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Identification of the Enzymatic Reactions Encoded by the *purG* and *purI* Genes of *Escherichia coli*

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The chromosomal locations of the genes *purG* and *purI* on the *Escherichia coli* linkage map are the opposites of those of *Salmonella typhimurium*. By methods which permit the identification of lesions in any of the five early enzymes of the purine de novo pathway, the gene-enzyme relationships of the *purG* and *purI* loci have been reevaluated in these two organisms. The results demonstrate that the relative locations of the genes encoding the two enzymes (phosphoribosylformylglycinamidine synthetase and phosphoribosylaminoimidazole synthetase) are similar in the two organisms. The gene products have been correctly determined in *S. typhimurium*. The gene products currently listed for the loci in *E. coli* are incorrect. The *E. coli purG* locus is equivalent to the *S. typhimurium purI* locus, and the *E. coli purI* locus is equivalent to the *S. typhimurium purG* locus.

In *Salmonella typhimurium* and in *Escherichia coli*, 14 enzymatic reactions are involved in the de novo biosynthesis of AMP and GMP. Twelve genes encoding the enzymes involved have been localized on the *S. typhimurium* chromosome. The genes occur mostly as unlinked single units or as small functional operons *guaA* and *B* and *purJ*, *H*, and *D* (1, 2, 8). One of the genes, *purB*, encodes a bifunctional enzyme known to catalyze two reactions of the pathway (2). In *E. coli*, analogous positions have been found for nine of the genes, and the positions for *purI* and *purG* are reversed (7, 11). *purJ* mutants have not been identified (1, 9). Mutants with a defective phosphoribosylglycinamide (GAR) formyltransferase (see Table 1) have not been found for either *S. typhimurium* or *E. coli*.

Mutations affecting the biosynthesis of inosine monophosphate can be classified into two groups. The first group includes defects in any of the five early enzymes, and the second group includes defects in any of the five late enzymes. Mutants which are defective in one of the five late enzymes excrete diazotizable amines when starved for purines (10, 12), whereas mutants blocked in one of the early steps (Table 1) do not. We now present evidence that the gene products assigned to the *purG* and *purI* loci are incorrect in *E. coli*. This was ascertained by determining phosphoribosylformylglycinamidine (FGAM) synthetase activity in *purG* and *purI* mutant strains, and by determining the accumulation of intermediary compounds (phosphoribosylformylglycinamide [FGAR] and FGAM) of the purine de novo pathway during purine starvation.

We observed that early purine auxotrophs, (*purF*, *purD*, *purG*, and *purI*) of *S. typhimurium* accumulated phosphoribosylpyrophosphate when starved for purines. The most dramatic accumulation is seen in *purF* mutants (4). Furthermore, *purG* mutants accumulate FGAR, and *purI* mutants accumulate both FGAR and FGAM, when starved for purines (Fig. 1A). Phosphoribosylamine accumulation is not observed with *purD* mutants, presumably owing to the lability of the compound (12). Mutants defective in the synthesis of the third enzyme of the pathway could be expected to accumulate GAR when starved for purines. When a similar approach was used with *E. coli* purine auxotrophs, there was a different pattern in the accumulation of FGAR and FGAM by particular mutants. Specifically, a purported *purG* mutant accumulated FGAR and FGAM, but a purported *purI* mutant accumulated only FGAR when starved for purines (Fig. 1B). To confirm the nature of the indicated enzyme deficiency, enzymatic analyses were carried out on cell-free extracts.

For the determination of FGAM formation in cell-free extracts, we developed a new assay which also allows the determination of FGAR formation. In principle, it is an improvement and simplification of the assay developed by Westby and Gots (12) for GAR and FGAR synthesis, an assay which involves the sequential action of the first enzymes of the de novo pathway.

The results for the mutant strains harboring defects in the early enzymes of the purine de

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TABLE 1. Gene enzyme relationships of the five initial reactions in the purine de novo biosynthetic pathway of *S. typhimurium* and *E. coli*

Enzyme	Reaction	Gene	Map position[a]	
			S. typhimurium	*E. coli*
	Phosphoribosylpyrophosphate			
Amidophosphoribosyltransferase (EC 2.4.2.14)	↓	*purF*	49	49
	Phosphoribosylamine			
GAR synthetase (EC 6.3.4.13)	↓	*purD*	90	90
	GAR			
GAR formyltransferase (EC 2.1.2.2)	↓	None		
	FGAR			
FGAM synthetase (EC 6.3.5.3)	↓	*purG*[b]	57	55
	FGAM			
Phosphoribosylaminoimidazole synthetase (EC 6.3.3.1)	↓	*purI*[c]	54	53
	Phosphoribosylaminoimidazole			

[a] Numbers refer to the unit scales on the linkage maps (1, 8).
[b] Now called *purL* in *E. coli* (formerly *purI*).
[c] Now called *purM* in *E. coli* (formerly *purG*).

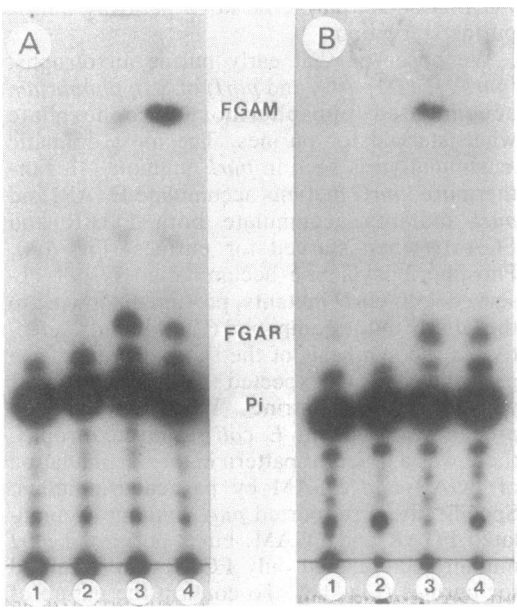

FIG. 1. Autoradiograms of one-dimensional chromatograms. (A) ^{32}P-labeled extracts of *S. typhimurium* mutant strains. Lane 1, *purF*; lane 2, *purD*; lane 3, *purG*; lane 4, *purI*. (B) ^{32}P-labeled extracts of *E. coli* mutants. Lane 1, *purF*; lane 2, *purD*; lane 3, *purG* (PC0631); lane 4, *purI* (PA3306). The original gene designations are used (see Table 2). Cells were grown exponentially in glucose minimal medium containing 0.3 mM phosphate (4). At an optical density at 436 nm of 1, the cells were harvested and suspended in fresh medium with no hypoxanthine added but containing $^{32}P_i$ (33 μCi/μmol). After 2 h of incubation, the cells were extracted with formic acid (4). The extracts (10 μl) were chromatographed on polyethyleneimine plates (20 by 20 cm) as described in Table 2, footnote *b*. For the identification of FGAR and FGAM, the presumed FGAR and FGAM spots were eluted with 1 M ammonium acetate and were lyophilized. The presumed FGAR and FGAM cochromatographed with authentic FGAR and FGAM (3) in several chromatographic systems, and both compounds could be ^{14}C labeled from [^{14}C]formate and [U-^{14}C]glycine.

novo pathway are given in Table 2. The *S. typhimurium purG* strain had no FGAM synthetase activity, as expected, but the *E. coli purG* strain showed activity. The reverse pattern was observed with the *purI* strains; the *purI* mutant of *S. typhimurium* possessed FGAM synthetase activity, but the *E. coli purI* mutant showed no activity. On the current *E. coli* linkage map, the purine de novo gene which is located close to *upp* is *purG* (11), and yet FGAM synthetase activity was observed in the *upp-purG* deletion mutant of *E. coli*. From the results, it is evident that the enzymatic defects of the *purG* and *purI* genes in *E. coli* have been misinterpreted. In agreement with our data, it has been observed that ColE1-*purI* plasmids which complemented *S. typhimurium purI* mutants also complemented *purG*, but not *purI*, mutants of *E. coli* (J. S. Gots, personal communication). We suggest that the locus of the allele in PA3306 (formerly *purI*) be called *purL* and that the locus of the allele in PC0631 and SØ1432 (formerly *purG*) be called *purM*.

The methods used in the present work may be applied to other microorganisms for the identifi-

TABLE 2. Bacteria used in this study

Strain	Relevant genotype[a]	Product formation[b] (nmol/min per mg of protein)		Source
		FGAR	FGAM	
E. coli				
AB1931	*purF1*	<0.1[c]	<0.1[c]	G. Eggertsson[d]
AB468	*purD13*	<0.1[c]	<0.1[c]	A. L. Taylor[d]
PC0631	*purG48*	65	15	P. G. de Haan[d]
PA3306	*purI66*	42	<0.1	Lavallé[d]
SØ1432	Δ (*upp-purG*)	83	42	J. Quinto and B. Mygind
S. typhimurium				
TT317	*purF*::Tn*10*	<0.1[c]	<0.1[c]	J. R. Roth via J. S. Gots
TT311	*purD*::Tn*10*	<0.1[c]	<0.1[c]	
TT293	*purG*::Tn*10*	94	<0.1	
TT11	*purI*::Tn*10*	103	52	

[a] In agreement with the results presented, *E. coli purG* should be designated *purM*, whereas *purI* should be named *purL*.

[b] Cultures for enzymatic analyses were grown in glucose minimal medium at 37°C (6). Amino acids were added to a final concentration of 40 μg/ml, hypoxanthine to 15 μg/ml, and thiamine to 1 μg/ml. Cell growth was monitored in an Eppendorf photometer at 436 nm. An optical density of 1 (1 cm light path) corresponds to about 3×10^8 cells per ml. Cells were grown exponentially for several generations. At an optical density of 0.6, the cells were transferred to fresh medium without hypoxanthine. After 2 h of purine starvation, which causes a derepression of the synthesis of the purine de novo enzymes, the cells were harvested and suspended in 30 mM potassium phosphate, pH 7.0, containing 1 mM EDTA and 1 mM dithiothreitol, disrupted by sonication, and centrifuged for 5 min at 6,000 × *g*. The supernatant was desalted by passage through a Sephadex G-25 column equilibrated with the extraction buffer. Protein was determined as described by Lowry et al. (5). FGAR and FGAM syntheses were determined in cell-free extracts as incorporation of [^{14}C]formate into FGAR and FGAM, respectively. The assay contained in a total volume of 100 μl Tris-hydrochloride (pH 8.0, 40 mM), KCl (20 mM), $MgCl_2$ (12 mM), [^{14}C]HCOONa (5 mM, 3.5 μC/μmol), glycine (3.3 mM), glutamine (6 mM), phosphoribosylpyrophosphate (5 mM), ATP (5 mM), phosphoenolpyruvate (8 mM), pyruvate kinase (1 μg/ml), and cell extract (1 to 2 mg of protein per ml). The mixture was incubated at 37°C, and 10-μl samples were withdrawn after 5, 10, and 30 min and applied to polyethyleneimine-impregnated cellulose on plastic sheets. The chromatograms were developed in methanol (2 cm)–1 M acetic acid (2 cm) followed by 0.3 M LiCl in 0.9 M acetic acid for another 15 cm. This system allows the separation of FGAR and FGAM from HCOONa. After autoradiography, the FGAR and FGAM spots were cut out, and the radioactivity was measured by scintillation counting. Substituting [U-^{14}C]glycine for [^{14}C]formate in the assay mixture and using 0.5 M HCOONa (pH 3.4) in the solvent system permits the measurement of GAR synthesis.

[c] FGAR and FGAM synthesis are not observed with *purF* and *purD* mutants because the assay procedure is a coupled assay which depends on the presence of both the *purF* and the *purD* encoded enzymes in the extract.

[d] Obtained through the *E. coli* Genetic Stock Center.

cation of defects in one of the five early enzymes of the purine biosynthetic pathway. Purine auxotrophs belonging to this group grow on hypoxanthine as the sole purine source and do not excrete diazotizable compounds when starved for purines. Mutants defective in either of the first two enzymes do not accumulate any intermediary compound of the pathway (Fig. 1), and no FGAR formation can be demonstrated in cell-free extracts. However, FGAR formation occurs when cell-free extracts of *purF* and *purD* mutants are combined, and thus by the use of cell extracts from known *purF* and *purD* mutant strains, the nature of the enzyme defect can be determined. Mutants defective in one of the three other enzymes can be identified by determining the intermediary compounds which accumulate during purine starvation (Fig. 1) and by determining GAR, FGAR, and FGAM formation in cell-free extracts.

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