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Precise physical mapping of the *Escherichia coli pheU* transcription unit

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We report the cloning by suppression of thermosensitive phenylalanyl-tRNA synthetase mutants of the second $tRNA^{Phe}$ gene and the precise physical mapping on the chromosome of its transcription unit in the near vicinity of the *cadAB* operon.

Escherichia coli; $tRNA^{Phe}$ gene

1. INTRODUCTION

We recently described the mapping of *pheV*, an *Escherichia coli* gene for $tRNA^{Phe}$ [1], that we cloned on three out of four plasmids by suppression of phenylalanyl-tRNA synthetase thermosensitive mutants [2]. Here we describe cloning, sequencing, transcription data, and precise physical mapping of *pheU*, the second $tRNA^{Phe}$ gene.

2. MATERIALS AND METHODS

The *Escherichia coli* strains, phages and plasmids used in this work are described in Table I. Sequencing was performed using the dideoxy-chain termination method as described [3]. The oligo primers used were either the universal M13 primer or a custom primer covering the 5′ end structural part of the $tRNA^{Phe}$ synthesized on a Gene Assembler (Pharmacia) (GCCCGGATAGCTCAGTC).

3. RESULTS AND DISCUSSION

By means of the previously described test involving the suppression of thermosensitive phenylalanyl-tRNA synthetase mutants, *pheU* has been cloned on the p5-43 cosmid [2]. In order to avoid difficulties due to large inserts in cosmids we subcloned fragments which still complement a thermosensitive *pheS* mutant. The DNA of the cosmid p5-43 was digested with *Eco*RI, ligated and used to transform IBPC1671, selecting for transformants at high temperature. The complementing plasmid, pEE5-34 carries a single *Eco*RI fragment (Fig. 1). A 5 kb *Eco*RI-*Bam*HI restriction fragment (the *Bam*HI site is reconstructed by ligation of a *Sau*3A site in the *Bam*HI site used as a cloning site in pHC79) has been subcloned in pBR322 to give pEB10. The latter plasmid has been shown to carry a $tRNA^{Phe}$ gene on a 1 kb *Cla*I-*Sal*I restriction fragment which has been subcloned in the pCS8 plasmid. The presence of $tRNA^{Phe}$ on the recombinant plasmids was demonstrated by Southern blotting experiments (data not shown) using the *pheV* gene cloned in M13 phage as probe.

The 1 kb *Eco*RI-*Sal*I restriction fragment of pCS8, cloned in M13mp8, has been used for sequencing from the *Sal*I site over 300 nucleotides as described in section 2. The $tRNA^{Phe}$ structural sequence is preceded by a promoter and followed by a rho-independent termina-

Table I

Escherichia coli strains, phages and plasmids used

Strain	Genotype and markers	Source
IBPC1671	F⁻ *thi-1, argE3, his-4, proA2, lacY1, galK2, mtl-1, xyl-5, tsx-29, supE44, recA1, λˢ, λ⁻, rpsL, pheS5*	M. Springer
JM101	*Δ(lac-pro), supE, thi-1,* [F⁻, *traD36, proAB, lacIᵠ, lacZDM15]*	J. Messing
M13 phages		
M13mp8PP15	*Pst*I-*Hpa*I fragment carrying *pheV* from pPP15 (Caillet et al.; 1985) cloned into *Pst*I and *Hin*cII sites of M13mp8	This work
M13mp8ES13	*Eco*RI-*Sal*I fragment carrying *pheU* from pCS8 cloned into M13mp8	This work
Plasmids		
p5-43	pHC79 cosmid derivative carrying a phenylalanyl-tRNA synthetase thermosensitive mutant suppressor	[2]
pEE5-34	shortened p5-43 cosmid	This work
pEB10	pBR322 derivative carrying a 5 kb *Eco*RI-*Bam*HI restriction fragment carrying *pheU*	This work
pCS8	pBR322 derivative carrying a 1 kb *Cla*I-*Sal*I restriction fragment carrying *pheU*	This work

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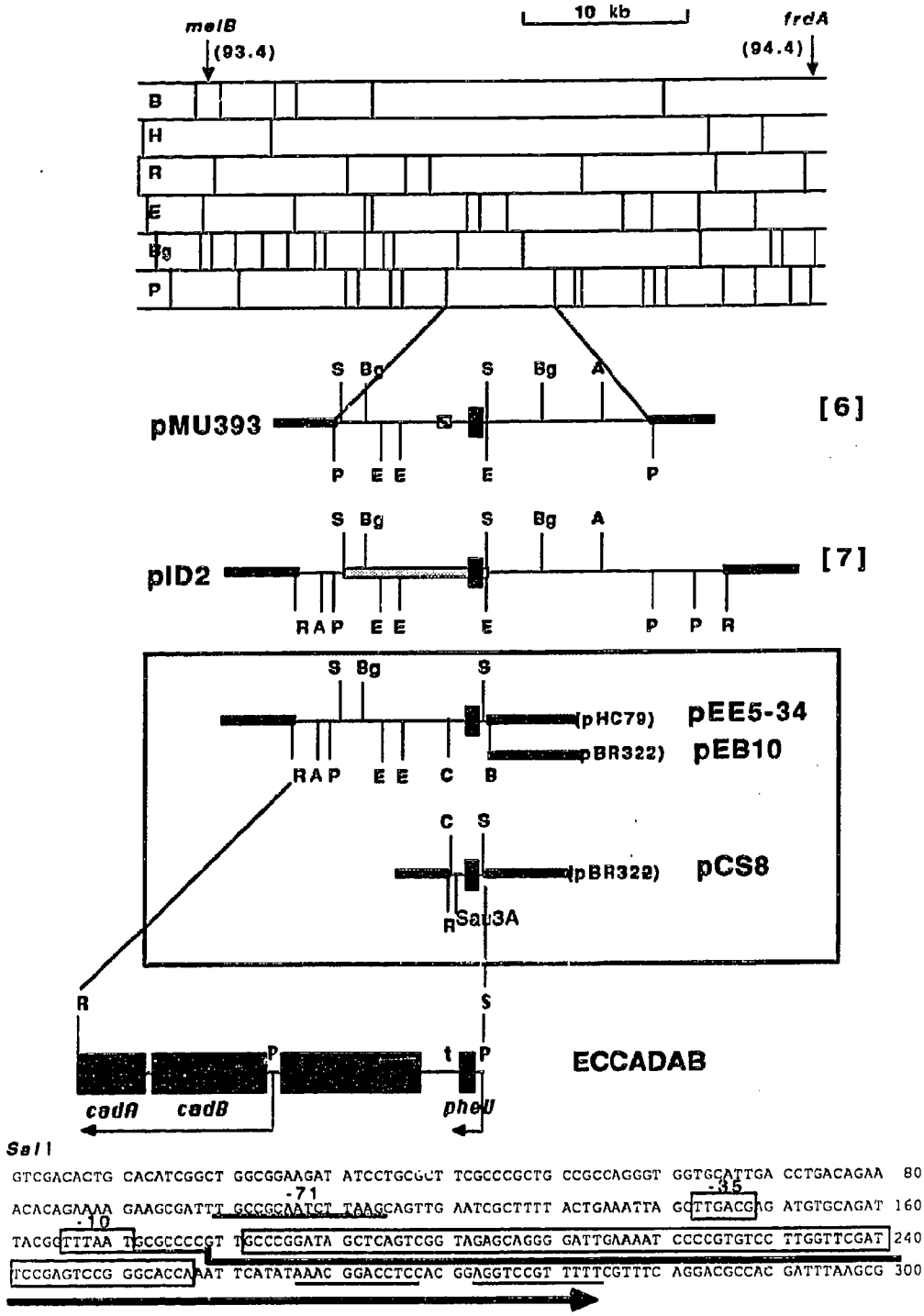

Fig. 1. Restriction maps of recombinant plasmids described in the text relative to the whole *Escherichia coli* chromosomal map as described [8,9]. The genes are represented by closed boxes. The 3.6 kb *Sal*I fragment in pID2 (shaded box) has been inversed from that shown in [7]. The restriction sites are: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; E, *Eco*RV; Bg, *Bgl*I; P, *Pst*I; S, *Sal*I; A, *Ava*I; C, *Cla*I. The nucleotide sequence of *pheU* and flanking regions: the mature $tRNA^{Phe}$ sequence is enclosed in a box, the sequences corresponding to the −35 and −10 regions of the promoter P are boxed and the sequences exhibiting dyad symmetry are underlined (t); the transcript is indicated with a bold arrow. The G/C-rich discriminator region required for the stringent control and the consensus FIS-binding site are underlined in bold.

tor (Fig. 1). The G/C-rich discriminator region upstream of the transcription start required for stringent control [4] and a very poor consensus FIS-binding site [5] at −71 are present. The processing of the primary transcript present a problem for the detection of transcripts in vivo but not in vitro. Therefore the *Eco*RI-

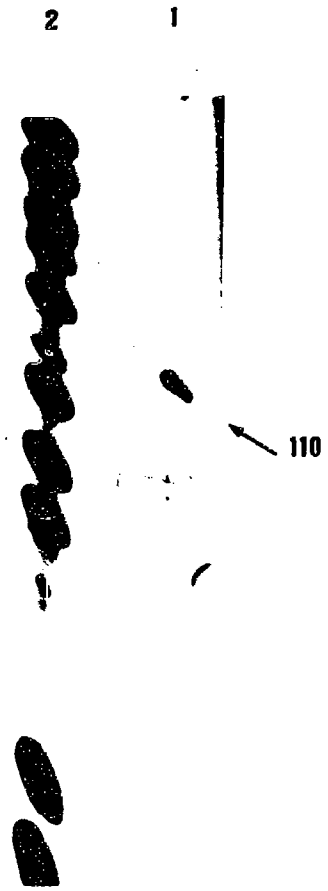

Fig. 2. Analysis of in vitro transcription products: using (1) pCS8 *Eco*RI-*Sal*I DNA restriction fragment. The experiment was performed as described in [12]. (2) Molecular weight markers: large *Eco*RI-*Hin*dIII fragment of pBR322 digested with *Hpa*II and 5′ end labelled.

*Sal*I restriction fragment of pCS8 has been used in in vitro transcription experiments (Fig. 2). Under our conditions, this fragment directs the synthesis of a 110-nucleotide-long RNA which probably corresponds to a transcript starting at the putative promoter and terminating at the putative rho-independent terminator.

The restriction map of the pEB10 plasmid is homologous to that one of pMU393 [6] and of the pID2 [7] except the inversion of a 3.6 kb *Sal*I fragment to be in accordance with the *Escherichia coli* chromosomal map [8]. The nucleotide sequence is identical to that one described [6,9] for the $tRNA^{Phe}$ gene mapped to 94 min. This sequence is also found but not characterized (nucleotide 1 to 300) in a sequence recently deposited in the EMBL Data Library (Olson, E.R., Watson, E.B. and Dunyak, D.S. – ECCADAB, accession number M67452) coding for the lysine decarboxylase.

There are only two $tRNA^{Phe}$ genes, *pheU* and *pheV* that code for identical products, on the *Escherichia coli* chromosome at 94 and 64 min respectively [9,10]. Here we provide details of the physical and genetic mapping of *pheU*, its transcription unit and confirm its nucleotide sequence. The genes *pheV* and *pheU* were characterized by four independent groups ([6,7,9] and this work). The gene *pheU*, called *pheR* in [6], was cloned using a screen based on the control of *pheA* expression. The gene *pheV* was cloned using the same screen as that described for *pheU* in the present paper, i.e. suppression of a thermosensitive *pheS* mutant [2]. Both *pheU* and *pheV* were found in a systematic screen for tRNA carrying phages from the ordered Kohara library [9]. However, one library gave two clones, called *pheW* [11] and *pheU* [7], with abberant sequences which probably derive from *pheU*.

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