JOURNAL OF BACTERIOLOGY, July 1988, p. 3243–3248
0021-9193/88/073243-06$02.00/0

Vol. 170, No. 7

# Structure of the Gene Encoding Phosphoribosylpyrophosphate Synthetase (*prsA*) in *Salmonella typhimurium*

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Received 14 October 1987/Accepted 15 April 1988

**The *Salmonella typhimurium* gene *prsA*, which encodes phosphoribosylpyrophosphate synthetase, has been cloned, and the nucleotide sequence has been determined. The amino acid sequence derived from the *S. typhimurium* gene is 99% identical to the derived *Escherichia coli* sequence and 47% identical to two rat isozyme sequences. Strains containing plasmid-borne *prsA* have been used to overproduce and purify the enzyme. The promoter for the *S. typhimurium prsA* gene was identified by deletion analysis and by similarity to the promoter for the *E. coli prsA* gene. The location of the *prsA* promoter results in a 416-base-pair 5′ untranslated leader in the *prsA* transcript, which was shown by deletion to be necessary for maximal synthesis of phosphoribosylpyrophosphate synthetase. The *S. typhimurium* leader contains a 115-base-pair insert relative to the *E. coli* leader. The insert appears to have no functional significance.**

The enzyme phosphoribosylpyrophosphate (PRPP) synthetase catalyzes a reaction at a key junction in intermediary metabolism. The enzyme diverts ribose 5-phosphate from energy generation by the pentose phosphate pathway to biosynthesis via the intermediate PRPP. PRPP is utilized in the biosynthesis of pyridine nucleotide coenzymes, the amino acids histidine and tryptophan, and the pyrimidine and purine nucleotides. From 70 to 80% of the carbon flow through PRPP synthetase is directed to nucleotide and nucleic acid synthesis (15).

The amount of PRPP synthetase activity in enteric bacteria is mediated at two levels: enzyme inhibition and regulation of synthesis. ADP is a potent inhibitor which binds at an allosteric site as well as competitively with ATP at the active site (5, 22). The ADP inhibition of PRPP synthetase at the allosteric site requires occupation of the active site. Thus, in the presence of ribose 5-phosphate, the activity of PRPP synthetase is mediated by the ratio of ADP to ATP. A pyrimidine, probably UDP or UTP, represses the synthesis of the enzyme 2- to 10-fold (17, 26). A *Salmonella typhimurium rpoBC* mutant which showed derepressed levels of aspartate transcarbamylase (*pyrBI*) and orotate phosphoribosyltransferase (*pyrE*), enzymes that have been shown to be regulated by attenuation mechanisms, also had a derepressed level of PRPP synthetase (8). This observation suggests that an attenuation mechanism may also function to regulate the gene encoding PRPP synthetase (*prsA*).

The catalytic mechanism of *S. typhimurium* PRPP synthetase has been studied extensively, whereas the genes encoding the *Escherichia coli* and rat enzymes have been cloned and sequenced (6, 7, 23). To initiate molecular genetic studies and to examine the regulation of *S. typhimurium* PRPP synthetase expression, the gene encoding the *S. typhimurium* PRPP synthetase has been cloned and the nucleotide sequence has been determined.

## MATERIALS AND METHODS

**Microbiology and molecular biology.** The strains used are listed in Table 1. Strain SB139 was produced from TR5878 and SB179 was produced from JL1002 by transduction with P22 grown on JL2943. Minimal medium was that of Vogel and Bonner (25) with 0.2% glucose. Rich medium was the LB medium of Miller (13). Plasmid pHO11 carries the *E. coli prsA* gene in a fragment of pBR322 (6). Standard molecular and microbiological methods were as described by Miller (13), Maniatis et al. (11), Davis et al. (3), Silhavy et al. (20), and Schleif and Wensink (19). The nucleotide sequence was determined by the method of Maxam and Gilbert (9, 12). Sequence determinations to confirm constructions and deletions were by a dideoxy termination method, with double-stranded plasmids as templates (2). Sequence analyses were performed with software by DNAstar, Inc.

**Enzyme assays and purification.** PRPP synthetase activity was assayed by a modification of the published method (21). The volume was reduced to allow assays to be run in 1.5-ml Microfuge tubes. A modification of the procedure described by Lupski et al. (10) was used to assay β-lactamase. Assays were run in 1 ml (final volume) of 0.1 M potassium phosphate (pH 7.0) at room temperature (23°C) and contained 20 μl of cephaloridine (1 mg/ml) and 2 to 10 μl of extract. The change in $A_{255}$ was monitored. Purification of PRPP synthetase followed the published protocol (21), except that strain SB139 containing plasmid pBS201 was used as the source and the last two steps of the purification were omitted.

**Construction of subclones and deletions.** pBS111R was constructed by digesting pBS111 with *Bam*HI and religation (Fig. 1). Analogous clones of the *E. coli* and *S. typhimurium prsA* genes were constructed in pUC18 and pUC19 which utilized the *Eco*RI sites at base pair (bp) 59 or bp 128 and the *Pvu*II sites at bp 1830 or bp 1780 in *S. typhimurium* and *E. coli*, respectively. A second *Eco*RI site in *S. typhimurium prsA* at bp 182 allowed simultaneous construction of similar plasmids which removed the putative *prsA* promoter with a 123-bp deletion. The constructed clones are represented in Fig. 1. Plasmid pBS202, constructed with pUC18, places the *prsA* gene in the same orientations as the *lac* promoter. Plasmids pBS201, pBS203, and pBS205, constructed with pUC19, place the *prsA* gene in the opposite orientation from the *lac* promoter. In plasmids pBS202 and pBS203, the *prsA* promoter has been removed. To establish the importance of the leader region, plasmids were derived from pBS201 and

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TABLE 1. Bacterial strains

| Strain | Description | Source or reference |
|---|---|---|
| *E. coli* | | |
| HO541 | *metB his trp leu rpsL prsA2*(Ts) | 6 |
| JM83 | *ara* Δ(*lac-pro*) *rpsL thi* φ80D *lacZ* ΔM15 | 28 |
| *S. typhimurium* | | |
| JL75 | *trp-196* | L. Bussey |
| JL1002 | *pyrA81* | L. Bussey |
| JL2943 | *srl-2*::Tn*10 recA1 rpsL* | L. Bussey |
| SB139 | *ilv-542 metA22 trpB2 strA120 flaA66 metE551 xyl-404 H1-b H2-e nml hsdLT6 hsdS29 recA1* | This work |
| SB179 | *pyrA81 srl-2*::Tn*10 recA1* | This work |
| SU422 | *met* | P. Sypherd |
| TR5878 | *ilv-542 metA22 trpB2 strA120 flaA66 metE551 xyl-404 H1-b H2-e nml hsdLT6 hsdS29* | J. Roth |

pBS202, which deleted most of the leader sequence. pBS214 was generated from pBS201 by partial *Eco*O109 digestion, *Sau*I digestion, DNA polymerase I large fragment treatment to generate blunt ends, and ligation. pBS215 was generated from pBS202 by *Eco*RI digestion, *Sau*I digestion, DNA polymerase I treatment, and ligation (Fig. 1). Plasmid pBS216 was generated by digestion of pBS111R with *Sma*I followed by *Nru*I and by blunt-end ligation. Restriction analysis and nucleotide sequencing across the deletion junctions confirmed proper constructions (data not shown).

## RESULTS

**Cloning of *S. typhimurium prsA*.** An *E. coli* strain with the temperature-sensitive *prsA2* mutation (HO541) was transformed with a library of *S. typhimurium* JL75 chromosomal DNA fragments generated by *Sau*3A partial digestion and was ligated into *Bam*HI-digested pUC13. Candidates were selected which grew at the nonpermissive temperature, 40°C. Instability of initial isolates suggested multiple transformation. Rapid-preparation plasmid DNA was fractionated by agarose gel electrophoresis, and individual bands were used to transform *E. coli* HO541. Candidates pBS111 and pBS119 were stable isolates, although they yielded very small colonies in strain HO541. Restriction analysis demonstrated that *Bam*HI sites were regenerated at each end for both isolates and that the inserts were 1.75 kbp for pBS111 and 1.95 kbp for pBS119.

**Nucleotide sequence of the *prsA* gene.** The nucleotide sequence of the *S. typhimurium* insert in pBS111 was determined by the chemical method of Maxam and Gilbert. The strategy and sites used to sequence both strands with complete overlaps are shown in Fig. 2. The nucleotide sequence and the derived amino acid sequence of the *S. typhimurium* gene and flanking regions are presented in Fig. 3. Differences in the DNA and derived amino acid sequences of *E. coli* from *S. typhimurium* sequences are indicated below the *S. typhimurium* sequences. PRPP synthetase is encoded by nucleotides 560 to 1504. The 315-codon reading frame initiates with GTG, as does the *E. coli* sequence, and ends with a TGA termination codon. Evidence indicating correct assignment of the reading frame includes (i) exact correspondence of residues 2 through 24 with the amino acid sequence of the mature protein (7); (ii) correct prediction of the COOH-terminal sequence, -Glu-His-COOH (7); (iii) correct alignment of the predicted amino acid sequence with the chemically determined amino acid sequence covering approximately 80% of the protein (K. W. Harlow, unpublished data); and (iv) near identity with the determined sequence of the *E. coli* gene (7). The predicted amino acid sequence of *S. typhimurium* PRPP synthetase differs from the *E. coli* sequence at only 2 of 315 amino acid residues, Thr278 (Ser in *E. coli*) and Ala283 (Ser in *E. coli*), and is 47% identical to the predicted sequence of the rat gene (23). Previous identi-

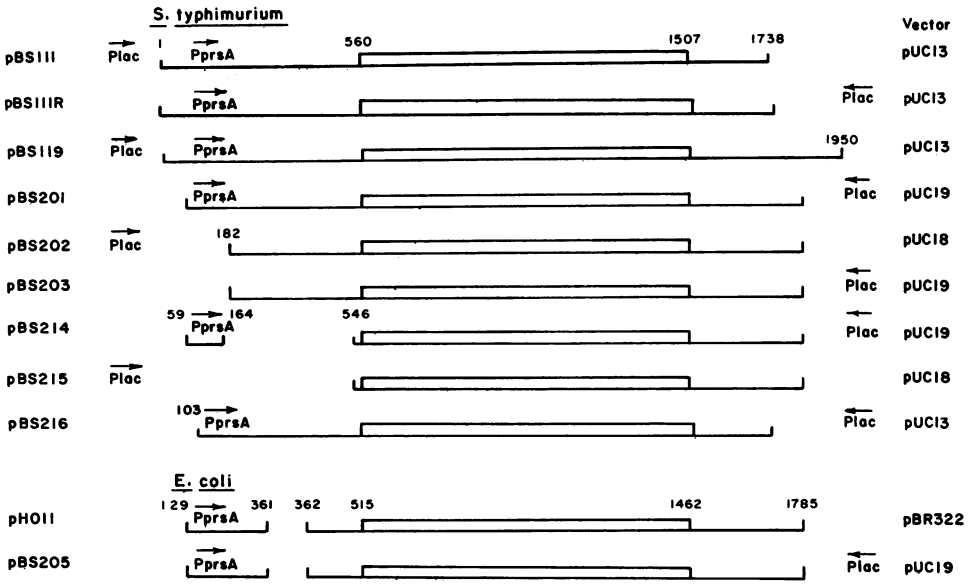


FIG. 1. Structures and alignment of analogous *prsA*-containing plasmids described in this study. *S. typhimurium* numbering is from Fig. 2. *E. coli* numbering is from reference 7. Vector pBR322 is the *Pvu*II (bp 2069)-to-*Eco*RI (bp 4362) fragment (18). Plac, Lactose promoter; $p_{prsA}$, *prsA* promoter.

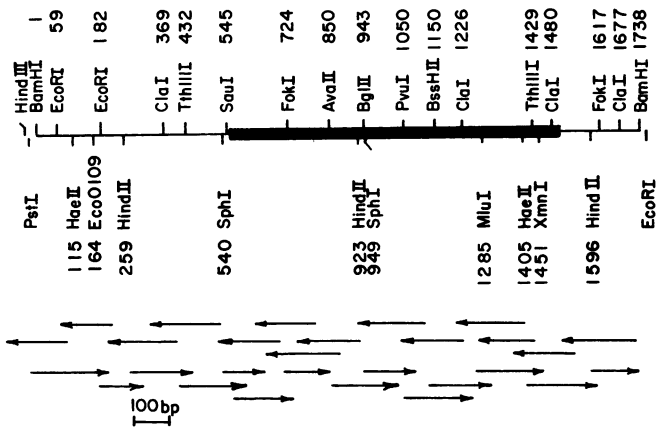


FIG. 2. Restriction map and sequencing strategy for the *S. typhimurium prsA* gene. The dark line indicates the region encoding the protein.

fication of *E. coli* residue 126 as Ile (ATA codon) (7) has been corrected to Thr (ACA codon) (unpublished data). Alignment of the N-terminal proline of PRPP synthetase as isolated with the second codon of the nucleotide sequence shows that the initiator fMet is processed from this protein. The nucleotide identity between the *S. typhimurium* and *E. coli* coding frames is 91%; 95% of the differences are in the third positions of codons. The high level of identity extends into the flanking regions; 230 bp on the 3′ side of the coding region are 92% identical. On the 5′ side, the identity is also 92%, except for a 115-bp insert in the *S. typhimurium* sequence relative to the *E. coli* sequence, which begins at bp 292 (Fig. 3).

A promoter driving the expression of the *prsA* gene in *E. coli* has been identified (7). The region of DNA encoding that promoter is essentially conserved in the *S. typhimurium* nucleotide sequence. The promoter sequence covers the region bp 108 through bp 144 in Fig. 3. The −10 and −35 regions identified in *E. coli* are completely conserved. The sequence, CGCGCC, between the −10 and transcription initiation site corresponds to a "discriminator sequence" identified as important in modulating the stringent response (24). The position of the putative *prsA* promoter relative to the translation start site (bp 560) results in a long transcribed leader, 302 bp in *E. coli* and 416 bp in *S. typhimurium*. The 115-bp insert in *S. typhimurium* relative to *E. coli* lies in this leader sequence. A sequence conserved near the promoters of *pyrC*, *pyrD*, and *carAB* (27), gGAAAACGtTT(c/g)-CGcttnt, is found just upstream of the *prsA* promoter at bp 90 to 103 and will be called the pyrimidine-conserved region. Upstream 40 bp from the −35 region of the *prsA* promoter is a consensus-binding site sequence for catabolite activator protein (CAP) (4): AAnTGTGAnnTnnnnC. Another noteworthy feature in the leader sequence is a "box A" sequence (16) at nucleotides 464 to 471 (Fig. 3). This sequence is downstream from the 115-bp insert and is present in both the *E. coli* and *S. typhimurium* sequences.

Downstream 16 bp from the termination codon of both *E. coli* and *S. typhimurium prsA* genes is a nearly identical region of hyphenated dyad symmetry followed by six T residues. Such structures can lead to the formation of hairpin loops in the mRNA and have been implicated as rho-independent terminators (29) and as blocks against 3′-to-5′ exonucleolytic activity (14). S1 mapping in *E. coli* has identified this site as the terminus of the isolated transcript (7).

There is a potential reading frame that extends from the 5′ cloning junction for more than 100 codons in both *E. coli* and *S. typhimurium*. The cloning junction for *E. coli* is 70 bp upstream from that of *S. typhimurium* and thus extends the open reading frame 23 codons. The 115-bp insert in the *S. typhimurium* sequence falls in the termination codon for the *E. coli* upstream open reading frame and extends the potential reading frame by 31 codons. Examination of the codon usage in the open reading frames showed no bias, suggesting high-level translation (data not shown). The strong promoter which normally expresses *prsA* lies within the open reading frame. A gap of about 160 bp lies between the open reading frame translation termination codon and the initiation codon for the *prsA* coding frame. There are no structures which suggest rho-independent termination between the open reading frame and the *prsA* gene. A search of the GenBank DNA sequence database (release no. 47, March 1987) and National Biomedical Research Foundation-Protein Identification Resource protein sequence database (release no. 12, April 1987) by using the DNAstar Nucscan and Proscan programs found no sequence with significant homology to the upstream open reading frame (data not shown). There is, at present, no evidence that the upstream open reading frame is actually expressed in either *E. coli* or *S. typhimurium*.

**PRPP synthetase activity of *E. coli* and *S. typhimurium* strains bearing homologous and heterogeneous *prsA* clones.** The *prsA* genes from *E. coli* and *S. typhimurium* were subcloned into pUC19 by using analogous restriction sites, generating pBS205 (*E. coli prsA* gene) and pBS201 (*S. typhimurium prsA* gene) (Fig. 1). Both genes were more highly expressed in *S. typhimurium* SB139 than in *E. coli* JM83, but expression appears normal for each in the heterologous background (Table 2). This suggests that the 115-bp insert in the leader region of the *S. typhimurium* gene has no effect on control of its expression.

**Localization of the *prsA* promoter by deletion.** Table 3 shows the levels of PRPP synthetase activity found in *S. typhimurium* SB179 containing various *prsA* plasmids. A comparison of the enzyme activity levels with plasmids pBS216 and pBS203 demonstrated that removal of the 79 nucleotides from bp 103 to 182 eliminated most of the plasmid-encoded activity. This result confirms the expected position of the promoter in *S. typhimurium*. Comparison of pBS201 to pBS216 showed that the 92-bp extension on the 3′ end has no effect on enzyme levels. pBS111R extended the sequence 59 bp 5′ to that present in pBS201 and increased the enzyme level threefold. An element 5′ to the promoter is thus essential for maximal expression.

**Effect of deletion of the 5′ untranslated leader region of *prsA*.** The 5′ untranslated leader is required for normal expression of the *prsA* gene. When the DNA from bp 165 to

TABLE 2. Expression of PRPP synthetase in *E. coli* and *S. typhimurium* strains bearing plasmids with homologous and heterologous *prsA* genes

| Plasmid | Description | Sp act[a] (μmol of PRPP/min per mg of protein) | |
|---|---|---|---|
| | | *E. coli* JM83 | *S. typhimurium* SB139 |
| pBS201 | *S. typhimurium prsA* in pUC19 | 1.9 | 5.8 |
| pBS205 | *E. coli prsA* in pUC19 | 2.4 | 9.1 |
| pUC19 | | 0.1 | 0.3 |

[a] Growth was in LB medium with ampicillin at 37°C.

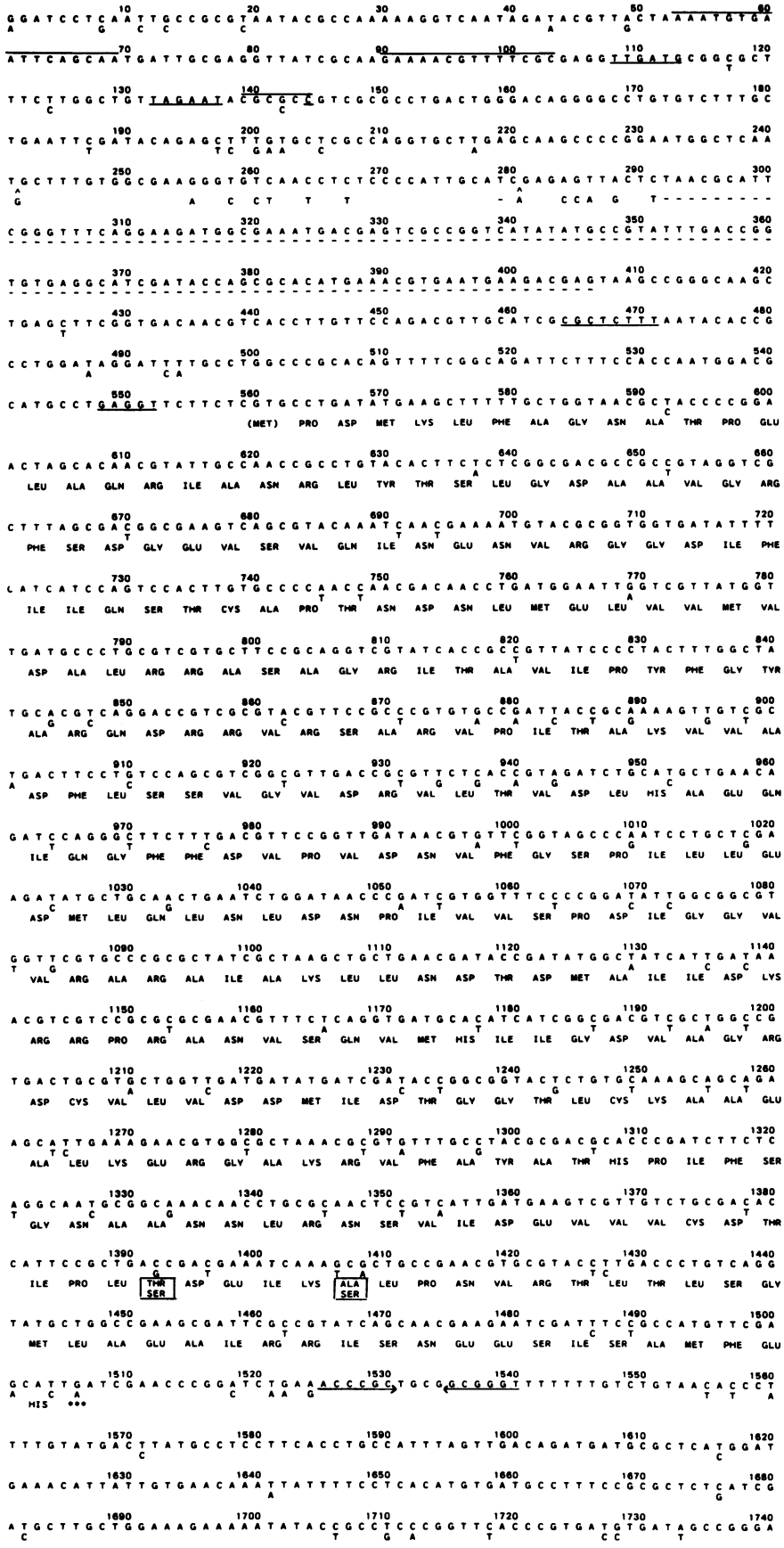

TABLE 3. Localization of elements essential for *S. typhimurium prsA* expression by deletion analysis

| Plasmid | Description | Sp act[a] (μmol of product/min per mg of protein) | |
|---|---|---|---|
| | | PRPP synthetase | β-Lactamase |
| pBS111R | bp 1–1738[b] | 4.7 | 3.7 |
| pBS201 | bp 59–1830 | 1.7 | 5.0 |
| pBS216 | bp 103–1738 | 1.4 | 4.4 |
| pBS203 | bp 182–1830 | 0.07 | NT |
| pUC19 | No insert | 0.04 | 6.5 |

[a] Growth was in Vogel-Bonner medium with 0.2% glucose at 30°C. The host strain was SB179. NT, Not tested.
[b] Inserts are in pUC19, i.e., with *prsA* transcription opposed to the plasmid-borne *lac* promoter.

TABLE 4. Evaluation of the role of the 5′ untranslated leader region of *prsA* by deletion analysis

| Plasmid | Description | Vector | Sp act[a] (μmol of product/min per mg of protein) | |
|---|---|---|---|---|
| | | | PRPP synthetase | β-Lactamase |
| pBS201 | bp 59–1830 | pUC19 | 1.7 | 5.0 |
| pBS214 | as pBS201 but bp 165–545 deleted | pUC19 | 0.04 | 4.8 |
| pBS202 | bp 182–1830 with $P_{lac}$ | pUC18 | 1.6 | 6.9 |
| pBS215 | bp 546–1830 with $P_{lac}$ | pUC18 | 0.11 | 5.2 |
| pUC19 | No insert | | 0.04 | 6.5 |

[a] Growth was in Vogel-Bonner medium with 0.2% glucose at 30°C. The host strain was SB179.

545 was removed from the *prsA*-bearing plasmids, expression was reduced to that attributable to the chromosomal gene (Table 4). This was true whether expression was driven by the *prsA* promoter (cf. pBS201 and pBS214) or by the *lac* promoter (cf. pBS202 and pBS215).

**Purification of PRPP synthetase.** *S. typhimurium* SB139 (*recA*) bearing plasmid pBS201 produced very high levels of PRPP synthetase at 42°C (22 μmol of PRPP per min per mg of protein). In one purification, 182 mg of pure enzyme was prepared from 2.5 liters of cell culture (6.3 g of cell paste) in a four-step procedure. This is a substantial improvement in comparison with 78 mg of enzyme from 200 liters of culture (500 g of cell paste) prepared by an earlier six-step procedure (21). An important element in obtaining such high levels of expression was growth of cells bearing pUC-derived plasmids at 42°C to stationary phase.

## DISCUSSION

The *S. typhimurium* gene encoding PRPP synthetase, *prsA*, has been cloned and sequenced. Strains containing plasmids with *prsA* could be induced to produce more than 200 times as much PRPP synthetase as is expressed by strain SU422, the strain previously used in enzyme purifications. Such strains have allowed purification of large amounts of enzyme required for chemical, enzymological, and structural studies.

The nucleotide sequence of the *S. typhimurium prsA* gene has allowed deduction of the amino acid sequence of PRPP synthetase. When compared with the deduced amino acid sequence for the *E. coli* protein (7), the two sequences are 99% identical. The two amino acid changes between the *E. coli* and *S. typhimurium* proteins are conservative replacements. A previous study has shown the physical, immunochemical, and kinetic properties of *E. coli* and *S. typhimurium* PRPP synthetases to be indistinguishable (7). A survey of 10 proteins whose genes have been sequenced from *E. coli* and *S. typhimurium* indicates that amino acid identity averages 92.4% (1). The amino acids which differ between the *E. coli* and *S. typhimurium* PRPP synthetases also diverge from the rat sequences. Thus, the *S. typhimurium* PRPP synthetase amino acid sequence, like that of *E. coli*, is 47% identical to the amino acid sequences of the two rat isozymes (23). These sequence comparisons indicate that PRPP synthetase is highly conserved over a considerable phylogenetic distance.

The region 5′ to the *S. typhimurium prsA* structural gene can be functionally divided into three sections: (i) upstream—*Bam*HI to *Nru*I (bp 1 to 103); (ii) promoter—*Nru*I to *Eco*O109 (bp 103 to 164); and (iii) leader—*Eco*O109 to *Sau*I (bp 164 to 545).

The upstream region contains a consensus CAP binding sequence and the pyrimidine conserved sequence. Constructions which alter or remove the CAP binding sequence reduce the expression of the *prsA* gene threefold. Whether the cyclic AMP-CAP system modulates the expression of the *prsA* gene has not yet been determined. The upstream region also contains the pyrimidine conserved region. Although this sequence was once postulated to be an operator, data now suggest that the pyrimidine conserved region is neither necessary nor sufficient for the nucleotide regulation of pyrimidine biosynthesis (C. L. Turnbough, Jr., personal communication).

The location of the promoter for the expression of the *E. coli prsA* gene has been identified by S1 mapping (7). The −35 and −10 regions and the transcription start site identified in *E. coli* are conserved in *S. typhimurium*. Comparison of PRPP synthetase expression from pBS201 and pBS203 (Table 3) confirms that the conserved region represents the major promoter for the *S. typhimurium prsA* gene.

The positioning of the *E. coli prsA* promoter results in a leader of 302 bp. The 115-bp insert in the *S. typhimurium* sequence extends the leader to 416 bp. Heterologous expression of the genes appears normal (Table 2), so the functional consequences of the insert, if any, are not clear. The leader region is required for maximal expression of the *prsA* gene. Expression from the *prsA* promoter is essentially eliminated when the leader sequence is removed. The previous observation that an *rpoBC* mutation results in derepression of

FIG. 3. DNA sequence of *S. typhimurium prsA* and deduced amino acid sequence of PRPP synthetase. Deviations in the *E. coli* nucleotide sequence are indicated below the corresponding bases in the *S. typhimurium* sequence. −, Deleted nucleotide; ∧, site of an inserted nucleotide; ***, termination codon. Deviations in the *E. coli* amino acid sequence are indicated below the corresponding *S. typhimurium* amino acids, and the residues are boxed. Promoter elements underlined are (i) −35 region, bp 108 to 113; (ii) −10 region, bp 132 to 137; and (iii) transcription start, bp 144 (7). The CAP consensus sequence, bp 53 to 69, pyrimidine conserved sequence, bp 90 to 103, and discriminator, bp 139 to 144, regions are overlined. A box A site, bp 464 to 471, and the ribosome-binding site, bp 548 to 552, are underlined. The dyad symmetry of a putative rho-independent terminator, bp 1525 to 1540, is marked with arrows.

PRPP synthetase (8), interpreted to indicate coupled transcription-translation control, is not yet explained. Deletion and mutational analyses of leader region function are now under way.

## ACKNOWLEDGMENTS

We thank John Roth and Charles L. Turnbough, Jr., for discussion and suggestions.

This work was supported by Public Health Service grant DK13488 from the National Institute of Diabetes and Digestive and Kidney Diseases.

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