JOURNAL OF BACTERIOLOGY, Aug. 1986, p. 749–753
0021-9193/86/080749-05$02.00/0
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Vol. 167, No. 2

Cloning of the *aroP* Gene and Identification of Its Product in *Escherichia coli* K-12

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Received 6 February 1986/Accepted 8 May 1986

The *aroP* gene of *Escherichia coli* K-12 was located in a ca. 1.2-kilobase region of DNA. The *aroP* gene product was identified as a membrane-bound protein with an apparent molecular weight of approximately 37,000.

In *Escherichia coli* and *Salmonella typhimurium*, the transport of aromatic amino acids can occur via a general transport system (1, 4, 22) or via systems that are specific for each amino acid (1, 4). The *aroP* gene codes for a component involved in general aromatic amino acid transport. Mutant strains defective in the transport of the aromatic amino acids via the general aromatic amino acid transport system (AroP$^-$) have been isolated in *E. coli* (4) and *S. typhimurium* (2), and *aroP* has been mapped at about minute 3 on both the *E. coli* chromosome (4) and the *S. typhimurium* chromosome (25). Guest (10) showed that the position of *aroP* in *E. coli* in relation to other genes within this region was *leu-azi-nadC-aroP-aceE-aceF-lpd-pan*.

In *E. coli* the expression of *aroP* is repressed by the TyrR protein in conjunction with phenylalanine, tyrosine, or tryptophan (29). The gene *tyrR* also regulates the tyrosine-specific transport system (29), the tryptophan-specific transport system (29), and some enzymes involved in aromatic amino acid biosynthesis (5, 7, 9, 16, 27).

Guest and Stephens (13) reported the construction of a λ *nadC* transducing phage (λG76N) by the incorporation of a 10.5-kilobase (kb) *Hin*dIII fragment of *E. coli* DNA in the λ vector NM761 (20). This 10.5-kb *Hin*dIII fragment was later cloned into the *Hin*dIII site of plasmid pBR322 to produce pGS15 (12). Plasmid pGS85 was derived from plasmid pGS15 by means of a *Bam*HI deletion (Fig. 1).

Strain JP3960 (*aroB351 pheP367 aroP*) was used to select for *aroP*$^+$ clones from plasmid pGS85 and its derivatives. In this strain phenylalanine-specific transport is absent as a result of a mutation in *pheP* (28), and the aromatic amino acid biosynthetic pathway is blocked by a mutation in the gene *aroB*. AroP$^+$ derivatives of strain JP3960 were distinguished by their ability to grow on minimal medium supplemented with the aromatic vitamins and low concentrations (2 $\times 10^{-5}$ M) of phenylalanine, tyrosine, and tryptophan and by their inability to grow on minimal medium or on medium supplemented with high levels of tyrosine and tryptophan (10^{-3} M) and low levels of phenylalanine. Failure to grow on the latter medium was caused by the repression of *aroP* and the saturation of any residual general aromatic amino acid transport activity with tyrosine and tryptophan. On the other hand, AroB$^+$ and PheP$^+$ clones would be expected to grow well on this medium, and AroB$^+$ clones should also be able to grow on minimal medium. Plasmid pGS85 and its derivative pMU1411, which carries the 5.9-kb *Bam*HI-*Hin*dIII *aroP*$^+$ fragment from pGS85 inserted in the low-copy-number vector pMU530 (Fig. 1), both conferred on strain JP3960 the growth characteristics expected for AroP$^+$ derivatives.

Transport assays were carried out by the procedure of Whip and Pittard (29) and confirmed that the ability to transport phenylalanine was repressed if cells were grown in the presence of aromatic amino acids. The results for strain JP3061(pMU1411) are shown in Fig. 2. The low-copy-number *aroP*$^+$ plasmid pMU1411 was used in these studies because, with high-copy-number plasmids, the greatly increased number of operator sites cannot be controlled by haploid levels of the TyrR protein.

Further mapping of *aroP* within the 5.9-kb *Bam*HI-*Hin*dIII fragment was achieved by using transposon Tn*1000* (previously designated [γδ] 14). The donor strain JP-3301(pGS85) (F$^+$ *recA* Tc^s Ap^r) was conjugated with strain JP3994 (F$^-$ *aroB351 pheP367 aroP recA* Tc^r Ap^s), and transconjugants carrying Tn*1000* insertional derivatives of plasmid pGS85 were selected on plates containing tetracycline and ampicillin. The AroP phenotype was determined by the method described above, and analysis of *Eco*RI and *Bam*HI single and double digests of these Tn*1000* derivatives showed the *aroP* gene to lie within a region of at least 1.2 kb to the left of the *Bam*HI-*Sph*I fragment (Fig. 3). Subsequently, plasmid pMU1410 was constructed by means of an *Sph*I deletion in plasmid pGS85 (Fig. 1). Transformation of strain JP3960 by plasmid pMU1410 showed that it retained the *aroP*$^+$ gene.

The maxicell method of Sancar et al. (23, 24) was used to detect plasmid-encoded proteins from derivatives of strain CSR603 transformed with plasmids pMU1410 and pGS85 and the Tn*1000* insertional derivatives of plasmid pGS85. A 12.5% sodium dodecyl sulfate-polyacrylamide gel (17) was used to separate the protein bands, which were then detected by autoradiography (Fig. 4). In addition to the three vector-encoded bands detected from plasmid pBR322 representing β-lactamases (31K and 28K) and the product of the tetracycline resistance gene (34K) (3, 19, 26), a polypeptide with an apparent molecular weight of 37,000 was detected from plasmids pMU1410 and pGS85 and the *AroP*$^+$ *Tn1000* insertional derivatives of plasmid pGS85 (pMU1416 and pMU1417). It was absent from the AroP$^-$ Tn*1000* insertional derivatives of plasmid pGS85 (pMU1414, pMU1429, pMU1432, pMU1433, and pMU1438). We propose that this

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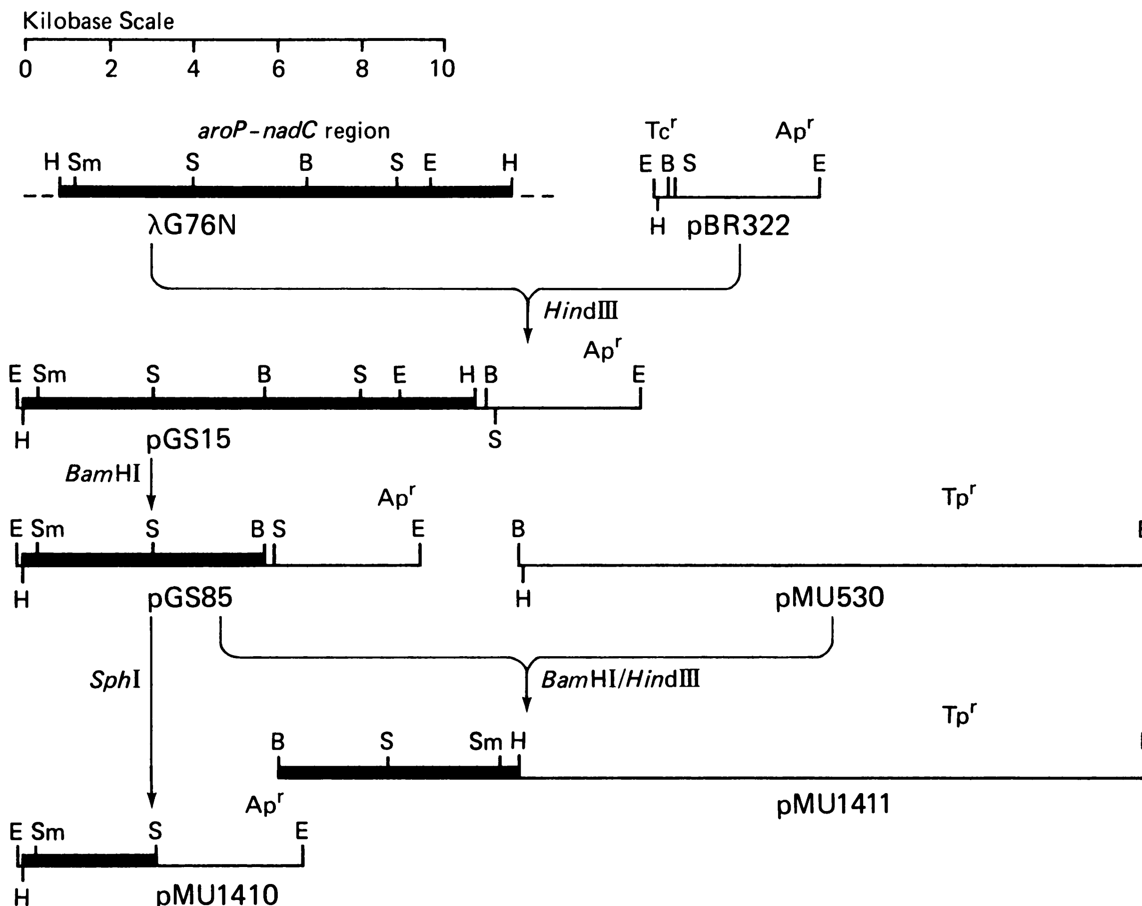

FIG. 1. Construction of the $aroP^+$ plasmids pGS15 (12), pGS85, pMU1410, and pMU1411. Plasmid pGS15 was constructed by cloning a 10.5-kb *Hin*dIII fragment into the *Hin*dIII site of plasmid pBR322 (12). Plasmid pGS85 was derived from pGS15 by means of a *Bam*HI deletion. Plasmid pMU1410 was derived from pGS85 by means of an *Sph*I deletion. The low-copy-number $aroP^+$ plasmid pMU1411 was constructed by cloning the 5.9-kb *Bam*HI-*Hin*dIII $aroP^+$ fragment from plasmid pGS85 into the low-copy-number plasmid pMU530. Plasmid pMU530 (P. Kasian, Ph.D. thesis, University of Melbourne, Melbourne, Australia, 1985) is a low-copy-number plasmid which contains both the replicon and trimethroprim resistance gene from plasmid pREG151 (S. Falkow). H, *Hin*dIII; B, *Bam*HI; S, *Sph*I; E, *Eco*RI; Sm, *Sma*I.

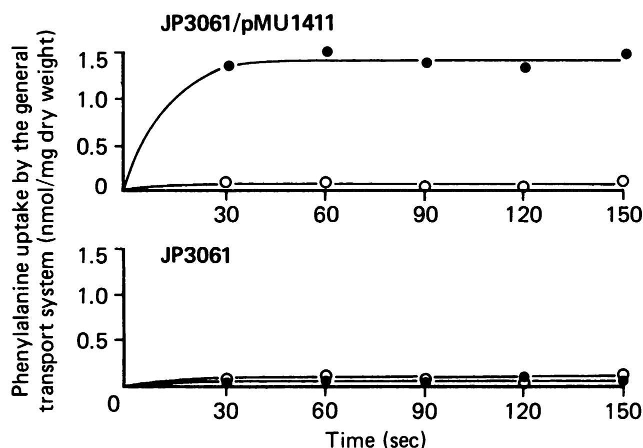

FIG. 2. Uptake of phenylalanine by the general aromatic amino acid transport system in JP3061 (*aroP*) and JP3061(pMU1411). Strains were grown in minimal medium (●) and in minimal medium supplemented with repressing concentrations of phenylalanine (10^{-3} M), tyrosine (10^{-3} M), and tryptophan (5×10^{-4} M) (○). Cells were assayed in the absence and in the presence of tryptophan (10^{-4} M), which competes with phenylalanine for transport by the general transport (*aroP*) system but has no effect on the phenylalanine-specific (*pheP*) system. Phenylalanine transport by the general transport system was calculated as the difference between total phenylalanine uptake and uptake by the phenylalanine-specific system.

polypeptide is the gene product of *aroP*. Previous studies with a λ *nadC* phage (λG78N), which contains the *nadC-aroP* region on a 10.1-kb *Eco*RI fragment, detected a 36.5K polypeptide which was postulated to be the gene product of either *aroP* or *nadC* (11).

Other polypeptides detected from plasmid pGS85 had molecular weights of 33,000 and 41,000. The 33K polypeptide was also encoded by plasmid pMU1410. Since the 41K polypeptide was absent in plasmids pMU1410 and pMU1417, in which Tn*1000* was inserted near the *Sph*I site

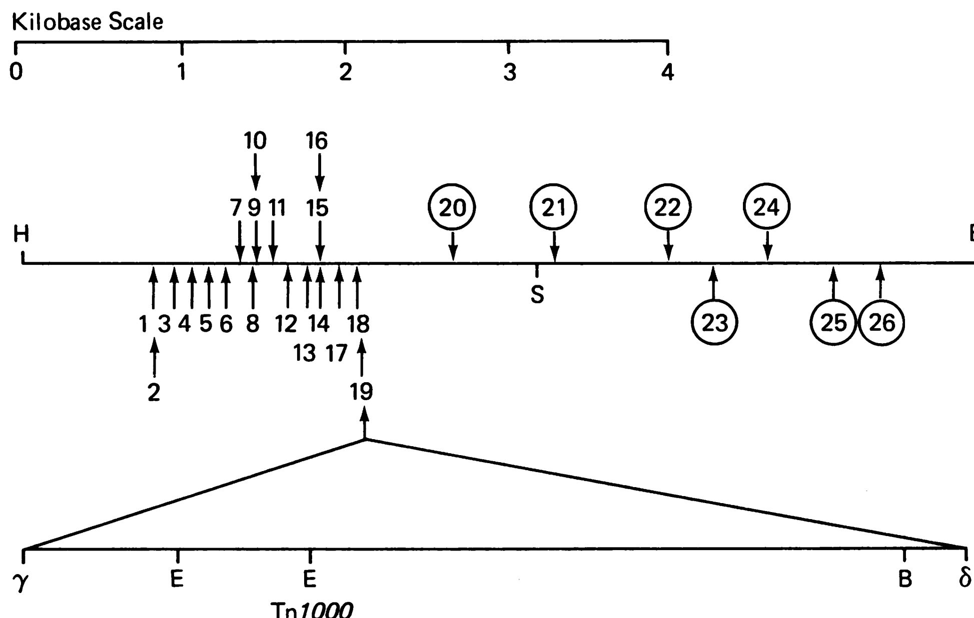

FIG. 3. Location and orientation of Tn*1000* (γδ) insertions within the 5.9-kb *Bam*HI-*Hin*dIII fragment from pGS85. Each arrow designates the position of a Tn*1000* insertion conferring an $AroP^-$ phenotype, and each arrow joined to an open circle designates the position of a Tn*1000* insertion in which the plasmid retained an $AroP^+$ phenotype. Arrows below the line represent Tn*1000* insertions in the orientation shown, and those above the line represent Tn*1000* insertions in the opposite orientation. The positions of restriction enzyme sites in Tn*1000* were obtained from Guyer (14). The numbers marked with each arrow correspond to the pMU plasmid numbers as follows: 1, pMU1429; 2, pMU1435; 3, pMU1427; 4, pMU1438; 5, pMU1431; 6, pMU1440; 7, pMU1428; 8, pMU1441; 9, pMU1430; 10, pMU1412; 11, pMU1414; 12, pMU1413; 13, pMU1437; 14, pMU1432; 15, pMU1434; 16, pMU1439; 17, pMU1426; 18, pMU1433; 19, pMU1436; 20, pMU1417; 21, pMU1422; 22, pMU1421; 23, pMU1418; 24, pMU1424; 25, pMU1423; and 26, pMU1419. Plasmids having Tn*1000* inserted at the same site were obtained from independent matings. E, *Eco*RI; H, *Hin*dIII; S, *Sph*I; B, *Bam*HI.

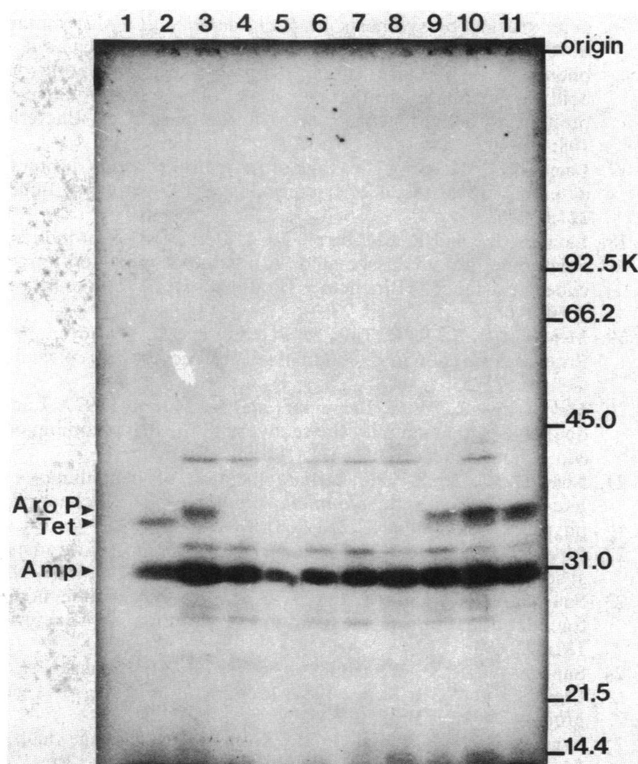

FIG. 4. Autoradiograph of [^{35}S]methionine-labeled proteins from maxicell preparations. Lanes: 1, CSR603; 2 through 11, derivatives of CSR603 carrying, respectively, the following plasmids: pBR322, pGS85 ($AroP^+$), pMU1429 ($AroP^-$), pMU1438 ($AroP^-$), pMU1414 ($AroP^-$), pMU1432 ($AroP^-$), pMU1433 ($AroP^-$), pMU1417 ($AroP^+$), pMU1416 ($AroP^+$), and pMU1410 ($AroP^+$). The positions of the molecular weight standards used (phosphorylase *b* [92.5K], bovine serum albumin [66.2K], ovalbumin [45K], carbonic anhydrase [31K], soybean trypsin inhibitor [21.5K], and lysozyme [14.4K]) are shown. The positions of the proteins encoded by the *aroP* gene (AroP), the tetracycline resistance gene (Tet), and the β-lactamase gene (Amp) are shown.

(Fig. 2), it is likely that the gene encoding this polypeptide spans the *Sph*I site. Furthermore, two independently isolated plasmids containing fragments flanking the *Sph*I site, pGS135 (containing the 3.2-kb *Hin*dIII-*Sph*I fragment which is identical to that in plasmid pMU1410) and pGS140 (containing the 2.7-kb *Sph*I-*Bam*HI fragment), were both *nadC* deficient (data not shown). It is possible that this 41K polypeptide is the *nadC* gene product.

Previous studies on the intracellular location of the *tyrP*-encoded polypeptide by use of ultracentrifugation to separate the membrane and soluble fractions of ^{35}S-labeled maxicells indicated that the *tyrP* gene product is in the membrane fraction of the cell (30). Similar experiments were carried out to examine the intracellular location of the *aroP*-encoded polypeptide. It was also found in the membrane fraction (Fig. 5). Plasmid pBR322 was used as a control; the products of the ampicillin resistance gene were found in the soluble fraction (21), while the product of the

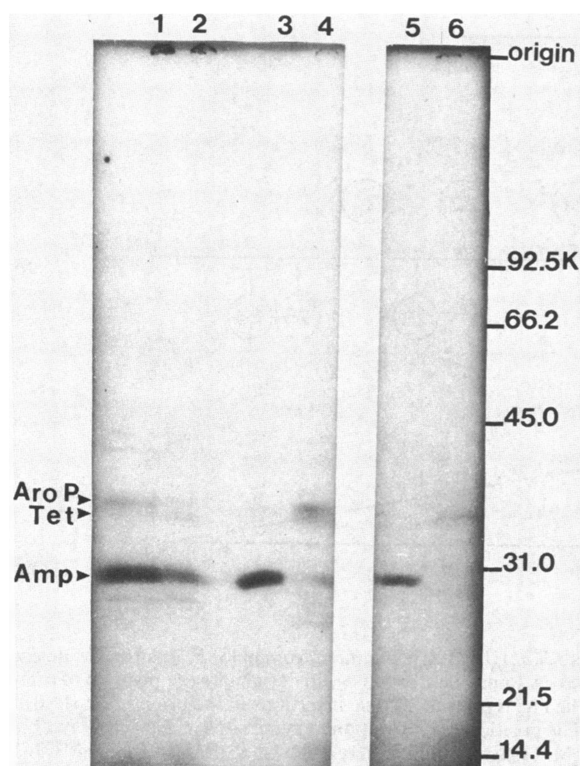

FIG. 5. Autoradiograph of [35S]methionine-labeled proteins from maxicell preparations of CSR603 derivative strains carrying plasmids pGS85 (lane 1, total cell protein; lane 3, soluble protein; and lane 4, membrane-associated protein) and pBR322 (lane 2, total cell protein; lane 5, soluble protein; and lane 6, membrane-associated protein). The positions of the proteins encoded by the *aroP* gene (AroP), the tetracycline resistance gene (Tet), and the β-lactamase gene (Amp) are shown. For a description of the molecular weight standards, see the legend to Fig. 4.

tetracycline resistance gene was located in the membrane fraction (18) (Fig. 5).

Although the *aroP* polypeptide has been identified in this study to have an apparent molecular weight of 37,000, its real molecular weight may be greater than this. In the case of a number of membrane proteins, it has been shown that the apparent molecular weight determined by gel electrophoresis is lower than the calculated molecular weight deduced from either the nucleotide sequence or the amino acid sequence (6, 8, 15).

We thank P. Kasian for helpful discussions and L. Vizard and Y. Jackson for technical assistance.

This work was supported by a grant from the Australian Research Grants Scheme. M.-L.C. is a recipient of a Commonwealth Scholarship and Fellowship Plan Award.

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