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Identification of the *purI* locus in *Escherichia coli* K-12

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A genetic locus has been identified in *Escherichia coli* that is analogous to the *purI* locus in *Salmonella*.

In an earlier publication (5), we reported the chromosomal location of a purineless mutation (*pur-4664*) in *Escherichia coli* K-12 to be between *glyA* and *nadB*, near minute 49 on the Taylor and Trotter (4) chromosomal map. It was also demonstrated through transduction experiments that this mutation did not involve *purG*, *purC*, or *purF*, the other purine loci known to map in that general area. We now offer proof that the *pur-4664* mutation involves a gene analogous to the *purI* locus in *Salmonella typhimurium*.

Stouthamer et al. (3) showed that *pur* mutants can be divided into three groups: adenine-specific, guanine-specific, and nonspecific. The nonspecific mutants grow on adenine, guanine, or hypoxanthine, showing that their defects occur before the synthesis of inosine monophosphate (Fig. 1). The purine mutation in UTH 4664 (*see* Table 1) falls into the nonspecific group.

This nonspecific group can be broken down into those which accumulate 5-aminoimidazol ribonucleotide (AIR; *purE* mutants), 5-amino-4-imidazolcarboxamide ribonucleotide (AICAR; *purH* mutants), and 5-amino-4-imidazol-*N*-succinocarboxamide ribonucleotide (SAICAR; mutants defective in the conversion of SAICAR to AICAR). A qualitative test for these imadazol derivatives can be achieved by utilizing the Bratton-Marshall reaction (1). The absorption maxima of the chromophores formed in this reaction are 502 nm for AIR and 540 nm for AICAR and SAICAR. The *purC* mutants also accumulate AIR in their culture fluids since 5-amino-4-imidazolcarboxylic acid ribonucleotide is unstable and decarboxylates spontaneously to AIR. When the Bratton-Marshall reaction was employed on the culture fluid of UTH 4664, no production of chromophore was noted. Thus, the mutant block does not affect any of the four steps in the conversion of AIR to 5-formamido-4-imidazolcarboxamide ribonucleotide (FAICAR).

The accumulation of FAICAR occurs in *purJ* mutants (2). The accumulation of FAICAR was tested for by acid hydrolysis of the culture fluid followed by a Bratton-Marshall reaction on AICAR. Acid-hydrolyzed culture fluids of UTH 4664 grown in a suboptimal concentration of adenine gave a positive Bratton-Marshall reaction as did those grown in an excess of adenine. An excess of adenine would be expected to repress the purine biosynthetic pathway and result in no accumulation of FAICAR. The chromophore formed in this reaction exhibited a greater absorption at 502 nm than at 540 nm, which also is not consistent with FAICAR being the reacting material. Since UTH 4664 also contains a *nadB* and an *argH* mutation, it was thought that either of these blocks might result in the accumulation of a material giving a positive Bratton-Marshall test. Therefore, the *pur-4664* mutation was transduced into UTH 4133 as a linked marker accompanying the removal of the *glyA* mutation in the recipient. This transductant was designated UTH 4142. It exhibited a negative Bratton-Marshall reaction for FAICAR, thus leading to the conclusion that the *pur-4664* mutation did not involve the *purJ* locus.

Since no imidazole derivatives accumulate, the block in purine biosynthesis in UTH 4664 must occur in one of the five enzymatic steps before the formation of AIR.

Our previous study (5) had demonstrated that the *pur-4664* mutation did not involve either *purF* or *purG*. In addition *purD* maps some 29 min from the *pur-4664* mutation (3, 5). Thus, the *pur-4664* mutation must involve either the gene responsible for the conversion of glycinamide

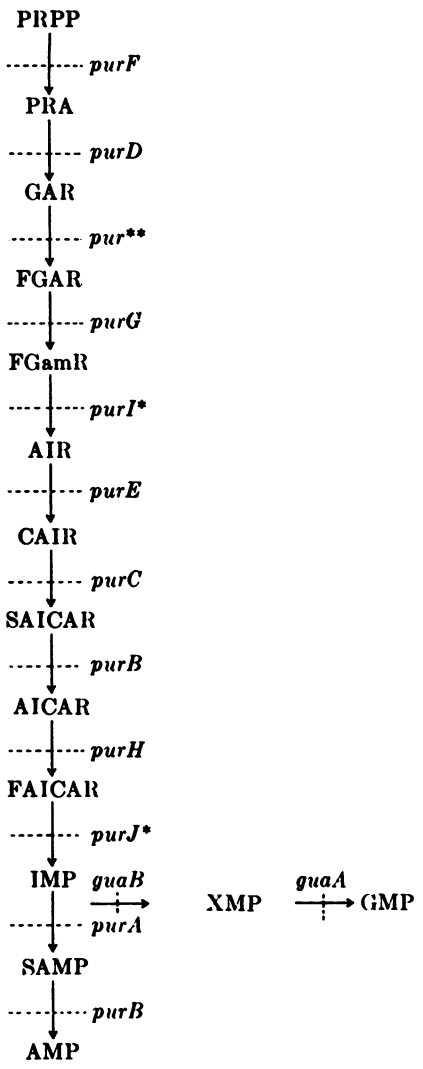

FIG. 1. *Purine biosynthetic pathway. PRPP, 5-phosphoribosyl-1-pyrophosphate; PRA, 5-phosphoribosylamine; GAR, glycinamide ribonucleotide; FGAR, formyl glycinamide ribonucleotide; FGamR, formyl glycinamidine ribonucleotide; AIR, 5-aminoimidazol ribonucleotide; CAIR, 5-amino-4-imidazolcarboxylic acid ribonucleotide; AICAR, 5-amino-4-imidazolcarboxamide ribonucleotide; SAICAR, 5-amino-4-imidazol-N-succinocarboxamide ribonucleotide; FAICAR, 5-formamido-4-imidazolcarboxamide ribonucleotide; IMP, inosinic acid; AMP, adenylic acid; SAMP, adenylsuccinic acid; XMP, xanthylic acid; GMP, guanylic acid. *These genes have been mapped in Salmonella but not in Escherichia coli. **This gene has not been mapped in either Salmonella or E. coli.*

TABLE 1. *Microorganisms used in this study*

Stock no.[a]	Species	Nutritional mutations present	Source
UTH 4035	*Escherichia coli*	*purF, aroC, arg*	E. McFall
UTH 4069	*E. coli*	*purC, guaB, hisC, tyrA, trp*	Phabagen Collection (HF 24)
UTH 4105	*E. coli*	*purG, hisC, trp*	Phabagen Collection
UTH 4116	*E. coli*	*purB, proA, hisG*	A. L. Taylor (AB 1325)
UTH 4118	*E. coli*	*purE, pyrB, proC, arg, met, pdx, his, ilv, thr*	A. L. Taylor (AB 3055)
UTH 4133	*E. coli*	*glyA*	A. L. Taylor (AT 2457)
UTH 4135	*E. coli*	*purC, hisC, tyrA, trp*	*guaB*$^+$ Spontaneous revertant of UTH 4069
UTH 4142	*E. coli*	*purI*	UTH 4664 × UTH 4133 transductant
UTH 4244	*Salmonella typhimurium*	*purI*	J. S. Gots (I305)
UTH 4245	*S. typhimurium*	*purJ*	J. S. Gots (H356)
UTH 4246	*S. typhimurium*	*purH*	J. S. Gots (H340)
UTH 4664	*E. coli*	*purI, nadB, argH*	A. L. Taylor (PA-3306)

[a] UTH designates University of Texas/Houston stock collection. All genetic symbols are those used by Taylor and Trotter (3) with the exception of *nad*, which designates a deficiency in the production of nicotinamide adenine dinucleotide.

ribonucleotide (GAR) to formyl glycinamide ribonucleotide (FGAR) or *purI*.

These alternatives can be distinguished on the basis of feeding experiments. A *purG* mutant should feed a mutant blocked in the conversion of GAR to FGAR and should not feed a *purI* mutant. A *purE* mutant should feed both. The results of such feeding experiments are summarized in Table 2. These data indicate that the enzymatic defect occurs in the conversion of formyl glycinamide ribonucleotide to AIR. Thus, the mutation was shown to involve the *purI* locus.

TABLE 2. *Cross-feeding by purineless mutants*[a]

Fed by stock no. (mutant locus)	Mutant being fed	
	UTH 4142 *Escherichia coli, pur-4664*	UTH 4244 *Salmonella typhimurium, purI*
UTH 4035 (*purF*)	−	−
UTH 4105 (*purG*)	−	−
UTH 4244 (*purI*)	−	−
UTH 4142 (*pur-4664*)	−	−
UTH 4118 (*purE*)	+	+
UTH 4135 (*purC*)	+	+
UTH 4246 (*purH*)	+	+
UTH 4245 (*purJ*)	+	+
UTH 4116 (*purB*)	+	+

[a] All cross-feeding experiments were performed on solid minimal medium supplemented with 0.1% (w/v) Casamino Acids (Difco) and 5.0 mg of thiamine per liter. The cultures to be tested were streaked parallel to each other and as close as possible without making contact. The plates were sealed with tape and incubated for 8 days at 37 C, after which cross-feeding was determined by the density of bacterial growth.

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