The defective phosphoribosyl diphosphate synthase in a temperature-sensitive *prs-2* mutant of *Escherichia coli* is compensated by increased enzyme synthesis

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An *Escherichia coli* strain which is temperature-sensitive for growth due to a mutation (*prs-2*) causing a defective phosphoribosyl diphosphate (PRPP) synthase has been characterized. The temperature-sensitive mutation was mapped to a 276 bp *Hind*III–*Bss*HI DNA fragment located within the open reading frame specifying the PRPP synthase polypeptide. Cloning and sequencing of the mutant allele revealed two mutations. One, a G → A transition, located in the ninth codon, was responsible for the temperature-conditional phenotype and resulted in a serine residue at this position. The wild-type codon at this position specified a glycine residue that is conserved among PRPP synthases across a broad phylogenetic range. Cells harbouring the glycine-to-serine alteration specified by a plasmid contained approximately 50% of the PRPP synthase activity of cells harbouring a plasmid-borne wild-type allele, both grown at 25 °C. The mutant enzyme had nearly normal heat stability, as long as it was synthesized at 25 °C. In contrast, there was hardly any PRPP synthase activity or anti-PRPP synthase antibody cross-reactive material present in cells harbouring the glycine to serine alteration following temperature shift to 42 °C. The other mutation was a C → T transition located 39 bp upstream of the G → A mutation, i.e. outside the coding sequence and close to the Shine–Dalgarno sequence. Cells harbouring only the C → T mutation in a plasmid contained approximately three times as much PRPP synthase activity as a strain harbouring a plasmid-borne wild-type *prs* allele. In cells harbouring both mutations, the C → T mutation appeared to compensate for the G → A mutation by increasing the amount of a partially defective enzyme at the permissive temperature.

Keywords: phosphoribosylpyrophosphate, PRPP synthase, RNA analysis, nucleotide synthesis, *prs* gene

INTRODUCTION

In *Escherichia coli* the compound 5-phospho- α -D-ribose 1-diphosphate (phosphoribosylpyrophosphate, PRPP) is a precursor in the biosynthesis of the purine, pyrimidine and pyridine nucleotides, as well as the amino acids histidine and tryptophan (Hove-Jensen, 1988, 1989). The

synthesis of PRPP is catalysed by PRPP synthase (ribose-phosphate pyrophosphokinase, EC 2.7.6.1): ribose-5-phosphate + ATP → PRPP + AMP. The gene specifying PRPP synthase of *E. coli* and *Salmonella typhimurium*, designated *prs*, has been the subject of extensive analysis. The gene from both organisms has been mapped (Hove-Jensen, 1983; Jochimsen *et al.*, 1985), cloned and sequenced (Bower *et al.*, 1988; Hove-Jensen, 1985; Hove-Jensen *et al.*, 1986). In *S. typhimurium* two mutants have been isolated by a physiological selection (Jochimsen *et al.*, 1985) or by screening mutants for temperature-sensitive PRPP synthase activity (Pandey & Switzer,

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Abbreviation: PRPP, 5-phospho- α -D-ribose 1-diphosphate.

Table 1. Bacterial strains

Strain	Genotype	Source/construction
HO336	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi</i>	Silhavy <i>et al.</i> (1984)
HO340	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi supF</i>	Nilsson & Hove-Jensen (1987)
HO371	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi supF hemA</i>	Nilsson & Hove-Jensen (1987)
HO447	<i>supF relA spoT rpsL lamB metB purE zbc::Tn10 deoD gsk-3</i>	Hove-Jensen (1989)
HO449	<i>supF relA spoT rpsL lamB metB deoD udp gsk-3 pncA hemA zjj::Tn10</i>	HO650 × P1(Sø1390), Tet ^r
HO497	<i>recB21 recC22 sbcB15 thr leu proA2 hisG argE thi rpsL zjj::Tn10 deoD</i>	JC7623 × P1(HO449), Tet ^r
HO498	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD thr zjj::Tn10</i>	HO336 × P1(HO497), Tet ^r
HO499	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD</i>	HO498 × P1(HO605), Thr ⁺
HO500	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD purE gsk-3 zbb::Tn10</i>	HO499 × P1(HO447), Tet ^r
HO541	<i>metB his trp_{am} leu_{am} lacZ_{am} galK_{am} galE rpsL sueB sueC supD43,74 prs-2</i>	Hove-Jensen (1985)
HO600	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD gsk-3</i>	HO500 × P1(HO605), Pur ⁺
HO605	<i>supF relA spoT rpsL lamB metB deoD udp::Tn5 gsk-3 pncA hemA</i>	Hove-Jensen (1988)
HO608	<i>supF relA spoT rpsL lamB metB deoD udp::Tn5 gsk-3 pncA</i>	Hove-Jensen (1989)
HO609	<i>supF relA spoT rpsL lamB metB deoD udp::Tn5 gsk-3 pncA prs-2</i>	Hove-Jensen (1988)
HO611	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi supF prs-2</i>	HO371 × P1(HO541), Hem ⁺
HO650	<i>supF relA spoT rpsL lamB metB deoD udp gsk-3 pncA hemA</i>	Hove-Jensen (1989)
HO697	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD gsk-3 udp::Tn5</i>	HO600 × P1(HO605), Kan ^r
HO700	<i>supF relA spoT rpsL lamB metB deoD udp gsk-3 pncA Δ(prs-3)::Kan^r</i>	Hove-Jensen (1989)
HO728	<i>supF relA spoT rpsL lamB metB deoD udp gsk-3 pncA Δ(prs-4)::Kan^r</i>	Hove-Jensen (1989)
HO764	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD gsk-3 udp</i>	HO765 × P1(Sø928), Ilv ⁺
HO765	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi deoD gsk-3 ilvD</i>	HO697 × P1(PS1500), Udp ⁺
HO770	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi supF deoD gsk-3 udp</i>	HO764 × P1(HO340), Trp ⁺
HO773	<i>araC_{am} araD Δ(lac)U169 trp_{am} rpsL relA thi supF udp deoD gsk-3 Δ(prs-4)::Kan^r</i>	HO770 × P1(HO728), Kan ^r
JC7623	<i>recB21 recC22 sbcB15 thr leu proA2 hisG argE thi rpsL</i>	Kushner <i>et al.</i> (1971)
JM83	<i>ara Δ(lac-proAB) rpsL thi φ80d lacZΔM15</i>	Yanisch-Perron <i>et al.</i> (1985)
PS1500	<i>ilvD pro metB argH thi lac gal mal rpsL supE rimC</i>	CGSC*
Sø928	<i>Δ(deoCABD) Δ(lac) thi upp udp ton</i>	Valentin-Hansen <i>et al.</i> (1978)
Sø1390	<i>leu thi zjj::Tn10</i>	K. Hammer†

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1982; Post & Switzer, 1991). In *E. coli* a number of *prs* mutants have been isolated and characterized. They include a mutant with altered kinetic properties of PRPP synthase (Bower *et al.*, 1989; Hove-Jensen & Nygaard, 1982) and genetically engineered *prs* deletion mutations constructed *in vitro* and subsequently transferred to the chromosome by homologous recombination (Hove-Jensen, 1988, 1989). In addition, a mutant that is

temperature-sensitive for growth due to a mutation in the *prs* gene has been isolated and characterized. The mutant allele responsible for the conditional growth is designated *prs-2* (Hove-Jensen, 1985). This mutant strain was isolated by localized mutagenesis of the *prs* region of the chromosome following the preparation of a bacteriophage P1 lysate in a *mutD*-mutator strain and transfer of the mutagenized DNA to the *E. coli* chromosome by trans-

duction. This report presents an analysis of the biochemical nature of the *prs-2* mutation.

METHODS

Bacterial strains, growth conditions and plasmid vectors. The *E. coli* strains used, and their derivation or source, are given in Table 1. Strains which harbour *prs* null mutations (Δ *prs-3* or Δ *prs-4*) are viable provided that guanosine, uridine, histidine, tryptophan and NAD are added to the growth medium (Hove-Jensen, 1989), whereas strains harbouring the *prs-2*_{ts} allele can be maintained at 25 °C without these additions (Hove-Jensen, 1988). Growth media were either LB medium (Miller, 1972) or AB minimal salts medium (Clark & Maaløe, 1967). The carbon source was glucose (0.2%). Thiamin (1 mg l⁻¹) was added routinely to all minimal media. When necessary, compounds were added to the following final concentrations: amino acids to 50 mg l⁻¹ each; uridine and guanosine to 40 mg l⁻¹ each. NAD was added to both LB and minimal media at 40 mg l⁻¹. Transduction was performed as described by Miller (1972). Plasmids were selected and maintained by the presence of ampicillin, 50 mg l⁻¹. Kanamycin and tetracycline were used at concentrations of 30 and 10 mg l⁻¹, respectively. pBR322 was used as a plasmid vector (Bolivar *et al.*, 1977).

Manipulation of nucleic acids. Procedures for the isolation of chromosomal DNA as well as plasmid DNA either on a small scale for screening or by buoyant-density centrifugation in CsCl gradients containing ethidium bromide, have been previously described (Birnboim & Doly, 1979; Sambrook *et al.*, 1989; Silhavy *et al.*, 1984). Transformation with plasmid DNA was performed as described by Mandel & Higa (1970). Restriction endonucleases were used as recommended by the suppliers (Boehringer Mannheim, Promega or New England Biolabs). Calf intestinal alkaline phosphatase and T4 DNA ligase were from Boehringer Mannheim. DNA fragments were isolated with the Gene Clean kit (Bio-101). Methods for Southern blot analysis (Sambrook *et al.*, 1989) and nucleotide sequencing (Post *et al.*, 1993; Sanger *et al.*, 1977) have been described previously. Procedures for the isolation of RNA, blotting of RNA onto nylon membranes, and hybridization with ³²P-labelled RNA probes, have been described previously (Post *et al.*, 1993).

Western blot analysis. The antibody preparation used was raised in a rabbit against highly purified *S. typhimurium* PRPP synthase (Post & Switzer, 1991). Cross-reactive material was determined by immunoblotting employing ¹²⁵I-protein A, according to the general procedure of Grandoni *et al.* (1989).

Enzyme assays. PRPP synthase activity was determined by the ³²P-transfer assay (Switzer & Gibson, 1978) with the modifications described previously (Bower *et al.*, 1988), except that the concentrations of the substrates were: 5 mM ribose 5-phosphate and 3 mM ATP. The Mg²⁺ concentration was 5 mM. β -Lactamase was assayed with cephaloridine as the substrate as previously described (Lupski *et al.*, 1984). The change in absorbance was monitored at 255 nm. Protein concentration was determined by the bicinchoninic acid procedure with chemicals provided by Pierce (Smith *et al.*, 1985).

RESULTS

Mapping of the temperature-sensitive lesion of *prs-2*

To map the lesion responsible for the temperature-sensitive growth of strain HO611 (*prs-2*) a series of *prs* non-complementing plasmids, containing deletions extending into the *prs* gene from either end of the coding sequence, was transformed into the *prs-2* strain followed

Fig. 1. Characterization of the *prs-2* allele. (a) Mapping of the temperature-sensitive lesion. A restriction map of pHO11 (Hove-Jensen, 1985) is shown at the top. Below are indicated the structure of the inserts of the plasmids pHO273, pHO271, pHO10 and pHO272, which were constructed as follows: pHO273 was constructed by digestion of pHO11 DNA with HindIII, followed by S1 nuclease treatment, PvuII digestion and ligation; pHO271 was constructed by digestion of pHO11 DNA with BssHII, S1 nuclease and PvuII followed by ligation; pHO272 was obtained by digestion of pHO11 DNA with EcoRI and BssHII, followed by S1 nuclease digestion and ligation; pHO10 was described before (Hove-Jensen, 1985). The coding sequence of the *prs* gene is shown as a black region, the promoter and leader regions are shaded, and flanking sequences are unshaded. On the right is indicated the ability of each plasmid to provide, by homologous recombination, a temperature-resistant *prs* allele following transformation of strain HO611 (*prs-2*_{ts}): +, ability to give temperature-resistant recombinants; –, inability to give temperature-resistant recombinants. (b) Nucleotide sequence of the *prs-2* allele. The stretch of nucleotides constitutes the wild-type sequence, with numbers referring to the previously published sequence (Hove-Jensen *et al.*, 1986). The amino acid sequence of the N-terminal end of the PRPP synthase subunit is shown below the nucleotide sequence. The two nucleotide alterations are given immediately below the wild-type nucleotide sequence, as well as the resulting alteration of the amino acid specified by the ninth codon. The Shine–Dalgarno sequence is underlined. Relevant restriction recognition sites are shown. (c) Separation of the two mutations of the *prs-2* allele. The structure of pHO11 with relevant restriction endonuclease recognition sites is shown at the top. The designation of the different regions is the same as that used in (a). The point mutations found in *prs-2* are indicated. The DNA fragments containing the mutations were subcloned from pDPH200 (*prs-2*) into pHO11 (similarly digested), to make a series of isogenic plasmids harbouring functional *prs* alleles with none, one or both of the point mutations as follows: pDPH201 contained the EcoRI–BssHII DNA fragment from pDPH200; pDPH202 contained the HindIII–BssHII DNA fragment from pDPH200; and pDPH203 contained the EcoRI–HindIII DNA fragment from pDPH200.

Table 2. PRPP synthase and β -lactamase activities in strains harbouring plasmids with various *prs* mutations

The data are from one experiment. Enzyme activities varied less than 20 % in duplicate experiments. ND, Not determined.

Plasmid*	Mutation(s) present	Growth temp. (°C)	PRPP synthase activity [$\mu\text{mol min}^{-1}$ (mg protein) $^{-1}$] $\dagger$		β -Lactamase activity $\ddagger$ [$\Delta A_{255} \text{ min}^{-1}$ (mg protein) $^{-1}$]
			25 °C	42 °C	
pHO11	None	25	0.76	3.6	2.2
		42	1.60	9.1	3.6
pDPH201	C $\rightarrow$ T, G $\rightarrow$ A	25	1.73	5.7	2.1
		42	0.05	0.13	3.0
pDPH202	G $\rightarrow$ A	25	0.39	1.1	2.1
		42	0.04	0.10	3.5
pDPH203	C $\rightarrow$ T	25	2.57	ND	1.5
		42	2.58	ND	3.7

* The host strain was HO700 ($\Delta prs-3$). Cells were grown in LB supplemented with guanosine, uridine and NAD.

$\dagger$ PRPP synthase activity was determined at the temperature indicated as described in Methods.

$\ddagger$ β -Lactamase activity was determined at 37 °C as described in Methods.

by a simultaneous selection for antibiotic-resistant and temperature-resistant (42 °C) recombinants, i.e. a selection for a wild-type *prs* allele. Thus, a plasmid with an insert which straddles the mutation causing the temperature-sensitive phenotype was expected to result in recombinants that were cured of this defect, whereas an insert which covered regions outside the mutation would be unable to cure the temperature defect. From the results given in Fig. 1(a), it appears that the lesion responsible for the temperature-sensitive phenotype was located between the *Hind*III and *Bss*HII restriction endonuclease recognition sites, i.e. a DNA fragment 276 bp in length. As expected, this location was within the open reading frame specifying the PRPP synthase polypeptide.

Cloning of the *prs-2* mutant allele

Southern blotting of DNA isolated from strain HO611 (*prs-2*) and hybridization with *prs* DNA as a probe revealed that the *prs-2* allele was located in a 2.3 kb DNA fragment generated by *Eco*RI (data not shown). This was expected from the restriction maps previously published (Hove-Jensen, 1985; Kohara *et al.*, 1987). Chromosomal DNA was then isolated from strain HO611 (*prs-2*) and digested by *Eco*RI, followed by fragment separation by agarose gel electrophoresis. A gel piece containing DNA fragments of 2.1–2.5 kb was excised and the DNA was purified. The fragments were ligated to pBR322 DNA that had been previously digested by *Eco*RI. Ligation products were amplified in strain JM83, and plasmid DNA was extracted from the pooled transformants and used to transform strain HO773 ($\Delta prs-4$) to *Prs*⁺ at 25 °C. One isolate, pDPH200, was used for further analysis.

Characterization of the *prs-2* allele

To determine the mutations in the isolated plasmids containing the *prs-2* allele, the region encompassing the *Eco*RI to *Bss*HII restriction sites in pDPH200 was sequenced (Fig. 1b). Two mutations were found in the DNA, one at bp 500 (C $\rightarrow$ T) and another at bp 539 (G $\rightarrow$ A), with numbers referring to the previously published sequence (Hove-Jensen *et al.*, 1986). The first mutation was 3 bp upstream of the Shine–Dalgarno sequence (Shine & Dalgarno, 1974); the second was the first base of the ninth codon and changed the glycine codon to a serine codon. Since the *prs-2* allele had a temperature-sensitive phenotype, we wanted to determine which of these mutations was responsible for the temperature sensitivity, and plasmids were constructed that contained either one or both of the mutations. Fortuitously there was a unique *Hind*III recognition site between the two mutations, which were separated by only 38 bp, facilitating the construction of the plasmids pDPH201, 202 and 203 (Fig. 1c). Strain HO700 ($\Delta prs-3$) harbouring each of these plasmids was grown at both 25 and 42 °C, and the cells were harvested in the exponential growth phase. The PRPP synthase activity in each plasmid-harbouring strain was then determined (Table 2). β -Lactamase activities were also determined to assess relative plasmid copy number. Both of the mutations had a substantial effect on the amount of PRPP synthase activity in the cell. When only the upstream mutation (C $\rightarrow$ T) was present, about 3.4-fold more PRPP synthase activity was present at 25 °C compared to that present in the wild-type. On the other hand, the presence of only the downstream mutation reduced the level of PRPP synthase activity to about 50 % of the wild-type activity. When both mutations were

Fig. 2. Northern blot analysis of *prs*⁺ and *prs-2*-specified mRNA transcripts. Five micrograms of RNA was loaded in each lane, electrophoresed and blotted as described in Methods. The blot was hybridized with a probe of the *E. coli prs* gene. The arrow indicates the 1300 nt *prs*-specified transcript. RNA was purified from a wild-type strain (HO608, lanes 1 and 3) and a *prs-2* strain (HO609, lanes 2 and 4) grown at 25 °C (lanes 1 and 2), or 42 °C (lanes 3 and 4).

present, the downstream mutation was epistatic to the upstream mutation, i.e. the downstream mutation reduced the activity produced by the upstream mutation – compare plasmids pDPH201 with pDPH203 grown at 25 °C – but the PRPP synthase activity was still about double that of the wild-type (pHO11) grown at 25 °C. This analysis also showed that the temperature-sensitivity of this PRPP synthase was caused by the downstream mutation located within the PRPP synthase reading frame (plasmids pDPH201 and pDPH202 compared with pHO11 and pDPH203 grown at 25 and 42 °C; Table 2). As shown previously for the chromosomal *prs-2* allele (Hove-Jensen, 1988), the plasmid-borne *prs-2* allele conferred temperature-sensitivity upon the host strain *in vivo*. Cells deleted for the chromosomal *prs* gene and harbouring a plasmid-borne *prs-2* allele were still able to grow at an elevated temperature (38 °C), but the amount of PRPP synthase was 30-fold lower than that of the same cells grown at 25 °C (data not shown). Since the mutation conferring temperature sensitivity was mapped to the polypeptide coding region, we would predict that the protein itself would be temperature-labile. However, if an extract of strain HO700 ($\Delta prs-3$), bearing a plasmid with the *prs-2* allele, was prepared from cells grown at 25 °C, and assayed for PRPP synthase activity at 42 °C, the *prs-2*-specified enzyme activity was almost as stable as the wild-type enzyme. Wild-type PRPP synthase activity increased four- to fivefold as the assay temperature was increased from 25 to 42 °C, whereas the *prs-2*-specified mutant enzyme activity increased by approximately threefold (Table 2: pDPH201 compared with pHO11). Thus, the mutant enzyme that had been synthesized at 25 °C appeared almost as stable *in vitro* as the wild-type enzyme.

Effects of temperature on the transcription and translation of the *prs-2* allele

Since the *prs-2* allele was a double mutation and one of the mutations was outside the coding sequence, we wished to determine if *prs* transcription or translation was altered in the *prs-2* strain. To investigate this possibility, *prs* mRNA was analysed by Northern blot analysis and PRPP

Fig. 3. Western immunoblot analysis of *E. coli prs*⁺ (HO608) and *prs-2* (HO609) strains. Western immunoblot analysis was carried out with 50 µg of crude extract from wild-type and mutant strains harvested in exponential and early stationary phases, from cultures grown at both 25 and 42 °C. Above each lane is indicated which strain was analysed (+, wild-type; –, *prs-2*), and the temperature and phase of growth (E, exponential; S, stationary) during which the sample was harvested. Both strains were initially grown at 25 °C (lanes 1–4); then a portion from each culture in stationary phase was inoculated into fresh medium at 42 °C. Samples were harvested from the 42 °C cultures in the exponential phase (lanes 5 and 6); and the stationary phase (lanes 7 and 8). The arrow indicates position of PRPP synthase.

synthase synthesis was analysed by Western immunoblot analysis with RNA and protein isolated from strains HO608 (*prs*⁺) and HO609 (*prs-2*). The results of the Northern analysis indicated that the *prs-2* strain contained two- to threefold more *prs*-specific transcript (Fig. 2) than the wild-type strain at 25 and 42 °C, respectively. Moreover, there was apparently no reduction in the amount of *prs-2*-specified mRNA in cells grown at 42 °C as compared to those grown at 25 °C. Thus, the temperature-sensitivity of cells harbouring *prs-2* was not caused by a temperature-sensitive transcription process.

The results of Western immunoblot analysis of extracts from strains HO608 and HO609 are shown in Fig. 3. It appeared that almost identical amounts of cross-reactive material were present in *prs*⁺ and *prs-2* cells grown at 25 °C. This was the case whether the cells were harvested in the exponential or in the stationary growth phase (Fig. 3, lanes 1–4). However, when cells were harvested several doubling times after a temperature shift to 42 °C, the amount of cross-reactive material in the *prs-2* strain was greatly reduced and eventually virtually undetectable, whereas the amount of cross-reactive material in the *prs*⁺ strain was hardly altered (Fig. 3, lanes 5–8). It is noteworthy that although the *prs-2* strain apparently contained more *prs*-specific transcript than the *prs*⁺ strain at 25 °C, it still had approximately the same amount of anti-PRPP synthase cross-reactive material as the wild-type (Fig. 3, lanes 1 and 2). Both cross-reactive material and enzyme activity were detected at greatly reduced levels in the *prs-2* strain following the temperature shift to 42 °C. This result indicated that the mutant enzyme was labile and possibly rapidly degraded at the elevated temperature.

DISCUSSION

Two separate point mutations were identified in the *prs-2* allele. The first mutation was located 3 bp upstream of the Shine–Dalgarno sequence. This mutation, a C → T tran-

E. c.	MPDMKLFAGNATPELAQRIANRLYTSGLGDAVGRFSDGEVSVQI	44
S. t.	MPDMKLFAGNATPELAQRIANRLYTSGLGDAVGRFSDGEVSVQI	44
B. s.	MSNQYGDKNLKFSLNSNPDLAKETADIVGVLGKCSVTRFSDGEVQINI	50
A. t.	KSVNRTNTRLLKFSOTANPALAQELAWYGLDLGKVNIRFADGEIVQL	81
L. d.	TEKRDYSRPFCLVSGNGNRPLAEAVALLMGTHHTHTSVTQYSNGEVNRI	87
C. e.	MPNIVFSGSSHPDLSTRICERLQLEVSKASLKKFSNKETNVEI	44
HI/HIII/RI	MPNIVFSGSSHPDLSTRICERLQLEVSKASLKKFSNKETNVEI	44
HII	MPNIVFSGSSHPDLSTRICERLQLEVSKASLKKFSNKETNVEI	44
RII	MPNIVFSGSSHPDLSTRICERLQLEVSKASLKKFSNKETNVEI	44

Fig. 4. Comparison of N-terminal sequences of PRPP synthases of various organisms. The sequences are aligned to give the highest degree of homology. Numbers to the right indicate the amino acid position of the residue at the right-hand end of each line. Abbreviations: *E. c.*, *E. coli* (Hove-Jensen *et al.*, 1986); *S. t.*, *S. typhimurium* (Bower *et al.*, 1988); *B. s.*, *Bacillus subtilis* (Nilsson *et al.*, 1989); *A. t.*, *Arabidopsis thaliana* (B. N. Krath & B. Hove-Jensen, EMBL accession no. X83764); *L. d.*, *Leishmania donovani* (Hendrickson *et al.*, 1993); *C. e.*, *Caenorhabditis elegans* (R. Wilson and others, GenBank accession no. U00036.); HI, HII and HIII, human PRPP synthase I, II and III, respectively (Roessler *et al.*, 1990; Taira *et al.*, 1990); RI and RII, rat PRPP synthase I and II, respectively (Taira *et al.*, 1987). All the sequences are deduced from nucleotide sequences. The *E. coli* and *S. typhimurium* sequences have also been determined by automated Edman degradation (Harlow & Switzer, 1990; Hove-Jensen *et al.*, 1986). The *L. donovani* enzyme has a 37 amino acid extension and the *A. thaliana* enzyme a 32 amino acid extension at their N-terminal ends, which are not shown. The sequences of human PRPP synthases I and III, as well as rat PRPP synthase I are identical within the segment shown. Filled circles indicate positions with identical amino acid among all the sequences; open circles indicate positions with conservative replacements.

sition, apparently did not create a better complementarity to the 16S rRNA sequence. Yet a strain carrying a plasmid-borne copy of this point mutation contained 3.4-fold more PRPP synthase activity than the same strain carrying the wild-type allele on an identical plasmid. Although the mechanism by which this mutation increased the synthesis of PRPP synthase remains unknown, it is possible that it improved ribosome binding by sequences in addition to the Shine-Dalgarno sequence. The second mutation was a G → A transition, which was situated in the ninth codon and resulted in the conversion of a glycine residue of the wild-type to a serine residue. This mutation was solely responsible for the temperature-sensitivity of the *prs-2* allele and was detrimental to production of the enzyme. A plasmid-borne copy of the latter mutation produced only about half as much PRPP synthase activity as was formed in cells carrying the wild-type gene on an identical plasmid. It was also observed that the downstream mutation was epistatic to the upstream mutation. That is, the downstream mutation caused a decrease in the amount of enzyme produced, when the upstream mutation was also present, from 3.4- to only 2-fold over wild-type PRPP synthase synthesis in cells grown at 25 °C. Also, the presence of the downstream mutation resulted in a temperature-sensitive phenotype, whether the upstream mutation was present or not. When a *prs-2* strain was grown at 25 °C, the PRPP synthase formed was almost as stable at 42 °C *in vitro* as the wild-type enzyme at 42 °C. However, Western immunoblots of crude extracts of a *prs-2* strain that had been grown at 42 °C showed very little cross-reactive material. The simplest explanation for the lack of mutant PRPP

synthase protein at 42 °C is degradation of the enzyme, although failure to synthesize the protein at 42 °C cannot be excluded. We believe that the G → A transition within the coding sequence appeared due to the screening procedure, i.e. temperature-sensitivity, and that the C → T transition appeared subsequently to compensate for a malfunctioning enzyme. Consequently, separation of the two mutations on the chromosome might result in the appearance of other secondary mutations. The separation of the two specific mutations as well as the analysis of their phenotypes was therefore performed on plasmids.

The function of various amino acid residues in PRPP synthases has been studied to some extent. The glycine residue that is altered by the *prs-2* allele is highly conserved, as indicated by the amino acid comparison shown in Fig. 4. All the PRPP synthase sequences, including those from Gram-negative and Gram-positive bacteria and various eukaryotic organisms, have a glycine residue at this position, except for *Bacillus subtilis*, which has a leucine residue. This conservation indicates an important function of the glycine residue, most likely in maintaining the structure of the polypeptide. It is therefore conceivable that the mutant enzyme did not fold correctly when it was synthesized at the restrictive temperature. Another indication of the importance of the N-terminal segment of PRPP synthase in maintaining the stability of the enzyme comes from the work of Ahmad *et al.* (1994), who used recombinant chimeric forms of human PRPP synthase isozymes to implicate amino acid residues 17, 18 and/or 41 as determining differences in the susceptibility of the isozymes to thermal denaturation and inactivation by proteolytic digestion.

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