae* crude extract against P$_i$-free buffer and in assay under P$_i$-free conditions and only 18% of the activity remained when the enzyme was assayed in the presence of 2 mM ADP relative to 100% in the absence of ADP (82). These properties differ from those of bacterial PRPP synthases.

(c) Physiological function. The complexity of *S. cerevisiae* PRPP synthase polypeptides may reflect their physiological function. Evidence for involvement of *PRS*-specified polypeptides in processes other than catalysis of PRPP synthesis originates from characterization of (i) *prs* mutants, (ii) mutants defective in other processes and from analyses of (iii) protein-protein interactions with *PRS*-specified polypeptides. First, Δ*prs3* mutant strains are highly pleiotropic and it was concluded that *PRS3* is required for cell wall integrity, cell cycle arrest following deprivation of nutrient, proper ion homeostasis, and proper actin cytoskeleton-organization, and cell size homeostasis (94-96). The cell wall integrity signaling pathway includes protein Slt2 and the transcription factor Rlm1 of the protein kinase C (Pkc1) mitogen-activated protein kinase cascade (97). Strains with *prs* lesions are sensitive to the purine analog 1,3,7-trimethylxanthine (caffeine), which interferes with Pkc-1 activated cell wall integrity signaling and the mitogen-activated protein kinase cascade (98), providing evidence for Prs polypeptides being involved in the cell wall integrity signaling (99). Second, the *pps1*-1 lesion of *S. cerevisiae*, isolated after screening for an elongated cell morphology, is an allele of *PRS1* (100). Third,
evidence of Prs polypeptides being involved in the stress and cell wall integrity signaling pathway comes from yeast two-hybrid analysis of Prs polypeptides with Slt2 (85, 101, 102).

A number of other protein-protein interactions with Prs polypeptides as partners have been identified. Rim11, a homolog of mammalian glycogen synthase kinase-3β interacts with Prs2, Prs3 and Prs5 as shown by affinity-capture mass spectrometry (103), and confirmed by manual yeast two-hybrid analysis with the RIM11 gene and each of the PRS genes (104). High through-put analyses revealed a number of protein-protein interactions of Prs polypeptides with other polypeptides (86, 88), although their physiological function has not been established (102). Interestingly, the interaction of the protein kinase Rim11 with Prs5 may be responsible for the phosphorylation of the latter protein (104). Phosphoproteome analysis by mass spectrometry revealed three phosphorylation sites in Prs5, 361-KTTpSTSpSTpSS-370, where p indicates phosphorylation of the following serine residues (105). This sequence is located within NHR5-2.

From the data given above it appears very likely that yeast PRS specified polypeptides share functions in addition to the catalysis of PRPP formation. Further investigations are necessary to establish the detailed mechanisms of this behaviour.

(d) Function of NHR. The function of the NHRs has been studied to some extent. Deletion of NHR1 prevented the interaction with Slt2 (84). It is therefore possible that yeast PRPP synthase may be a bifunctional enzyme involved in nucleotide and amino acid biosynthesis as well as in maintaining cell wall integrity, the latter function involving the NHR1 (84, 85, 101, 102).

Finally, the deletion of NHR5-1 resulted in a polypeptide with altered interaction with other Prs polypeptides. The simultaneous expression in an E. coli Δprs host
strain of PRS3 and prs5 ΔNHR5-1 or of PRS3 and prs5 ΔNHR5-1 ΔNHR5-2 resulted
in complementation of the E. coli Δprs allele, and, thus, production of active PRPP
synthase, whereas no complementation was observed by the simultaneous expression
of the wild-type alleles PRS3 and PRS5. This result may indicate that the NHR5-1
prevents the formation of an active enzyme rather than preventing interaction of Prs3
and Prs5, as interaction of these polypeptides has been previously established (89,

(e) Ribonucleotide pool sizes and PRPP synthase activity. The apparent
interaction of Prs polypeptides with proteins of other cellular processes suggests an
involvement of Prs polypeptides in linking metabolism to various stress situations. It
remains to be established, however, if other physiological phenomena, such as
perturbation of the pool sizes of ribonucleotides or PRPP, are also involved in the
transmission of these metabolic effects. Single PRS knock-outs are either unaffected
in growth rate (μ) as observed for Δprs2, Δprs4 and Δprs5 harboring strains or
reduced in growth rate as observed for Δprs1 and Δprs3 harboring strains (8, 81, 106).
Along with reduced growth rates, the pool sizes of adenylate nucleotides (AMP, ADP
and ATP) were reduced to approximately 50 % of those of wild-type strains in Δprs1
and Δprs3 strains, whereas less reduced adenylate nucleotide pool sizes were observed
in Δprs2, Δprs4 and Δprs5 strains (106). Correspondingly, PRPP synthase activity
was lower in Δprs1 or Δprs3 strains than in Δprs2 or Δprs4 strains (81). A Δprs1
Δprs3 strain (i.e. synthesizing Prs2, Prs4 and Prs5) contained approximately 40 % of
the adenylate nucleotides and 2-3 % of the PRPP synthase activity compared to the
values of the wild-type strain. A Δprs2 Δprs4 Δprs5 strain (i.e. synthesizing Prs1 and
Prs3) had almost normal growth rate, 90 % adenylate nucleotide pools and 16 %
PRPP synthase activity compared to the values of the wild-type strain (83). The
relatively low PRPP synthase activities determined in the various mutant strains may indicate that non-optimal assay conditions were employed or that PRPP synthase may unstable in vitro when one or more PRS gene products are absent, and thus may not accurately reflect PRPP synthase activity in vivo. Altogether, a hierarchy exists among prs lesions. Thus, Δprs3 or Δprs1 deletants appear to be more severely affected than Δprs3, Δprs4 or Δprs5 deletants.

In summary, much focus has been devoted to the analysis of Prs polypeptide interactions with one another as well as with other polypeptides. However, the enzymology of yeast PRPP synthase is far from solved. The characterization in the future of a few mutants suggested from studies of the well characterized bacterial PRPP synthases might contribute significantly to our understanding of the oligomerization of this enzyme. The *B. subtilis* PRPP synthase amino acid residues His135, Asp174 and Lys197 are all very important for catalysis. These amino acid residues are identical in the five yeast Prs polypeptides except for Prs1, which has Asn171 and Arg196 rather than aspartate and lysine residues, respectively, found in *B. subtilis* PRPP synthase (Table S3). Thus, a replacement of the Lys197 analogs with alanine followed by complementation and enzyme activity analysis might prove valuable in establishing which Prs polypeptide, i.e. PRPP synthase subunit, is important for enzyme activity. It is generally believed that an active PRPP synthase is composed of Prs1 and Prs3 or of Prs2 Prs4 and Prs5 (83). It is furthermore very likely that these polypeptides form a propeller-like hexameric structure similar to that of *B. subtilis* PRPP synthase. It would therefore be of interest to analyze variants such as Prs2Lys197Ala Prs4 Prs5, Prs2 Prs4Lys197Ala, Prs2 Prs4 Prs5Lys308Ala, Prs1Arg196Ala Prs3 or Prs1 Prs3Lys196Ala as well as some double variants for complementation and enzymatic properties. Indeed, the multiple, heterologous-subunits composition of yeast PRPP
synthase provides a unique system to study alterations in a single subunit of the hexameric enzyme, which is not possible with other PRPP synthases, which are also hexamers, but composed of a single species of subunit.

*S. cerevisiae* PRPP synthase is an intriguing enzyme. The expression of more than one ortholog necessary for PRPP synthase activity. Also the presence of HNRs imposes interesting questions about the structure and function of PRPP synthase. Do NHRs have ordered structures or are they intrinsically disordered regions? Could they confer Hub-protein character to PRPP synthase, thus facilitating heterologous protein interactions such as those described above?

(ii) *S. pombe*. *S. pombe* contains three PRPP synthase orthologs, *PRS1*, *PRS2* and *PRS3*, presumably specifying class I PRPP synthases, as judged from their deduced amino acid sequences. The three genes are unlinked with *PRS1* located in chromosome I, *PRS2* in chromosome II, and *PRS3* in chromosome III. As *PRS*-specified polypeptides of *S. cerevisiae*, two of the *PRS*-specified polypeptides of *S. pombe* contain NHRs (Fig. 5). Similar to the NHRs of *S. cerevisiae* Prs1 and Prs5, the NHRs of *S. pombe* are located within the catalytic flexible loop (Prs1 NHR1) or within the regulatory flexible loop (Prs2 NHR2). Amino acid sequence alignments with *B. subtilis* PRPP synthase and *S. pombe* *PRS* specified amino acid sequences reveal an identity for Prs1 of 29% (37% when the NHR1 is removed), for Prs2 of 39% (42% when the NHR2 is removed), and for Prs3 45%. A comparison of amino acid residues presumed to be important for active site formation and catalysis is included in Table S3. Specifically, these amino acid residues of Prs3 are highly conserved, 88%, compared to 63 and 75% of those of Prs1 and Prs2, respectively. In addition, only Prs2 and Prs3 contain both of the important lysine and arginine residues of the catalytic flexible loop.
The three *S. pombe* PRS genes have been cloned in a plasmid vector and expressed in an *E. coli* Δprs strain for complementation analysis (Table S4). The results showed that neither PRS1, PRS2 nor PRS3 alone complemented the *prs* null allele. In contrast, simultaneous expression of PRS3 and PRS1 or PRS2 resulted in complementation, whereas simultaneous expression of PRS1 with PRS2 did not result in complementation. Furthermore, very low PRPP synthase activity could be measured in extracts of cells harboring *PRS1* *PRS3* or *PRS2* *PRS3*. The activity, however, increased more than 10-fold in extracts of cells harboring *PRS1* *PRS3* in one plasmid and *PRS2* in a second compatible plasmid or, vice versa, *PRS2* *PRS3* in one plasmid and *PRS1* in a second compatible plasmid. These data suggest that the presence of all three *PRS* gene products may have a stabilizing effect on the enzyme activity (BH-J, unpublished data).

Heterozygous diploid deletion mutations have been constructed for essentially all of the genes of *S. pombe*. Strains containing deletion mutations in *PRS1* (also designated SPAC4A8.14) or *PRS2* (also designated SPBC3D6.06c) are viable. In contrast, the *PRS3* (also designated SPCC1620.06c) is essential (107). Additionally, two amino acids, Tyr168 and Ser172, of the *PRS3* specified amino acid sequence were shown to be phosphorylated (108). Ser172 corresponds to Ser172 of *B. subtilis* PRPP synthase, a fairly conserved amino acid residue. It remains to be established, if phosphorylation affects the activity of PRPP synthase.

The combined conclusion of the complementation and deletion analyses is that PRPP synthase activity requires the *PRS3* gene product as well as the gene product of either *PRS1* or *PRS2*.

(iii) PRPP synthase of other fungi. The association of multiple PRPP synthase subunits occurs in other species of fungi as well. The filamentous fungus *Aspergillus*
*A. nidulans* contains a mycelium of multinucleate cells that are partitioned by septa (reviewed by Harris (109)). Briefly, the formation of septa is regulated by a protein kinase cascade, which involves a *sepH*-specified protein kinase, which is a positive regulator of the septation initiation network. Thus, a phenotype of *sepH* mutations is a reduction or lack of septation. A number of suppressors of *sepH* mutation has been isolated and characterized, among which are altered levels of PRPP synthase activity. The authors offered a somewhat speculative model, in which competition of PRPP synthase and SepH for ATP might be the trigger of septation (110).

*A. nidulans* contains three PRPP synthase orthologs, *PRS1* (located on chromosome I), *PRS2* (chromosome VI) and *PRS3* (chromosome VII). Prs1 (Genbank CBF69369.1) (489 amino acid residues) resembles *S. cerevisiae* Prs1 as there is an addition of 169 amino acid residues inserted within the catalytic flexible loop, Prs2 (435 amino acid residues) (Genbank CBF83270.1) has an insertion of 116 amino acid residues at position 115, i.e. far from the catalytic flexible loop, and Prs2 may represent a new variant of PRPP synthases, and Prs3 (320 amino acid residues) (Genbank CBF85908.1) appears to be a “normal” PRPP synthase polypeptide. Each of the three amino acid sequences reveals all of the characteristic features for class I PRPP synthases, i.e. amino acids involved in subunit-subunit interactions, substrate binding and catalysis, and allosteric regulation. Yeast two hybrid analysis revealed interaction of Prs1 with Prs2, Prs1 with Prs3, and Prs2 with Prs3. Thus, the enzyme may contain all three polypeptides in an unknown stoichiometry (110).

The filamentous fungus *Eremothecium (Ashbya) gossypii* contains four PRPP synthase-specifying orthologs (111, 112). Two of these genes, both located on chromosome VII, have been analyzed: Agl080c (accession NP_986586), a homolog of *S. cerevisiae PRS2* or *PRS4*, and Agr371c (accession NP_987037) a homolog of *S.*
cerevisiae PRS3. The two other orthologs are homologs of S. cerevisiae PRS1 (accession NP_984943) and PRS5 (accession NP_984410). Both of these E. gossypii gene products have NHRs at positions similar to those of S. cerevisiae Prs1 and Prs5 although their length varies. All four E. gossypii genes may specify typical class I PRPP synthases (112). Similar to the situation in S. cerevisiae, the knockout of the PRS3 homolog had more dramatic effects on various growth parameters than that of the knockout of the PRS2/PRS4 homolog. The presence of only four PRS orthologs in E. gossypii contrary to five in S. cerevisiae may indicate some redundancy in the PRS2 and PRS4 genes of S. cerevisiae, as only one of these is present in E. gossypii. It was furthermore shown that introduction of the amino acid alteration Leu133Ile or Leu132Ile into the PRS2/PRS4 or the PRS3 homolog, respectively, resulted in reduction of feed-back inhibition of PRPP synthase activity. Similar effects were observed by His196Gln or His195Gln alterations of the PRS2/PRS4 or the PRS3 homolog, respectively. These amino acid alterations of PRPP synthase were introduced to desensitize the pathway for riboflavin biosynthesis for which purine nucleotides, and, thus, PRPP are precursors (112). We shall return to explain the effect of the amino acid alterations on PRPP synthase activity in the section “Regulation of PRPP Synthase Activity”.

It is obvious from the description above that the physiology of yeast PRPP synthase differs from that of bacteria. Although also belonging to the class I PRPP synthases, major differences exist such as the presence of the amino acid sequences NHR, the requirement of heteromeric oligomerization, and the involvement of PRS-specified polypeptides in cellular processes other than PRPP synthesis.

Mammalian PRPP synthase and PRPP synthase associated proteins. As described above, yeasts contain PRPP synthase-specifying homologs with NHRs
inserted at specific positions as well as “ordinary” PRPP synthase-specifying homologs without these insertions. Similar proteins are found in mammalian organisms, where apparent PRPP synthases have insertions similar to the yeast NHRs. In mammals a PRPP synthase polypeptide with an insertion has been designated PRPP synthase-associated protein or PAP. The description below of each type, PRPP synthase and PAP, complements previously published reviews (18, 20).

(i) PRPP synthase. Among PRPP synthases of mammalian origin, only those of humans and rat have been characterized. Both organisms contain three genes, \( PRPS1-3 \) encoding PRPP synthase isozymes 1-3. \( PRPS1 \) and \( PRPS2 \) are located on the X-chromosome, whereas \( PRPS3 \) is located on chromosome 7. The latter gene is expressed only in testes, whereas both of the former genes are expressed in all the tissues examined (11).

(a) Enzyme characteristics. Human and rat PRPP synthases also belong to class I, and as such their properties resemble the microbial class I PRPP synthases with respect to their restricted diphosphoryl donor capacity (ATP and dATP) and allosteric regulation by P\(_i\) and ADP (18, 20). The amino acid sequence identity among the three human PRPP synthase isozymes is 91-95 %, whereas the identities of \( B. subtilis \) PRPP synthase and the three human isozymes are 45 or 46 %. Exclusion chromatography of PRPP synthase activity isolated from rat liver revealed a protein complex with a molecular mass of 1000 kDa or more. The protein complex consisted of four different polypeptides, the two catalytic PRPP synthase isozymes 1 and 2 as well as the two so-called PAP-39 and PAP-41 estimated to contain 20 molecules of isozyme 1, five of isozyme 2, eight and one of PAP-39 and PAP-41, respectively (113). Previous characterization of human PRPP synthase may have been performed on mixtures of isozyme 1 and 2, although erythrocytes, the source of enzyme in those
early studies, contain predominantly isozyme 1 (114), and similar to the rat liver enzyme, human PRPP synthase was isolated as large oligomeric structures (115, 116). Human isozyme 1 and 2 differ in 15 amino acid residues, human and rat isozyme 1 are identical, whereas human and rat isozyme 2 differ in only three amino acid residues.

Although native PRPP synthase isolated from tissues may better represent the physiological state of the enzyme, efforts have been spent also to characterize the individual isozymes. Isozymes 1 and 2 of both humans and rat have been purified and characterized after expression of their recombinant genes in *E. coli* (71, 73). As expected from the close identity of the four enzymes they shared many similarities in physico-chemical properties. They all oligomerize to very large structures with molecular masses around 1000 kDa (human isozyme 1 and 2, rat isozyme 1) or approximately 550 kDa (rat isozyme 2) as determined by exclusion chromatography. Even though they are quite similar in primary structure, there are also differences between human isozyme 1 and 2. Different purification procedures were employed for the two isozymes; isozyme 2 was much more labile to heat inactivation than isozyme 1; isozyme 1 was saturated by lower concentrations of ATP, ribose 5-phosphate and Mg$^{2+}$ than isozyme 2; and isozyme 1 was more prone to inhibition by ribonucleoside diphosphates than isozyme 2. Comparison of human and rat enzymes revealed very few differences (71, 73). Some kinetic properties of PRPP synthases of humans and rat are listed in Table 2.

(b) Three-dimensional structure. Human PRPP synthase isozyme 1 three-dimensional structure has been determined from a number of crystal forms (Table 1). (a) hPRS1 wild-type-PRPP synthase with three SO$_4^{2-}$ bound, one at the phosphate position of ribose 5-phosphate, one at the position of the $\alpha$-phosphate of ADP at the
allosteric site, and one at a new, third position at the parallel dimer interface, determined at 2.0 Å resolution (PDB code 2h06); (b) \(ATP/\text{SO}_4^{2-}/\text{Cd}^{2+}-\text{PRPP synthase}\) (also wild-type) with three \(\text{SO}_4^{2-}\) bound as in the hPRS1 wild-type-PRPP synthase crystal form, ATP (modelled to only AMP) at the active site and one \(\text{Cd}^{2+}\) per monomer, determined at 2.2 Å resolution (PDB code 2hcr) (51); (c) \(PRS1\) (wild-type PRPP synthase) with \(\text{SO}_4^{2-}\) bound, determined at 2.0 Å resolution (PDB code 3s5j) (117, 118). In addition, three-dimensional structures of some mutant variants have been determine as well and are described below. From the 46 % identity among \(B.\) \textit{subtilis} and human PRPP synthase isozyme 1 amino acid sequences, it comes as no surprise that the three-dimensional structure of the two enzymes are essentially identical. Superimposition of hPRS1 wild-type-PRPP synthase and various \(B.\) \textit{subtilis} PRPP synthase structures revealed root mean-square deviation values of 1.0 to 1.2 Å. As expected, also the human PRPP synthase isozyme 1 has the hexameric propeller structure of \(B.\) \textit{subtilis} PRPP synthase (Fig. 3C) (51). In addition, the structure of human PRPP synthase isozyme 1 has been visualized by negative stain electron microscopy. The apo and the ADP-bound forms have a three-leaf clover structure, into which the crystal structure of isozyme 1 could be docked (118).

\(c\) Mutant PRPP synthases. A number of variants of human PRPP synthase isozyme 1 have been discovered because of their severe clinical effects on the affected individuals. As reviewed previously (119), these pathological effects are caused either by increased activity of PRPP synthase isozyme 1, i.e. “superactivity” of the enzyme or overexpression of the \(PRPS1\) gene, which results in an array of physiological effects including hyperuricemia, gouty arthritis or neurosensory defects, or by reduced activity of PRPP synthase isozyme 1, resulting in neuropathy, deafness or intellectual disabilities. Historically, these altered PRPP synthases were described by the
somewhat imprecise designations “gain of function” or “loss of function”. Sixteen
mutant variants affecting 15 amino acid positions have been discovered and are
summarized in Table 4. (No mutant forms of PRPP synthase isozyme 2 or PAP have
been described). The loss of function of PRPSI variants (reduced activity in Table 4)
are the variants Asp64Asn, Ala86Thr, Met114Thr, Gln132Pro, Val141Leu,
Leu151Pro, Ile289Thr and Gly305Arg variants, which had 40 to 70 % of the specific
activity of normal PRPP synthase (120-122). In Fig. 2 each of these amino acid
positions is indicated by blue arrow heads, which point to the homologous position in
B. subtilis PRPP synthase. It is evident that none of these amino acid alterations are
within positions that are directly involved in catalysis, which presumably would have
produced inactive enzyme forms. Asp64 and Met114, equivalent to B. subtilis PRPP
synthase Glu70 and Leu120, respectively, are located at stretches of amino acids
involved in the formation of the bent dimer, Val141 equivalent to B. subtilis PRPP
synthase Ile147, is located close to sequences that are involved the formation of the
parallel dimer, and Gln132, Ile289 and Gly305, equivalent to B. subtilis PRPP
synthase Gln138, Ser293 and Gln308 are within or close to positions involved in
allosteric regulation of PRPP synthase activity. Finally, Ala86, equivalent to B.
subtilis PRPP synthase Ile92 is located close to the regulatory flexible loop.
Altogether, the amino acid positions causing reduced activity of PRPP synthase
isozyme 1 are located at positions secondary to catalysis, and, rather, their effect may
be to alter the secondary structure, resulting in poorer enzymatic function.
The gain of function variants (increased activity in Table 4) are the variants
Asp51His, Asn113Ser, Leu128Ile, Asp182His, Ala189Val and His192Leu. The
variant forms of PRPP synthase have increased activity primarily because of their
reciprocal effects on allosteric regulation by ADP and P, namely, reduced inhibition
by the former and increased stimulation by the latter (72, 123). Studies of these PRPP synthase mutant variants have proven valuable in the elucidation of the allosteric regulation of the enzyme, and we shall return to a further description of them in the section “Regulation of PRPP synthase activity”.

The structure of three mutant variants has been determined. One is the Asp51His variant, which has SO$_4^{2-}$ bound at the position of the α-phosphate of ADP at the allosteric site as well as Mg$^{2+}$ (PDB code 4f8e) (124). Patients containing the Asp51His variant suffer from hyperuricemia and severe gout. The other structures are those of the Ser131Ala (PDB code 2h07) and Tyr145Met (PDB code 2h08) variants, each with three SO$_4^{2-}$ bound as described for the hPRS1wild-type-PRPP synthase structure. The authors used the observation of these SO$_4^{2-}$ as indicative of a second allosteric P$_i$-binding site (51).

(d) PRPP synthase isozyme 2 and Myc-driven cancers. As mentioned above, no mutants of human PRPP synthase isozyme 2, specified by the \textit{PRPS2} gene, have been described. Remarkably, however, the activity of isozyme 2 has been shown to be critical in developing and maintaining cancers caused by Myc-transcription factor. The eukaryotic translation initiation factor 4E, is also involved in the development of this type of cancer. The \textit{PRPS2} gene, but not the \textit{PRPS1} gene, contains a pyrimidine-rich translational element in the 5'-untranslated region. Myc-driven hyperactivation results in an interaction of the eukaryotic translation initiation factor 4E and possibly other factors with the pyrimidine-rich translational element and results in an increase of translation of \textit{PRPS2}-specific mRNA, which results in increased PRPP synthase isozyme 2-synthesis and, thus, increased nucleotide production. The increase in nucleotide synthesis inhibits PRPP synthase isozyme 1, whereas isozyme 2 is less sensitive to feedback inhibition by ribonucleotides. Also, the lack of the pyrimidine-
rich translational element within the PRPS1 mRNA prevents the stimulation of synthesis of isozyme 1. Altogether, compelling evidence shows that PRPP synthase isozyme 2 plays an important role in the metabolism of cells with Myc-driven cancers (125).

(ii) PAP. Mammalian PAP-39 and PAP-41 may be variants of the S. cerevisiae and S. pombe PRS1-specified gene products described previously, i.e. PRPP synthase-like proteins containing an NHR. The amino acid sequence of human PRPP synthase isozyme 1 is 41% identical to both PAP-39 and PAP-41; the amino acid sequence of PAP-39 is 76% identical to that of PAP-41. The NHRs are both located within the catalytic flexible loop and are 29 and 30 amino acid residues in length, respectively. Thus, the additions are located at a position similar to NHR1 of S. cerevisiae Prs1, NHR5-2 of S. cerevisiae Prs5 and the NHR of S. pombe Prs1, but differ in length (Fig. 5). The identity of B. subtilis PRPP synthase and PAP-39 and PAP-41 is 31 and 34 %, respectively. After removal of the additions, these values increase to only 34 and 37 %, respectively. Inspection of important amino acid residues reveal that the ribose 5-phosphate binding site is well-preserved in PAP-39 and PAP-41. In contrast, residues involved in the binding of ATP and residues important for catalysis are poorly conserved. Specifically, the two important residues of the catalytic flexible loop (Lys197 and Arg199 in B. subtilis) are absent in the two PAPs. Data for PAP-39 is included in Table S3. Originally, the PAPs were identified as subunits co-purifying with rat PRPP synthase. Interaction of PRPP synthase and PAP has been demonstrated by several methodologies, such as crosslinking and co-immunoprecipitation. The PAP could be removed from PRPP synthase isolated from rat liver by exclusion chromatography in the presence of one molar magnesium chloride. Both of these latter processes yielded PRPP synthase with increased activity,
which suggested that the PAPs play a negative regulatory role on PRPP synthase activity (113). Furthermore, a protein devoid of PRPP synthase activity was obtained after expression of the PAP-39 specifying gene in *E. coli*. Partial reconstitution of rat PRPP synthase activity could be achieved by co-expression of recombinant genes specifying PRPP synthase isozymes 1 and 2 and PAP-39 in *E. coli*, which resulted in an active oligomeric enzyme eluting near the void volume, i.e. an enzyme consisting of a large number of subunits (126).

Mammalian and yeast PRPP synthase structures resemble one another in interesting ways. Both mammalian and yeast PRPP synthases have constituent subunits of “ordinary” PRPP synthase polypeptides, cf. i.e. isozymes 1 and 2 of humans or rat and Prs2, Prs3 and Prs5 of *S. cerevisiae*, as well as the NHR containing polypeptides PAP-39 and PAP-41 of humans or rat and Prs1 and Prs5 of *S. cerevisiae* as described above. Indeed, the presence of multiple, heterogenous subunits including subunits with NHR sequences may be widespread among eukaryotic organisms. The studies with yeast suggest that such heterologous structures may play roles in cellular regulation that are not yet well-defined.

The crystal structures of human PAP-39 and PAP-41 have been determined at 2.7 and 2.6 Å resolution (PDB codes 2c4k and 2ji4, respectively). We found that superimposition of these two structures with that of *B. subtilis* PRPP synthase (PDB code 1dku) revealed root mean-square deviation values of 1.1 and 0.97 Å for PAP-39 and PAP-41, respectively, which indicate that the three-dimensional structures of the PAPs and human and *B. subtilis* PRPP synthases are essentially identical.

**Other class I PRPP synthases.** Some interest has been devoted to PRPP synthase of the mosquito *Culex pipiens pallens*. This mosquito is the major vector for various roundworms causing lymphatic filariasis (elephantiasis) and for the Japanese
encephalitis virus. An important insecticide used in controlling *C. ppiens pallens* and other insects as well is the pyrethroid ester deltamethrin. Many insect species develop deltamethrin-resistance by knock-out mutations, and increased expression of specific genes may assist in providing resistance. Expression of the *PRPS1* gene is one example of this phenomenon. The *PRPS1* encoded enzyme is a typical class I PRPP synthase. The exact mechanism of the contribution of overexpression of *PRPS1* to deltamethrin-resistance remains to be established (127).

Planarians, with *Dugesia japonica* as an example, are studied because of their remarkable ability to regenerate from injuries such as lost body parts. A PRPP synthase-specifying ortholog (*DjPRPS*) of *D. japonica* has been cloned. The deduced amino acid sequence reveals a typical class I PRPP synthase, with 37% identity to the amino acid sequence of *B. subtilis* PRPP synthase (128).

**Class II PRPP Synthases**

*S. oleracea* isozymes 3 and 4 were proposed to constitute a second class of PRPP synthases, class II, based on a comparison of the biochemical properties of the enzymes (14, 15). Both isozyme 3 and 4 are active in the absence of Pi, and both enzymes are inhibited by the ribonucleoside diphosphates ADP and GDP in a competitive manner, i.e. they compete with ATP for binding at the active site. Thus, no allosteric inhibition by either of these ribonucleoside diphosphates was detected. Kinetic parameters are given in Table 2. Both of the *S. oleracea* isozymes are able to use ATP, dATP, GTP, CTP and UTP as diphosphoryl donors. The apparent Michaelis constants for the other diphosphoryl donors for isozyme 3 and isozyme 4 varied between 85 and 680 μM. Exclusion chromatography of isozyme 4 revealed a molecular mass of 110 kDa. Identical results were obtained in Tris- or phosphate ion-
based buffer and in the presence or absence of MgATP. Recombinant *S. oleracea*

isozyme 3 eluted identically to isozyme 4 in exclusion chromatography. With a
calculated molecular mass of the subunit as 35.4 kDa, this value of 110 kDa is
consistent with a homotrimeric quaternary structure (46, 47). Some controversy exists
on this point, as characterization of sugarcane class II PRPP synthase revealed a
molecular mass of 214 kDa, i.e. a value consistent with a hexameric quaternary
structure. Like *S. oleracea* PRPP synthase isozyme 3 and 4, the activity of sugarcane
PRPP synthase is independent of P$_i$ (129).

Amino acid sequence alignments of PRPP synthase isozyme 3 or 4 with the
amino acid sequence of *B. subtilis* PRPP synthase revealed a conservation of amino
acids along the polypeptide. Thus, a small part of the regulatory flexible loop (Phe97-
Thr114 of isozyme 4), the PRPP binding site consisting of the PP loop (Asp183-
Ala186 of isozyme 4) and the ribose 5-phosphate binding loop (Gly222-Thr237 of
isozyme 4) are extensively conserved. The catalytic flexible loop (Lys204-Leu215) is
less conserved, but the apparently indispensable residues, Lys204 and Arg206, are
retained. The amino acid residues important for binding of the adenine moiety of
ATP, Phe41, Asp43 and Gln47, and the catalytically important residues His141 are
also conserved (Fig. 2 and Tables S1 and S2). Altogether, it is therefore likely that the
mechanism of catalysis of class II PRPP synthases is identical or similar to that of
class I PRPP synthases.

The amino acid residues involved in the formation of the two types of subunit-
subunit interactions identified in *B. subtilis* PRPP synthase, i.e. the bent dimer and the
parallel dimer are poorly conserved in the class II PRPP synthases (Table 3). In class
II PRPP synthases represented by the *S. oleracea* PRPP synthase isozyme 4, only
three of the eight residues involved in the formation of the *B. subtilis* PRPP synthase
bent dimer are conserved, and only four of the 12 residues involved in the formation of the *B. subtilis* PRPP synthase parallel dimer are conserved. It is likely, therefore, that the quaternary structure of class II PRPP synthases differs from that of class I enzymes. Also, the amino acid residues identified as involved in allosteric regulation of *B. subtilis* PRPP synthase are poorly conserved in *S. oleracea* PRPP synthase isozyme 4; among the 20 residues listed in Table S2 only six are conserved, which is consistent with the lack of allosteric regulation of class II PRPP synthases.

The amino acid sequences of *S. oleracea* PRPP synthase isozyme 2 (class I) and isozyme 3 (class II) contain extensions of 76 and 87 amino acids, respectively, at the N-terminus as compared to isozyme 4. For isozyme 2 this extension contains an amino acid sequence that indicates its localization in chloroplasts, whereas for isozyme 3 the extension contains an amino acid sequence that indicates its localization in mitochondria. Indeed, in vitro expression of *PRS2* and synthesis of isozyme 2 in the presence of chloroplasts showed that this isozyme was transported to chloroplasts (15). Unlike *S. oleracea* PRPP synthase isozyme 2 and 3, the amino acid length of isozyme 4 is similar to that of other PRPP synthases (318 compared to 316 of *B. subtilis* PRPP synthase), and, thus, isozyme 4 is believed to be located in the cytoplasm (15).

Interestingly, previous analyses of plant PRPP synthases may provide some additional information on the class II enzymes. PRPP synthase from spinach leaves was partially purified. The activity was independent of Pi. In fact, Pi inhibited the activity with half-maximal inhibition at 28 μM. The apparent $K_M$ values for ATP, ribose 5-phosphate and Mg$^{2+}$ were 36 μM, 10 μM and 1 mM, respectively. ATP was by far the best diphosphoryl donor, but GTP, UTP and CTP also were diphosphoryl donors. The PRPP synthase activity was found predominantly in the cytosol (>95% of
the total activity), with minor contributions by the nuclear, chloroplast and mitochondrial fraction (1 to 2%) (130). These data have to be reevaluated in light of the present knowledge of multiple isozymes in spinach. Also, rubber tree (Hevea brasiliensis) latex PRPP synthase has been partially purified and characterized. Exclusion chromatography revealed a molecular mass of 200 kDa. The authors estimated the subunit molecular mass as 56 kDa following gel electrophoresis under denaturing conditions. Actually, a band corresponding to a polypeptide of molecular mass 35 kDa is also visible in the photograph of the gel. This size, 35 kDa, is the “standard” size of PRPP synthases. Activity was obtained in the absence of Pi. ATP functioned as diphosphoryl donor, whereas neither GTP, CTP nor UTP functioned as diphosphoryl donor. Apparent $K_M$ values for ATP and ribose 5-phosphate were determined as 0.2 M and 40 μM, respectively (131). As before, the data should be reevaluated with the current knowledge of multiple forms of PRPP synthase. It remains unknown if the latex fraction contains a specific PRPP synthase. BLAST analysis with the amino acid sequences of S. oleracea or A. thaliana PRPP synthase isozyme 3 or 4 as the queries revealed the presence of these enzymes also in green algae species such as Chlamydomonas reinhardtii (accession XP_001694483 [an isozyme 3-like sequence]) (132), Chlorella variabilis (XP_005848636 [isozyme 3-like]) (133), Coccomyxa subellipsoidea (XP_005643144.1 [353 amino acid sequence, isozyme 4-like]) (134), Ostreococcus lucimarinus (XP_001421441.1 [305 amino acid sequence, isozyme 4-like]) and XP_001421590 [304 amino acid sequence, isozyme 4-like]) (135), Ostreococcus tauri (XP_003083594.1 [396 amino acid sequence, isozyme 3-like] and XP_003083246.1 [552 amino acid sequence]) (136), and Volvox carteri (XP_002955183 [398 amino acid sequence, isozyme 3-like]) (137). BLAST analysis with S. oleracea PRPP
synthase isoenzyme 1 as the query revealed the presence of class I PRPP synthase also in *O. lucimarinus, C. variabilis, C. subellipsoidea,* and *V. carteri.* Thus, in general the class II PRPP synthases seem to be an addition to the classical, class I, PRPP synthases. It should be added that the completion of the *A. thaliana* genome sequencing revealed the presence of a gene encoding a class I PRPP synthase isozyme 5 (16).

**Archaeal PRPP Synthases**

The biochemical characterization of PRPP synthase of *M. jannaschii* persuaded the authors to propose the existence of a third class of PRPP synthases. The activity of this enzyme is activated by Pi, and it has restricted diphosphoryl donor capacity (ATP and dATP only), which are properties of class I PRPP synthases. However, ADP binds to only the active site, and the enzyme appears to be unregulated by an allosteric mechanism involving purine ribonucleoside diphosphates, which is a property of class II PRPP synthases. Additionally, the quaternary structure of *M. jannaschii* PRPP synthase is tetrameric (48). In light of three-dimensional structures of PRPP synthase of *T. volcanium,* and *S. solfataricus,* which are dimers, it seems more reasonable to define this class of PRPP synthases as “archaeal PRPP synthases” rather than class III.

Amino acid sequence alignments of PRPP synthase of the three archaeal species, *M. jannaschii,* *T. volcanium,* and *S. solfataricus,* with the amino acid sequence of *B. subtilis* PRPP synthase revealed conservation of amino acids along the polypeptide. The regulatory flexible loop, the PP loop and the ribose 5-phosphate binding loop are extensively conserved; the catalytic flexible loop is less conserved, but the apparent indispensable lysine and arginine residues are retained. The amino acid residues important for binding of the adenine moiety and the catalytically important histidine residues are also conserved (Table S1). As with the class II PRPP synthases, it is
likely that the archaean PRPP synthases and class I PRPP synthases share identical catalytic mechanisms.

**M. jannaschii.** The amino acid sequences involved in various functions are conserved in *M. jannaschii* PRPP synthase, i.e. the regulatory flexible loop, Leu88-Ser104, the PP loop, Asp163-Ala166, the catalytic flexible loop, Lys186-Ala197, the ribose 5-phosphate binding loop, Asp205-Thr220 (Table S1). Also, the two-domain structure of the *M. jannaschii* PRPP synthase subunit resembles that of the *B. subtilis* enzyme.

Biochemical characterization of PRPP synthase from *M. jannaschii* revealed properties unlike those described above for class I and class II PRPP synthases (Table 2). Apparently, the poor affinity for both substrates may be compensated by a very large $V_{\text{max}}$ value. Additionally, the activity of *M. jannaschii* PRPP synthase is stimulated by P$_i$, diphosphoryl donors are ATP and dATP, ADP is a competitive inhibitor, and ADP binds to a single site per subunit, hence the active site. These properties partly point to a class I enzyme, i.e. restricted diphosphoryl donor capacity and activation by P$_i$, and partly to a class II enzyme, i.e. lack of allosteric regulation. The quaternary structure of *M. jannaschii* PRPP synthase was furthermore found to be tetrameric (48).

The structure of *M. jannaschii* PRPP synthase, as deduced from x-ray crystallographic analysis, is considerably more compact than that of *B. subtilis* PRPP synthase, primarily because of truncations at the N- and C-terminal ends as well as one shorter loop in the interior of the sequence. As with *B. subtilis* PRPP synthase, the structure of *M. jannaschii* PRPP synthase revealed two different types of subunit-subunit interactions; these were revealed by the structure of Apo-PRPP synthase structure (PDB code 1u9y), and the structure of the AMP/R5P-PRPP synthase...
complex (with AMP and ribose 5-phosphate bound, PDB code 1u9z). The tetrameric
*M. jannaschii* PRPP synthase could be superimposed easily with two of the subunits
of *B. subtilis* PRPP synthase to form almost identical bent dimers. Consistent with the
bent dimer-organization of *M. jannaschii* PRPP synthase, six of the eight amino acid
residues selected in Table 3 were identical or conserved among *B. subtilis* and *M.
jannaschii* PRPP synthases. Interestingly, the interface of the bent dimer contains
residues involved in the binding of the adenine moiety of ATP, namely Phe32B
(Phe40D of *B. subtilis* PRPP synthase), Asp34B (Asp42D), Glu36B (Glu44D) and
Arg92A (Arg101A). The rest of the active site, i.e. the binding of the remaining part
of ATP and ribose 5-phosphate, is provided entirely by one subunit of a bent dimer,
indicating that substrate recognition and catalytic machinery of *B. subtilis* and *M.
jannaschii* PRPP synthases are identical. The conservation of amino acid residues
involved in substrate binding and catalysis is apparent from the amino acid
comparison of Table S1. In contrast, the other subunit-subunit interaction of *M.
jannaschii* did not superimpose with the linear dimers of the *B. subtilis* enzyme. Only
four of 12 amino acid residues involved in the parallel-dimer formation of *B. subtilis
PRPP synthase* were identical or conserved (Table 3). The allosteric effectors of *B.
subtilis* PRPP synthase, ADP and P_i, bind to residues at the interface of the parallel
dimer, which is not conserved among the two enzymes, and very few if any amino
acid residues involved in allosteric regulation of *B. subtilis* PRPP synthase are
conserved in *M. jannaschii* PRPP synthase (Table S2). This is consistent with the lack
of allosteric regulation in vitro of *M. jannaschii* PRPP synthase, which binds only a
single molecule of ADP per subunit (48).

*T. volcanium.* As was described above for *M. jannaschii* PRPP synthase, amino
acids involved in the formation of the bent dimer are relatively well-conserved
relative to the homologous amino acid residues of *B. subtilis* PRPP synthase, whereas
the amino acid residues involved in the formation of the parallel dimer are much less
conserved. Amino acid residues involved in substrate binding and catalysis are well-
conserved (Table S1), whereas only few amino acid residues involved in allosteric
regulation of *B. subtilis* PRPP synthase are conserved in *T. volcanium* PRPP synthase
(Tables S2).

Structures of several crystal forms of *T. volcanium* PRPP synthase have been
described. (a) *ADP/SO_4^{2-}-PRPP synthase* with ADP and SO_4^{2-} present at the active
site representing ATP and the phosphoryl moiety of ribose 5-phosphate, determined at
1.5 Å resolution (PDB code 3lrt). The active site is located in the cleft between the
two domains as in the *B. subtilis* enzyme. The binding of ADP is well defined; the
adenine moiety is stacked between Arg91 and Phe32 and N6 is bound to Asp34 and
Glu36, the ribose hydroxyls are bound to Glu92, His93 and Tyr96. In addition, ADP
interacts with the side chains of amino acids residues Asp34, Glu36, Arg91 and
Ser214 through water molecules. The crystal form has an open conformation, i.e. the
catalytic flexible loop is open, resulting in full accessibility of the active site for
substrates. The side chain nitrogens of the catalytic flexible loop-Lys184 are located
14 to 16 Å away from the β-phosphate oxygen of ADP (138); (b) *mATP/SO_4^{2-}-PRPP synthase*
determined at 1.8 Å resolution (PDB code 3lpn). In this crystal form the
ATP analog α,β-methylene ATP replaces ADP of the ADP/SO_4^{2-}-PRPP synthase
described above. The binding of α,β-methylene ATP, including the triphosphate chain
is well defined and shows His93 interacting with the oxygens of the β- and γ-
 phosphates, and Tyr96, His124 and Asp125 interacting with the γ-phosphate. The
catalytic flexible loop remained open (138). (c) *ADP-Mg^{2+}/R5P-PRPP synthase*
determined 1.8 Å resolution (PDB code 3mbi). Here MgADP and ribose 5-phosphate
are located at the active site. Mg$^{2+}$ coordinates the polypeptide through His124, the α- and β-phosphates as well as three water molecules. Thus, the binding of this Mg$^{2+}$ ion resembles that of the MG2 site of *B. subtilis* PRPP synthase, except for the coordination of Mg$^{2+}$ to the γ-phosphate rather than water in the *B. subtilis* enzyme. Furthermore, the catalytic flexible loop is closed, i.e. it is bent approximately 45° towards the bound ligands, MgADP and ribose 5-phosphate, compared to the open form; this results in the placement of Lys184 and Arg186 close to the β-phosphate of ADP (and presumably of ATP), and in shielding of the active site and the transition state from the solvent (138). The importance of this closure of the catalytic flexible loop for catalysis is discussed further below in the section “Mechanism of Catalysis”.

The crystal structure as well as molecular sieving revealed that *T. volcanium* PRPP synthase is a dimer, which does not form higher symmetry oligomers (hexamers or tetramers) like those described for the class I PRPP synthases (138). Also the crystal structure with α,β-methylene ATP/ADP and ribose 5-phosphate present revealed a dimeric quaternary structure. However, molecular sieving in the presence of ATP (or α,β-methylene ATP) and ribose 5-phosphate has not been performed, so formation of a tetramer resembling that of *M. jannaschii* PRPP synthase under some conditions cannot be ruled out.

*S. solfataricus*. *S. solfataricus* PRPP synthase resembles PRPP synthases from *M. jannaschii* and *T. volcanium* with respect to conservation or lack of conservation of amino acid residues involved in dimer formation (Table 3), substrate binding and catalysis (Table S1), and allosteric regulation (Table S2). Structural information for *S. solfataricus* PRPP synthase is available from analysis of a crystal form with AMP and sulfate representing the phosphoryl group of ribose 5-phosphate at 2.8 Å resolution (PDB code 4twb). The enzyme appears to be active as a dimer like that described for
T. volcanium PRPP synthase. Amino acid sequence identity of S. solfataricus and T. volcanium PRPP synthases is 29%. The fold of the subunit resembles that of other PRPP synthases, and the binding of the nucleotide substrate ATP (modeled to only the AMP portion) is highly conserved in comparison to the PRPP synthases of T. volcanium, M. jannaschii and B. subtilis (139).

**Other archaeal PRPP synthases.** The gene encoding PRPP synthase of the hyperthermophilic archaeon *Thermococcus kodakaraensis* has been expressed in *E. coli* and PRPP synthase purified to near homogeneity. Recombinant *T. kodakaraensis* PRPP synthase accepts ATP, CTP and GTP as diphosphoryl donors, with ATP as the preferred diphosphoryl donor. The enzyme eluted as a monomer in exclusion chromatography (140). Chromatography was performed without the presence of substrates. It is therefore possible that the active enzyme consists of more than one subunit. Indeed, comparison of amino acid residues involved in the formation of the bent dimer of *M. jannaschii* with those of *T. kodakaraensis* revealed an almost exact match, suggesting that active *T. kodakaraensis* PRPP synthase may constitute a tetramer similar to that of *M. jannaschii* PRPP synthase. The similarity of the amino acid sequence of *T. kodakaraensis* PRPP synthase with those of *M. jannaschii*, *S. sulfolobus* and *T. volcanium* is 52 to 58% (identity 32 to 40%), where with *B. subtilis* PRPP synthase the values are 47% (similarity) and 28% (identity).

The genomes of the following archaeal species appear to contain two *prs* genes: *Archaeoglobus fulgidus, Archaeoglobus veneficus, Ferroglobus placidus, Halorhabdus utahensis, Methanocella conradii, Thermofilum pendens, Thermogladius cellulolyticus*. No biochemical information is available on the properties of the corresponding proteins. Amino acid sequence comparison of two putative *A. fulgidus* PRPP synthases and *B. subtilis* PRPP synthase reveals a high
degree of conservation of amino acid residues involved in substrate binding and catalysis. Specifically, the two important lysine and arginine residues are present in both sequences, Lys172 and Arg175 in sequence 1, Lys186 and Arg 188 in sequence 2 (Table S3). Indeed, amino acid sequence comparison reveals that *A. veneficus*, *F. placidus*, *M. conradii*, *T. pendens*, and *T. cellulolyticus* may encode two bonafide PRPP synthases as evaluated by the presence of conserved or identical amino acids at positions homologous to *B. subtilis* PRPP synthase residues Phe40, His135, Asp174 of the ribose 5-phosphate binding loop and Lys197 and Arg199 of the catalytic flexible loop. In contrast, one of the putative PRPP synthases of *H. utahensis* has aspartate and threonine rather than lysine and arginine in the catalytic flexible loop, and, thus, very likely does not represent an active PRPP synthase.

In spite of large variations in protein structure among class I, class II and archaeal PRPP synthases as a result of the different oligomerization properties described above, it obvious that the catalytic mechanism essentially is the same among the three PRPP synthase classes as evaluated by the conservation of the catalytically important amino acid residues in the three classes.

**MECHANISM OF CATALYSIS**

The three-dimensional structure of the important catalytic flexible loop, Lys197 to Met208 of *B. subtilis* PRPP synthase, Lys186 to Ala197 of the *M. jannaschii* enzyme, Lys188 to Ile199 of the *S. solfataricus* enzyme, and Lys194 to Met205 of human PRPP synthase isozyme 1, remained unresolved in studies of the structures of these enzymes (48-51, 139). However, the solution of the *T. volcanium* PRPP synthase structure revealed that the binding of the substrate ribose 5-phosphate and the substrate analog MgADP resulted in a closed conformation of the catalytic flexible
loop, in which strands β10 and β11 move 17 Å toward the substrates and bring the important catalytic residues, Lys184 and Arg186, into proximity with ribose 5-phosphate and the triphosphate chain of the nucleotide (Fig. 6A). According to this structure, Lys184 and Arg186 bind to oxygen(s) of the β-phosphate of ATP. Additionally, Mg$^{2+}$ coordinates to a nitrogen of His124 as well as to oxygens of the α- and β-phosphates of ADP, and, presumably of ATP. In the open conformation, the C5’, the α-phosphorus and the β-phosphorus of ADP or ATP are essentially linearly arranged, whereas in the closed conformation the β-phosphate is bent approximately 90° relative to the C5’, and the α-phosphorus, and, thus locates the β-phosphorus in ideal position for nucleophilic attack by the O1 of ribose 5-phosphate (Fig. 6B) (138). Only a single Mg$^{2+}$ was identified at the active site of *T. volcanium* PRPP synthase, and biochemical information as to the number of Mg$^{2+}$ necessary for catalysis has not been published. This is important because dual roles for Mg$^{2+}$ have been identified in the reaction mechanism of other PRPP synthases.

Additional information was obtained from studies of two structures of activated *B. subtilis* PRPP synthase. In the first of these structures, a transition-state analog of phosphoryltransfer reactions, AlF$_3$, was employed in the presence of the product AMP and the substrate ribose 5-phosphate, which generated a transition-state bound complex (AlF$_3$-PRPP synthase structure) (Fig. 6C). Here Al$^{3+}$ represents the β-phosphorus, whereas the three F$^-$ represent the three oxygens of the β-phosphate, and the α- and γ-phosphates were provided by two AMP molecules as described before. In the second of these structures, the substrate analog α,β-methylene ATP and the substrate ribose 5-phosphate were employed to generate an active-state quaternary complex (mATP/R5P-PRPP synthase structure). In the former structure containing AlF$_3$, the catalytic flexible loop was closed and revealed contacts between Lys197 and
Arg199 with the β-phosphoryl group of ATP, whereas in the second, mATP/R5P structure, the catalytic flexible loop was open. Thus, the contacts between Lys197 and Arg199 observed in the AlF3-structure appear to be lost in the active state quaternary complex (mATP/R5P), where the flexible catalytic loop remains unresolved. Thus, loop closure appears to be transient and specific for only the transition state. Al3+ of the AlF3 structure has an octahedral coordination with the C1-oxygen of ribose 5-phosphate, the three F as well as one oxygen from each of the α- and γ-phosphoryl groups. The distances from the Al3+, which corresponds to the β-phosphorus of ATP, to the C1 oxygen of ribose 5-phosphate and the oxygen of the α-phosphoryl group are 2.0 and 1.9 Å, respectively, and, thus these cooordinations have partial bond character. In the AlF3 structure Mg2+ is located on both sides of the triphosphate chain. As observed with \textit{T. volcanium} PRPP synthase, the binding of Mg2+ at the MG2 site of \textit{B. subtilis} PRPP synthase results in bending of the triphosphate chain of ATP. This bent conformation is caused by the tridentate conformation, α,β,γ-MgATP. Furthermore, the Mg2+ of the MG1 site coordinates to the γ-phosphate (as well as to ribose 5-phosphate and Asp174) and, thus, the two Mg2+ are responsible for a tight conformation of the triphosphate chain of ATP, and a perfect inline geometry of the C1 oxygen of ribose 5-phosphate, the β-phosphate and the α-phosphate is created. Furthermore, the bent conformation of the triphosphate chain results in electrostatic repulsion among the phosphoryl groups, whereas the coordination of Mg2+ to a nitrogen of His135 may withdraw charge from the phosphoryls coordinated to the Mg2+ at the MG2 site (54).

Although the Al3+-generated transition-state adopts a hexacoordinated octahedral bipyrimidal structure rather than the pentacoordinated trigonal-bipyrimidal transition-state expected for a phosphotransfer mechanism, the data are in agreement with an
associative nucleophilic substitution, \( S_N^2 \), mechanism. This mechanism is supported
by the perfect inline orientation of the nucleophile (the C1 oxygen of ribose 5-
phosphate), the partial bond character between the attacking nucleophile and the
transferred group (represented by \( \text{Al}^{3+} \)), a transient charge of -2 (provided by the two
“free” \( \gamma \)-phosphoryl oxygens and represented by fluorides). An effect of the two
basic amino acid residues of the catalytic flexible loop, Lys197 and Arg199, may be
stabilization of the transient negative charge. The \( S_N^2 \) mechanism is supported by
previous studies of \textit{S. enterica} PRPP synthase, which showed that the product of
reaction of ATP with [1-\(^{18}\text{O}\)]ribose 5-phosphate was \([^{18}\text{O}]\text{PRPP}\) rather than
\([^{18}\text{O}]\text{AMP}\) (66, 141).

\section*{REGULATION OF PRPP SYNTHASE ACTIVITY}

All PRPP synthases analyzed, class I from microbes (\textit{S. enterica} (69), \textit{B. subtilis} (70),
\textit{E. coli} (45, 75), humans (72, 142), rat (143, 144)), class II (spinach isozymes 3 and 4
(46, 47)) as well as the archaeon \textit{M. jannaschii} (48), are inhibited in a competitive
manner by ADP, which binds in competition with ATP at the active site.
Superimposed on the competition of ADP and ATP at the active site is allosteric
inhibition by ADP. This allosteric inhibition is well documented. However, let us first
mention some results of a steady state kinetic analysis of the binding of allosteric
effectors to \textit{E. coli} PRPP synthase, which showed that \( \text{P}_1 \) and ADP compete for
binding to the same site (75). Additionally, \( \text{P}_1 \) is required for the activity of the class I
PRPP synthases of \textit{S. enterica} (35, 56), \textit{E. coli} (45), \textit{B. subtilis} (70), \textit{B. caldolyticus}
(55), humans (73), and rat (71), as well as for the stability in the absence of MgATP
of \textit{S. enterica} and \textit{E. coli} PRPP synthases (35, 45). Finally, \( \text{P}_1 \) can be replaced as an
activator by high concentration of \( \text{SO}_4^{2-} \) (49, 71, 73). Altogether, it is therefore more
correct to say that that PRPP synthase is allosterically regulated with P\textsubscript{i} as an allosteric activator and ADP as an allosteric inhibitor (75).

Allosteric inhibition of PRPP synthase by ADP was demonstrated by detailed steady state kinetic analysis. A distinctive feature of allosteric binding by ADP is that it requires the active site to be fully occupied by both ATP or ADP and ribose 5-phosphate. This was concluded from the observation that ADP inhibition becomes stronger as the enzyme approaches saturation with ribose 5-phosphate; this is explained by the ordered binding of substrates in which ribose 5-phosphate binds last, i.e., ADP binding at the active (ATP) site induces apparent substrate inhibition by ribose 5-phosphate (69). The presence of a separate ADP binding site was demonstrated directly by equilibrium binding dialysis. Thus, ATP (actually the unreactive analog α,β-methylene ATP) binds to a single site per molecule, the active site, in the presence of ribose 5-phosphate. In the absence of ribose 5-phosphate ADP binds to the same site as ATP, whereas in the presence of ribose 5-phosphate ADP binds to the additional allosteric site to yield two bound ADP per monomer (68).

The synthesis of PRPP was furthermore studied in vivo. Purine starvation of \textit{S. \textit{enterica}} resulted in accumulation of PRPP, whereas purine nucleotides were depleted suggesting that these conditions caused reduction of an inhibitor of PRPP synthesis. This inhibitor could very well be ADP (145). Also, the addition of adenine to cultures of \textit{S. \textit{enterica}} caused a dramatic reduction in the PRPP pool, which only slowly returned to the previous level. Adenine and PRPP react in vivo to form AMP followed by the formation ADP, which causes inhibition of PRPP synthase. Therefore, the PRPP pool only slowly returns to the level before adenine-addition (146). PRPP synthase from some organisms such as \textit{B. subtilis}, \textit{B. caldolyticus} and mammals are also allosterically inhibited by GDP (55, 70, 71, 73).
Characterization of several mutations in human PRPP synthase isozyme 1 demonstrated the existence and physiological importance of the allosteric site in human PRPP synthase. The mutant forms were identified in the human PRS1 genes of individuals with inherited defects in purine metabolism (72). The positions of the amino acid replacements identified, Asp51His, Asn113Ser, Leu128Ile, Asp182His, Ala189Val, and His192Gln, are indicated by arrows above the deduced B. subtilis PRPP synthase amino acid sequence of Fig. 2. The affected individuals exhibit hyperuricemia, gouty arthritis, and in some cases neurosensory defects, with an X-linked pattern of inheritance. Six different mutations in human PRPP synthase isozyme 1 have been characterized at the molecular level. The kinetics of inhibition of the purified recombinant mutant enzymes by ADP and GDP confirmed that they were much less sensitive to inhibition than the wild-type isozyme and that the pattern of inhibition by ADP with respect to ATP was altered from a complex pattern of inhibition of the wild-type enzyme to simple competitive. Thus, the existence of a distinct allosteric mode of inhibition by ADP and GDP was demonstrated. The fact that desensitization to allosteric inhibition results in metabolic defects that are readily explained by overproduction of PRPP in human cells is strong evidence that this inhibition is critical for normal regulation of PRPP synthesis. The mutations that destroy allosteric inhibition in human PRPP synthase are scattered throughout the primary sequence, but localised in the three-dimensional structure almost exclusively in the interface between dimers in the hexamer.

In molecular terms the mechanism of allosteric inhibition may be described by comparing the three-dimensional structures of various PRPP synthase complexes. Thus, the B. subtilis SO4$^{2-}$-, ADP-, GDP- and mGDP-PRPP synthase complexes are characterized by tight packing of both the N- and C-terminal domains and represent an inactive form (the T-state) of the enzyme, whereas the B. subtilis AlF$_3$- and mATP/R5P-
PRPP synthase complexes are less tightly packed and represent an active form (the R-state) of the enzyme. Of particular importance is a comparison of the crystal structures of the transition-state complex, AlF3-PRPP synthase, and the inhibited-state quaternary complex, mGDP-PRPP synthase. The latter complex contained Mg$^{2+}$, α,β-methylene ATP and ribose 5-phosphate in the active site as well as methylene GDP in the allosteric site. The binding of ribose 5-phosphate, the adenine base of ATP, the γ-phosphate as well as Mg$^{2+}$ of the MG1 site is virtually identical in the two complexes. In contrast, Mg$^{2+}$ at the MG2 site was displaced, and the torsional angle of the glycosidic bond of ATP differed by a 55° rotation among the two structures, 72° (in mGDP-PRPP synthase) versus 127° (in AlF3-PRPP synthase). This rotation displaces the α- and β-phosphates of the nucleotide bound to the active site and appears to prevent the inline arrangement of the C1 oxygen of ribose 5-phosphate and the β- and α-phosphates of ATP necessary for nucleophilic attack. The important amino acid residues involved in these alterations are located within the regulatory flexible loop (Tyr97-Thr113, Fig. 2). Thus, for the binding of the ribose moiety of ATP a shift in hydrogen bonding of the C2’ oxygen and Gln102 to hydrogen bonding of the C3’ oxygen and Glu110 occurred. Additionally, the MG2-coordinated water molecules changed from coordinating to Arg101, Asp103 and Arg104 to coordinate only Asp103, whereas Arg104 changed to form hydrogen bonding to oxygens of the α-phosphoryl. Arg101 no longer formed hydrogen bonding in the T-state (54).

As expected from the analysis of the mutant variants of human PRPP synthase isozyme 1 described above, these altered substrate-binding capabilities are accompanied by transitions in the hexameric structure. Reciprocal interactions between two C-terminal domain amino acid residues Val178-Asp195, Arg198 and Asn209-Val211 result in a tightly packed interface. This stretch of amino acid
residues straddles the catalytic flexible loop (Lys197-Met208). Particularly important is a hydrophobic pocket consisting of Ile192, Ile194 and Val211. Altogether, the interactions prevent loop closure. The binding of substrates at the active site as well as $\text{SO}_4^{2-}$ (and hence presumably $\text{P}_i$) at the allosteric site decouples these C-terminal amino acid interactions and permits loop closure. On the other hand, when ADP or GDP is bound at the allosteric site, interactions of the C2’ hydroxyl of the bound ADP or GDP with Gln140, which interacts with the imidazole moiety of His149 through a water molecule and hence interaction with Leu134 results in maintaining the hydrophobic pocket and the tight C-terminal domain interaction. All these interactions are lost in the transition-state like complex, AlF$_3$. (54).

Site-directed mutagenesis of the *B. subtilis* prs gene was performed to generate mutant variants with similar amino acid alterations equivalent to the naturally occurring mutations in human PRPP synthase isozyme 1 describe above (54). As observed for the human counterparts, the kinetic properties of $\text{P}_i$ activation among the mutant variants were generally shifted toward an increased affinity for $\text{P}_i$. The mutant *B. subtilis* enzymes displayed about 10-fold decrease or more in the half-saturation concentrations for $\text{P}_i$ when compared to the wild-type form. Except for the Asp57His variant (homologous to human Asp51His variant), whose $\text{P}_i$ dependence on activity was similar to that of the wild-type, and Asp196Gln (homologous to human His192Gln), whose activity appeared independent of $\text{P}_i$ within the range of $\text{P}_i$ concentrations applied.

The *B. subtilis* PRPP synthase variants described above were also analyzed with respect to inhibition by the nucleoside diphosphate GDP, which is less complex than ADP inhibition as GDP only binds to the regulatory site and not the active site. When inhibition by GDP of each of the mutant PRPP synthases was analyzed at a $\text{P}_i$
concentration corresponding to the individual half-saturation concentration value for P_i, similar $K_i^{\text{GDP}}$ values were obtained for the wild-type and the following variants

Asn119Ser (homologous to human Asn113Ser), Leu134Ile (homologous to human Leu128Ile), Asp186His (homologous to human Asp182His) and Ala193Val (homologous to human Ala189Val). This observation indicates that PRPP synthase may represent the $V$-system of the Monod, Wyman and Changeux model for allosteric systems (147), where the activator (P_i) and the inhibitor (GDP) compete for binding to the same site to alter the equilibrium between the R and T conformer of the enzyme.

The mutant enzymes described above that have increased affinity for P_i all have changed equilibrium between conformers that further the R-conformer compared to wild type enzyme.

Mapping of each of the amino acid substitutions in the structure (cf. Fig. 2) that influence this equilibrium between active and inactive conformer shows that three amino acid residues, Asp186, Ala193 and Asp186 are located within the region of the C-terminal domain interaction, which is important for transition of the allosteric signal, i.e. the binding of ribonucleoside diphosphate at the allosteric site.

Additionally, Asn199 is located at the N-terminal interface of the bent dimer, and likewise may perturb the binding of ribonucleoside diphosphate at the allosteric signal as described above. Finally, Leu134 is involved in the transmission of the allosteric signal. Almost all of the amino acid substitutions are located on the interface within a dimer or between dimers in the hexamer. These positions are in accordance with the existence of an equilibrium between the active and the inactive form of class I PRPP synthase that can be altered by side chain substitutions that stabilize any of the two conformers, the active R or the inactive T conformer.
A large number of mutations have furthermore been identified in the gene specifying human PRPP synthase isozyme 1 of patients suffering from relapse of acute lymphoblastic leukemia. Negative feedback-defective PRPP synthase resulted from the mutations that arose after treatment with 6-mercaptopurine and 6-thioguanine as chemotherapeutics. Twenty-nine lesions affecting 14 amino acid positions have been identified. Some of these positions were already known from the previous analysis of PRPP synthase from patients with gout or uric acid overproduction, Asn113, Asp182 and Ala189. At the latter position two variants were identified (Ala189Thr, 10 patients, and Ala189Val, one patient). Mapping of the amino acid alterations on the three-dimensional structure of human PRPP synthase isozyme 1 revealed the location of the variations at the allosteric site (Ser102, Asn143, Thr302) or at the dimer interface (Asn113, Gly173, Lys175, Asp182, Ala189, Leu190). The conclusion from this analysis is that the cytotoxic effect of administering compounds such as 6-mercaptopurine and 6-thioguanine may be counteracted by mutations that accelerate the production of PRPP and hence accelerating the de novo synthesis of purine nucleotides, which competes with the cytotoxic compounds (148).

Fortuitously, the presence of Ca$^{2+}$ during crystallization of B. subtilis PRPP synthase allowed important conclusions about the mechanism of inhibition by this ion to be drawn (54). Ca$^{2+}$ was identified as a hepta-coordinating metal ion at the MG1 site of the GDP-PRPP synthase complex, whereas Mg$^{2+}$ occupied the MG2 site (Fig. 6D). In contrast to this Mg$^{2+}$/Ca$^{2+}$ arrangement, two Mg$^{2+}$ were found at the other active site of the asymmetric unit of the GDP-PRPP synthase complex. The ligands coordinated to Ca$^{2+}$ resembled those coordinated to Mg$^{2+}$: Asp174, oxygens of C1, C2 and C3 of ribose 5-phosphate, oxygen of the $\gamma$-phosphate, a water molecule, with an
additional seventh coordination to oxygen of the β-phosphate of ATP. In particular, this latter coordination of Ca$^{2+}$ causes perturbation of the inline arrangement of the C1 oxygen of ribose 5-phosphate and the β- and α-phosphates of ATP described above (54). Previous kinetic analysis revealed that Ca$^{2+}$, which does not support PRPP synthase activity alone, is an inhibitor of PRPP synthase activity even in the presence of a very large excess of Mg$^{2+}$ ions (56, 57).

The detailed molecular description of the regulation of PRPP formation has prompted the application of this knowledge for the improvement of commercially important products of biochemical pathways for which PRPP is an intermediate. The previously discussed improvement of riboflavin synthesis by *E. gossypii* harboring feedback-resistant variants of PRPP synthase is one example (112). Another example is the production of purine nucleosides by *Bacillus amyloliquefaciens*. The following PRPP synthase variants were constructed with the equivalent variant of human PRPP synthase isozyme variant shown in parenthesis: Asp58His (Asp51His), Asn119Ser (Asn113Ser) and Leu134Ile (Leu128Ile). As expected, the *B. amyloliquefaciens* PRPP synthase variants Asn119Ser and Leu134Ile were activated at lower Pi concentrations and were relatively more resistant to inhibition by ADP and GDP compared to the wild type, and, indeed, both *B. amyloliquefaciens* and *B. subtilis* strain carrying the mutations specifying Asn119Ser or Leu134Ile had improved production of purine nucleosides presumably due to increased purine de novo synthesis. Kinetic constants of *B. amyloliquefaciens* PRPP synthase are given in Table 2 (149).

**PHOSPHORIBOSYL BISPHOSPHATE PHOSPHOKINASE**

Mutants that are defective in the *prs* gene have been isolated in *E. coli* and *S. enterica* as described further below. Strains with knockout mutations (Δ*prs*) have a
simultaneous requirement for five metabolites: purine and pyrimidine nucleosides, histidine, tryptophan and NAD (2, 3). Mutants that suppress the requirement for NAD can be easily isolated. These mutants retain the other four requirements; their genetic defect maps to the \textit{pstSCAB-phoU} operon (150, 151). Lesions in this operon result in constitutive expression of the Pho regulon operons, which are involved in the assimilation of \( P_i \) and the acquisition of \( P_i \) from other sources (152). Genetic analysis revealed that suppression of the NAD requirement suppression phenotype originated from increased expression of the \textit{phnN} cistron of the tetrakaidecacistronic \textit{phnC-P} operon (150). The gene products of this operon are involved in the catabolism of phosphonates (152). It was furthermore shown that the \textit{phnN} cistron specifies a phosphokinase with the ability to use ribosyl 1,5-bisphosphate as a substrate: ribosyl 1,5-bisphosphate + ATP \( \rightarrow \) PRPP + ADP, i.e. a ribosyl bisphosphate phosphokinase (EC 2.7.4.23) (Fig. 7A). It was shown that the enzyme was able to convert ribosyl 1,5-bisphosphate to a product that functioned as a substrate for purified xanthine phosphoribosyltransferase and that the product was xanthosine 5’-monophosphate according to the chemical equation xanthine + PRPP \( \rightarrow \) xanthosine 5’-monophosphate + PP\(_i\) (151). Subsequently, it was shown that ribosyl 1,5-bisphosphate is an intermediate in phosphonate catabolism (153). However, ribosyl 1,5-bisphosphate is also a metabolite in cells that are not thriving on phosphonate, although the origin and metabolic role of the compound under these conditions remain unclear. Even though the reaction product of ribosyl bisphosphate phosphokinase is PRPP, the amount synthesized is insufficient to replace the activity of PRPP synthase even in cells growing with phosphonate as their \( P_i \) source (B.H.-J., unpublished result) or in cells that express \textit{phnN} in a multi-copy plasmid (151). The amino acid sequences of ribosyl bisphosphate phosphokinase and PRPP synthase only show low similarity,
approximately 25%. Among the amino acid residues of \textit{B. subtilis} PRPP synthase with annotated functions from the crystal structure, very few if any at all, can be recognized in the amino acid sequence of ribosyl bisphosphate phosphokinase. Likewise, the similarity of ribosyl bisphosphate phosphokinase to type I phosphoribosyltransferase and type II phosphoribosyltransferase is very low, 16% with adenine phosphoribosyltransferase (type I) and with quinolinate phosphoribosyltransferase (type II). Thus, the evolutionary origin and mechanism of phosphoribosyl bisphosphate phosphokinase remain enigmatic; a deeper understanding awaits the determination of the three-dimensional structure of the enzyme. The physiologic importance of phosphoribosyl bisphosphate phosphokinase in the context of phosphonate catabolism has been discussed and previously reviewed (154, 155).

\textbf{DIPHOSPHORYL-, NUCLEOTIDYL- AND PHOSPHORYLTRANSFER}

In spite of a large number of di- or triphosphate-containing compounds, very few enzymes catalyze the transfer an intact diphosphoryl group, i.e. attack the β-phosphorus of ribonucleoside triphosphates, in contrast to enzymes that catalyze the transfer of a phosphoryl group, i.e. attack the γ-phosphorus, and enzymes that catalyze the transfer of a nucleotidyl group, i.e. attack the α-phosphorus. These latter two groups of enzymes are much more abundant than the diphosphoryltransferases. Below we shall give examples of the mechanism of catalysis of each of these three enzyme classes as a means of comparing catalytic strategies for nucleophilic substitutions on the α-, β- and γ-phosphorus atoms of nucleoside triphosphate substrates.

\textbf{Substitution Reactions at α-, β- or γ-Phosphates}
**Diphosphoryltransfer**

The diphosphoryltransferases other than PRPP synthase are 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropterin diphosphokinase (ATP:2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropterin 6′-diphosphotransferase, EC 2.7.6.3) encoded by *folK* in *E. coli* (156), the stringent factor or GTP/GDP 3′-diphosphokinase (ATP:nucleoside-5′-phosphate pyrophosphotransferase, EC 2.7.6.5) encoded by *relA* in *E. coli* (157, 158), and in some organisms thiamine diphosphokinase (ATP:thiamine diphosphotransferase, EC 2.7.6.2) (159), and nucleotide diphosphokinase (ATP:nucleoside-5′-phosphate diphosphotransferase, EC 2.7.6.4) (160) (Fig. 7B-F). In addition, a number of other enzymes, primarily diphosphohydrolases, catalyze reactions with nucleophilic attack on the β-phosphorus of (deoxy)ribonucleoside triphosphates with the release of PPi and the corresponding (deoxy)ribonucleoside 5′-monophosphate, and are members of the so-called Nudix enzyme superfamily as reviewed previously (161-163).

(i) **Hydroxymethylidihydropterin diphosphokinase.** In addition to PRPP synthase, the crystal structure of a number of enzymes catalyzing diphosphoryltransfer reactions has been determined. These allow the comparison of the active sites and catalytic mechanism of these enzymes.

Hydroxymethylidihydropterin diphosphokinase of *E. coli* contains 159 amino acid residues, is monomeric with a molecular mass of 18 kDa, and catalyzes the diphosphorylation of 6-hydroxymethyl 7,8-dihydropterin, the committed step in folate biosynthesis (Fig. 7C). The structure of *E. coli* hydroxymethylidihydropterin diphosphokinase with a variety of ligands has been determined: these include the ternary complex with a substrate analog and a substrate, enzyme-Mg²⁺-α,β-methylene ATP-hydroxydihydropterin complex (PDB code 1q0n) (164). Additional reports on
crystal or solution structures of hydroxymethyldihydropterin diphosphokinase of *E. coli* and *Haemophilus influenzae* have been published (165-167). Structural and mechanistic properties of this enzyme have been previously reviewed (168). Only 23 amino acid residues are identical between *E. coli* hydroxymethyldihydropterin diphosphokinase and PRPP synthase, and the folds of the two polypeptides are very different. The former enzyme adopts a fold comprised of four α-helices and six β-strands, β₁α₁β₂α₂β₃α₃α₄. Substrate recognition is mediated by three loop regions located between β₁ and α₁ (loop L₁), between β₂ and β₃ (loop L₂) and between α₂ and β₄ (loop L₃). ATP binds first to hydroxymethyldihydropterin diphosphokinase, which results in large structural changes in the three loops, in particular L₂ and L₃, and allows the binding of the second substrate, hydroxymethyldihydropterin (164, 169, 170). Furthermore, two Mg²⁺ are bound within active site (Fig. 8A). One Mg²⁺, Mgₐ, coordinates to oxygens of the α- and β-phosphates of ATP, to Asp₉₅ and Asp₉₇ as well as to two water molecules. A second Mg²⁺, Mg₉, coordinates to oxygens of the β- and γ-phosphates of ATP, to the hydroxyl of the substrate hydroxymethyldihydropterin, to Asp₉₅ and Asp₉₇, as does the first Mg²⁺, and finally to a water molecule. Mg₉ thus coordinates both substrates. This arrangement of Mg²⁺, substrate and amino acid residues places the hydroxymethyldihydropterin hydroxyl at a distance of 3.2 Å to β-phosphorus of ATP (164). The triphosphate chain of ATP and the hydroxyl of hydroxymethyldihydropterin are organized in a head to head arrangement much like that of ribose 5-phosphate and ATP of PRPP synthase with the γ-phosphate "bent" away, rather than in a linear arrangement, which allows attack of the hydroxymethyldihydropterin hydroxyl on the β-phosphate of ATP (Fig. 8A).

**(ii) Thiamine diphosphokinase.** Thiamine diphosphate is the active cofactor in a number of C-C bond formation and scission processes (171). Some organisms
synthesize thiamine diphosphate by diphosphoryltransfer of the $\beta,\gamma$-diphosphoryl group of ATP to thiamine (Fig. 7F), whereas others phosphorylate thiamine phosphate in a single phosphoryl transfer reaction. The crystal structure of mouse thiamine diphosphokinase, which utilizes the former mechanism, has been solved; the enzyme structure contained AMP and pyrithiamine diphosphate, i.e. the products of the reaction of ATP with pyrithiamine, a thiamine analog which contains a substituted pyrimidine moiety rather than a substituted thiazole moiety. The structure also contained one $\text{Mg}^{2+}$, which coordinated to oxygens of the AMP-phosphate, to the $\alpha$-phosphate of pyrithiamine diphosphate and to the carboxyls of Asp46, Asp71, Asp73 and Asp100 (PDB code 2f17). The coordination of $\text{Mg}^{2+}$ to the $\alpha$-phosphate of pyrithiamine diphosphate is consistent with (but does not demonstrate) a bent structure of the triphosphate chain of the substrate ATP, as was observed in PRPP synthase and hydroxymethyldihydropterin diphosphokinase. However, the authors also suggested that the data permitted a linear arrangement of the triphosphate chain and a more or less perpendicular orientation of the pyrithiamine with the hydroxyl group oriented for nucleophilic attack on a $\beta$-phosphate oxygen (172). The structure of thiamine diphosphokinase from a few other organisms have been solved. These include the structure of the *Streptococcus mutans* enzyme with thiamine diphosphate and two $\text{Mg}^{2+}$ present, but without an adenylyl moiety present (PDB code 3ihk), *Candida albicans* with thiaminyl imidodiphosphate present (173), and *S. cerevisiae* with thiamine present (PDB code 1ig0) (174). The exact architecture of the active site with substrates and $\text{Mg}^{2+}$ cannot presently be clearly established.

(iii) **GTP/GDP 3’-diphosphokinase.** The structure of the third diphosphoryltransferase, GTP/GDP 3’-diphosphokinase (Fig. 7D), is poorly characterized, presumably because the C-terminus associates with the ribosome.
association may hamper crystallization because that region may be disordered when not bound. However, important conclusions have been obtained from the studies of the bifunctional RelA/SpoT homolog (Rel$_{Seq}$) of *Streptococcus dysgalactiae* subsp. *equisimilis* in which the diphosphoryltransferase (RelA) and diphosphohydrolytic (SpoT) activities are located in the same polypeptide but in different domains (175). The diphosphoryltransferase domain of Rel$_{Seq}$ bears some structural resemblance to the so-called palm domain of human DNA polymerase β, in which two Mg$^{2+}$ intricately position the substrates, primer and deoxyribonucleoside triphosphate, through coordination to the 3’-hydroxyl of the nucleophilic primer and to oxygens of each of the α-, β- and γ-phosphates of the incipient deoxyribonucleoside triphosphate, and by means of coordination to three invariably conserved aspartate residues (Asp190, Asp192, Asp256) (176). Unlike DNA polymerase β and PRPP synthase, Rel$_{Seq}$ appears to carry out catalysis by the assistance of only a single Mg$^{2+}$. Thus, the dependence of the diphosphoryltransfer reaction on the Mg$^{2+}$ concentration parallels the ribonucleoside triphosphate concentration; that is, there is no stimulation of diphosphoryltransfer activity by excess Mg$^{2+}$ (177). Rel$_{Seq}$ contains only two of the three acidic residues: Asp264, which is homologous to Asp190 of DNA polymerase, and Glu323, which is homologous to Asp256. The lack of a homologue of the third aspartate residue may imply inability to bind a second Mg$^{2+}$ (175). However, this view that GTP/GDP 3’-diphosphokinase requires only as single Mg$^{2+}$ has been challenged. The activity of monofunctional *E. coli* GTP/GDP 3’-diphosphokinase is stimulated by Mg$^{2+}$ in excess of the ribonucleoside triphosphate (ATP and GTP) concentration; this is unlike the activity of RelA/SpoT bifunctional *M. tuberculosis* GTP/GDP 3’-diphosphokinase, which was inhibited at increased Mg$^{2+}$ concentrations. Analysis of mutant variants of these two GTP/GDP 3’-diphosphokinases revealed the
importance of the amino acid sequence 306-Glu-Any-Asp-Asp-309 in *E. coli*

GTP/GDP 3’-diphosphokinase (homologous to 348-Arg-Any-Lys-Asp-351 in *M. tuberculosis* GTP/GDP 3’-diphosphokinase). After swapping these motifs, the activity of *E. coli* GTP/GDP 3’-diphosphokinase is inhibited by excess Mg$^{2+}$, whereas the activity of *M. tuberculosis* GTP/GDP 3’-diphosphokinase chimera is stimulated by Mg$^{2+}$ (178). It is possible that the mechanism of diphosphoryltransfer of bifunctional GTP/GDP 3’-diphosphokinases, such as those of *S. dysgalactiae* or *M. tuberculosis*, may deviate from that of PRPP synthase and hydroxymethylidihydropterin diphosphokinase by the apparent requirement of only a single Mg$^{2+}$, whereas the mechanism of diphosphoryltransfer of monofunctional GTP/GDP 3’-diphosphokinases, such as that of *E. coli*, may resemble other diphosphoryltransferases in requiring two Mg$^{2+}$ for activity.

(iv) MutT. For the sake of completeness, we include a brief description of one member of the Nudix hydrolases, MutT, encoded by the *mutT* gene in *E. coli*. Like the diphosphoryltransferases, MutT catalyzes a nucleophilic substitution reaction on the β-phosphorus but using water as the nucleophile. MutT contains 129 amino acid residues, has a molecular mass of 15 kDa and is active as a monomer. The solution structure has been determined (179). The enzyme is also designated 8-oxo-dGTP diphosphohydrolase because 8-oxo-dGTP appears to be the best substrate in vitro, and is probably also the most important substrate in vivo. Other (deoxy)ribonucleoside triphosphates are also hydrolyzed, albeit at much higher $K_M$ values (180). 8-Oxo-dGTP results from oxidation of dGTP, and may be misincorpoated into DNA opposite deoxyadenylate residues, which would result in AT → GC transversions (181). The frequency of such transversions increases by 100 to 1000 fold in a *mutT* strain (182). Like PRPP synthase and other diphosphoryltransferases, MutT requires two divalent
metal ions (Mg$^{2+}$ or Mn$^{2+}$ with a preference for Mg$^{2+}$), for activity. One ion is
enzyme-bound, and the other is bound to the (deoxy)ribonucleoside triphosphate. The
enzyme-bound Mg$^{2+}$/Mn$^{2+}$ coordinates to the nucleophilic water, to Gly38, Glu56,
Glu57 and Glu98 and to a second water molecule, whereas the nucleotide-bound
Mg$^{2+}$/Mn$^{2+}$ coordinates to oxygens of the β- and γ-phosphates (162, 183). Catalysis
involves the abstraction of a proton of the nucleophilic water by Glu53, followed by
attack on the β-phosphorus and displacement of 8-oxo-dGMP and MgPP$_i$ (162). The
biochemistry, physiology and genetics of MutT as well as other members of the
Nudix enzyme family have been previously reviewed (161, 162).

**Nucleotidyltransfer.** Nucleotidyltransferases catalyze reactions of the type
acceptor + (deoxy)ribonucleoside-P-P-P → acceptor-P-(deoxy)ribonucleoside + PP$_i$.
Examples include reactions catalyzed by nucleic acid polymerases (DNA$_n$ + dNTP →
DNA$_{n+1}$ + PP$_i$, or RNA$_n$ + NTP → RNA$_{n+1}$ + PP$_i$, or glucose 1-phosphate
uridylyltransferase (glucose 1-phosphate + UTP → UDP-glucose + PP$_i$). Indeed,
nucleic acid polymerases and sugar phosphate nucleotidyltransferases (sometimes
called sugar nucleoside diphosphate diphosphorylases) are the most prominent
members of this class of enzymes. These reactions have substitutions with
nucleophilic attack on the α-phosphorus of ribonucleoside triphosphate or
deoxyribonucleoside triphosphate in common. The structure of the active sites of
RNA and DNA polymerases are quite similar. Here we shall return to the human
DNA polymerase β, briefly described above, to further describe the active site and the
mechanism of catalysis, which involves the intricate positioning of two Mg$^{2+}$ ions by
means of coordination to three conserved aspartate residues, as was originally
suggested by Steitz and Steitz for DNA polymerase I and other nucleic acid
polymerases (184). Fig. 8B shows the binding of the two Mg$^{2+}$ to the active site of
DNA polymerase β. Mg\textsubscript{A} coordinates to oxygens of the α-, β- and γ-phosphates, to Asp190 and Asp192 and to a water molecule, whereas Mg\textsubscript{B} coordinates to an oxygen of the α-phosphate, to the two carboxylate oxygens of Asp190, and to Asp192 and Asp256. The sixth coordination of Mg\textsubscript{B} is not detected. Presumably this coordination normally involves the 3’-hydroxyl of the deoxyribose that receives the nucleotidyl moiety. This 3’-hydroxyl is missing in 2’,3’-dideoxyribonucleotides, and, possibly, the crystal packaging does not allow space for a water molecule (176). Similar geometric arrangements are found in other DNA polymerases, such as DNA polymerase II (185). Extensive reviews of the structure and function of DNA polymerase β and other DNA polymerases have been previously published (186, 187).

An example of a sugar phosphate nucleotidyltransferases is the \textit{glmU} gene product, a bifunctional enzyme that catalyzes the acetylation of 2-amino-glucose 1-phosphate and the uridylation of N-acetyl-2-amino 1-phosphate to yield UDP-N-acetylglucosamine. The two activities are located in different domains, with the uridylyltransferase allocated to the N-terminal domain (190). UDP-N-acetylglucosamine is a precursor of cell wall synthesis in many bacterial and fungal species as well as a substrate in glycosylation reactions in eukaryotic species (191). The crystal structure of GlmU of \textit{M. tuberculosis} contains two Mg\textsuperscript{2+}. One (Mg\textsubscript{A}\textsuperscript{2+}) coordinates to Asn239 and Asp114, to an oxygen of the phosphate of N-acetylglucosamine 1-phosphate, and to an oxygen of the α-phosphate of UTP, and thus, Mg\textsubscript{A}\textsuperscript{2+} bridges the two substrates. The other (Mg\textsubscript{B}\textsuperscript{2+}) coordinates to oxygens of the α-, β- and γ-phosphates of UTP and to three water molecules. Thus, Mg\textsubscript{B}\textsuperscript{2+}
apparently does not coordinate to any amino acid residue (and formally there is no Mg\(_{B}^{2+}\) binding-site). Rather, the uridylylation domain of GlmU appears to harbor a single enzyme-bound Mg\(_{A}^{2+}\), whereas the other Mg\(_{A}^{2+}\) enters as MgUTP during the catalytic cycle. This two-Mg\(_{A}^{2+}\)-mediated mechanism is believed to be general in sugar nucleotide synthesis (192), as similar active site arrangements are observed for 2-keto-3-deoxymanno-octulonate cytidylyltransferase of *E. coli* (PDB code 3k8d) (193), glucose 1-phosphate uridylyltransferase of *Corynebacterium glutamicum* (PDB code 2pa4) (194), 2-C-methylerythritol 4-phosphate cytidylyltransferase of *Mycobacterium smegmatis* (PDB code 2xwl) and *M. tuberculosis* (PDB code 2xwn) (195).

A exception to this two-Mg\(_{A}^{2+}\) mediated mechanism is *N*-acetylglucosamine uridylyltransferase of *C. albicans*, which contains only a single Mg\(_{A}^{2+}\) presumably coordinated to oxygens of the α-, β- and γ-phosphates of UTP, i.e. Mg\(_{B}^{2+}\) in analogy with *M. tuberculosis* GlmU. Furthermore, the function of Mg\(_{A}^{2+}\) may be performed by Lys421, because the ε-N of this residue coordinates oxygens of the α- and β-phosphates of UDP-*N*-acetylglucosamine after product formation, and, thus, it also presumably coordinates with oxygens of the phosphate of *N*-acetylglucosamine and of the α-phosphate of UTP during catalysis. Arg116 may also participate in the function performed Mg\(_{A}^{2+}\) in other sugar phosphate nucleotidyltransferases (196).

**Phosphoryltransfer.** In general, enzymes catalyzing phosphoryl transfer reactions also require two Mg\(_{A}^{2+}\), as demonstrated from studies of *Pyrococcus furiosus* UMP kinase (PDB code 2bmu) (197), *Staphylococcus aureus* tagatose 6-phosphate kinase (PDB code 2jg1) (198), *Bifidobacterium longum* *N*-acetylhexosamine kinase (PDB code 4wh3) (199), mammalian cyclin-dependent kinase 2 (PDB code 3qhw) (200), or cAMP-dependent protein kinase A (PDB codes 1l3r and 4hpu) (201, 202).
We shall use protein kinase A for a more detailed description. Crystals of the enzyme were obtained as a complex with reaction-inert β,γ-imido ATP, Mg\(^{2+}\) and a serine-containing peptide substrate. Of the two Mg\(^{2+}\), Mg\(_A\)\(^{2+}\) coordinates to oxygens of the α- and γ-phosphates, to the bridging N of the β- and γ-phosphates of β,γ-imido ATP, to Asn171 and Asp184, as well as to a water molecule, whereas the other, Mg\(_B\)\(^{2+}\), coordinates to oxygens of the β- and γ-phosphates, to both of the oxygens of Asp184, as well as to two water molecules (Fig. 8C). Unlike the examples of diphosphoryl and nucleotidyltransfer reactions described above, there is apparently no coordination between Mg\(^{2+}\) and the nucleophilic hydroxyl of the serine residue of the peptide substrate. However, this oxygen appears to be held firmly in place by hydrogen bonding to Lys168 and Asp166 (202).

The number of Mg\(^{2+}\) in kinase-catalyzed reactions may vary from enzyme to enzyme. Typically, nucleoside diphosphokinases contains only a single Mg\(^{2+}\).

Adenylate kinase catalyzes the reversible reactions AMP + ATP → 2 ADP or dAMP + ATP → dADP + ADP (203). The enzyme contains a loop, which closes upon substrate binding, and, thus, prevents nucleophilic attack by water on the ATP triphosphate chain. The structure of the E. coli enzyme has been probed with β,γ-imido ATP (PDB code 1ank). The phosphoryl group of AMP and the phosphate chain of β,γ-imido ATP face one another in an almost co-linear arrangement. One oxygen of the phosphoryl group of AMP is in close proximity to the γ-phosphorus of β,γ-imido ATP, and, thus, ideally placed for an inline attack with the subsequent formation of ADP (204). This collinear structure of the phosphoryl groups resembles the structure of adenylate kinase ligated to the inhibitor di(adenosine-5’)pentaphosphate, except for the presence of an additional phosphoryl group (205). Mg\(^{2+}\), which is necessary for adenylate kinase activity, does not coordinate to side chains of the enzyme, but rather...
coordinates to two oxygens of di(adenosine-5’)-pentaphosphate as well as to four water molecules (206). This binding of Mg$^{2+}$ to di(adenosine-5’)-pentaphosphate is consistent with β,γ-MgATP as the substrate for adenylate kinase (207), and Mg$^{2+}$ coordination to oxygens of both of the β-phosphates of two ADP molecules of M. tuberculosis adenylate kinase, i.e. the reaction in the direction of ATP synthesis (PDB code 2cdn) (208). Furthermore, in comparison with diphosphoryl and nucleotidyltransfer reactions described above, adenylate kinase may represent an alternative means of performing the function played by the second Mg$^{2+}$ in other phosphotransferases. The stabilizing and neutralizing effect of a second Mg$^{2+}$ may be replaced by one or more arginine and/or lysine residues such as those found near the triphosphate chain and AMP in adenylate kinase (PDB codes 1ank and 2cdb) (204, 208).

Catalytic strategies. The triphosphate chain of (deoxy)ribonucleoside triphosphates is quite electron rich and, hence, negatively charged, so that chemical reactions involving this chain require special conditions. In addition, an activated, i.e. deprotonated, nucleophile may contribute additional negative charge to the transition-state. As described above, these difficulties are circumvented at least in part by the presence of Mg$^{2+}$ in the active site. Fig. 9 shows a schematic representation of part of the active sites of enzymes or groups of enzymes that catalyze substitution reactions at the α-, β- or γ-phosphates of (deoxy)ribonucleoside triphosphates and that contain two Mg$^{2+}$. The triphosphate chain, the coordination of each Mg$^{2+}$, and the nucleophile are shown for each enzyme. Additional amino acid residues may be involved in the binding of the substrates but are not shown in Fig. 9. The two Mg$^{2+}$ may have multiple functions that include exact positioning of the substrates for transition-state formation, electrostatic stabilization of the nucleophile and the transition-state, and
electrostatic stabilization of the product(s) or leaving group(s). As described before for PRPP synthase, one Mg$^{2+}$ (Mg$_B$ of Fig. 9) presumably is involved in the stabilization of the nucleophile, i.e. the acceptor of the diphosphoryl group, the C1 hydroxyl of ribose 5-phosphate. Similarly, Mg$_B$ of nucleotidyltransferases may participate in stabilization of the nucleophiles, i.e. hexose phosphate (Fig. 9C) and deprotonated DNA primer (Fig. 9D) (189). In contrast, no coordination is observed between Mg$_B$ and the $\gamma$-phosphate-attacking nucleophilic serine residue in protein kinase A (Fig. 9A), and nucleophile positioning involves amino acid residues. As in the case of Mg$_B$ in PRPP synthase, Mg$_B$ of nucleotidyltransferases binds the nucleophile, and may stabilize the deprotonated nucleophile (Fig. 9C and D). Interestingly, Mg$_B$ of PRPP synthase and the nucleotidyltransferases coordinate both of the substrates, i.e. ribose 5-phosphate and ATP in PRPP synthase (Fig. 9B), N-acetylglucosamine 1-phosphate and UTP in N-acetylglucosamine 1-phosphate uridylyltransferase (Fig. 9C), and the DNA template and deoxyribonucleoside triphosphate in DNA polymerases (Fig. 9D). The $\gamma$-phosphate of nucleoside triphosphates carries two negative charges at physiologic pH, whereas the $\alpha$- and $\beta$- phosphates carry one negative charge, each. These charges are neutralized by Mg$^{2+}$. The second Mg$^{2+}$, Mg$_A$, in the enzymes under discussion typically binds the substrate (deoxy)ribonucleoside triphosphate. Thus, the form bound to the enzyme in all the cases shown appears to be the tridentate $\alpha,\beta,\gamma$-MgATP. The Mg$_A$ therefore not only partly neutralizes the triphosphate chain, but also provides the proper conformation of the substrate to the enzyme. The general belief is that the functioning of Mg$_A$ includes a stabilization of the transition state. It is noteworthy that the two Mg$_A$ and Mg$_B$ are localized on both sides of the triphosphate chain except in the nucleic acid.
polymerases, where the two Mg$^{2+}$ are located on the same side of the triphosphate chain.

**UTILIZATION OF PRPP**

The concentration of PRPP is approximately 0.5 mM in cells of *S. enterica* growing exponentially in minimal salts medium with glucose as carbon source. This value may be compared to the concentrations of ATP, GTP, UTP and CTP, which are 3, 0.9, 0.9, and 0.5 mM, respectively (19). PRPP is utilized in nucleotide, amino acid and co-factor biosynthetic pathways. The relative consumption of PRPP for each of these processes in *E. coli* has been estimated. Thus, purine and pyrimidine nucleotide biosynthesis consumes 30-40% of the PRPP synthesized, each, whereas histidine and tryptophan biosynthesis consumes 10-15% of the PRPP synthesized, each, and NAD, NADP biosynthesis consumes approximately 1% of the PRPP synthesized (1).

**Reactions at the Anomeric Carbon of PRPP**

The reactions occurring at the anomeric carbon of PRPP greatly outnumber the reactions occurring at the diphosphoryl chain. The reactions at the anomeric carbon are catalyzed by phosphoribosyltransferases, whereas hydrolases catalyze the reactions at the diphosphoryl chain. Phosphoribosyltransferases catalyze reactions in which N-, C- or O-glycosidic bonds are formed with the concomittant formation of PP, (Fig. 1). We shall review first the reactions occurring at the anomeric carbon, and then the reactions occurring at the diphosphate chain.

**Comparison of phosphoribosyltransferase and PRPP synthase structures.**

The structure of phosphoribosyltransferases vary to some extent. The most widespread structure, present in the so-called type I phosphoribosyltransferase,
consists of a core of a five-stranded β-sheet surrounded by a number of helices, and, additionally, a loop structure, the hood, which participates in the binding of the nucleobase substrate, and a (catalytic) flexible loop, which remains unresolved until the binding of PRPP, when it closes the active site for catalysis (209, 210). The type I phosphoribosyltransferases utilize purine or pyrimidine bases or glutamine-derived ammonia as the phosphoribosyl acceptor. Remarkably, in spite of only approximately 20 % amino acid sequence identity among type I phosphoribosyltransferases and PRPP synthase, the three dimensional structures of type I phosphoribosyltransferases strongly resemble that of each domain of PRPP synthase, as shown in Fig. 10A for *T. volcanium* PRPP synthase and *Toxoplasma gondii* hypoxanthine-guanine phosphoribosyltransferase. We shall use the *T. gondii* hypoxanthine-guanine phosphoribosyltransferase as a structural “prototype” of type I phosphoribosyltransferase, as many of the structural features of this enzyme apply to most other type I phosphoribosyltransferases as well. The homology of the central five-stranded β-sheet and the surrounding helices is evident in both structures, as is also the homologous position of the catalytic flexible loop of PRPP synthase and the flexible loop of the phosphoribosyltransferase.

A comparison of amino acid residues of PRPP synthase and various type I phosphoribosyltransferases reveals only a single conserved region, a series of hydrophobic residues, usually three, followed by one or two aspartate residues, two or three hydrophobic residues, and a four-residue sequence characteristic of sharp turns in the polypeptide chain, such as Thr-Gly-Gly-Thr (44, 45, 211). This region in *B. subtilis* PRPP synthase (216-Gly-Lys-Thr-Ala-Ile-Leu-Ile-Asp-Asp-Ile-Ile-Asp-Thr-Ala-Gly-Thr-232) is the ribose 5-phosphate binding loop described above (45), whereas the corresponding region in *T. gondii* hypoxanthine-guanine
phosphoribosyltransferase (139-Asp-Lys-His-Val-Leu-Ile-Val-Glu-Asp-Ile-Val-Asp-
Thr-Gly-Phe-Thr-154) is the PRPP binding loop (212). Additionally, the PP loop 79-
Lys-Glu-80 in *T. gondii* hypoxanthine-guanine phosphoribosyltransferase) and amino
acid residues of the flexible catalytic loop (residues 114 to 134 in in *T. gondii*
hypoxanthine-guanine phosphoribosyltransferase) are involved in the binding of
PRPP or MgPRPP in other phosphoribosyltransferases; collectively, these sequences
constitute the PRPP binding site (209, 210). The thee-dimensional structure of the
ribose 5-phosphate binding loop of PRPP synthase and the PRPP binding loop of
phosphoribosyltransferases are very much alike as shown in Fig. 10B, which shows a
superimposition of parts of the active site of *B. subtilis* PRPP synthase and *T. gondii*
hypoxanthine-guanine phosphoribosyltransferase: the binding of ribose 5-phosphate,
the β- and γ-phosphates of ATP and two Mg$^{2+}$ at the active site of *B. subtilis* PRPP
synthase and the binding of PRPP and the Mg$^{2+}$ at the active site of *T. gondii*
hypoxanthine-guanine phosphoribosyltransferase (54, 212).

**N-Glycosidic bond formation.** By far the most abundant reactions involving
PRPP are formation of N-glycosidic bonds. The prototype of reactions involving
PRPP is shown in Fig. 1, and examples of the products containing carbons of PRPP
are shown in Fig. 11. A nitrogen-containing compound displaces the diphosphoryl
group of PRPP in a nucleophilic reaction with inversion of the anomeric carbon (for
example: adenine + PRPP $\rightarrow$ AMP + PP$_i$). In general, the nitrogen-containing
compounds are aromatic bases. In purine and pyrimidine nucleotide biosynthesis the
ribosyl group of PRPP remains the ribosyl or deoxyribosyl group of the nucleotides.
Similarly, in co-factor (NAD and NADP) biosynthesis the ribosyl group of PRPP
remains the ribosyl groups of the co-factors. In contrast, in tryptophan and histidine
biosynthesis only some of the ribosyl carbons are built into the products. Thus, in
tryptophan biosynthesis carbon two and three of the indolyl moiety originate from carbon one and two, respectively, of the ribosyl moiety of PRPP, whereas five of the six carbons of histidine originate from the ribosyl moiety of PRPP with the carboxyl group originating from C5 of PRPP. The nitrogen-containing compounds utilized by phosphoribosyltransferases may be the purine bases adenine, hypoxanthine, xanthine or guanine, the pyrimidine bases may be orotate or uracil, the pyridine bases may be quinolinate or nicotinate, or the bases may be anthranilate or ATP. A variant among phosphoribosyltransferases, amidophosphoribosyltransferase, the first enzyme of de novo purine biosynthesis, utilizes glutamine-derived ammonia. All together, 10 enzymes utilize PRPP for \( \text{N} \)-glycosidic bond formation with 11 products in organisms such as \textit{E. coli} or \textit{B. subtilis}, Fig. 11.

As we shall see below, PRPP is a very versatile compound for the delivery of phosphoribosyl moieties. A large number of phosphoribosyltransferases have been described. Although they all use PRPP as phosphoribosyl donor and, presumably, they follow similar chemical mechanisms, they differ widely in three-dimensional structure. The enzymes range from the type I phosphoribosyltransferases, which are primarily involved in purine and pyrimidine nucleotide biosynthesis, type II phosphoribosyltransferases, which are involved in pyridine nucleotide biosynthesis, to type III and type IV phosphoribosyltransferase, which are involved in histidine and tryptophan biosynthesis, respectively. The three-dimensional structures of a large number of phosphoribosyltransferases have been determined. Detailed reviews on the biochemistry of phosphoribosyltransferases have been published previously (209, 210, 213).

\textbf{(i) Purine nucleotide biosynthesis.} Nucleotide biosynthesis occurs via two sets of reactions: de novo reactions in which nucleotides are generated by the addition of
atoms one by one to the starting material, and the so-called salvage or auxiliary reactions by which preformed nucleobases are utilized for nucleotide biosynthesis.

(a) Purine de novo synthesis. De novo purine nucleotide biosynthesis comences with PRPP. The purine ring is built on the phosphoribosyl moiety atom by atom beginning with the addition to C1 of the ribosyl moiety of the nitrogen of glutamine derived NH3, which subsequently becomes N9 of the complete purine moiety. This reaction is catalyzed by amidophosphoribosyltransferase (5-phospho-β-D-ribosylamine:phospho-α-D-ribosyltransferase (glutamate-amidating), EC 2.4.2.14) encoded by purF in E. coli, glutamine + PRPP + H2O $\rightarrow$ glutamate + 5-phosphoribosyl 1-amine + PPi (Fig. 11, compound a) (214). Amidophosphoribosyltransferase contains a domain that catalyzes the hydrolysis of glutamine to form glutamate and ammonia. Subsequently, the ammonia is transferred through a channel of 20 Å long to the phosphoribosyltransferase domain active site, where PRPP is already postioned for further reaction (215). The process, ammonia channeling in amidophosphoribosyltransferases, has been previously reviewed (216).

Although it is unusual for a phosphoribosyltransferase to have an additional enzyme activity such as the glutaminase, amidophosphoribosyltransferase has a typical type I phosphoribosyltransferase fold with the five-stranded β-sheets surrounded by two helices on either side, a hood, a catalytic flexible loop as well as a MgPRPP binding site consisting of the PRPP binding loop and a PP loop. The enzyme binds PRPP in a manner similar to that described above for T. gondii hypoxanthine-guanine phosphoribosyltransferase (Fig. 10B).

(b) Purine salvage synthesis. The purine salvage or auxiliary reactions consists of several enzymes that phosphoribosylate adenine, or the 6-oxopurines hypoxanthine, xanthine or guanine. The products of these phosphoribosylations reactions are AMP,
inosine 5′-monophosphate, xanthosine 5′-monophosphate, and GMP, respectively (Fig. 11, compound f to i). In general, in most organisms there is a single phosphoribosyltransferase that is specific for adenine, and at least one additional phosphoribosyltransferase with specificity toward 6-oxopurine. *E. coli* and *S. enterica* both contain *apt*-specified adenine phosphoribosyltransferase, *hpt*-specified hypoxanthine phosphoribosyltransferase, and *gpt*-specified xanthine-guanine phosphoribosyltransferase (19), whereas *S. solfataricus* contains *pgT-1*-specified adenine phosphoribosyltransferase and *pgT-2*-specified 6-oxopurine phosphoribosyltransferase with affinity for hypoxanthine, xanthine and guanine (217). The purine phosphoribosyltransferase-catalyzed reactions are responsible for the utilization of the purine compounds adenine, hypoxanthine, xanthine and guanine as purine source by purine auxotrophic strains.

The specificity of purine phosphoribosyltransferases are not readily deduced from their amino acid sequences. The PRPP binding loop of adenine phosphoribosyltransferase (EC 2.4.2.7) (adenine + PRPP → AMP + PPi) of *E. coli* is 126-Asp-Asp-Leu-Leu-Ala-Thr-131. The sequence Asp-Asp-hyd-hyd-Ala-Thr (hyd indicating hydrophobic residues) is found in a large majority of adenine phosphoribosyltransferases. An exception is *S. solfataricus* adenine phosphoribosyltransferase, which has 95-Asp-Asp-Ile-Thr-Asp-Thr-100. On the other hand, hypoxanthine and hypoxanthine-guanine phosphoribosyltransferases contain the homologous sequence Glu-Asp-Ile-Ile-Asp-Thr/Ser. That is, a glutamate residue replaces the first acidic residue of the Asp-Asp-dipeptide and the Asp typed in italic replaces an alanine residue of adenine phosphoribosyltransferase, exemplified by Ala130 of the *E. coli* adenine phosphoribosyltransferase. *E. coli gpt*-specified xanthine-guanine phosphoribosyltransferase rather has the sequence 88-Asp-Asp-Leu-
Val-Asp-Thr-93, i.e. an Asp-Asp dipeptide as in adenine phosphoribosyltransferase and Asp93 as in oxopurine phosphoribosyltransferase (217). The length of the adenine phosphoribosyltransferases shows some heterogeneity. The enzyme has a basal size of approximately 180 amino acid residues, as seen in the enzymes from *E. coli*, the purine de novo synthesis-lacking protozoan parasite *Giardia lamblia*, *S. cerevisiae* and humans, whereas the enzymes from the protozoan parasite *Leishmania donovani* and the archeon *S. solfataricus* have 237 and 210 mino acid residue, respectively. Nevertheless all of them share the common core structure of type I phosphoribosyltransferases (218-220). The additional amino acid residues of *S. solfataricus* and *L. donovani* adenine phosphoribosyltransferases protrude from both the N- and C-termini relative to the 180-amino acid adenine phosphoribosyltransferases and may contribute to structural variations of the hood (221, 222). The structures have been determined with the apo-forms or with adenine or AMP bound in the active site. Particularly interesting is the structure of the *G. lamblia* enzyme with the purine analog 9-deazaadenine and MgPRPP bound at the active site. In this structure the hydroxyls of the ribose moiety coordinate to Mg$^{2+}$ and to the 124-Glu-Asp-125 acidic dipeptide, whereas the 5-phosphate is bound to the 128-Ala-Thr-Gly-Gly-Thr-132 subset of the PRPP binding loop, and the diphosphate chain ligates to Mg$^{2+}$ and to Arg63, which is part of the PP loop. Superimposition of the 9-deazaadenine and MgPRPP-containing structure and an AMP-containing structure revealed a 2.1 Å movement of the anomeric carbon during catalysis, whereas the 5- or 5’-phosphate and adenine moieties essentially remained fixed (218). As already mentioned, the specificity of phosphoribosyltransferases with affinity for 6-oxopurine varies depending on the organism. Any given 6-oxopurine phosphoribosyltransferase has some affinity for all three 6-oxopurines (hypoxanthine,
xanthine and guanine), but in general, has a preference for one or two of the
compounds. The discrimination between 6-amino and 6-oxo purines is based on
hydrogen bonding to the nitrogen and oxygen of the purine ring (209, 213). Thus, the
allocation of a phosphoribosyltransferase to the EC 2.4.2.8 (hypoxanthine
phosphoribosyltransferase) class as opposed to the EC 2.4.2.22 (xanthine
phosphoribosyltransferase) class may be a somewhat superficial and misleading
description. The binding of PRPP to the 6-oxypurine phosphoribosyltransferases is
similar to that described above for adenine phosphoribosyltransferase; that is, in
involves a PRPP binding site consisting of a PRPP binding loop with a carboxylate
dipeptide, Asp-Asp or Glu-Asp, a PP loop and a Thr/Ser-Gly-Glu-Thr consensus
sequence involved in the binding of the 5-phosphate moiety.

(ii) Pyrimidine nucleotide biosynthesis. Unlike de novo purine nucleotide
biosynthesis, in de novo pyrimidine nucleotide biosynthesis the pyrimidine moiety is
first synthesized, and the product, orotate, and PRPP are assembled to form orotidine
5’-monophosphate (Fig. 11, compound b) in a reaction catalyzed by orotate
phosphoribosyltransferase (orotidine-5’-phosphate:diphosphate phospho-α-D-ribosyl-
transferase, EC 2.4.2.10, orotate + PRPP $\rightarrow$ orotidine 5’-monophosphate + PP$_i$).
Orotate phosphoribosyltransferase, a typical type I phosphoribosyltransferase,
contains the “usual” structural elements, a five-stranded β-sheet surrounded by a
number of helices, usually four, a hood structure involved in the binding of the
substrate orotate and a flexible catalytic loop, which closes the active site on catalysis.
The structures of orotate phosphoribosyltransferase from a variety of sources,
including S. enterica (223), E. coli (224), S. mutans (225), Plasmodium falciparum
(226) and L. donovani (227) have been published.
The three-dimensional structure of *S. enterica* orotate phosphoribosyltransferase in complex with orotate and MgPRPP revealed an asymmetric structure of the dimer, because both substrates bind to the active site of one subunit (B), whereas only orotate was bound to the subunit A. The flexible catalytic loop of subunit A closes the active site of subunit B, whose flexible catalytic loop remains unstructured and presumably open. Orotate is bound by amino acids of the hood, and PRPP is bound in the PRPP binding loop, which includes the 124-Asp-Asp-125 dipeptide (coordinating to hydroxyls of ribose C2 and C3), the sequence 128-Thr-Ala-Gly-Thr-131 (binding the 5-phosphate), Tyr72 and Lys73 (the PP loop). A single Mg\(^{2+}\) coordinates the oxygens of ribosyl C1, C2 and C3, an oxygen of the β-phosphate as well as two water molecules. On catalytic flexible loop closure, lysine, arginine and histidine residues of this loop bind to the diphosphate chain, and, together with the Mg\(^{2+}\), participate in neutralizing the diphosphate chain during catalysis (228). The structure reveals the presence of two shared active sites at the interface of the dimer, which has been also demonstrated by formation of a heterodimer composed of one subunit defective in PRPP binding and one subunit defective in the catalytic flexible loop (229).

The structure of *S. cerevisiae* orotate phosphoribosyltransferase resembles that of *S. enterica* (230). As mentioned, the dimeric structure is asymmetric. This asymmetry, together with the movement of the catalytic flexible loops during (i.e. before and after) catalysis, as well as kinetic analysis prompted the authors to suggest an alternating active site catalytic mechanism, i.e. only one active site is functional at any given time (230, 231).

Unlike the purine salvage reactions, pyrimidine salvage involves the activity of only a single phosphoribosyltransferase: uracil phosphoribosyltransferase (UMP:diphosphate phospho-α-D-ribosyltransferase, EC 2.4.2.9), uracil + PRPP →
UMP + PPi (Fig. 11, compound j). Uracil phosphoribosyltransferase allows organisms to utilize uracil formed endogenously by nucleic acid catabolism or supplied by the environment. Uracil phosphoribosyltransferase requires Mg$^{2+}$ for activity. In general, the activity of the enzyme is allosterically activated by GTP. GTP causes association of subunits to tetramers, as observed for uracil phosphoribosyltransferase from *E. coli* (232) and *T. gondii* (233). Alternatively, GTP functions as an allosteric activator of the oligomeric uracil phosphoribosyltransferase of *Sulfolobus* species (234-237). The enzyme from *M. tuberculosis* differs from the aforementioned in being unaffected by GTP (238, 239), and the enzyme from *G. intestinalis* dimerizes in the presence of GTP (240).

The PRPP binding motif of uracil phosphoribosyltransferase differs markedly from that of other phosphoribosyltransferases or PRPP synthase in that the typical acidic dipeptide (Asp-Asp or Glu-Asp) is replaced by Asp-Pro (highlighted in bold), as illustrated here with the *E. coli* uracil phosphoribosyltransferase amino acid sequence: 123-Glu-Arg-Met-Ala-Leu-Ile-Val-Asp-Pro-Met-Leu-Ala-Thr-Gly-Gly-Ser-138 (241). The Asp-Pro dipeptide appears to be universal among uracil phosphoribosyltransferase, as it is present in all the enzymes from the organisms mentioned above as well as yeasts (242), bacilli (243, 244), and lactococci (245).

Analysis of a mutant variant that had this particular proline residue replaced by an aspartate revealed a 100-fold increase in the $K_M$ value for uracil and a 50-fold reduction in $k_{cat}$. The $K_M$ value for PRPP was reduced in the mutant, whereas $k_{cat}/K_M$ was almost unchanged. It was therefore concluded that the proline residue of the PRPP binding loop of uracil phosphoribosyltransferase “is of little importance for binding of PRPP to the free enzyme, but is critical for binding of uracil to the enzyme-PRPP complex and for the catalytic rate” (246).
Two variant forms of uracil phosphoribosyltransferase are found in Gram-positive organisms such as *B. caldolyticus* and *B. subtilis*. Both genes were shown to complement *E. coli* *upp* lesions and therefore encode active enzymes. One of these (*upp*) specified uracil phosphoribosyltransferase, whereas the other (*pyrR*) specified a regulatory protein involved in regulation of pyrimidine gene expression, PyrR (247, 248). We shall return to PyrR in the section “PRPP as Mediator of Metabolic Regulation”.

Finally, a phosphoribosyltransferase activity for dioxotetrahydropyrimidine (dioxotetrahydropyrimidine phosphoribosyltransferase, EC 2.4.2.20) has been previously characterized (249). Whether it is a separate activity or a variant of other pyrimidine phosphoribosyltransferases remains unresolved.

(iii) **NAD biosynthesis.** The phosphoribosyltransferases of NAD biosynthesis are members of the so-called type II phosphoribosyltransferases. De novo pyridine nucleotide biosynthesis resembles de novo pyrimidine nucleotide biosynthesis in that the aromatic base is synthesized prior to phosphoribosylation (250). The reaction with PRPP and quinolinate is catalyzed by quinolinate phosphoribosyltransferase (β-nicotinate-β-D-ribonucleotide:diphosphate phospho-α-D-ribosyltransferase) encoded by *nadC* in *E. coli*: quinolinate + PRPP → 5′-phospho-β-D-ribosyl-β-nicotinate + PPi + CO2. 5′-Phosphoribosylnicotinate is also designated nicotinate mononucleotide (Fig. 11, compound c). The reaction is unusual in producing carbon dioxide (251, 252). In pyridine co-factor salvage synthesis, nicotinate phosphoribosyltransferase (5-phospho-α-D-ribose 1-diphosphate:nicotinate ligase (ADP, diphosphate-forming), EC 6.3.4.21), encoded by *pncB* in *E. coli*, catalyzes the reaction nicotinate + PRPP (+ ATP) → 5′-phospho-β-D-ribosyl-nicotinate + PPi (+ ADP + P1). This reaction is unusual in that the enzyme is
stimulated by ATP in a stoichiometric manner. That is, the enzyme is phosphorylated by ATP at His219, which results in the formation of a high-affinity form, with $K_M$ values for nicotinate and PRPP 200-fold lower than those of the unphosphorylated enzyme. Also, the coupling of ATP hydrolysis to the phosphoribosyltransferase reaction causes a change in the equilibrium value from 0.67 to 1100 (253-255). Mutational replacement of His219 completely abrogates phosphorylation and stimulation by ATP (256). Mammalian organisms such as mice and humans contain yet a different pyridine phosphoribosyltransferase, nicotinamide phosphoribosyltransferase (nicotinamide-D-ribonucleotide:diphosphate phosphor-α-D-ribosyltransferase, E.C. 2.4.2.12), nicotinamide + PRPP $\rightarrow$ 5’-phospho-β-D-ribosyl-nicotinamide (nicotinamide mononucleotide, NMN) + PPI (257, 258). As with purine and pyrimidine nucleotide biosynthesis, PRPP is used in both the de novo reactions of NAD synthesis (quinolinate phosphoribosyltransferase) and in NAD salvage reactions (nicotinate and nicotinamide phosphoribosyltransferases).

(a) Quinolinate phosphoribosyltransferase. The structures of quinolinate phosphoribosyltransferase from a number of sources have been determined. These include *M. tuberculosis* (259), *S. enterica* (211), *Helicobacter pylori* (260), *Thermotoga maritima* (261), and *S. cerevisiae* (262). The structures of these enzymes resemble one another. The structure of the ternary complex of *M. tuberculosis* quinolinate phosphoribosyltransferase with a quinolinate analog (phthalate) and a PRPP analog (5-phosphoribosyl-1-[β-methylene]diphosphate, i.e. the two phosphorus atoms are connected by a methylene moiety) reveals the binding of quinolinate and PRPP together with two divalent metal ions (in this case Mn$^{2+}$). In the ternary complex the ribosyl moiety of PRPP is hydrogen bonded to Glu201 and Asp222 through the C2 and C3 hydroxyls, respectively. The two divalent metal ions
coordinate to the same two hydroxyls, to the diphosphate chain or to water molecules; the ions do not coordinate to enzyme amino acid residues. The diphosphate chain forms hydrogen bonds to arginine, lysine and aspartate residues. The 5-phosphate is hydrogen bonded, to among other residues Gly270 and Gly249. Thus, although the fold of quinolinate phosphoribosyltransferase (a type II phosphoribosyltransferase) is very different from the fold of type I phosphoribosyltransferases, some similarities are evident. PRPP binding involves two acidic residues (Glu201 and Asp222) reminiscent of the Asp-Asp dipeptide of type I phosphoribosyltransferases and of PRPP synthase; the 5-phosphate binding involves glycine residues, and diphosphate binding involves several basic residues. Although different in structures, similar arrangements have evolved for the binding of the common substrate PRPP, which suggests a common theme in the design of active sites.

(b) Nicotinate phosphoribosyltransferase. The structure of nicotinate phosphoribosyltransferase of the archaeon Thermoplasma acidophilum revealed a three domain arrangement, where the structures of the N-terminal and central domains resemble the two domains of quinolinate phosphoribosyltransferases, and a unique C-terminal domain is added. Only a few differences were observed in the number of β-strands between nicotinate and quinolinate phosphoribosyltransferases. The phosphate group of PRPP binds to T. acidophilum nicotinate phosphoribosyltransferase in a mode similar to the binding of the PRPP analog to M. tuberculosis quinolinate phosphoribosyltransferase, and the structures of the active sites of the two enzymes are very similar in spite of little sequence conservation between them (263).

The C-terminal domain, approximately 100 amino acid residues in length, is composed of seven β-strands and one helix. Enzymological data for T. acidophilum nicotinate phosphoribosyltransferase are not available, so it remains unknown if the
activity of this enzyme is stimulated by ATP. It is possible that the C-terminal domain
participates in phosphorylation and ATP hydrolysis, and, by analogy, the
phosphorylation site may be present in the central domain, although a His219
homolog is not found in *T. acidophilum* nicotinate phosphoribosyltransferase. The C-
terminal domain of *S. enterica* nicotinate phosphoribosyltransferase is exposed to the
solvent; trypsin treatment removed 24 C-terminal amino acid residues and inactivated
the ATPase activity. This inactivation was prevented by ATP binding, which provides
evidence for the involvement of the C-terminal domain in energy coupling (264).

(c) Nicotinamide phosphoribosyltransferase. Vertebrates lack nicotinamide
deamidase and, thus, do not produce nicotinate from nicotinamide. Rather, they
salvage nicotinamide by means of nicotinamide phosphoribosyltransferase.

The sandwich and α/β-barrel structure described above for *M. tuberculosis*
quinoxylate phosphoribosyltransferase is repeated in human nicotinamide
phosphoribosyltransferase. The position of 5'- or 5'-phosphate maps identically in
structures with 5'-phospho β-D-ribosylnicotinamide or with PRPP bound, whereas the
ribose moieties are displaced relative to one another. The 5'-phosphate of PRPP is
hydrogen bound to Gly383 and Gly384 (both of the A subunit), which are part of the
loop 381-Gly-Ser-Gly-Gly-Gly-385 that is similar to the corresponding loop of *T.
acidophilum* nicotinate phosphoribosyltransferase (263). The diphosphate of PRPP is
hydrogen bonded to Arg39 (B subunit), Arg40 (B subunit), Arg196 (A subunit) and
Lys400 (B subunit), and the C2 and C3 hydroxyls are hydrogen bonded to Arg311
and Asp313 (both of the A subunit). Thus, the binding of PRPP to human
nicotinamide phosphoribosyltransferase is homologous to the binding of PRPP to the
other type II phosphoribosyltransferases (265).
As expected, nicotinamide phosphoribosyltransferase from mouse and rat have structures similar to that of the human enzyme (266, 267). There is extensive structural resemblance between the type II phosphoribosyltransferases, namely, quinolinate, nicotinate and nicotinamide phosphoribosyltransferases. Chappie and coworkers offer a theory for the evolution of the type II phosphoribosyltransferases, in which nicotinamide phosphoribosyltransferase evolved from nicotinate phosphoribosyltransferase, which in turn evolved from quinolinate phosphoribosyltransferase (268).

Studies of human nicotinamide phosphoribosyltransferase have attracted interest, because the enzyme has been identified as a growth factor for early stage B cells (hence called “pre-B cell colony-enhancing factor”) and has been associated with binding to and activation of the insulin receptor and has been also called “visfatin”. For a review of the many apparent functions of human nicotinamide phosphoribosyltransferase, consult the article by Garten and colleagues (269). It is unclear if the many functions may be ascribed simply to variations in the NAD pool. However, because of the increased synthesis of nicotinamide phosphoribosyltransferase in tumor tissues, studies have been directed toward the isolation of inhibitors of the enzyme. Several of these inhibitors could be converted enzymatically to phosphoribosylated derivatives. Analysis of the structure of a ribosylated inhibitor bound to nicotinamide phosphoribosyltransferase confirmed the binding of its 5-phosphoribosyl moiety (270).

(iv) Histidine biosynthesis. In histidine biosynthesis all of the five carbons of PRPP are retained. The carboxylate, the Cα and the Cβ of the amino propanoic side chain of histidine are generated from C5, C4 and C3, respectively of PRPP, whereas C4 and C5 of the imidazole moiety are derived from C2 and C1 of PRPP. In the first
step of histidine biosynthesis, PRPP and ATP react with the formation of PP\(_i\) and N-
(5’-phospho-β-D-ribosyl)-ATP, which has an N-glycosidic bond of C1 of the ribosyl
moiety to N1 of ATP (Fig. 11, compound d) (271). The reaction is catalyzed by ATP
phosphoribosyltransferase (ATP phosphoribosyltransferase, EC 2.4.2.17), which is
encoded by \textit{hisG} in \textit{E. coli} (272, 273).

There are two subfamilies of ATP phosphoribosyltransferases. They have
different quaternary structures, hexa- or octameric (274-276), but similar molecular
architecture of their catalytic domains. The \textit{E. coli} ATP phosphoribosyltransferase
polypeptide contains three domains. Domain I consists of four parallel and two
antiparallel β-strands flanked by two α-helices on each side; domain II is constructed
of four parallel and one antiparallel β-strand flanked by one helix on one side and two
helices on the other side. Domain III is a histidine binding domain (277). Domains I
and II constitute the catalytic fold of ATP phosphoribosyltransferase. Thus, although
this fold appears to be reminiscent of the type I phosphoribosyltransferase and PRPP
synthase domain fold, it represents a unique fold. Furthermore, ATP
phosphoribosyltransferases lack the hood and the catalytic flexible loop characteristic
of type I phosphoribosyltransferases. Additionally, the active form of both type of
enzymes are dimers or higher order oligomers.

The active form of \textit{E. coli} ATP phosphoribosyltransferase is a dimer of the three-
domain polypeptide. Activity is inhibited by AMP, which prevents the binding of
ATP and PRPP. The binding of the inhibitors histidine and eventually also guanosine
3’-diphosphate 5’-diphosphate result in the formation of a hexameric, inactive
quaternary structure (277-279). There is only 27% sequence identity between \textit{E. coli}
guanine-xanthine phosphoribosyltransferase and ATP phosphoribosyltransferase. A
PRPP binding loop located within domain II, however, is easily identified in the ATP
phosphoribosyltransferase sequence: 162-Gly-Leu-Ala-Asp-Ala-Ile-Cys-Asp-Leu-Val-Ser-Thr-Gly-Ala-Thr-176. The structure of ATP phosphoribosyltransferase from *M. tuberculosis* is similar to that of *E. coli*; in this case, the PRPP binding loop was identified as 147-Gly-Val-Ala-Asp-Ala-Ile-Ala-Asp-Ala-Gly-Arg-Thr-Leu-Ser-162 (280). ATP phosphoribosyltransferases such as those of *E. coli* and *M. tuberculosis* are sometimes designated HisG<sub>L</sub> with L designating “long” form. This is in contrast to the HisG<sub>S</sub> with S designating “short” form in for example *L. lactis*. HisG<sub>S</sub> ATP phosphoribosyltransferases lack domain III, and instead associate with a distinct polypeptide, HisZ, to form active octameric oligomers, whereas the isolated HisG and HisZ polypeptides associate into inactives dimers. The catalytic fold of HisG<sub>S</sub> ATP phosphoribosyltransferases is almost identical to that of HisG<sub>L</sub> ATP phosphoribosyltransferases, and the active site is located, once again, in a cleft between domain I and II. PRPP binding loops have been identified in ATP phosphoribosyltransferases of *L. lactis* (148-Gly-Leu-Ala-Asp-Ala-Ile-Val-Asp-Ile-Val-Glu-Thr-Gly-Asn-Thr-162) (281), and of the hyperthermophilic bacterium *T. maritima* (140-Gly-Leu-Ser-Asp-Leu-Ile-Val-Asp-Ile-Thr-Glu-Thr-Gly-Arg-Thr-155) (282). Mutations that replaced Thr<sub>159</sub> or Thr<sub>162</sub> of *L. lactis* ATP phosphoribosyltransferase confirmed the importance of this sequence in PRPP binding (283).

(v) **Tryptophan biosynthesis.** Contrary to the situation in histidine biosynthesis, only two carbons of PRPP are retained in tryptophan. C2 and C3 of the indole moiety of tryptophan originate from C1 and C2, respectively, of PRPP. The tryptophan biosynthetic intermediate anthranilate reacts with PRPP in a reaction catalyzed by anthranilate phosphoribosyltransferase (EC 2.4.2.18), encoded by *trpD*, which results in the formation of *N*-(5-phospho-β-D-riboyl)-anthranilate (Fig. 11, compound e) and
The anthranilate phosphoribosyltransferase isolated from *E. coli* is bifunctional: it also contains glutamine chorismate amidotransferase activity. Consequently, the *E. coli trpD* gene product is considerably larger, a total of 531 amino acid residues, than monofunctional anthranilate phosphoribosyltransferases, such as those from *S. solfataricus* or *M. tuberculosis*, which have 345 or 370 amino acid residues, respectively (284, 285). Other tryptophan biosynthetic enzymes from various species have been shown to harbor two activities. The overall three-dimensional structure of anthranilate phosphoribosyltransferase is similar among all of the species so far analyzed, i.e. the enterobacterium *Pectobacterium carotovorum* (286), *S. solfataricus* (284), *M. tuberculosis* (285), *Xanthomonas campestris* (PDB code 4hkm), the Gram-negative bacterium *Thermus thermophilus* (PDB code 1v8g) and a cyanobacterial species (*Nostoc sp.*) (PDB code 1vqu). The two-domain structure consists of a smaller N-terminal domain of six helices (the α-domain) and a larger C-terminal domain of six parallel β-sheets and one antiparallel β-sheet as well as eight or more helices (the α/β-domain). Although the five-stranded parallel β-sheet characteristic of type I phosphoribosyltransferases is recognizable, anthranilate phosphoribosyltransferase contains additional structural features, and, thus constitutes a separate fold sometimes designated as type IV phosphoribosyltransferase (287). The enzyme is active as a homodimer; the α-domain is responsible for the dimerization. Binding of PRPP and anthranilate is mediated in a cleft of the α/β-domain. The binding of PRPP is promoted by two Mg\(^{2+}\) or Mn\(^{2+}\), which accounts for a requirement of Mg\(^{2+}\) for activity (288). The binding of PRPP is well mapped in the structures of *S. solfataricus* and *M. tuberculosis* anthranilate phosphoribosyltransferase. The amino acid residues involved are the sequence 79-Gly-Thr-Gly-Gly-Asp-83, Lys106 and 223-Asp-Glu-224 of the *S. solfataricus*...
enzyme (107-Gly-Thr-Gly-Gly-Asp-111, Lys136 and 251-Asp-Glu-252, respectively, of the *M. tuberculosis* enzyme). Remarkably, in spite of this sequence conservation, the binding of PRPP in the two enzymes is very different. In common, one divalent cation (MG1) coordinates to the α- and β-phosphates of PRPP. Another divalent cation (MG2) coordinates to the two acidic residues (Asp-Glu). The Gly-Thr-Gly-Gly-Asp “wraps around” PRPP with the amino terminal glycine binding to the diphosphate chain and the carboxy terminal aspartate or glycine binding to the 5-phosphate. Specifically, Gly79 forms hydrogen bond to the β-phosphate in the *S. solfataricus* enzyme. Lys106 of the latter enzyme binds to the β-phosphate, whereas Lys135 of the *M. tuberculosis* enzyme binds to the α-phosphate. Thus, this sequence may be compared to the PPi loop of type I phosphoribosyltransferases and of PRPP synthases. Among the differences in PRPP binding between the two enzymes are the binding properties of Asp-Glu, which bind to MG2 and to the oxygen of C5 (Asp residue only) in *S. solfataricus* anthranilate phosphoribosyltransferase. MG2 furthermore coordinates to the 5-phosphate and to the oxygens of C4 and C5 of ribose 5-phosphate (289). The corresponding aspartate and glutamate residues of the *M. tuberculosis* enzyme are coordinated to MG1 (Glu252) or to MG2 (Asp251). The PRPP-divalent cation-coordinations of the latter enzyme are directed exclusively toward the diphosphate chain (285). The conserved glycine-rich sequence 79-Gly-Thr-Gly-Gly-Asp-83 resembles the subsequence Asp-Asp-Xxx-Xxx-Thr-Gly-Gly-Thr of several phosphoribosyltransferases and the glycine-containing subsequence of the PRPP binding loop of PRPP synthase (228-Thr-Ala-Gly-Thr-232, 234-Ser-Gly-Gly-Thr-237 or 217-Thr-Gly-Gly-Thr-220 of *B. subtilis*, *S. solfataricus* or *M. jannaschii* PRPP synthase, respectively). These sequences of phosphoribosyltransferases and
PRPP synthases are involved in the binding of the 5-phosphate moiety of PRPP or ribose 5-phosphate.

Each subunit of anthranilate phosphoribosyltransferase binds two anthranilate molecules in addition to one PRPP molecule and two Mg$^{2+}$. Three sites for anthranilate are responsible for tunnel movement of anthranilate with a concomitant 180° flipping of the compound toward MgPRPP, which at this point is presumably already present. However, anthranilate may bind to the outer end of the tunnel before MgPRPP is bound, and binding of the latter compound may participate in tunnel formation (290). Interestingly, in *S. solfataricus* anthranilate phosphoribosyltransferase Gly79 also forms a hydrogen bond to the anthranilate nitrogen. Thus, this residue may be particularly important in positioning anthranilate and PRPP for inline attack of anthranilate on C1 of PRPP (289).

Tryptophan biosynthesis in *M. tuberculosis* has attracted much attention, as trp strains of this organism are avirulent (291). Since the organism’s host, humans, are unable to synthesize tryptophan a search for inhibitors of tryptophan biosynthetic enzymes, including anthranilate phosphoribosyltransferase has been conducted in hopes of identifying an antimicrobial agent (287, 290, 292).

**(vi) Phosphoribosyl transfer without participation of PRPP.** For the sake of completeness, the *cobT* encoded nicotinate nucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase (EC 2.4.2.21) of *S. enterica* and *E. coli* should be mentioned at this point. Altough this enzyme is a phosphoribosyltransferase, it does not utilize PRPP as a substrate. Rather, the 5-phosphoribosyl moiety is detached from the nicotinamide mononucleotide and added to the 5,6-dimethylbenzimidazole, and, thus, one N-glycoside bond is replaced by a second N-glycoside bond (293, 294). A variant of this enzyme (EC 2.4.2.55) has been characterized from the bacterium
Sporomusa ovata. In contrast to nicotinate nucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase, the S. ovata enzyme (EC 2.4.2.55) also utilizes phenol derivatives as acceptors in the transfer of the 5-phosphoribosyl moiety (295).

C-Glycosidic bond formation.

(i) Tetrahydromethanopterin biosynthesis. Tetrahydromethanopterin is one of a number of co-factors involved in the methanogenesis and sulfate-reduction in a variety of archaeal species (296, 297), and methylotrophic bacteria (298, 299). The pathway of tetrahydromethanopterin biosynthesis has been deduced from the structures of a series of metabolites meticulously solved by White and colleagues (4, 300-303). The function of methanopterin is analogous to that of dihydrofolate, i.e. it is a carrier of one-carbon molecules; formyl, methylene and methyl groups. Both methanopterin and folic acid contain a substituted pteridyl moiety as well as an aryl component apparently derived from 4-aminobenzoate. However, methanopterin contains both deoxyribulosyl and phosphoribosyl moieties that are not present in folate. Both of these pentose moieties are derived from PRPP. The two PRPP-utilizing biochemical reactions of methanopterin biosynthesis are shown in Fig. 12A. Initially, and regarded as the first committed step in tetrahydromethanopterin biosynthesis, 4-hydroxybenzoate condenses with PRPP with the formation of 5′-phospho-β-D-ribosyl 4-hydroxybenzene as well as PPi and carbon dioxide (Fig. 12A, reaction 1). The product 5′-phospho-β-D-ribosyl 4-hydroxybenzene contains a carbon-carbon bond between the ribosyl and aryl moieties (Fig. 12A, compound \( m \)). The enzyme catalyzing this reaction has affinity for both 4-hydroxy- and 4-aminobenzoate (304, 305). In vitro and in vivo analyses used 4-aminobenzoate to elucidate the metabolic pathway. The enzyme has been designated 4-(β-D-ribofuranosyl)aminobenzene-5′-phosphate synthase, and, in M. jannaschii, is encoded by the MJ1427 gene. Although
4-hydroxybenzoate rather than 4-aminobenzoate appears to be the origin of the aryl moiety of methanopterin, at least in *M. jannaschii* (304), we shall use the designation aminobenzoate phosphoribosyltransferase for this enzyme, because most studies have used 4-aminobenzoate as the substrate. Indeed, the product of the aminobenzoate phosphoribosyltransferase-catalyzed reaction with 4-hydroxybenzoate as the substrate, 5’-phospho-β-D-riboyl 4-hydroxybenzene, is transformed in a subsequent enzymic step to the amino-derivative 5’-phospho-β-D-riboyl 4-aminobenzene (Fig. 12A, compound n).

Aminobenzoate phosphoribosyltransferase of the methanogenic archaea *Methanosarcina thermophila*, *M. jannaschii* and *Methanothermobacter thermautotrophicus* as well as the hyperthermophilic, non-methanogenic (sulfate-reducing) archaeon *A. fulgidus* have been purified and studied. Edman degradation of aminobenzoate phosphoribosyltransferase isolated from cell extracts of *M. thermophila* strain TM-1 was used to establish the sequence of the 20 N-terminal amino acid residues, which were then used to identify aminobenzoate phosphoribosyltransferase-encoding genes in a variety of organisms (299). The molecular mass of the native enzyme is 63.5 kDa, that of the subunit is 32.6 kDa, i.e. the enzyme is a homodimer. PP, and 5’-phospho-β-D-riboyl 4-aminobenzene have been demonstrated as products of the reaction. The reaction requires Mg$^{2+}$ or Mn$^{2+}$, the latter ion being the most efficient. The optimal pH is 4.8, the optimal temperature 50 °C. Some kinetic constants were determined; an apparent $K_M$ for 4-aminobenzoate was 58 μM and an apparent $K_M^{PP}$ was 3.6 mM. Pyridoxal phosphate is suggested as involved in the reaction, but data are inconclusive in this respect, and it is suggested that pyridoxal phosphate may be involved in the reaction in a way that does not require the carbonyl of pyridoxal phosphate (301, 305).
The *M. jannaschii* aminobenzoate phosphoribosyltransferase, encoded by the gene MJ1427, resembles that of *M. thermophila* (306). Product inhibition studies established the reaction as an ordered Bi-Ter mechanism with binding of PRPP followed by 4-aminobenzoate, and release of CO₂, then 4-(β-D-ribofuranosyl)aminobenzene-5'-phosphate and finally PPᵢ. As with the enzyme from *M. thermophila*, the affinity for PRPP is low ($K_{M}^{PRPP}$ 1.5 mM), whereas that for 4-aminobenzoate was lower ($K_{M}^{4\text{-aminobenzoate}}$ 0.15 mM). A $k_{cat}$ value was determined as 0.23 s⁻¹. Phosphoribosyltransferase catalyzed reactions are thought to proceed with the intermediate formation of a ribooxocarbenium ion as well as a negatively charged nucleophile. Aminobenzoate phosphoribosyltransferase poses a special problem for this generalization. How does the enzyme stabilize negative charge at C1 of the nucleophile when the substrate already carries a negatively charged carboxyl group (4-aminobenzoate or 4-hydrozybenzoate)? No prosthetic group, neither pyridoxal 5-phosphate nor a pyruvoyl moiety, appear to be present, indicating that enzyme side-chains only are involved in the catalytic process (306).

In *A. fulgidus* the gene AF2089 encodes aminobenzoate phosphoribosyltransferase. Following cloning and expression of the gene in *E. coli*, the enzyme was partly purified. It has a temperature optimum at 70 °C, and maximal activity at pH 5.3. Gel filtration revealed a molecular mass of 57 kDa, corresponding to a homodimer, because the molecular mass of the subunit was established as 35.5 kDa (307). Finally, a procedure for the purification of aminobenzoate phosphoribosyltransferase from *M. thermautotrophicus* has been published (307). Some archaeal species appear to contain two aminobenzoate phosphoribosyltransferase-encoding genes. These are *Pyrococcus abyssi*, where the aminobenzoate phosphoribosyltransferases (sequence designations PAB0141 and
PAB1694) show 30% amino acid sequence identity, *Pyrococcus horikoshii* (sequence designations PH0227 and PH1228) whose enzymes show 29% amino sequence identity, and *Aeropyrum pernix* (sequence designations APE2425 and APE1512) whose enzymes show 28% amino acid sequence identity (299). None of the aminobenzoate phosphoribosyltransferases contains a classical PRPP binding site, as is the case for type II phosphoribosyltransferases. As mentioned, the binding of PRPP to type II phosphoribosyltransferases occurs by interaction of a few amino acid residues to the phosphate moieties of PRPP. Amino acid sequence comparison of aminobenzoate phosphoribosyltransferases with *M. tuberculosis* or *T. acidophilum* nicotinate and quinolinate phosphoribosyltransferases did not reveal conserved amino acid residues between these phosphoribosyltransferases.

**O-Glycosidic bond formation.**

(i) Tetrahydromethanopterin biosynthesis. Another reaction of archaeal methanopterin biosynthesis utilizes PRPP as a substrate; interestingly, this reaction results in the formation of an O-glycosidic bond (Fig. 12A, reaction 2). The phosphoribosyl acceptor substrate of this reaction is \(N'\text{-(7,8-dihydropterin-6-yl)methyl}\)-4-(1-deoxy-D-ribulosyl)aminobenzene (Fig. 12A, compound o), and the product is 1-(4-{\(N'\text{-(7,8-dihydropterin-6-yl)methyl}\)amino}phenyl)-5-(5-phospho-\(\alpha\text{-D-}\)ribulosyl)-1-deoxyribitol (Fig. 12A, compound p), i.e. C1 of the phosphoribosyl moiety is attached to a hydroxyl group of the ribulosyl moiety of compound o, with inversion of the anomeric carbon of the phosphoribosyl moiety, as usual (301). The enzyme catalyzing this reaction has not been identified. One additional reaction is necessary to convert compound p to the final cofactor as shown in Fig. 12A and is described in the legend.
(ii) **Arabinosyl monophosphodecaprenol biosynthesis.** Arabinogalactan is an important constituent of the cell wall of mycobacteria such as *M. tuberculosis* and *M. smegmatis*. Arabinogalactan contains D-arabinosyl and D-galactosyl moieties. Polymerization of the arabinosyl moieties is catalyzed by a number of arabinosyltransferases, for which the donor is a lipid-linked arabinosyl donor, β-D-arabinosyl monophosphodecaprenol [reviewed previously (5, 308)]. Tracer methodology applied to membrane preparation of *M. smegmatis* to study the origin of the arabinosyl derivatives revealed that ribulose 5-phosphate was a precursor, but that ribulose 5-phosphate was not converted directly to arabinose 5-phosphate (309). Rather, the pentosyl phosphate donor was shown to be PRPP. Thus, following incubation of *M. smegmatis* membrane preparations with [14C]PRPP, the production of [14C]ribosyl phosphopolyprenol, [14C]arabinosyl phosphopolyprenol, 5-phospho-[14C]ribosyl phosphopolyprenol, and 5-phospho-[14C]arabinosyl phosphopolyprenol was demonstrated, which showed the involvement of PRPP in the synthesis of arabinose derivatives for cell wall formation in this organism, Fig. 12B (310). Subsequently, an *M. tuberculosis* gene (*Rv3806c*) encoding an enzyme that phosphoribosylates decaprenyl phosphate was identified, cloned and expressed in *E. coli*. The enzyme is an integral membrane protein; membrane fractions of an *E. coli* strain hosting *Rv3806c* were able to convert PRPP and decaprenyl phosphate into 5-phospho-β-D-ribosyl 1-phosphoryldecaprenol (311). The enzyme is designated 5-phospho-α-D-ribosyl-1-diphosphate:decaprenyl-phosphate 5-phosphoribosyltransferase. In analogy with other phosphoribosyltransferases, we shall use the name decaprenyl phosphate phosphoribosyltransferase. Inspection of the amino acid sequence of decaprenyl phosphate phosphoribosyltransferase does not reveal the classical PRPP binding site that is
characteristic of PRPP synthases and type I phosphoribosyltransferases. As mentioned, decaprenyl phosphate phosphoribosyltransferase is a membrane bound enzyme, which is consistent with both a membrane soluble substrate (decaprenyl phosphate) and a soluble substrate (PRPP). A topology with nine transmembrane domains was suggested for the structure of *M. tuberculosis* decaprenyl phosphate phosphoribosyltransferase by molecular modeling studies. According to this model, the active site may involve the cytoplasmic loop II. From a search of amino acid sequence alignments with other phosphoribosyltransferases, a sequence, 73-Asn-Asp-x-x-Asp-77 (where x indicates any amino acid), was suggested as important for PRPP binding and enzymatic function (312). Indeed, the *M. tuberculosis* decaprenyl phosphate phosphoribosyltransferase amino acid sequence 69-Val-Tyr-Leu-Val-Asn-Asp-Val-Arg-Asp-77 resembles a large part of the PRPP binding loop of *E. coli* hypoxanthine-guanine phosphoribosyltransferase, 95-Val-Leu-Ile-Val-Glu-Asp-Ile-Ile-Asp-103, as well as the corresponding amino acid sequence of other phosphoribosyltransferases (213, 313). These amino acids are located within the cytoplasmic loop II and the junction of this loop and the transmembrane 2-domain.

Analysis of the decaprenyl phosphate phosphoribosyltransferase replacement mutants Asn73Gln and Asp77Glu revealed significantly increased $K_M$ values for PRPP, providing evidence that this sequence is involved in PRPP binding (312).

(iii) Aminoglycoside antibiotic biosynthesis. A third reaction that forms an O-glycosidic bond with PRPP as the phosphoribosyl donor occurs in the biosynthesis of the aminoglycoside antibiotic butirosin. The 26-ORF *btr* gene cluster specifies the biosynthetic pathway for butirosin in *Bacillus circulans* (314, 315). The *btrL* cistron encodes neamine phosphoribosyltransferase (neamine:5-phospho-$\alpha$-D-ribose 1-diphosphate phosphoribosyltransferase, EC 2.4.2.49), which phosphoribosylates
neamine with the formation of 5''-phosphoribostamycin, Fig. 12C (316). The *B. circulans* neamine phosphoribosyltransferase contains a well-conserved PRPP binding site 212-Asp-Ile-Val-Leu-Glu-Asp-Gln-Pro-His-Thr-Gly-Gly-Thr-225, which may be compared to the *E. coli* hypoxanthine phosphoribosyltransferase sequence, 94-Asp-Val-Leu-Ile-Val-Glu-Asp-Ile-Ile-Asp-Ser-Gly-Asn-Thr-107. Neamine phosphoribosyltransferase contains 604 amino acid residues and is much larger than other type I phosphoribosyltransferases (316), such as *E. coli* hypoxanthine and guanine-xanthine phosphoribosyltransferases, which contain 178 and 152 amino acids, respectively. It is also larger than type II phosphoribosyltransferases such as *E. coli* quinolinate and nicotinate phosphoribosyltransferases, which contain 297 and 400 amino acids, respectively. It is possible that the neamine phosphoribosyltransferase is a bifunctional enzyme, although a second activity has not been discovered. This putative second activity is not the phosphatase that hydrolyzes the phosphate ester of 5''-phosphoribostamycin to ribostamycin, as this activity is encoded by the *btrP* cistron (316).

In addition to butirosin, a number of other aminoglycoside antibiotics contain a pseudo-disaccharide resembling that of butirosin as well as a ribosyl moiety. Examples of these antibiotics are neomycin B, paromomycin, and lividomycin B. The neomycin B biosynthesis-specifying gene cluster of *Streptomyces fradiae* contains the *neoL* reading frame (accession CAF33322), whose deduced amino acid sequence shows only 22% amino acid sequence identity with that of neamine phosphoribosyltransferase. However, certain stretches of amino acids along the sequences appears highly conserved, among these the PRPP binding site (317). Similarly, a putative neamine phosphoribosyltransferase may be present in *Streptomyces lividus*, because an ORF (accession CAG38706) of the lividomycin B
biosynthetic cluster of *S. lividus* is 29% identical to the *B. circulans* neamine phosphoribosyltransferase amino acid sequence. Details on the genetics of aminoglycoside antibiotic biosynthesis have been previously reviewed (318).

Reactions at the Diphosphoryl Moiety of PRPP

A few enzymes have been shown to hydrolyse the phosphoric anhydride bond of the diphosphoryl moiety of PRPP: PRPP + H₂O → ribosyl 1,5-bisphosphate + Pᵢ. A number of archaeal species, such as *M. jannaschii*, *Methanosarcina acetivorans*, *A. fulgidus*, and *T. kodakaraensis* contain a form III ribulose 1,5-bisphosphate carboxylase/oxygenase, which catalyzes the carboxylation of ribulose 1,5-bisphosphate. Usually ribulose 1,5-bisphosphate is formed by ATP-dependent phosphorylation of ribulose 5-phosphate. However, in *M. jannaschii* and *M. acetivorans* ribulose 1,5-bisphosphate originates from PRPP, and it was suggested that PRPP was hydrolyzed to ribose 5-phosphate followed by NAD⁺-dependent oxidation to ribulose 1,5-bisphosphate. Alternatively, PRPP may be transformed to ribosyl 1,2-cyclic phosphate and then to ribulose 1,5-bisphosphate. The physiologic function of this pathway remains to be established (319). In addition, certain diphosphoryl (Nudix) hydrolases are able to hydrolyze PRPP with the formation of ribosyl 1,5-bisphosphate and Pᵢ (320). Finally, a PRPP pyrophosphatase activity has been identified in macrophages exposed to hypoxia (321).

Co-Factor Biosynthesis

**Thiamine diphosphate.** Thiamine diphosphate synthesis utilizes 5’-phosphoribosyl 5-aminoimidazole as a precursor. This compound is also a precursor of purine nucleotide biosynthesis. 5’-Phosphoribosyl 5-aminoimidazole is converted to a
pyrimidine moiety by thiC-encoded phosphomethylpyrimidine synthase. As such, some of the carbons of PRPP end up in the pyrimidine moiety of thiamine diphosphate. Thus, C2 of PRPP becomes the methyl group of the pyrimidine moiety, C4 of PRPP becomes C5 of the pyrimidine moiety, whereas C5 of PRPP becomes the methylene group that connects the pyrimidine and thiazole moieties of thiamine diphosphate. C1 and C3 of PRPP are lost as formate and carbon dioxide (322). Flavins. The ribitol moiety of the flavin-containing co-factors FAD and FMN are derived from the ribosyl moiety of GTP, and, thus, originally from PRPP (323, 324). Pterins. The pterin moiety of folate derivatives are synthesized from GTP. In tetrahydrofolate, the C7, C6 and the methylene attached to C6 of the pterin moiety are derived from C1, C2 and C3 of PRPP. C4 and C5 of PRPP are lost as glyoxylate. The synthesis of pterin and methanopterin follow identical pathways at this stage (325, 326). Also, the molybdopterin is synthesized from GTP with similar utilization of carbons of PRPP (327).

Carbon-Phosphorus Lyase Pathway

As mentioned previously, PRPP can be synthesized in a reaction catalyzed by phosphoribosyl bisphosphate phosphokinase. This reaction is part of the carbon-phosphorus lyase pathway, by which phosphonates are catabolized by means of a radical-mediated process. This catabolic pathway is specified by the 14-cistron operon phnCDEFGHIJKLMNOP found in numerous microorganisms including E. coli (154). Phosphoribosyl bisphosphate phosphokinase is specified by the phnN cistron and catalyzes the reaction ribosyl 1,5-bisphosphate + ATP → PRPP + AMP, which is the final chemical reaction specified by the phnC-D operon (151, 328). Thus, catabolism of phosphonates by the carbon-phosphorus lyase pathway produces PRPP, in which
the phosphorus of phosphonate is represented by the α-phosphate at the C1 position.

The utilization of phosphonate-derived phosphorus requires one or more of the above described phosphoribosyltransferases, which produces PPi, which can by hydrolyzed to P_i by diphosphatase (154, 329).

PRPP AS MEDIATOR OF METABOLIC REGULATION

Until now this review has presented enzymes that produce PRPP and enzymes that consume PRPP with the formation of N-, O- or C-glycosidic bonds. The following section is dedicated to the regulatory functions played by PRPP. These functions include binding to the PyrR regulator, which is involved in translational control of pyrimidine biosynthesis gene expression; binding to the PRPP responsive PurR regulator of Gram-positive organisms with low GC content; and allosteric activation by PRPP of carbamoylphosphate synthetase.

The Enigma of PRPP as a Regulator of Metabolic Activity

Bacterial enzymes that utilize PRPP generally have $K_M$ values at least one order of magnitude lower than the normal intracellular PRPP concentration. Thus, the PRPP concentration is unlikely to be a rate-determining factor for phosphoribosyltransferases. No regulatory function, such as allosteric inhibition, of phosphoribosyltransferase activity by PRPP at a metabolic level under normal growth conditions has been identified experimentally. Traditionally, PRPP pool sizes are expressed as millimol per gram dry weight; this unit is dependent on the cell composition and is not directly translatable into intracellular molar concentration for comparison between different species. However, it appears safe to state that the PRPP concentration of bacterial cells is in the millimolar range (330, 331). Bacteria regulate
their PRPP pools in response to exogenous purine sources. Thus, the PRPP pool of B. subtilis, L. lactis, E. coli or S. enterica is reduced two to four-fold by the addition of adenine or hypoxanthine (Table 5). This reduction in PRPP pool sizes may be caused by allosteric and isosteric regulation of PRPP synthase by purine nucleotides, i.e. ADP and GDP, as described above.

The situation is different in mammals, where the PRPP concentration is rate-limiting for purine biosynthesis de novo and for salvage reactions (332), which suggests that the rates of PRPP-utilizing enzymes may be determined by substrate saturation kinetics. The PRPP concentration of mammalian cells is much lower than that of bacteria, 5-30 µM, although with variation throughout the cell cycle (332, 333).

We are therefore faced with the enigma of explaining how the high intracellular PRPP levels of microbial organisms are sensed by regulator proteins, which presumably should be saturated with PRPP under most physiological conditions. The enigma is only partly resolved, and further research will be needed to fill in the missing information. Details of the mechanism of PRPP-mediated regulation of pyrimidine and purine nucleotide synthesis by the PyrR and PurR regulatory proteins are described in the next section.

Regulation of Pyrimidine Metabolism by the RNA Binding PyrR Protein

Pyrimidine biosynthetic genes are present in most organisms, and their expression is subject to control by different mechanisms. Regulation of bacterial pyrimidine metabolism has been extensively reviewed previously (334-336). Here we shall concentrate on a description of the structure of PyrR and the involvement of PRPP in
Three-dimensional structure of PyrR. The three-dimensional structure of PyrR from three microbial sources have been published: *B. subtilis* (337), *B. caldolyticus* (338) and *M. tuberculosis* (339). The three-dimensional structure of *T. thermophilus* PyrR has also been solved (PDB code 1ufr). The overall structure of the four PyrR proteins is identical, and contains the typical type I phosphoribosyltransferase fold, a five-stranded β-sheet surrounded by three helices, and the typical sequence elements are easily identified: a PRPP binding site, i.e. a PRPP binding loop (100-Val-Ile-Leu-Val-Asp-Asp-Val-Leu-Phe-Thr-Gly-Arg-Thr-112 of *B. caldolyticus* PyrR), a PP loop (37-Gly-Ile-Lys-Thr-Arg-41 of *B. caldolyticus* PyrR), a flexible loop (Thr71 to Asn83 of *B. caldolyticus* PyrR), and a hood structural element. The binding of UMP and GMP to *B. caldolyticus* PyrR involves identical amino acid residues, Asp104, Asp105, Thr109 and Arg137. The 104-Asp-Asp-105 dipeptide of the PRPP binding loop binds to the nucleotide via Mg$^{2+}$. Altogether, the binding of PRPP and nucleotides to PyrR is similar to that of type I phosphoribosyltransferases. PyrR from *B. caldolyticus* has been proposed to exist as a dimer or tetramer (338, 340, 341), although *B. subtilis* PyrR has been shown to exist in a hexameric state (342). The dimerization pattern of PyrR is different from that of type I phosphoribosyltransferases and the RNA binding activity of PyrR has been proposed to be due to the exposure of a region of basic amino acid residues in the dimer. Effectors of the quaternary structure are PRPP, uridine ribonucleotides, GTP and pyr leader mRNA. A close resemblance of the crystal structure of GMP-bound *B. caldolyticus* PyrR to an evolutionarily distant hypoxanthine-guanine phosphoribosyltransferase suggests that the two proteins share ancestry (338).
However, PyrR from both *B. subtilis* and *B. caldolyticus* have a low uracil phosphoribosyltransferase activity that can support slow growth of a pyrimidine requiring strain on uracil (248, 343).

**Mechanism of PRPP-mediated regulation by PyrR.** The *B. subtilis pyr* gene cluster encodes all of the enzymes required for the biosynthesis of UMP, a uracil transporter, and the regulatory protein PyrR (343). Transcription is controlled by pyrimidine availability through an attenuation mechanism. There are three terminator-antiterminator structures in *pyr* mRNA. PyrR binds to a conserved region in the *pyr* operon leader resulting in disruption of an antiterminator that is the dominant RNA structure in the absence of PyrR binding. The negative effect of PyrR is observed only when pyrimidine precursors are available in the growth medium (342-344).

The active RNA binding form of PyrR appears to be a dimer (Fig. 13A), which is capable of recognizing and binding a specific RNA structure present in the leader of mRNA transcripts of PyrR regulated genes (341). Dimer-formation and RNA binding occurs when UMP, UTP, or PRPP is bound to PyrR, resulting in transcriptional termination. GTP counteracts the binding of UTP (and possibly UMP), resulting in multimer formation and presumably transcriptional read-through because the RNA binding sites are sequestered in the tetramer. UMP, UTP, and GTP bind to PyrR at physiological concentrations, while PRPP binding has an apparent dissociation constant at sub-physiological concentrations, as discussed above (341).

The physiological relevance of PRPP binding to *L. lactis* PyrR was demonstrated by transcriptomic analysis of nucleotide metabolism. *L. lactis pyr* gene expression is regulated by PyrR-dependent attenuation with a mechanism similar to that of *B. subtilis* (345-347). The mRNA levels under PyrR attenuation control showed an inverse correlation with the intracellular PRPP concentrations (348), in accordance
with an increased RNA binding and attenuation upon PRPP binding to PyrR. It is reasonable to suspect that PyrR from *L. lactis* also binds RNA as a dimer, and that dimers are stabilized in relation to tetramers by binding of PRPP. An increase in PRPP concentration is a consequence of purine shortage and hence a diminished demand for pyrimidine synthesis; thus, the inverse correlation of PRPP and *pyr* gene expression serves a valuable physiological function.

Proteins with homology to PyrR are found among many bacterial species. With few exceptions, PyrR is found among firmicutes, cyanobacteria and actinobacteria. Apparently, PyrR is absent from the α-proteobacteria, but it is present in some orders of the other classes of proteobacteria. PyrR is absent from enterobacteriales, but present in the Pseudomonadales order. *B. subtilis* has two different enzymatic activities that catalyze the formation of UMP from uracil and PRPP, PyrR and uracil phosphoribosyltransferase encoded by *upp* (248). PyrR and uracil phosphoribosyltransferase share 23% identity, which suggests that PyrR, aside from its close ancestry to the hypoxanthine-guanine phosphoribosyltransferases, also shares ancestry with uracil phosphoribosyltransferases.

**Regulation of Purine Metabolism by PRPP-Responsive, DNA Binding PurR Proteins**

PRPP responsive PurR transcriptional regulators are found only in Low-GC Gram-positive bacteria, but the members of this small protein family differ widely in their mechanism of regulation. The *B. subtilis* PurR, hereafter designated PurR<sub>Bs</sub>, is a negative regulator, whereas the *L. lactis* PurR, hereafter designated PurR<sub>Ll</sub>, is an activator of purine metabolism gene expression. In contrast to the PRPP-responsive PurR regulators from Gram-positive bacteria, a LacI-type PurR repressor protein is
present in *E. coli* and a variety of other bacterial species. The LacI-type PurR proteins do not respond to PRPP, but repress the transcription of purine genes when bound to the co-repressors hypoxanthine or guanine (349, 350). In the following sections we shall describe in more detail the structure and function of *B. subtilis* and *L. lactis* PurR.

**Three-dimensional structure of *B. subtilis* PurR.** *B. subtilis* PurR contains 285 amino acid residues and has a homodimeric quaternary structure (351). A C-terminal domain of the subunit (residues 77 to 285) has the characteristic fold of type I phosphoribosyltransferases and PRPP synthases described previously, a central parallel β sheet flanked by α helices. Hood and flexible loop structural elements are also present. The C-terminal domain of PurR<sub>Bs</sub> also contains a PRPP binding site, i.e. a PRPP binding loop (196-Gly-Ser-Asn-Val-Leu-Ile-Ile-Asp-Asp-Phe-Met-Lys-Ala-Gly-Gly-Thr-211) and a PP loop (139-Thr-Lys-Gly-Ile-142) (352). Gel mobility shift analysis showed that binding of PRPP to PurR<sub>Bs</sub> changes its affinity towards its DNA binding sites (351, 353). X-ray structural analysis of PurR<sub>Bs</sub> crystals containing the PRPP analog cPRPP revealed that the compound binds to the PRPP and PP loops analogous to PRPP binding in type 1 phosphoribosyltransferases. Contacting amino acid residues were Lys 140 and Asp203, Asp204 of the PP and PRPP binding loop, respectively, as well as residues in the flexible loop (354). Curiously, the binding of cPRPP occurred without the participation of Mg<sup>2+</sup>, which may suggest relatively weak binding of cPRPP. DNA binding of two mutant variants of PurR<sub> Bs</sub>, Asp203Ala and Asp204Ala, could not be inhibited by PRPP in vitro, and the alterations caused a super-repression phenotype of pur gene expression consistent with poor binding of PRPP to the altered PurR<sub> Bs</sub> proteins (353).
Altogether, binding of PRPP to PurR₈₅ strongly resembles the binding of PRPP to type I phosphoribosyltransferases.

In spite of the high sequence similarity to type I phosphoribosyltransferases, PurR₈₅ is inactive as a phosphoribosyltransferase and does not even bind purine bases. This deficiency is explained by the interactions of the large side chains of well-conserved Tyr102 and Phe205, which prevent the binding of a nucleophile in proper position relative to C1 of PRPP. The presence of these bulky amino acid side chains also explains the inability of compounds containing nucleobases to inhibit PurR₈₅-PurBox interactions (352). Interestingly, xanthine phosphoribosyltransferases contain phenylalanine in the analogous position to Phe205, which indicates that the Phe205-Tyr102 interaction is crucial to blocking access of purines to the active site of PurR.

The N-terminal domain (residues 1 to 74) of PurR₈₅ forms a winged-helix domain, a subdivision of the DNA binding helix-turn-helix domains (352), and is attached to the C-terminal domain by a three-residue linker. This domain is responsible for the binding of PurR₈₅ to so-called PurBox sites located upstream of the PurR regulated operons (354). Functionally, PurR₈₅ may be regarded as a crippled phosphoribosyltransferase, which has lost the catalytic activity but retained the PRPP binding capability and has gained an N-terminal DNA binding domain.

**Mechanism of PRPP-mediated regulation of purine biosynthesis in B. subtilis.** The addition of adenine to cultures of B. subtilis causes repression of transcription of a large number of operons including genes for purine nucleotide de novo biosynthetic enzymes, genes specifying purine transport proteins, genes for enzymes in purine nucleotide interconversion and certain co-factor biosynthetic genes (351, 355, 356). Similar to the situation in enteric organisms (146), the addition of adenine to cultures of B. subtilis causes depletion of the PRPP pool (357). Induction
of expression of PurR regulated genes by PRPP binding has been evaluated by PurR\textsubscript{Bs}-DNA binding assays in vitro (351). PurR\textsubscript{Bs} binds to a region 20 to 150 bp upstream of the \textit{B. subtilis} pur operon (358). This DNA region contains the operator region: \textasciitilde81 AACACGAACATTA (PurBox1)-16 nucleotides-TATCGTTCGATAAT\textasciitilde38 (PurBox2), numbered relative to the transcription start site. PurBox1 and PurBox2 have similarity to the consensus PurBox (AWWWCCGAACWWT) although PurBox2 is inverted (355, 359). Functional dissection has defined a minimal control region and revealed the importance of the two PurBoxes in the overall regulatory process. Based on the affinity of PurR\textsubscript{Bs} for the PurBoxes, PurBox1 was designated “strong”, and PurBox2 was designated “weak”. High-affinity binding of PurR to the control region was shown to require at least one strong PurBox, whereas the induction of PurR\textsubscript{Bs} repression by PRPP requires at least one weak PurBox. The binding of PurR\textsubscript{Bs} to the operator is cooperative with a stoichiometry of two PurR\textsubscript{Bs} dimers binding to the operator, which suggests that one PurR\textsubscript{Bs} dimer binds to PurBox1 and another PurR\textsubscript{Bs} dimer to PurBox2 with wrapping of the DNA around the two PurR\textsubscript{Bs} dimers (Fig. 13B) (354).

In \textit{B. subtilis} a guanine-responsive attenuation mechanism is superimposed on the repression of transcription initiation by the PurR regulatory system, which results in a complex regulatory pattern. Thus, the addition of guanine to cultures of \textit{B. subtilis} causes premature transcription termination in the mRNA leader region of \textit{purE} (the upstream cistron of the major 12-cistron \textit{pur} operon) (360). This premature transcription termination is caused by the purine riboswitch mechanism, which regulates the \textit{pur, xpt, pbuG} and \textit{yxjA} operons (361). This riboswitch mechanism involves the binding of guanine or hypoxanthine to the untranslated leader sequences.
but does not involve PRPP. Details of the mechanisms of riboswitches have been previously reviewed (362, 363).

**Mechanism of PRPP-mediated regulation of purine biosynthesis in *L. lactis***.

Overall the effect of purine addition has the same effect on *L. lactis* as that on *B. subtilis*: the PRPP level drops and the expression of genes of the PurR regulon is lowered (364). However, a different mechanism of action became apparent when the *L. lactis purR* gene was identified among purine auxotrophic strains obtained after transposon mutagenesis. The *purR* insertion mutation caused a reduced transcription of *purD*, which was complemented by *purR*+. These and other data showed that *L. lactis purR* encodes an activator of purine gene expression. *PurR*<sub>Ll</sub> contains 271 amino acid residues, close to the length of *PurR*<sub>Bs</sub> (285 amino acids). Remarkably, *PurR*<sub>Ll</sub> and *PurR*<sub>Bs</sub> are 48% identical (69% similar), and yet their mechanisms are very different. Like *PurR*<sub> Bs</sub>, *PurR*<sub>Ll</sub> contains a helix-turn-helix domain at the N-terminal end. It also contains the typical type I phosphoribosyltransferase structural elements, a PRPP binding site, i.e. a PRPP binding loop (194-Gly-Gln-Asn-Val-Leu-Ile-Val-Asp-Asp-Phe-Met-Lys-Gly-Ala-Gly-Thr-209), a PP loop (137-Thr-Lys-Gly-Ile-140), and a flexible loop (359).

*PurR*<sub>Ll</sub> recognizes PurBox sequences similar to the consensus PurBox sequence AWWWCCAACWWT (365). *PurR*<sub>Ll</sub> binds to PurBoxes under both activating (high PRPP) and non-activating conditions. Activation of transcription requires that the PurBox sequence is located at an exact position relative to the -10 promoter region, presumably in order to bind and position RNA polymerase correctly for open complex formation (359) (Fig. 13C). The standard -35 promoter elements of constitutive promoters are usually absent from PurR activated promoters. In *L. lactis* different topologies of PurBoxes have been identified in which the binding sites are located in...
tandem or head-to-head, which suggests that PurR may form different multimeric
conformations (364).

In vivo kinetics of the PRPP-modulated activation of transcription by PurR<sub>Ll</sub> was
studied by a combination of temporal mRNA and intracellular PRPP quantifications
during a metabolic downshift (348). Saturation curves for the coupled process of
PRPP binding to PurR<sub>Ll</sub> and subsequent activation of mRNA synthesis were obtained
for all genes belonging to the PurR regulon in <i>L. lactis</i>. Interestingly, most saturation
curves appeared sigmoid, suggesting mechanisms employing cooperativity, but more
classical Michaelis-Menten curve forms were apparent for some PurR-activated genes
involved in purine salvage and folate metabolism (348). To our knowledge, this work
represents the first example of a kinetic analysis of the action of a regulatory protein
performed in a living organism.

**Evolution of PurR.** PurR proteins from various organisms share extensive
sequence similarity, but the differences in regulatory mechanism between PurR<sub> Bs</sub> and
PurR<sub>Ll</sub> show that they have diverged considerably during evolution. Fig. 14 shows the
phylogenetic relationship among members of the PurR family. The branching pattern
of the PurR phylogeny resemble the branching pattern for the whole genome bacterial
phylogeny (366), indicating that the PurR protein was present during most of the
evolution of the low-GC branch of Gram-positive bacteria. The <i>Bacillus</i> branch and
the <i>Streptococcus</i> branch share a common ancestor, which is not shared by any other
species. It is apparent that the bacilli and streptococci use the <i>B. subtilis</i> type PurBox,
while all the others use the <i>L. lactis</i> type PurBox (Fig. 14A), except the clostridia, for
which no information is available. According to the unrooted phylogenetic tree, it is
likely that the activating PurR<sub>Ll</sub> type evolved first and that the PurR<sub>Bs</sub> type diverged
early in the evolution of the Low-GC Gram-positive bacteria. As a consequence, the
L. lactis PurBox must simultaneously have evolved into the B. subtilis PurBox. When the sequences in the logo plots for the two PurBox types are compared (Fig. 14B), it is easily recognized that the B. subtilis PurBox consists of two L. lactis type PurBox sequences located as inverted repeats with a spacing of 16 nucleotides, as previously described (355).

To further analyze the relationship of PurR with enzymes containing a type I phosphoribosyltransferase fold, a phylogenetic analysis was performed for homologues of B. subtilis class 1 phosphoribosyltransferases with all the major bacterial lineages, spanning an evolution of at least three billion years. Included in the analysis are also logo-plots of conserved amino acids in the PP loop and the PRPP binding loop (Fig. 15). It is apparent that PurR and xanthine phosphoribosyltransferase are close relatives (367), and that PyrR and hypoxanthine-guanine phosphoribosyltransferases are also closely related (338), and that they share ancestry with uracil phosphoribosyltransferases (337). As noted in Fig. 15, PurR and xanthine phosphoribosyltransferase homologues are only found in the low-GC-Gram-positive bacterial lineage. PurR and adenine phosphoribosyltransferase has previously been inferred to be structurally related, based upon the common structure of the flexible loop which forms a two-stranded antiparallel β-ribbon structure (210). A similar structure is also present in the flexible loop of B. subtilis xanthine phosphoribosyltransferase (367). Thus PurR, xanthine phosphoribosyltransferase and adenine phosphoribosyltransferase are all structurally related.

If PurR arose by evolution from a xanthine phosphoribosyltransferase, perhaps it could have exploited some of the peculiarities of this enzyme. Of special interest is the fact that the active form of xanthine phosphoribosyltransferase is a dimer, and that binding of PRPP is necessary for stabilization of the dimeric conformation (367).
Most other type I phosphoribosyltransferases have active tetrameric rather than dimeric structures. Dimer formation could be important for PRPP modulation of PurR$_{Ll}$ as it is for PurR$_{Bs}$, perhaps by exposing interaction sites needed for RNA polymerase capture.

**In search of a common mechanism for PRPP modulation of PurR**

PurR is a family of proteins that share extensive similarity, but which are divided into at least two phylogenetic branches with different PurR functionalities, a *B. subtilis*-like branch and an *L. lactis*-like branch. Members of both branches appear to have rigid DNA binding domains with hydrophobic cores that are not readily modulated by PRPP binding, and both appear to harbor extensive positively charged domains that may bind the DNA backbone and result in protection of large DNA stretches. Yet regulators from one family represented by PurR$_{Ll}$ appear to bind to single PurBox motifs with unknown stoichiometry. Presumably the binding of PRPP and closure of the flexible loop expose a binding site that is used to recruit RNA polymerase. Regulators from the second family represented by PurR$_{Bs}$, recognize two PurBoxes in a stoichiometry of one PurR dimer per PurBox and in a conformation that involves formation of PurR tetramers (Fig. 13B and C). Occupancy of the PRPP binding site reduces unspecific DNA binding and prevents PurR repression of transcription initiation. It is likely that the closing of the flexible loop upon PRPP binding, which is also shared by all type I phosphoribosyltransferases, is the mechanistic basis for both effects. There is currently no evidence that all PurR regulators in one family share a common mechanism, and it is not clear whether PurR$_{Ll}$ and PurR$_{Bs}$ may substitute one another. However, *B. subtilis*-like tandem-PurBoxes are found only in the *Bacillus* and *Staphylococcus* branches and single PurBoxes are found in the *Lactococcus, Streptococcus* and *Lactobacillus* branches. A
search for evolutionary “cross-over” organisms with PurR proteins that possess both repression and activation ability may be very useful in this respect.

**Allosteric Activation of Carbamoylphosphate Synthetase Activity by PRPP**

Carbamoyl phosphate is a precursor for the synthesis of pyrimidine nucleotides and the amino acid arginine. While bacterial species like *E. coli* and *S. enterica* contain only a single carbamoyl phosphate synthetase, other bacterial species like *B. subtilis* contain specific enzymes for each pathway, pyrimidine nucleotides and arginine. The activity of pyrimidine nucleotide-specific enzyme was found to be stimulated by PRPP (368, 369).

In animals and fungi two or three of the six enzymes of de novo pyrimidine nucleotide biosynthesis are grouped in di- or trifunctional enzymes. Animals contain the trifunctional fusion of carbamoylphosphate synthase, aspartate transcarbamoylase and dihydroororase activities, called CAD. CAD, thus, catalyzes the ATP-dependent conversion of glutamine, bicarbonate and aspartate to dihydroorotate. CAD function and structure have been previously reviewed (370, 371). Dihydroorotate in turn is oxidized to orotate, which is then phosphoribosylated to orotidine 5’-monophosphate catalyzed by orotate phosphoribosyltransferase. In the present context, the important activity of CAD is carbamoyl phosphate synthase. The activity of this enzyme is regulated by UTP and PRPP. UTP is a feedback inhibitor, whereas PRPP is an allosteric activator of the enzyme, as found for the *B. subtilis* pyrimidine specific carbamoyl phosphate synthetase (369, 372). CAD activity is modulated further by protein phosphorylation mediated by the MAP kinase (373), cAMP dependent protein kinase (373), and mTORC1 (374). The expression of the *cad* genes are also subject to
regulation. Upon transition from G1 to S phase, the human \textit{cad} mRNA level increased approximately 10-fold, which is mediated by the global regulator c-MYC (374, 375).

\textbf{prs GENES, MUTANTS, AND GENE REGULATION}

PRPP synthase is believed to be present in all free living organisms. A number of \textit{prs} mutants with alterations in the enzyme have been isolated and characterized. In addition, a few knockout mutant alleles have been constructed in vitro, and, following recombination, gene conversions have yielded null-alleles. Also mutant variants of PRPP synthase have been characterized in a wide variety of organisms, including humans. We review the properties of these mutant variants of bacterial origin in this section.

\textit{E. coli} And \textit{S. enterica}

\textit{E. coli}, \textit{prs-1} and \textit{prs-2}. A number of \textit{prs} mutants have been isolated in \textit{E. coli}. First, the \textit{prs-1} allele appeared in a genetic selection designed to isolate mutants with improved utilization of guanosine as purine source (28). Briefly, five mutants were characterized; all of them had a lesion in the \textit{gsk} gene as well as a lesion in a gene specifying genes of an early part of the purine nucleotide biosynthesis de novo pathway, i.e. mutants with the genotype \textit{purF gsk} and \textit{purM gsk}. The appearance of the \textit{prs-1 gsk} mutant strain may be explained along this line by extending the purine nucleotide biosynthesis de novo pathway to include \textit{prs} in addition to \textit{purF, purD, purL} etc. The \textit{prs-1} allele specifies Asp128Ala. This residue is only two residues away from His130, equivalent to His135 of \textit{B. subtilis} PRPP synthase. As described before, His135 is important for the coordination of the MG2 site, i.e. maintaining the \textit{\alpha,\beta,\gamma}-tridentate complex of MgATP. Consistent with this, PRPP synthase specified by
*prs-l* had a 27-fold increase in the $K_M$ for ATP, whereas the $K_M$ for ribose 5-phosphate was essentially unaltered (376).

A temperature-labile PRPP synthase, specified by the *prs-2* allele, was isolated by localized mutagenesis of the *hemA* region (2). The *prs-2* allele contained two mutations, one responsible for a Gly8Ser alteration, the other, a cytidylate to thymidylate transition that results in an alteration close to the Shine-Dalgarno sequence. The latter mutation resulted in increased synthesis of PRPP synthase, and, thus, in part compensated for a poorly-functioning enzyme. The Gly8Ser alteration was responsible for the temperature-labile PRPP synthase (377).

**In vitro generated knockout *prs* alleles.** A *prs* knockout mutation would render the strain prototrophic for purine, pyrimidine and pyridine nucleotides as well as histidine and tryptophan. Any scheme to isolate such a strain must included the following considerations. NAD, or its metabolite nicotinamide mononucleotide, may be utilized intact without catabolism to satisfy the pyridine nucleotide requirement (378). The requirement of PRPP for tryptophan and histidine synthesis can be eliminated by the addition of these two amino acids to the growth medium. In contrast, PRPP is normally essential for both de novo and salvage purine and pyrimidine nucleotide reactions. PRPP consumption in de novo synthesis of purine and pyrimidine nucleotides is mediated by *purF*-specified amidophosphoribosyltransferase and *pyrE*-specified orotate phosphoribosyltransferase, whereas the salvage reactions of purine and pyrimidine nucleotides are mediated by adenine, hypoxanthine, xanthine, guanine and uracil phosphoribosyltransferases. Some organisms, such as *E. coli* and *S. enterica* also contain nucleoside kinases, which phosphorylate nucleosides such as inosine, guanosine and uridine to inosine 5'-monophosphate, GMP and UMP, respectively.
Uptake of these nucleosides via these kinase reactions would eliminate the requirement for PRPP. However, nucleosides are predominantly phosphorolyzed by nucleoside phosphorylases such as purine nucleoside phosphorylase I (deoD) with specificity for adenosine, inosine and guanosine, purine nucleoside phosphorylase II (xapA) with specificity for inosine, xanthosine and guanosine, and uridine phosphorylase (udp) with specificity for uridine (19). Thus, knockout of deoD and udp is necessary to permit the salvage of nucleosides by phosphorylation of guanosine by guanosine kinase (gsk) and uridine by uridine kinase (udk). With respect to guanosine utilization, yet another genetic lesion was necessary to permit isolation of a Δprs strain. This lesion, for example gsk-3, improved the activity of guanosine kinase by rendering the enzyme insensitive to feedback inhibition by GTP and presumably GDP (379, 380).

Altogether, a background strain deoD udp gsk-3 was used for the successful recombination of in vitro generated, plasmid-borne Δprs allele into the chromosome of E. coli. (The growth medium included guanosine, uridine, histidine, tryptohan and NAD.) Two alleles Δprs-3::Kan' and Δprs-4::Kan' were recombined into the chromosome (3). Also the temperature sensitivity of the prs-2 allele could be rescued in the deoD udp gsk-3 genetic background (2).

dnaR. PRPP synthase has been shown to be involved in DNA replication, but it is unclear whether the effect is direct or indirect. A genetic lesion of E. coli, dnaR130, was shown to be an allele of the prs gene, hereafter designated prs-130. A strain harboring prs-130 was temperature sensitive for initiation of DNA replication, whereas elongation was unaffected. Only replication initiating at chromosome-borne chromosomal origin of replication, oriC, was affected by prs-130. Thus, replication of an oriC-harboring plasmid, stable DNA synthesis, i.e. replication independent of RNase HI (specified by rnhA), and replication of bacteriophage λ were all unaffected.
by prs-130. The fact that bacteriophage λ DNA replication was unaffected by
temperature and that identical bacteriophage λ burst sizes of prs-130 and prs+ strains
was observed, prompted the author to suggest that the temperature-sensitive DNA
replication of the prs-130 strain was not caused by lack of precursors for nucleic acids
or proteins. Also the decline in PRPP synthase activity specified by prs-130 paralleled
the decline in DNA replication upon temperature shift. The prs-130 lesion is located
within a 274 bp HindIII-AvaII DNA fragment of the prs coding sequence. The entire
prs-130 allele has been sequenced, but the sequence cannot be found in the available
data bases (381, 382).

A number of mutant alleles of genes other than prs were able to suppress the
temperature sensitivity caused by the prs-130 allele. These extragenic suppressors of
prs-130 include dnaA (383), certain rpoB alleles specifying rifampicin resistant
variants of the β-subunit of RNA polymerase (384), rpe specifying D-ribulose 5-
phosphate 3-epimerase. Suppression of prs-130 by mutations in the dnaA gene
prompted the author to suggest a direct physical interaction of DnaA and PRPP
synthase, whereas the appearance of prs-130 suppressors in RNA polymerase
suggested an involvement of PRPP synthase in a transcriptional event in DNA
replication at oriC. Remarkably, D-ribulose 5-phosphate 3-epimerase (specified by the
rpe gene) also suppressed the temperature sensitive phenotype of the prs-130 strain
(385). Ribulose phosphate epimerase catalyzes the interconversion of ribulose 5-
phosphate and xylulose 5-phosphate and the enzyme is part of the non-oxidative
pentose phosphate pathway (386), which involves ribose 5-phosphate, the precursor
of PRPP. This fact leads us to ask whether a knock-out of rpe results in an increase of
ribose 5-phosphate isomerase activity? This activity catalyzes the interconversion of
ribose 5-phosphate, which might lead to improved PRPP synthesis. Altogether, these various observations demonstrate an involvement of PRPP synthase in some aspects of DNA synthesis in *E. coli*, but the detailed biochemistry of this effect remains unclear.

**ssrA.** The *ssrA* gene specifies the SsrA-RNA, which together with the SmpB protein is involved in rescuing stalled ribosomes. SsrA-RNA functions both as a tRNA and as an mRNA molecule, SsrA-RNA function results in the synthesis of an Ssr-polypeptide fusion that is destined for degradation and the liberation of the stalled ribosome (387, 388). Two temperature-sensitive mutants were isolated in a Δ*ssrA* genetic background. Both mutations mapped within the *prs* gene, one of which specified Cys215Tyr. The temperature-sensitive phenotype was suppressed by reinsertion of a wild-type *ssrA* gene (389). Similar mutants were isolated in the *thyA* gene (390), and, thus the restoration of activity of SsrA is not restricted to misexpression of the *prs* gene (388).

However, using SmpB as bait in pull-down assays, SsrA-RNA and a number of polypeptides were copurified. One of these polypeptides was PRPP synthase. Half maximal binding of PRPP synthase to SsrA-RNA was obtained at 1-2 μM PRPP synthase; this is compared to half maximal binding of ribosomal protein S1 to SsrA-RNA at 0.030 μM, which indicates relatively poor binding of PRPP synthase to SsrA-RNA. The binding of PRPP synthase was therefore relatively unspecific, so that physiologically significant binding of PRPP synthase to an SsrA-RNA particle, if it occurs at all, may require additional protein factors (391).

**S. enterica, prs-100.** The *prs-100* allele, previously designated *prsB* (392), was isolated by combined screening of ethyl methanesulfonate-mutagenized cells for...
temperature-sensitive growth and lack of PRPP synthase activity (392). The prs-100
lesion causes an Arg78Cys alteration, and is located within a stretch of amino acid
residues that are involved in the formation of the bent dimer (Table 3 amino acid
residues Leu76 and Ile77). This supports the conclusion of the authors “that the
mutation alters the enzyme’s kinetic properties through substantial structural
alterations rather than by specific perturbation of substrate binding or catalysis” (27).

**Thiamine biosynthesis.** A genetic screen for mutants of *S. enterica* with
improved biosynthesis of thiamine was conducted in a strain with *purF* and *gnd*
lesions, i.e. a strain lacking amidophosphoribosyltransferase and glucose 6-phosphate
dehydrogenase activities. Normally, amidophosphoribosyltransferase (specified by
*purF*) is essential for thiamine diphosphate synthesis, but six thiamine-prototrophic
mutants lacking amidophosphoribosyltransferase arose at a low frequency
(approximately 4 \times 10^{-8}). All of the mutants had decreased activity of PRPP synthase,
which presumably resulted in an increase in the ribose 5-phosphate pool, and, thus, a
stimulation of the amidophosphoribosyltransferase-independent, spontaneous (i.e.
nonenzymic) amination of ribose 5-phosphate to 5-phosphoribosyl 1-amine, and
subsequent thiamine diphosphate synthesis. One mutation mapped within the Shine-
Dalgarno sequence whereas the remaining five mapped within the *prs* coding region.
The in vitro PRPP synthase activity of these latter mutants was 20-60 % of the wild-
type activity. Interestingly, one PRPP synthase variant, Asp224Ala, i.e. an alteration
within the PRPP binding loop, retained approximately 30% of the wild-type PRPP
synthase activity. As pointed out by the authors, the selection procedure yielded only
mutants with residual activity of PRPP synthase. Because the mutations were acquired
by positive selection (growth in the absence of thiamine) and all of the mutations
negatively affected the activity of PRPP synthase, this procedure could be valuable in
a screen for mutations that test the importance of various amino acid residues in PRPP synthase activity (393).

**prs Gene Organization and Regulation of prs Gene Expression**

*E. coli and S. enterica.* The *prs* gene is a member of the purine regulon, because *prs* gene expression is under the control of the *purR* gene product (a LacI-type). The Pur box sequence of the *prs* gene is identical and located at identical positions in *E. coli* and *S. enterica*: -56AAGAAAACGTTTTCGC−41 (with the first cytidylate residue of the transcript numbered as +1). Four nucleotides separate the Pur box and the -35 region. PurR caused a three-fold repression of *prs* gene expression in vivo in experiments that measured expression of *prs-lacZ* operon fusions with or without the presence of *purR*. Furthermore, electrophoretic mobility shift analysis of PurR binding to DNA containing the *prs* Pur box demonstrated that PurR bound to the DNA. Finally, protection of the *prs* Pur box by PurR was demonstrated by DNase I footprinting (394). The synthesis of PRPP synthase is derepressed approximately 10 fold during pyrimidine starvation (395), and uridine derivatives specifically cause repression of PRPP synthase synthesis (396).

The genetic organization of the *prs* region in *E. coli* and *S. enterica* is identical. The *prs* gene is located downstream of the *lolB* gene (encoding outer membrane lipoprotein required for localization of lipoproteins) (397), and *ispE* (encoding 4-diphosphocytidyl 2-C-methylerythritol kinase) (398). The *prs* gene is transcribed from two promoters. A larger transcript is initiated two genes upstream of *prs* and comprises *lolB-ispE-prs*. A smaller transcript initiates within the *ispE* coding sequence and contains the last 152 nucleotides of the *ispE* coding sequence in both organisms. The transcription start site of the smaller transcript and a rho-independent
termination site downstream of the *prs* coding sequence have been mapped (45, 399). The smaller transcript is 20-fold more abundant than the larger transcript. Pyrimidine-mediated *prs* gene regulation occurs by increase of the amount of the smaller transcript (399). The coding region of the *prs* gene is preceded by a leader sequence 302 nucleotides long in *E. coli*, 417 nucleotides long in *S. enterica* (45, 399, 400). Comparison of the two leader sequences reveals 149 almost identical nucleotides of the 5'-end of the transcripts of the two organisms, followed by 115 nucleotides in the *S. enterica* *prs* leader, which are not present in the *E. coli* *prs* leader, and 152 nucleotides, which are almost identical in the leaders of the two transcripts, i.e. an a-b-c arrangement in *S. enterica* and an a-c arrangement in *E. coli*.

Altogether, the expression of the *prs* gene of *E. coli* and *S. enterica* is controlled by both purine and pyrimidine compounds. Purine-mediated regulation occurs by means of the PurR repressor binding to the Pur box located within the upstream *ispE* gene. Several lines of experimental results provide evidence for the involvement of the *prs* leader in regulation of the synthesis of PRPP synthase. First, two *prs-galK* operon fusions were analyzed for production of galactokinase activity. The first gene fusion contained the *prs* promoter as well as 24 of the 5'-nucleotides of the *prs* transcript, the second contained the *prs* promoter, the entire *prs* leader and 13 nucleotides of the *prs* coding sequence. Cells containing gene fusion one contained five times as much galactokinase activity as cells containing gene fusion two. Thus, nucleotides of the *prs* leader contain structure(s) that reduce the production of galactokinase of the gene fusion, and, presumably of PRPP synthase under native conditions (401). In another study a mutant variant of RNA polymerase (*rpoBC*) of *S. enterica* that had been selected as resistant to pyrimidine fluoroanalogues was constitutive in expression of the *pyrE* gene specifying orotate phosphoribosyltransferase. The
activity of orotate phosphoribosyltransferase was elevated 40-fold, but the activity of PRPP synthase was also elevated more than two-fold (402). The defective RNA polymerase was shown to increase the coupling of transcription and translation at the pyrE attenuator (403). It is possible therefore that the increase in prs gene expression is also caused by increased coupling of transcription and translation within the prs leader. In other experiments, whole transcriptome shotgun sequencing has revealed a short transcript originating from the *E. coli* prs promotor. Although the ends of this transcript have not been mapped, the analysis provides additional evidence for the existence of a prematurely terminated prs transcript (M. A. Sørensen, University of Copenhagen, personal communication). Indeed, a number of potential leaders may be formed from the prs leader, and quite a number of secondary structures, including hairpins and loops, are predicted from the nucleotide sequence. One possible secondary structure of the *S. enterica* prs leader is shown in Fig. 16. Unlike the pyrE attenuator, which is followed by eight uridylate residues and has been shown to be regulated by premature transcription termination in the leader sequence (404), the putative *E. coli* prs leader stem loops are followed by none or a few uridylate residues. This may explain the difference in fold of pyrimidine-mediated regulation of the two operons: 40-fold in pyrE gene expression, 2.3-fold in prs gene expression.

**Gram-positive organisms.** Genetic mapping and nucleotide sequencing showed that the prs gene of *B. subtilis* is located between the gcaD (tms-26) and ctc genes (43, 405). The former gene encodes the bifunctional enzyme α-D-glucosamine 1-phosphate acetyltransferase, *N*-acetylglucosamine 1-phosphate uridylyltransferase (406), whereas the latter gene encodes a general stress protein similar to *E. coli* ribosomal protein L25 (407). In vegetative cells of *B. subtilis* gcaD, prs, and ctc are expressed as a tricistronic operon from a promoter immediately upstream of gcaD (408). Similar
arrangements of *gcaD-prs-cts* are found in other Gram-positive organisms, Clostridiaceae, Corynebacteriaceae, Listeriaceae, Staphylococcaceae species. On the other hand, Streptococcaceae such as *Streptococcus pyogenes, Enterococcus faecalis* and *L. lactis* have other more heterogeneous genetic organizations of *prs* genes. Streptococcaceae generally contain two *prs* genes, as described previously for *L. lactis*. In contrast to the situation in enterobacteria, the regulation of *prs* gene expression in Gram-positive organisms is essentially unknown.

**FUTURE PERSPECTIVES**

Although extensive efforts have been spent on elucidation of the various aspects of PRPP metabolism, a number of important aspects remain to be disclosed. Perhaps most important is the function or functions of the NHR found in many eukaryotic PRPP synthases, and the functions of the PAPs in human and rat PRPP synthases. For example purification of PRPP synthase from crude extracts of wild-type *S. cerevisiae* should be possible and would elucidate the quaternary structure of the native enzyme or enzymes. Additionally, the fact that *S. cerevisiae* PRPP synthase is a multimeric enzyme consisting of different subunits, such as Prs1 and Prs3 and possibly higher order structures, makes possible studies of mutants with alteration of various amino acid residues in one subunit, while the other subunit(s) retain(s) their wild-type phenotype. Candidates for such an analysis are, with amino acid numbers referring to *B. subtilis* PRPP synthase, His135, Asp174 and Lys197 residues. Similar experiments may be possible with mammalian PRPP synthase(s). As mentioned previously, there is compelling evidence for the involvement of yeast PRPP synthase-polypeptides in a variety of cellular processes other than amino acid and nucleotide synthesis, and there is some evidence that NHRs play roles in this involvement. PAPs may also be
involved in cellular processes other than nucleotide synthesis, and studies in mammalian cell lines might reveal roles of the PAPs in such other physiological processes and cross-regulation. Studies of mouse cell lines (and live animals) with the PAPs knocked out, individually and together, could be of very great interest in this respect. As described above, a specific role for human PRPP synthase isozyme 2 in cancer cells has been documented (125). Although PAPs were not implicated, they could very well participate in mediating the effects.

Another aspect of interest is the three-dimensional structure of class II PRPP synthases. The available data on their quaternary structure is conflicting. On one hand, circular dichroism and gel filtration analyses predict the structure of sugarcane PRPP synthase to be hexameric similar to that of *B. subtilis* PRPP synthase (129), whereas recombinant spinach PRPP synthase isozymes 3 and 4 eluted as trimers in gel filtration chromatography (47).

Furthermore, the three-dimensional structure of PRPP-utilizing enzymes other than those of purine, pyrimidine, pyridine, histidine and tryptophan biosynthesis, i.e. enzymes catalyzing the formation of O- and C-glycosidic bonds is worth solving as they may contribute additional information regarding the versatility of PRPP in intermediary metabolism.

Regulation of PRPP synthase activity in vivo is presently poorly understood. In vitro, the activity is intricately regulated by the energy charge with ATP being a substrate, AMP a product, and ADP a potent negative, allosteric and isosteric (i.e. competitive at the ATP site) regulator of PRPP synthase activity. Understanding how these properties contribute to physiological in vivo regulation requires further investigation.
ACKNOWLEDGEMENTS

K.R.A. is funded by The Danish Diabetes Academy, which is supported by the Novo Nordisk Foundation, a Sapere Aude grant from the Danish Council for Independent Research, and a grant from the Lundbeck Foundation.
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superactivity and recurrent infections is caused by a p.Val142Leu mutation in


Bjarne Hove-Jensen began graduate studies at the Enzyme Division, Institute of Biological Chemistry B, University of Copenhagen in 1976, where he worked on various aspects of purine nucleotide salvage metabolism in *E. coli*. He serendipitously discovered a mutant with an altered phosphoribosyl diphosphate (PRPP) synthase. After receiving his Ph.D. degree in Biochemistry in 1983 at the University of Copenhagen he continued studies of nucleotide metabolism in various organisms with emphasis on the physiological role of PRPP synthase. He became Associate Professor of Biochemistry in 1988 at the University of Copenhagen. Fortuitously, around 2000 he discovered that PRPP is also an intermediate in the catabolic pathway by which phosphonic acid-phosphorus is assimilated by the carbon phosphorus-lyase pathway. Important parts of his research project were conducted on sabbaticals with Switzer at University of Illinois at Urbana-Champaign, at Queen’s University, Kingston, Ontario and at Aarhus University. He is now Associate Professor Emeritus at Aarhus University and continues studies of the physiological role of PRPP.

Kasper R. Andersen received his Ph.D. degree in structural biology in 2009 at Aarhus University. He worked with Nobel Laureate V. Ramakrishnan and his laborarory at the Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK in an effort to explore the mechanism of mRNA cleavage by the bacterial toxin RelE. From the crystal structures of RelE bound to the ribosomal A-site in both the pre- and post-cleaved state they described how the RelE endonuclease performs its ribosome-dependent mRNA cleavage. In 2010 he began his postdoctoral studies at Massachusetts Institute of Technology, where he determined the structure of the largest nucleoporins Nup188 and Nup192 by using a combination of X-ray
crystallography and electron microscopy and revealed that the structures have distant
similarity to nuclear transport receptors. In 2014 he returned to Denmark as an
Assistant Professor at Aarhus University, where he studies kinase-mediated signaling.

**Mogens Kilstrup** received his Master degree in biochemistry in 1986, his Ph.D.
degree in molecular biology in 1990 both at the Institute of Biological Chemistry B,
University of Copenhagen. He worked on multivalent regulation of pyrimidine gene
expression in enteric bacteria. He discovered the guanine responsive PurR regulator
and characterized the first *purR* mutant of *E. coli*. After a short postdoctoral period he
was employed at the Technical University of Denmark, where he studied mechanisms
of stress response in *L. lactis*. Kilstrup became Associate Professor and Reader at
DTU-Bioengineering, the Technical University of Denmark in 2000 and 2008,
respectively. Kilstrup and Martinussen collaborate and have jointly discovered and
characterized the first activating PRPP-responsive PurR regulator protein of *L. lactis*,
and published the first in vivo kinetic analysis of allosteric interactions in bacteria, by
quantifying and correlating the intracellular concentrations of PRPP and all *pur*
mRNA levels during a purine downshift.

**Jan Martinussen** received his Ph.D. degree from the Department of Molecular Biology,
University of Southern Denmark in 1992. His research area was control of gene
expression in *E. coli* by using the cAMP/CRP, CytR/DeoR regulons and the *hok/sok*
antisense system as model systems. From 1992 to 1997 he was Postdoctoral Researcher
at the Danish Center for Lactic Acid Bacteria, the Technical University of Denmark,
where he investigated pyrimidine nucleotide metabolism in *L. lactis*. He became
Associate Professor at DTU-Bioengineering, Technical University of Denmark in 1997,
and his work has ever since been focused on the genetics and physiology of Gram-positive organisms with emphasis on nucleotide metabolism in lactic acid bacteria.

Robert L. Switzer received his Ph.D. degree in biochemistry from the University of California, Berkeley in 1966 under the direction of Professor H. A. Barker. He began his studies on the regulation and mechanistic enzymology of bacterial PRPP synthase at the suggestion of Earl R. Stadtman while a post-doctoral fellow at the National Institutes of Health from 1966 to 1968. After joining the Department of Biochemistry at the University of Illinois at Urbana-Champaign in 1968, he continued research on PRPP synthase for more than 25 years, a period that included fruitful collaborations with Hove-Jensen and Michael Becker. He also led research on the regulation of numerous enzymes and genes of bacterial pyrimidine and purine biosynthesis. His group discovered the RNA-binding regulatory protein PyrR. He remained on the Illinois Biochemistry faculty until retirement as Professor Emeritus in 2002. He was a Guggenheim Fellow and is a Fellow of the American Academy of Microbiology.

Martin Willemoës received his Ph.D. degree from the University of Copenhagen in 1996 while working with Hove-Jensen on structure-function analysis of the PRPP binding motif in PRPP synthase and the enzyme kinetics of allosteric regulation of this enzyme. As both Assistant and Associate Professor at the Centre for Crystallographic Studies, Department of Chemistry, University of Copenhagen (1999-2003) and at the Section for Biomolecular Sciences, Department of Biology (from 2005). He has worked with structure-function analysis of catalysis and regulation of enzymes of pyrimidine nucleotide metabolism. Over the last seven years his research interests has gradually moved toward the area of protein and enzyme design as a
member of the Linderstrøm-Lang Centre for Protein Science at Department of Biology, University of Copenhagen.
FIG 1 Biochemical function of PRPP. The β,γ-diphosphoryl group of ATP is transferred to ribose 5-phosphate with the generation of PRPP in a reaction catalyzed by PRPP synthase, encoded by a prs gene. PRPP is a substrate in a number of substitution reactions most of which involve a nitrogen-containing compound. The reaction occurs at C1 of the ribosyl moiety and proceeds with inversion of the configuration of this carbon and with PPi, as a leaving group.

FIG 2 Alignment of amino acid sequences of PRPP synthases from *B. subtilis* (*B.s.*), *S. oleracea* (isozyme 4) (*S.o.*) and *M. jannaschii* (*M.j.*). The *B. subtilis* PRPP synthase amino acid sequence is numbered from the N-terminal serine, as the original N-terminal methionine is removed in the mature protein. Functional elements of the *B. subtilis* PRPP synthase is indicated by bars above the *B. subtilis* amino acid sequence. Amino acid residues identical in all three sequences are indicated by asterisks below the *M. jannaschii* PRPP synthase amino acid sequence, whereas conserved amino acid residues are indicated by a dot. Conserved amino acids are valine, leucine and isoleucine; phenylalanine and tyrosine; aspartate and glutamate; arginine and lysine; serine and threonine; glycine and alanine. The division of the N- and C-terminal domains (150-Leu-Met-151) of *B. subtilis* PRPP synthase is indicated by a vertical line. *B. subtilis* PRPP synthase amino acid residues, which are located at the active site are typed in red, those which are located at the allosteric, regulatory site are typed in blue, whereas those involved in subunit-subunit interactions are typed in green. Amino acid residues involved in formation of the bent dimer are typed in bold, whereas those involved in formation of the parallel dimer are typed in italic. Whenever amino acid residues of PRPP synthase of *S. oleracea* or *M. jannaschii* are
identical to those of the *B. subtilis* enzyme, the color code of the latter enzyme is applied to the *S. oleracea* and *M. jannaschii* residues as well. The underlined amino acid residues, Val178-Asp196, Arg198, and Asn209-Val211 are involved in the formation of a tightly packed interface necessary for allosteric inhibition. Amino acid residues were selected on the basis of the three-dimensional structures previously published (49, 50, 54). Vertical arrow heads point to *B. subtilis* PRPP synthase amino acids, which are homologous to amino acids altered in the human PRPP synthase isozyme 1 due to point mutations in the *PRSP1* gene. Red arrow heads point to amino acid alterations resulting in increased PRPP synthase activity, whereas blue arrow heads point to amino acid alterations resulting in decreased PRPP synthase activity. The amino acid alterations and properties of the human PRPP synthase variants are described further in the text and summarized in Table 4.

FIG 3 Three-dimensional structure of *B. subtilis* PRPP synthase. (A) Monomer drawn on the basis of the SO$_4^{2-}$-PRPP synthase structure (PDB code 1dkr) (49). The N-terminal domain is at the top. Shown are the five-stranded parallel β-sheets (red), helices (blue), the flag region (green), the regulatory flexible (RF) loop, the ribose 5-phosphate (R5P) loop, and the PP loop (yellow). The unresolved catalytic flexible (CF) loop is shown as a dotted line. (B) Bent and parallel dimers drawn on the basis of the Cd$^{2+}$-PRPP synthase structure (PDB code 1ibs) (50). Subunit A is colored as the monomer in (A). Shown are Cd$^{2+}$ (black), AMP of the active site (red), sulfate bound at the position of the phosphate moiety of ribose 5-phosphate and at the position of the α-phosphate of ADP of the allosteric site (red). (C) Hexameric propeller structure drawn on the basis of the mADP-PRPP synthase structure (PDB code 1dku) (49). Subunit A (as well as subunit C and E) is colored as the monomer in
(A). Shown are the positions of the methylene ADP moieties (red) and methylene ADP molecules (green), both modeled to only AMP, of the ATP binding sites and the allosteric sites, respectively.

FIG 4 Allosteric site of *B. subtilis* PRPP synthase. Stereo view based on the mADP-PRPP synthase structure (PDB code 1dku) (49). The site is occupied by methylene ADP. Amino acid residues contributing to methylene ADP binding are provide by three subunits labelled A, B and D as in Fig. 3. Amino acid residues of subunit A are shown in blue, amino acid residues of subunit B in light gray and amino acid residues of subunit D are shown in dark gray.

FIG 5 Positions of non-homologous regions. Each line of bars represents polypeptides of a PRPP synthase with the amino-terminus at the left-hand end. *S. c. 1, S. cerevisiae* Prs1; *S. c. 3, S. cerevisiae* Prs3; *S. c. 5, S. cerevisiae* Prs5; *S. p. 1, S. pombe* Prs1; *S. p. 2, S. pombe* Prs2; *S. p. 3, S. pombe* Prs3; PAP39, human PRPP synthase-associated protein 39; PAP41, human PRPP synthase-associated protein 41. *S. cerevisiae* and *S. pombe* Prs3 are shown at the top as a bar consisting of three segments (I, II and III) represented by different blue shadings. This bar could represent most class I PRPP synthases. The left- and right-hand ends of segment II are located within the regulatory and catalytic flexible loops, respectively. A non-homologous region (NHR) is shown in red shading. The number of amino acid residues of each NHR is shown below the bars. The three NHRs of *S. cerevisiae* Prs1 and 5 are designated NHR1, NHR5-1 and NHR5-2. According to this nomenclature *S. cerevisiae* Prs1 has the structure segment 1-segment 2-NHR1-segment 3, *S. cerevisiae* Prs5 has the structure segment 1-NHR5-1-segment 2-NHR1-segment 3, *S. pombe* PRPP synthase 1 has the
structure segment I-segment II-NHR-segment III, *S. pombe* PRPP synthase 2 has the
structure segment I-NHR-segment II-segment III, and human PAP39 and 41 have the
structure segment I-segment II-NHR-segment III.

**FIG 6** Catalytic mechanism of PRPP synthase. (A) Closure of the catalytic flexible
loop of *T. volcanium* PRPP synthase by superimposition of the open and closed
structures (PDB code 3lrt and 3mbi, respectively). Structural elements are colored as
in Fig. 3A. A 17 Å movement of the flexible catalytic loop, consisting of the β10 and
β11 strands, results in the closed conformation necessary for catalysis. (B) Close up of
the binding of substrates at the active site of *T. volcanium* PRPP synthase with open
and closed catalytic flexible loop. In the open conformation the triphosphate chain of
ATP, modeled here to only ADP, forms a more or less linear arrangement. In the
closed conformation the triphosphate chain, again modeled to only ADP, bends with
the β-phosphate obtaining a position ideal for attack of O1 of ribose 5-phosphate on
the β-phosphorus. An Mg\(^{2+}\) of the closed conformation is shown as a black sphere
(138). (C) Stereo view of the binding of ribose 5-phosphate, Mg\(^{2+}\) ions and the
transition state analog AlF\(_3\) to the active site of *B. subtilis* PRPP synthase, AlF\(_3\)-PRPP
synthase (54). α indicates the α-phosphate of ATP provided by an AMP molecule, β
indicates the β-phosphate of ATP provided by Al\(^{3+}\) (bound to three F\(^{-}\)), γ indicates the
γ-phosphate of ATP provided by the phosphate of a second AMP molecule. The two
Mg\(^{2+}\) are indicated by MG1 and MG2. Relevant amino acid residues, His135,
Asp174, Lys197 and Arg199 are included as well. Reprinted with permission (54).
(D) Stereo view of the binding of ribose 5-phosphate, α,β-methylene ATP, Mg\(^{2+}\) and
Ca\(^{2+}\) ions to the active site of *B. subtilis* PRPP synthase in the GDP-PRPP synthase
complex. Ca\(^{2+}\) (designated CA1) coordinates to the hydroxyls at C1, C2 and C3 of
ribose 5-phosphate, oxygen of the β- and γ-phosphates of α,β-methylene ATP,

Asp174 as well as a water molecule. The Mg\(^{2+}\) (designated MG2) coordinates to the

oxxygen of C2’ of the ribosyl moiety as well as oxygen of the α- and γ-phosphates of

α,β-methylene ATP, as well as to three water molecules. Thus, there is no

coordination to oxygen of the β-phosphate of α,β-methylene ATP. Reprinted with

permission (54).

FIG 7 Reactions catalyzed by diphosphoryltransferases and alternative biosynthesis of

PRPP. In some cases ATP may be replaced by dATP. The diphosphoryl and

phosphoryl moieties of the products are shown in red and blue, respectively. (A)

Reaction catalyzed by phosphoribosyl bisphosphate phosphokinase. The substrate is

ribose 1,5-bisphosphate, the product is PRPP; (B) reaction catalyzed by PRPP

synthase. The substrate is ribose 5-phosphate, the product is PRPP; (C) 2-amino-4-

hydroxy-6-hydroxymethylidihydropterin diphosphokinase. The substrate is 2-amino-4-

hydroxy-6-hydroxymethylidihydropterin, the product is 2-amino-4-hydroxy-6-

hydroxymethylidihydropterin diphosphate; (D) GTP/GDP 3’-diphosphokinase

(stringent factor). R may be a hydrogen or a phosphoryl group, i.e. the substrate is

GDP or GTP, respectively, and the product is guanosine 3’-diphosphate 5’-

diphosphate (ppGpp) or guanosine 3’-diphosphate 5’-triphosphate (pppGpp),

respectively; (E) nucleotide diphosphokinase. R\(_1\) may be an adenyl, a guanyl or a

hypoxanthyl univalent radical, whereas R\(_2\) may be a hydrogen, a phosphoryl or a

diphosphoryl moiety; (F) thiamine diphosphokinase. The substrate is thiamine, the

product is thiamine diphosphate.
FIG 8 Diphosphoryl-, nucleotidyl- and phosphoryltransfer reactions. Water molecules are indicated by Wat. (A) The active site of the ternary complex of *E. coli* hydroxymethyldihydropterin diphosphokinase. AMPCPP, α,β-methylene ATP; HP; hydroxydihydropterin (PDB code 1q0n) (164). (B) The active site of DNA polymerase β (PDB code 1bpy) (176). (C) The active site of cAMP protein kinase A. AMPPNP, β,γ-imido ATP (PDB code 4hpu) (201).

FIG 9 Catalytic strategies. The coordination of Mg$^{2+}$ (shown as blue spheres) of the active site to the substrates, to amino acid residues and to water molecules (shown as red spheres) is schematically illustrated by blue lines. (A) cAMP-dependent protein kinase A (PDB code 4hpu) (201). (B) PRPP synthase. R5P, ribose 5-phosphate. Mg$_{\text{A}}$ was designated MG2, and Mg$_{\text{B}}$ was designated MG1 before (54). (C) Hexose 1-phosphate nucleotidyltransferase based on the structure of N-acetylglucosamine 1-phosphate uridylyltransferase (PDB code 4g87). Mg$_{\text{B}}$ coordinates to aspartate and asparagine residues (192). (D) DNA polymerase based on the structure of DNA polymerase β (PDB code 1bpy) (176). The three acidic residues are usually aspartates, but occasionally one aspartate is substituted by glutamate (185, 186). The structure is valid also for RNA polymerases (187).

FIG 10 Similarity of the folds of the PRPP synthase domain and type I phosphoribosyltransferases. (A) Superimposition of the C-terminal domain (amino acids 151 to 286) of *T. volcanium* PRPP synthase (blue, PDB code 3mbi) (138) and *T. gondii* hypoxanthine-guanine phosphoribosyltransferase (green, PDB code 1fsg) (212). The PP, ribose 5-phosphate (R5P) and catalytic flexible (CF) loops are indicated. (B) Substrate binding at the active sites of *B. subtilis* PRPP synthase and *T.
B. subtilis PRPP synthase transition state analog (cf Fig. 6C) consisting of the phosphoryl moiety of AMP (indicated by α), AlF₃ (indicated by β), the phosphoryl moiety of a second AMP molecule (indicated by γ) and ribose 5-phosphate as well as Asp223 and Asp224 are shown as thick lines, and the two Mg²⁺ as green spheres (54). Residues from the structure of T. gondii hypoxanthine-guanine phosphoribosyltransferase in complex with 9-deazaguanine (not shown), PRPP and Mg²⁺ as well as Glu146 and Asp147 (PDB code 1fsg) (212), is shown as thin yellow lines or spheres superimposed on the AlF₃-PRPP synthase structure. Reprinted with permission (54).

FIG 11 N-glycosidic bond formation with PRPP. PRPP and atoms of the products derived from PRPP are shown in red. Each reaction produces an N-glycosidic 5’-phosphoribosyl compound and PPᵢ. The reaction with quinolinate also produces carbon dioxide. The compounds a to e are products of de novo reactions: a, 5-phosphoribosyl 1-amine; b, orotidine 5’-monophosphate; c, 5’-phosphoribosynicotinate; d, 5’-phosphoribosyl-ATP; e, 5’-phosphoribosylanthranilate. The compounds f to k are products of salvage reactions: f, AMP; g, inosine 5’-monophosphate; h, xanthosine 5’-monophosphate; i, GMP; j, UMP; k, 5’-phosphoribosynicotinate.

FIG 12 C- and O-glycosidic bond formation with PRPP. The phosphoribosyl donor PRPP is shown in red, and atoms derived from PRPP in the intermediates and products are also shown in red. (A) Biosynthesis of tetrahydromethanopterin. Compounds: l, 4-hydroxybenzoate; m, 5’-phosphate-β-D-ribosyl 4-hydroxybenzene; n, 5’-phosphate-β-D-ribosyl 4-aminobenzene; o, N’-[7,8-dihydropterin-6-yl)methyl]-
4-(1-deoxy-D-ribulosyl)aminobenzene; \( p \), 1-(4-\{N-[7,8-dihydropterin-6-yl]methyl\}amino)phenyl)-5-(5-phospho-\( \alpha \)-D-ribulosyl)-1-deoxyribitol. Enzyme 1, 4-aminobenzoate phosphoribosyltransferase (5-phospho-\( \alpha \)-D-ribose 1-diphosphate:4-aminobenzoate 5-phospho-\( \beta \)-D-ribofuranosyltransferase (decarboxylating), EC 2.4.2.54); enzyme 2, 1-(4-\{N-[7,8-dihydropterin-6-yl]methyl\}amino)phenyl)-5-(5-phospho-\( \alpha \)-D-ribulosyl)-1-deoxyribitol synthase. This enzyme activity has not been identified. The four reactions leading from compound \( m \) to compound \( n \) in effect convert a hydroxy group to an amino group and involve the formation of phosphate ester and the addition of an aspartyl residue, the removal of \( P_i \) and fumarate (304). The three reactions leading from compound \( n \) to compound \( o \) involve the attachment of a pterin derivative (\( R = N-[7,8-dihydropterin-6-yl]methyl \)) to the nitrogen of compound \( n \) followed by opening of the ribosyl moiety and isomerization to a ribulose derivative and dephosphorylation to form compound \( o \). The boxed compound is the product of the pathway, 5,6,7,8-tetrahydromethanopterin (with a complete structure of the pteridyl moiety), the active co-factor in transformation of carbon dioxide to methane in methanogenic Archaea (301), and is formed form compound \( p \) by attachment of a glutamyl moiety to the phosphate group followed by dehydrogenation of the pteridyl moiety. (B) Biosynthesis of arabinosyl monophosphodecaprenol. Compounds: \( q \), decaprenyl phosphate; \( r \), 5-phospho-\( \beta \)-D-ribosyl 1-\( O \)-monophosphodecaprenol (decaprenylphospho-\( \beta \)-D-ribosyl 5-phosphate). Enzyme 3: decaprenyl phosphate phosphoribosyltransferase (5-phospho-\( \alpha \)-D-ribosyl 1-diphosphate:decaprenyl-phosphate 5-phosphoribosyltransferase, EC 2.4.2.45). The boxed compound is the arabinosyl donor arabinosyl monophosphodecaprenol, which is formed from compound \( r \) by dephosphorylation of the ester at C5 of the ribosyl moiety followed by epimerization. The latter two reactions occur on the outside of the
cell (5). (C) Biosynthesis of butirosin. Compounds: s, neamine; t, 5″-
phosphoribostamycin. Enzyme 4: neamine phosphoribosyltransferase. The boxed
compound is butirosin. Two isomers are synthesized, one contains a ribosyl moiety
(shown) a second contains an arabinosyl moiety (not shown). Other aminoglycoside
antibiotics derived from neamine, i.e. neomycin B, paromomycin, and lividomycin B,
lack the 4-amino-2-hydroxybutyryl side chain but contain an N-acetylamino-glucosyl
moiety attached to C3″ of the ribosyl moiety, and the pseudo-disaccharides of the
three compounds are differently decorated with hydroxyl and amino groups (318).

FIG 13 Mechanism of PRPP-mediated regulation of transcription by PyrR and PurR.
PyrR and Pur R are shown as orange spheres. (A) Model of PyrR regulation based on
the combined data from B. subtilis and B. caldolyticus. RNA (green hairpin) binds to
dimeric PyrR, which is stabilized by UMP, UTP or PRPP. The tetrameric
conformation is stabilized by GMP, and does not bind RNA. See text for details. (B)
Model of PurR₈₈ repression. DNA (blue line) containing one strong (solid green line)
and one weak (green punctuated line) PurBox binds to two PurR₈₈ dimers (forming a
weak tetramer) in the absence of PRPP. In the presence of PRPP the DNA binding is
prevented, and the tetramerization is lost. (C) Model of PurR₉₈ activation. PurR₉₈ binds
to PurBox sequences, irrespectively of PRPP binding, presumably as a dimer. Binding
of PRPP is hypothesized to expose a binding site for RNA polymerase. RNA
polymerase is positioned correctly relative to the -10 region of the promoter.

FIG 14 Phylogenetic analysis of PurR from low-GC Gram-positive bacteria. (A) PurR
sequences from representatives of the major bacterial lineages in the low-GC Gram-
positive bacteria were aligned using the Clustal Omega program, and a phylogenetic
tree was constructed using the ClustalW2 Phylogeny program. (B) The type of PurBox used by each species was identified at the RegPrecise website (http://regprecise.lbl.gov/RegPrecise/collection_tffam.jsp?tffamily_id=53), and the two types of logo-plots were constructed from the PurBox sequences presented for the *Streptococcales* and *Bacilliales*, respectively, using the weblogo service (http://weblogo.berkeley.edu/logo.cgi).

FIG 15 Common phylogeny of PyrR, PurR, type 1 phosphoribosyltransferases and class I PRPP synthases in all major bacterial lineages. Homologous protein sequences were deduced and identified from representative genome sequences of all major bacterial lineages, for the following proteins: adenine phosphoribosyltransferase (APRTase); hypoxanthine-guanine phosphoribosyltransferase (HPRTase); orotate phosphoribosyltransferase (OPRTase); uracil phosphoribosyltransferase (UPRTase); xanthine phosphoribosyltransferase (XPRTase). The collected sequences were aligned using the Clustal Omega program, and a phylogenetic tree was constructed using the ClustalW2 Phylogeny program. Left panel, phylogenetic tree with protein sequences belonging to same enzyme families grouped together as triangles, starting at the point of the first branching of the individual protein sequences and labeled with the enzyme abbreviations shown above. The triangle marked OPRTase* is a group of orotate phosphoribosyltransferase sequences that appears to form a separate branch with a distinct set of amino acid sequences in the PP and PRPP binding loops. Sequences with homology to the xanthine phosphoribosyltransferase and PurR proteins were only found among the low-GC Gram-positive bacteria, as indicated. Middle panel, logo plot of the frequency of amino acids in the PP loop identified in the Clustal Omega alignment and constructed using the weblogo service.
amino acids in the PRPP binding loop identified in the Clustal Omega alignment and constructed using the weblogo service.

FIG 16 Possible secondary structure of the *S. enterica* *prs* leader. The sequence ranges from nucleotide one, indicated by 5’, to nucleotide 417, which is immediately followed by the initiator codon-specifying guanylate-uridylate-guanylate triplet and indicated by 3’ (45). The ΔG of the structure is -540 kJ mol⁻¹. The leader *prs* sequences of *S. enterica* and *E. coli* are identical except for the framed sequence, which is present only in the *S. enterica* *prs* leader. The nucleotides circled in red represent the coding of a possible leader peptide, the red arrows points to the nucleotides of a possible Shine-Dalgarno sequence. Possible leader peptide amino sequences are indicated for *E. coli* and *S. enterica*. Identical amino acid residues of the two possible leader peptides are underlined.
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<td>5001</td>
<td></td>
<td></td>
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<tr>
<td>5002</td>
<td>B. pseudomallei</td>
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<tr>
<td>5003</td>
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<td>E. coli PRPPS</td>
<td>2.7</td>
<td>Mg(^{2+})</td>
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</tr>
<tr>
<td>5004</td>
<td>M. jannaschii</td>
<td>Apo</td>
<td>2.7</td>
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<tr>
<td>5005</td>
<td>Ternary</td>
<td>2.9</td>
<td>AMP, ribose 5-phosphate</td>
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<tr>
<td>5006</td>
<td>S. solfataricus</td>
<td>2.8</td>
<td>AMP</td>
<td></td>
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<tr>
<td>5007</td>
<td>T. volcanium</td>
<td>ADP/SO(_4)</td>
<td>1.5</td>
<td>ADP, SO(_4^{2-})</td>
<td></td>
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<tr>
<td>5008</td>
<td>ADP/SO(_4)</td>
<td>1.8</td>
<td>ADP, SO(_4^{2-})</td>
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<td></td>
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<tr>
<td>5009</td>
<td>mATP/SO(_4)</td>
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<td>mATP, SO(_4^{2-})</td>
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<tr>
<td>5010</td>
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<td>ADP, Mg(^{2+}), Pi, ribose 5-phosphate</td>
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<tr>
<td>5011</td>
<td>Human</td>
<td>hPRS1 wild-type(^a)</td>
<td>2.6</td>
<td>SO(_4^{2-})</td>
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<tr>
<td>5012</td>
<td>hPRS1 ATPSO(_4^{2-})Cd(^a)</td>
<td>2.2</td>
<td>AMP, SO(_4^{2-}), Cd(^{2+})</td>
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<td></td>
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<tr>
<td>5013</td>
<td>hPRS1S132A(^b)</td>
<td>2.2</td>
<td>SO(_4^{2-})</td>
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<td></td>
</tr>
</tbody>
</table>

\(^a\) 

\(^b\)
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5014</td>
<td>hPRS1Y146M&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5</td>
<td>SO₄²⁻</td>
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<tr>
<td>5015</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5016</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5017</td>
<td>-&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.3</td>
<td>Mg²⁺, SO₄²⁻</td>
</tr>
<tr>
<td>5018</td>
<td>PRS1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5019</td>
<td>E43T&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.0</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5020</td>
<td>D65N&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.1</td>
<td>SO₄²⁻</td>
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<tr>
<td>5021</td>
<td>A87T&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3.3</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5022</td>
<td>M115T&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2.1</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5023</td>
<td>Q133P&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2.7</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5024</td>
<td>PAP39&lt;sup&gt;j&lt;/sup&gt;</td>
<td>2.7</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>5025</td>
<td>PAP41&lt;sup&gt;k&lt;/sup&gt;</td>
<td>2.6</td>
<td>SO₄²⁻</td>
</tr>
</tbody>
</table>

<sup>a</sup> PRPP synthase isozyme 1.

<sup>b</sup> Ser132Ala mutant variant of PRPP synthase isozyme 1.

<sup>c</sup> Tyr146Met mutant variant of PRPP synthase isozyme 1.
Asp52His mutant variant of PRPP synthase isozyme 1.

Glu43Thr mutant variant of PRPP synthase isozyme 1.

Asp65Asn mutant variant of PRPP synthase isozyme 1.

Ala87Thr mutant variant of PRPP synthase isozyme 1.

Met115Thr mutant variant of PRPP synthase isozyme 1.

Gln133Pro mutant variant of PRPP synthase isozyme 1.

PRPP synthase-associated protein 39

PRPP synthase-associated protein 41
<table>
<thead>
<tr>
<th>Organism</th>
<th>PRPP synthase</th>
<th>$K_M$ (μM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; or specific activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>I</td>
<td>480 660</td>
<td>190</td>
<td>(70)</td>
</tr>
<tr>
<td>B. caldolyticus</td>
<td>I</td>
<td>530 310</td>
<td>400</td>
<td>(55)</td>
</tr>
<tr>
<td>S. enterica</td>
<td>I</td>
<td>160 50</td>
<td>130</td>
<td>(56)</td>
</tr>
<tr>
<td>E. coli</td>
<td>I</td>
<td>203 113</td>
<td>181</td>
<td>(57)</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>I</td>
<td>8.2-71 1-25</td>
<td>1.4-530</td>
<td>(77-79)</td>
</tr>
<tr>
<td>B. amyloliquefaciens</td>
<td>I</td>
<td>105 50</td>
<td>37</td>
<td>(149)</td>
</tr>
<tr>
<td>Human isozyme 1</td>
<td>I</td>
<td>52 21</td>
<td>25</td>
<td>(73)</td>
</tr>
<tr>
<td>Human isozyme 2</td>
<td>I</td>
<td>83 70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36</td>
<td>(73)</td>
</tr>
<tr>
<td>Rat isozyme 1</td>
<td>I</td>
<td>40 44</td>
<td>39</td>
<td>(71)</td>
</tr>
<tr>
<td>Rat isozyme 2</td>
<td>I</td>
<td>73 60</td>
<td>35</td>
<td>(71)</td>
</tr>
<tr>
<td>Rat, liver enzyme</td>
<td>I</td>
<td>64 49</td>
<td>16</td>
<td>(143)</td>
</tr>
<tr>
<td>S. oleracea, isozyme 3</td>
<td>II</td>
<td>110 170 233 650 116 137 13.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5053</td>
<td><em>S. oleracea</em>, isozyme 4</td>
<td>II</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>5054</td>
<td><em>M. jannaschii</em></td>
<td>Archaea</td>
<td>2800</td>
<td>2600</td>
</tr>
<tr>
<td>5055</td>
<td><em>T. kodakaraensis</em></td>
<td>Archaea</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> *S*<sub>0.5</sub> value.

<sup>b</sup> Values varied between 1.2 and 6.9 with GTP, CTP or UTP as the diphosphoryl donor.

<sup>c</sup> Values varied between 4.2 and 6.6 with GTP, CTP or UTP as the diphosphoryl donor.
TABLE 3 Comparison of amino acid residues involved in dimer association of *B. subtilis* PRPP synthase with amino acids of PRPP synthase of other organisms

<table>
<thead>
<tr>
<th></th>
<th><em>M. tuberculosis</em></th>
<th>Spinach</th>
<th><em>M. jannaschii</em></th>
<th><em>T. volcatus</em></th>
<th><em>S. solfataricus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td><em>E. coli</em> isozyme 4</td>
<td>Bent dimer (B. subtilis PRPP synthase, N-terminal domain interactions)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N69</td>
<td>N64</td>
<td>N72</td>
<td>I73</td>
<td>N60</td>
<td>E61</td>
</tr>
<tr>
<td>E70</td>
<td>D65</td>
<td>R73</td>
<td>F74</td>
<td>D61</td>
<td>V62</td>
</tr>
<tr>
<td>I72</td>
<td>L67</td>
<td>L75</td>
<td>Q76</td>
<td>I63</td>
<td>E64</td>
</tr>
<tr>
<td>M73</td>
<td>M68</td>
<td>M76</td>
<td>L77</td>
<td>V64</td>
<td>M65</td>
</tr>
<tr>
<td>L76</td>
<td>L70</td>
<td>L79</td>
<td>I80</td>
<td>I67</td>
<td>T68</td>
</tr>
<tr>
<td>I77</td>
<td>V72</td>
<td>I80</td>
<td>Y81</td>
<td>L68</td>
<td>L69</td>
</tr>
<tr>
<td>L116</td>
<td>V112</td>
<td>L119</td>
<td>L122</td>
<td>A107</td>
<td>I106</td>
</tr>
<tr>
<td>L120</td>
<td>F116</td>
<td>L123</td>
<td>V127</td>
<td>I111</td>
<td>I110</td>
</tr>
</tbody>
</table>

Parallel dimer (B. subtilis PRPP synthase, N- and C-terminal domain interactions)

<table>
<thead>
<tr>
<th></th>
<th><em>B. subtilis</em></th>
<th><em>E. coli</em></th>
<th><em>M. jannaschii</em></th>
<th><em>T. volcatus</em></th>
<th><em>S. solfataricus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>K115</td>
<td>K111</td>
<td>R118</td>
<td>R120</td>
<td>R106</td>
<td>Q105</td>
</tr>
<tr>
<td>Q138</td>
<td>Q134</td>
<td>Q141</td>
<td>Q144</td>
<td>H128</td>
<td>T128</td>
</tr>
<tr>
<td>I139</td>
<td>I135</td>
<td>I142</td>
<td>E145</td>
<td>I129</td>
<td>L129</td>
</tr>
<tr>
<td>F142</td>
<td>F138</td>
<td>F145</td>
<td>F139</td>
<td>F132</td>
<td>S132</td>
</tr>
<tr>
<td>D144</td>
<td>D140</td>
<td>D147</td>
<td>S150</td>
<td>T134</td>
<td>V144</td>
</tr>
<tr>
<td>V178</td>
<td>V174</td>
<td>V182</td>
<td>G185</td>
<td>V167</td>
<td>L165</td>
</tr>
<tr>
<td>D186</td>
<td>K182</td>
<td>D190</td>
<td>Q183</td>
<td>K175</td>
<td>A173</td>
</tr>
<tr>
<td>I192</td>
<td>M189</td>
<td>L197</td>
<td>M199</td>
<td>Y181</td>
<td>H179</td>
</tr>
<tr>
<td>A193</td>
<td>A190</td>
<td>A198</td>
<td>V200</td>
<td>D182</td>
<td>F180</td>
</tr>
<tr>
<td>I194</td>
<td>I192</td>
<td>F199</td>
<td>V201</td>
<td>Y183</td>
<td>F181</td>
</tr>
</tbody>
</table>
The annotated amino acid residues involved in the formation of the two types of dimer (bent and parallel, see text for details) of *B. subtilis* PRPP synthase are listed at the left-hand column according to the previously published three-dimensional structure (49). The *B. subtilis* PRPP synthase amino acid sequence was then aligned pairwise with the amino acid sequences of PRPP synthases of *E. coli* (accession U00096) (313); *M. tuberculosis* (accession AL123456) (410); *S. oleracea* isozyme 4 (15); *M. jannaschii* (accession L77117) (411); *T. volcanium* GSS1 (accession BA000011) (412) and *S. solfataricus* P2 (accession AE006641) (413), and amino acid residues at similar positions of PRPP synthases of these latter six organisms are listed in the other columns. Amino acid residues that are identical or conserved relative to the *B. subtilis* enzyme are shown in bold, whereas non-conserved residues are shown in red.
<table>
<thead>
<tr>
<th>Amino acid alteration</th>
<th>Homologous position in</th>
<th>B. subtilis PRPP synthase</th>
<th>Enzyme property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased activity $^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp51His</td>
<td>Asp57</td>
<td></td>
<td>Reduced inhibition by ADP, increased stimulation by $P_i$ (72)</td>
</tr>
<tr>
<td>Asn113Ser</td>
<td>Asn119</td>
<td></td>
<td>Reduced inhibition by ADP, increased stimulation by $P_i$ (72)</td>
</tr>
<tr>
<td>Leu128Ile</td>
<td>Leu134</td>
<td></td>
<td>Reduced inhibition by ADP, increased stimulation by $P_i$ (72)</td>
</tr>
<tr>
<td>Asp182His</td>
<td>Asp186</td>
<td></td>
<td>Reduced inhibition by ADP, increased stimulation by $P_i$ (72)</td>
</tr>
<tr>
<td>Ala189Val</td>
<td>Ala193</td>
<td></td>
<td>Reduced inhibition by ADP, increased stimulation by $P_i$ (72)</td>
</tr>
<tr>
<td>His192Leu</td>
<td>Asp196</td>
<td></td>
<td>Reduced inhibition by ADP, increased stimulation by $P_i$, reduced stability (72, 123)</td>
</tr>
<tr>
<td>Reduced activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu42Asp</td>
<td>Asn48</td>
<td></td>
<td>Not characterized (120)</td>
</tr>
<tr>
<td>Asp64Asn</td>
<td>Glu70</td>
<td></td>
<td>Reduced specific activity in crude erythrocyte extract (121)</td>
</tr>
<tr>
<td>Ala86Thr</td>
<td>Ile92</td>
<td></td>
<td>Reduced specific activity in crude erythrocyte extract (121)</td>
</tr>
</tbody>
</table>

$^a$ Table 4 Human PRPP synthase isozyme 1 variants

$^b$
| 5116 | Met114Thr | L119 | Reduced specific activity in crude fibroblast extract (120) |
| 5117 | Gln132Pro | Gln138 | Reduced specific activity in crude erythrocyte and fibroblast extracts (122) |
| 5118 | Val141Leu | Ile147 | Reduced activation by $P_i$ (414) |
| 5119 | Leu151Pro | Leu156 | Reduced specific activity in crude erythrocyte and fibroblast extracts (122) |
| 5120 | Ile289Thr | Ser293 | Reduced specific activity in crude erythrocyte extract (121) |
| 5121 | Gly305Arg | Gln308 | Reduced specific activity in crude erythrocyte extract (121) |

5122

5123 Acute lymphoblastic leukemia relapse-specific$^c$

<p>| 5124 | Val52Ala | Cys58 | (148) |
| 5125 | Ile71Val | Ile77 | (148) |
| 5126 | Cys76Ser | Leu82 | (148) |
| 5127 | Ser102Asn | Ser108 | (148) |
| 5128 | Asn113Asp | Asn119 | (148) |
| 5129 | Asp138Gly | Asp144 | (148) |
| 5130 | Asn143Ser | Asp148 | (148) |</p>
<table>
<thead>
<tr>
<th>Amino Acid Residues</th>
<th>Numbered From</th>
<th>Original N-Terminal Methionine is Removed in the Mature Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly173Glu Gly177</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Lys175Asn Thr179</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Asp182Glu Asp186</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Ala189Thr/Val Ala193</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Leu190Ile Ile194</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Thr302Ser Val305</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Tyr310Cys Tyr313</td>
<td>(148)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Amino acid residues are numbered from the N-terminal proline residue, as the original N-terminal methionine is removed in the mature protein (72).

\(^b\) A comprehensive review of the various diseases caused by mutations in the human PRPP synthase isozyme 1-encoding gene has been published previously (415).

\(^c\) Relapse-specific PRPP synthase variants have not been characterized enzymatically.
### TABLE 5 PRPP pool sizes in various bacterial species

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Purine added</th>
<th>PRPP pool size (mmol [g dry weight]⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>None</td>
<td>1.1 ±0.1</td>
<td>(357)</td>
</tr>
<tr>
<td></td>
<td>Adenine</td>
<td>0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(357)</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td>None</td>
<td>2.6 ±0.6</td>
<td>(416)</td>
</tr>
<tr>
<td></td>
<td>Hypoxanthine</td>
<td>0.7 ±0.2</td>
<td>(416)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>None</td>
<td>4.4</td>
<td>(417)</td>
</tr>
<tr>
<td></td>
<td>Hypoxanthine</td>
<td>1.2</td>
<td>(417)</td>
</tr>
<tr>
<td><em>S. enterica</em></td>
<td>None</td>
<td>1.2</td>
<td>(36)</td>
</tr>
<tr>
<td></td>
<td>Hypoxanthine</td>
<td>0.3</td>
<td>(36)</td>
</tr>
</tbody>
</table>

<sup>a</sup> No repression observed by hypoxanthine.
Phylogenetic relationship

HPRTase

PyrR

PRPP synthase

UPRTase

APRTase

OPRTase

OPRTase*

XPRTase

Only found in low-GC gram-positive bacteria

PurR

PP loop

PRPP binding loop
E. coli (38 amino acid residues)
MetAlaLeuTrpArgLysAlaLeullePheHisCysThr
GluProCysPheLysProGlyLysLeuSerPheGlyAsp
AsnValThrLeuPheGlnThrLeuHisArgAlaLeu

S. enterica (25 amino acid residues)
MetLysThrSerLysProGlyLysLeuSerPheGlyAsp
AsnValThrLeuPheGlnThrLeuHisArgAlaLeu