NOTES

Cloning and Characterization of *Escherichia coli* K-12 Regulator Gene *tyrR*

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The *Escherichia coli* K-12 regulator gene *tyrR* was cloned into the multicopy plasmid pBR322 from a λ (Tn10)*tyrR*⁺ specialized transducing phage. Further subcloning localized the gene within a 2.1-kilobase region. Analysis of plasmid-coded proteins showed that the *tyrR* gene codes for a 63,000-dalton polypeptide.

The gene *tyrR* is located at about min 29 on the *Escherichia coli* chromosome (1) and codes for a major regulatory protein involved in transcriptional control of aromatic amino acid biosynthesis (21). The amino acids tyrosine, phenylalanine, and tryptophan interact with the *tyrR* protein to control the expression of at least eight different transcriptional units concerned with aromatic amino acid biosynthesis and transport (4-6, 10-12, 21, 22).

We describe here the construction of pBR322 derivatives carrying the *tyrR* gene cloned from the specialized transducing phage λ (Tn10)*tyrR*⁺ (7). DNA isolated from λ (Tn10)*tyrR*⁺ was digested with *Pst*I and *Eco*RI and ligated with a *Pst*I-*Eco*RI digest of pBR322. After *E. coli* JP2144 cells had been transformed with the ligation mix, strains carrying chimeric plasmids were identified by their tetracycline-resistant (Tc^r), ampicillin-sensitive (Ap^s) phenotype. Strain JP2144 is a *tyrR* mutant and is therefore resistant to the tyrosine analog 3-fluorotyrosine (5) and sensitive to the phenylalanine analog β -thienylalanine (6). Approximately 1% of Ap^s Tc^r transformants were sensitive to 3-fluorotyrosine and resistant to β -thienylalanine, indicating a *tyrR*⁺ genotype. Plasmid DNA was purified from one such transformant (14) and designated pMU352 (Fig. 1).

Digestion of pMU352 with restriction endonucleases *Pst*I and *Eco*RI showed that two fragments had been cloned into pBR322: a 7.8-kilobase (kb) *Pst*I-*Eco*RI fragment predicted to carry the *tyrR* gene, and a 0.3-kb *Pst*I fragment probably originating from the λ DNA portion of λ (Tn10)*tyrR*⁺ (7). Further digestion of pMU352 with restriction endonuclease *Bgl*II generated four fragments, 7.4, 2.9, 1.2, and 0.3 kb (Fig. 1).

To construct a smaller *tyrR*⁺ plasmid, we religated these *Bgl*II fragments and used them to transform JP2144 to tetracycline resistance. Both *tyrR*⁺ and *tyrR* transformants were identified, and plasmids from these two phenotypes were examined by *Bgl*II restriction analysis (data not shown). We identified *tyrR*⁺ plasmids that had lost the 2.9-kb *Bgl*II fragment, and one such plasmid was designated pMU355. This plasmid was mapped with restriction endonucleases *Sac*II, *Hind*III, *Rsa*I, and *Pvu*II (Fig. 1). Our analysis of the 1.2-kb *Eco*RI-*Bgl*II fragment with respect to *Sac*II and *Hind*III sites is in agreement with the Tn10 restriction map published by Jorgensen et al. (13). We concluded therefore that the *tyrR* gene must lie between the *Pst*I site on the 7.8-kb insert and the most distal *Bgl*II site.

Analysis of other plasmids derived from the religation of the *Bgl*II digest of pMU352 demonstrated that plasmids lacking either the 1.2- or the 0.3-kb *Bgl*II fragment did not carry the intact *tyrR*⁺ gene. Plasmid pMU353, also constructed in this religation, was shown to have the same complement of *Bgl*II fragments as did pMU355, but did not confer a *TyrR*⁺ phenotype. Comparison of *Rsa*I and *Rsa*I-*Sac*II digests of the two plasmids (data not shown) showed that they differed in the orientation of the 1.2-kb *Bgl*II fragment (Fig. 1). The orientation of the 0.3-kb *Bgl*II fragment in pMU353 could not be determined readily. The inference from these observations was that the *tyrR* gene extended over one or both of the *Bgl*II digestion sites delimiting the 0.3-kb fragment and into the 1.2-kb *Bgl*II fragment.

To subclone the *tyrR* gene further, we digested pMU355 with *Pvu*II and *Hind*III, religated it

FIG. 1. Construction of *tyrR*⁺ plasmids pMU352, pMU355, and pMU400 and the *tyrR* plasmid pMU353. λ (Tn10)*tyrR*⁺ was digested with *Pst*I and *Eco*RI, and the 7.8-kb fragment carrying *tyrR*⁺ was ligated into pBR322. Conditions used for restriction endonuclease digestions, ligation, and transformation have been described elsewhere (8). Digestion of pMU352 with *Bgl*II gave four fragments of 7.4, 2.9, 1.2, and 0.3 kb. Plasmids pMU355 and pMU353 were constructed by digesting pMU352 with *Bgl*II and religating. These two plasmids are identical in their complement of *Bgl*II fragments; however, the 1.2-kb *Bgl*II fragment in pMU353 is in the opposite orientation. Plasmid pMU400 was constructed by cloning the 2.4-kb *Pvu*II-*Hind*III fragment from pMU355 into pBR322 as described in the text. The *Pvu*II cut site was lost during this subcloning. The thick lines in the plasmids are those regions derived from pBR322. The *Rsa*I cut sites shown for pMU353 and pMU355 show the different orientations of the 1.2-kb *Bgl*II fragment in these two plasmids; both plasmids contain other *Rsa*I cut sites which are not shown.

with a similar digest of pBR322, and transformed it into JP2144. Transformants that were *tyrR*⁺ and Ap^r were selected. The 4.7-kb plasmid pMU400 was purified from one of the transformants and had the restriction map shown in Fig. 1. The location of the *tyrR* gene was therefore

more closely defined as being on the 2.1 kb of DNA between the *Pvu*II site and the distal *Bgl*III site in pMU355. The *Pvu*II site was found to be missing from pMU400, which suggests that pMU400 resulted from the transformation of a linear DNA molecule that had not been ligated at the blunt-ended *Pvu*II site. Subsequent in vivo ligation can be associated with a deletion in the region of the ends of the linear molecule (20). The extent of such a deletion in pMU400 cannot be large, since the *Rsa*I fragment spanning this region is not detectably smaller than the 280 base pairs predicted from *Rsa*I mapping of pMU355 and pBR322.

tyrR mutants carrying multicopy *tyrR*⁺ plasmids have been shown to have repressed levels of the tyrosine- and phenylalanine-inhibitable isoenzymes of 3-deoxy-D-arabinoheptulosonate 7-phosphate synthetase as would be predicted for a *tyrR*⁺ genotype (6).

To characterize the plasmid-coded proteins, we used the maxicell technique of Sancar et al. (17, 18) with strain CSR603 derivatives containing either pBR322 or one of the four plasmids described above. Electrophoresis of extracts from CSR603 containing pBR322 gave four protein bands containing [³⁵S]methionine (Fig. 2, lane A). On the basis of molecular weight estimations, it was concluded that bands 4 and 5 (31,000 and 28,000 daltons, respectively) were products of the ampicillin resistance gene and that band 3 (34,000 daltons) was a product of the tetracycline resistance gene (2, 16, 19). Band 6, which migrated considerably faster than did the 21,000-dalton marker, was also a product of the tetracycline resistance gene since it was not encoded by the Ap^r Tc^s plasmid derivative of pBR322, pMU400 (Fig. 2, lane E). It probably includes the 14,000- and 12,000-dalton proteins related to tetracycline resistance observed by other workers (16, 19). The *tyrR*⁺ plasmid pMU352 coded for two polypeptides with molecular weights of approximately 41,000 and 63,000 daltons in addition to the above-mentioned tetracycline resistance proteins. The 41,000-dalton protein was not synthesized by strains carrying plasmids pMU353, pMU355, or pMU400, which lack the 2.9-kb *Bgl*II fragment. Since pMU355 and pMU400 specify a Tyr^r phenotype, it can be concluded that this 41,000-dalton polypeptide is not the *tyrR* gene product. The 63,000-dalton polypeptide was coded for by *tyrR*⁺ plasmids pMU355 and pMU400, but not by pMU353, which as already mentioned fails to confer a Tyr^r phenotype. We propose therefore that the *tyrR* gene codes for a polypeptide with a molecular weight of approximately 63,000. Assuming an average amino acid residue molecular weight of 110, 1.7 kb of DNA would be needed to encode such a polypeptide. The

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [35 S]methionine-labeled proteins produced in maxicells by the *tyrR*⁺ plasmid pMU352 and its derivatives. Samples were run on 10 to 20% polyacrylamide gradient slab gels, and the radiolabeled protein bands were detected by fluorography as described by Laemmli (15). Lanes: (A) pBR322; (B) pMU352; (C) pMU355; (D) pMU353; (E) pMU400. The molecular weight markers used were SA11 rotavirus polypeptides of molecular weights 113,000, 96,000, 84,000, 42,000, 33,000, 31,000, and 21,000 (9). The migration position of four of these markers is indicated on the right.

alternate possibility, consistent with the above data, that the *tyrR* protein is a low-molecular-weight protein that was undetected by the maxicell system, can be excluded by a consideration of the restriction data. Such an arrangement would require that both the *tyrR* protein and the 63,000-dalton polypeptide be encoded within the 2.1-kb *PvuII*-*BglII* region. It has been shown that the 0.3-kb *BglII* fragment (Fig. 1) is essential for the expression of *tyrR*⁺, and therefore the *tyrR* gene must span either one or both of the *BglII* sites that define this fragment. Given this constraint, it is not possible for a small gene containing even one of the *BglII* sites and the minimum of 1.7 kb of DNA required to specify the 63,000-dalton polypeptide to be accommodated within this 2.1-kb region. Hence, we conclude that the 63,000-dalton polypeptide synthesized by the maxicells is the *tyrR* gene product.

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