Genetic Studies on Ribose 5-Phosphate Isomerase Mutants of *Escherichia coli* K-12

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A gene for the constitutive ribosephosphate isomerase (rpiA) is highly cotransducible with *serA* at 56.2 min on the genetic linkage map of *Escherichia coli* K-12. Suppression of ribosephosphate isomerase A-negative mutants can occur by a regulator gene mutation permitting constitutive synthesis of the normally inducible ribosephosphate isomerase B.

We have previously described the selection and properties of *Escherichia coli* K-12 mutants lacking the constitutive ribose 5-phosphate isomerase (EC 5.3.1.6) (4). These mutants can use as sole carbon source only those compounds, such as ribose, adenosine, and uridine, whose catabolism leads to the production of an inducible ribosephosphate isomerase. For growth on compounds such as glucose, gluconate, glycerol, pyruvate, succinate, and acetate, the additional presence of a small amount of ribose or ribonucleoside is essential. In this paper we describe both the genetic mapping of a locus for the constitutive ribosephosphate isomerase (rpiA) and the nature of a secondary mutation that leads to phenotypic suppression of rpiA.

**Bacterial strains.** Table 1 lists the strains used. The symbol rpiA has been chosen for the gene specifying the constitutive ribosephosphate isomerase, rpiB has been chosen for the gene specifying the inducible ribosephosphate isomerase, and rpiR has been chosen for a regulator gene controlling rpiB (4).

**Media.** Bacteria were maintained on Oxoid nutrient agar slopes supplemented with adenine (40 µg/ml). Selective plates contained minimal medium (2) solidified with 1.5% Oxoid no. 1 agar, 25 mM gluconate plus 5 mM ribose as carbon source, and the appropriate nutritional supplements. The ribose was omitted from the plates used to score rpiA. The media used for phage P1 transduction experiments were as described previously (1).

**Conjugation experiments.** Log-phase cells growing on nutrient broth were mixed at 37 C to give approximately 10⁸ Hfr cells and 5 × 10⁸ F⁻ cells per ml. After 5 min of gentle agitation, the mating mixture was diluted 20-fold into nutrient broth and incubated at 37 C for 120 min.

Samples were then spread on to the appropriate selective plates containing streptomycin sulfate (100 µg/ml) to select against the Hfr cells.

**Transduction.** The procedures used were as described earlier (1).

**Enzyme assays.** Ribosephosphate isomerase was assayed as described previously (4). One unit of ribosephosphate isomerase is defined as that amount of enzyme catalyzing the formation of 1 µmol of ribulose 5-phosphate per min at 37 C.

**Mapping of the rpiA locus.** Two mutants, AS11 and AS131, carrying the mutations rpiA1 and rpiA2 were isolated from strains DF2001 and AB2297, respectively (4). Strain AS11 was used for the preliminary conjugation experiments to determine the approximate map location of rpiA. For this, strain AS11 was mated for 120 min with F⁻ strA strains carrying various auxotrophic markers, and streptomycin-resistant recombinants that had received the wild-type allele for a particular auxotrophic marker were selected. When his⁺ and tyrA⁺ recombinants were selected from a cross with strain SA51, approximately 5% of the his⁺ and 25% of the tyrA⁺ recombinants had inherited the rpiA allele. This suggested that rpiA was located in the region of 40 to 60 min on the *E. coli* linkage map (5). Since a gene for transketolase (*tkt*), another enzyme of the nonoxidative branch of the pentose phosphate pathway, had been tentatively located (3) in this region (at 53 to 56.5 min), we wondered whether rpiA and *tkt* were closely linked. Accordingly, we decided to look for cotransduction of rpiA with *lysA* (54.7 min), *argA* (53.6 min), and *cysC* (52.5 min). With the rpiA1 mutant AS11 as phage P1 donor and strain AT713 as recipient, no cotransduction between rpiA and *argA* or *cysC* was detected, but a very low linkage was observed with *lysA* (Table 2). These results suggested that rpiA was clockwise (as the chromosome is usually repre-

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Table 1. Characteristics of the Escherichia coli K-12 strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF2001</td>
<td>HfrC</td>
<td>zuf, thi(?)</td>
<td>D. G. Fraenkel</td>
</tr>
<tr>
<td>AB2297</td>
<td>Hfr</td>
<td>ilc, ade, xyl</td>
<td>E. A. Adelberg</td>
</tr>
<tr>
<td>AS11</td>
<td>HfrC</td>
<td>zuf, thi(?)</td>
<td>From DF2001 by mutagenesis(4)</td>
</tr>
<tr>
<td>AS131</td>
<td>Hfr</td>
<td>ilc, ade, xyl</td>
<td>From AB2297 by mutagenesis(4)</td>
</tr>
<tr>
<td>SA51</td>
<td>F⁻</td>
<td>tyrA, his, arg, strA</td>
<td>From S. I. Ahmad</td>
</tr>
<tr>
<td>AT713</td>
<td>F⁻</td>
<td>cysC39, argA21, lysA10, mal, mtl, xyl, thi(?)</td>
<td>From A. L. Taylor</td>
</tr>
<tr>
<td>AB856</td>
<td>Hfr</td>
<td>serA, lac(?)</td>
<td>From B. J. Bachmann</td>
</tr>
<tr>
<td>AS113</td>
<td>HfrC</td>
<td>zuf, thi(?)</td>
<td>This paper</td>
</tr>
<tr>
<td>AS114</td>
<td>HfrC</td>
<td>zuf, thi(?)</td>
<td>This paper</td>
</tr>
</tbody>
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Table 2. Transduction of rpiA

<table>
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<tr>
<th>Expt</th>
<th>Donor</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>No. scored</th>
<th>No. of rpiA</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>AS11 (rpiA)</td>
<td>AT713 (cysC, argA, lysA)</td>
<td>cysC⁺</td>
<td>260</td>
<td>0</td>
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<tr>
<td>2</td>
<td>AS11 (rpiA)</td>
<td>AT713 (cysC, argA, lysA)</td>
<td>argA⁺</td>
<td>260</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>AS11 (rpiA)</td>
<td>AT713 (cysC, argA, lysA)</td>
<td>lysA⁺</td>
<td>262</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>AS11 (rpiA)</td>
<td>AB856 (serA)</td>
<td>serA⁺</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>AS131 (rpiA2)</td>
<td>AB856 (serA)</td>
<td>serA⁺</td>
<td>102</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>AS113 (rpiA1, rpiR1)</td>
<td>AB856 (serA)</td>
<td>serA⁺</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>AS114 (rpiA1, rpiR2)</td>
<td>AB856 (serA)</td>
<td>serA⁺</td>
<td>100</td>
<td>99</td>
</tr>
</tbody>
</table>

*Selection of nutritional markers was on appropriately supplemented gluconate plus ribose minimal plates. rpiA was scored on appropriately supplemented gluconate minimal plates.

sented [5]) from lysA and, by using the function derived by Wu [6] to relate frequency of joint transduction to distance in time units between the markers, 1.5% linkage suggested a map location 1.5 min from lysA, that is, at 56.2 min, close to serA. With strain AS11 as phage P1 donor and strain AB856 as recipient (Table 2), 97% cotransduction between rpiA1 and serA was observed. With the rpiA2 mutant AS131 as the phage P1 donor and strain AB856 as recipient, a 100% cotransduction between rpiA2 and serA was obtained (Table 2).

Crude extracts were prepared from a number of the rpiA transductants after growth on nutrient broth in the presence and absence of 5 mM ribose and analyzed for ribosephosphate isomerase. As with strain AS11, there was hardly any activity detectable in nutrient broth-grown cells, but significant activity was present after growth in the presence of ribose. Under both conditions strain AB856 showed the same high specific activity (2.4 U/mg of protein) as strain DF2001 (4).

Properties of secondary mutants of strain AS11. It was reported previously (4) that secondary mutants of strain AS11 that had regained ribosephosphate isomerase activity and no longer required ribose or a ribonucleoside for growth were readily obtained. The specific activity of ribosephosphate isomerase in such mutants (0.3 to 1.0 U/mg of protein) was intermediate between that of fully induced ribosephosphate isomerase B (0.1 U/mg of protein) and the constitutive ribosephosphate isomerase A (3.0 U/mg of protein). When the Michaelis constant of the enzyme from such a mutant was measured, the $K_m$ for ribose-5-phosphate (0.4 mM) was more like that of ribosephosphate isomerase B (0.95 mM) than that of ribosephosphate isomerase A (6.2 mM). This observation and other properties of the enzyme in the secondary mutant (M. K. Eisenberg and R. A. Cooper, manuscript in preparation) suggested that it might, in fact, be ribosephosphate isomerase B rather than a mutant form of ribosephosphate isomerase A. This conclusion was supported by the results of experiments in which two such secondary mutants, AS13 and AS114, were used as donors in phage P1-mediated transduction experiments with strain AB856 as recipient. When the serA⁺ transductants were analyzed, the number that were rpiA was the same as with strain AS11 itself (Table 2), showing that, despite the presence of ribosephosphate isomerase, these secondary mutants still contained the nonfunctional rpiA gene.

The high frequency of spontaneous mutation to ribose independence (10⁻⁸ to 10⁻⁹) for the
RpiA mutants is characteristic of a loss of function mutation. This, coupled with the presence of the nonfunctional rpiA gene in the secondary mutants, suggests that reappearance of ribosephosphate isomerase activity is due to a regulator gene mutation permitting constitutive synthesis of the normally inducible ribosephosphate isomerase B. The ready suppression of rpiA has so far prevented the identification of the rpiA locus as a structural gene.

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LITERATURE CITED