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Sequence of the *Escherichia coli pheST* Operon and Identification of the *himA* Gene

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The complete nucleotide sequence of the *Escherichia coli pheST* operon coding for the two subunits of phenylalanyl-tRNA synthetase (an $\alpha_2\beta_2$-type enzyme) has been determined. Another open reading frame (*prp*) was revealed downstream from *pheT* which was identified as *himA*, the gene for the α subunit of the integration host factor.

The *Escherichia coli pheST* operon codes for the small (β) and large (α) subunits of phenylalanyl-tRNA synthetase, a tetrameric enzyme of the $\alpha_2\beta_2$ type (5). These two genes, which map at 38 min on the chromosome (3), were isolated on a λ transducing phage, λp2, and subcloned in pBR322 to give plasmid pB1 (Fig. 1) (8, 16). In vitro and in vivo studies (6, 23) strongly suggested that *pheST* transcription was controlled by a phenylalanine-dependent attenuation mechanism. More recently, the physiological behavior of mutants isolated in vivo or obtained in vitro by the creation of short deletions or insertions or of site-directed point mutations firmly established the presence of an attenuation mechanism (10, 22; J. F. Mayaux, G. Fayat, M. Panvert, M. Springer, M. Grunberg-Manago, and S. Blanquet, J. Mol. Biol., in press).

In this study, the sequence of the 2,688-base-pair (bp) *Pst*I-*Hin*dIII region of pB1 was resolved by the dideoxy chain termination method (20), with recombinant M13mp8 and M13mp9 phage DNA used as the template (11) (Fig. 2). The first open reading frame to the right of the *Pst*I site (nucleotides 1 to 221, Fig. 3) corresponds to the 3′ end of the *pheS* structural gene, as previously reported (6). The second open reading frame, starting at nucleotide 239, previously identified as the beginning of the *pheT* structural gene (6), was 2,385 nucleotides long and accounted for a polypeptide of M_r 87,024. The deduced M_r of phenylalanyl-tRNA synthetase, 247,562, was in good agreement with previous determinations done by equilibrium centrifugation (240,000 ± 10,000 [5]) and neutron scattering (227,000 ± 20,000 [4]).

To search for a recognizable transcription termination signal downstream from the *pheST* operon, an 850-bp *Sac*II-*Sac*II fragment from the *pheST* transducing phage λp2 (Table 1) was cloned into the single *Sac*II site of plasmid pHL78 to give recombinant plasmid pHIII-2 (Fig. 1). This plasmid carries the 3′-terminal end of *pheT* (153 bp) and a large 3′ flanking region (700 bp). The nucleotide sequence of the λp2 *Sac*II fragment was determined and combined with the sequence of the *Pst*I-*Hin*dIII fragment of pB1 (Fig. 3). Downstream from *pheT*, on the same DNA coding strand and in a different coding phase, an open reading frame (*prp*) of 297 nucleotides was found. The first possible AUG initiator codon for this *prp* open reading frame was separated by only 7 nucleotides from the UGA stop codon of *pheT* and was preceded 10 bases upstream by a GAGG sequence internal to *pheT*, indicating a strong translation initiation signal (21). In addition, 15 nucleotides downstream from the stop codon of *prp*, there was a region of dyad symmetry followed by a stretch of T's resembling a rho-independent transcription terminator (18).

It is known (14) that λp2 expresses one of the two subunits of the integration host factor (IHF) coded by *himA*, the other subunit being encoded by the *himD* gene (also called *hip* [13]). On the other hand, λp2 suppresses the HimA⁻ phenotype (12). Moreover, *pheST* and *himA* are 95% cotransducible by phage P1 (14). Thus, *himA* should be located very close to the *pheST* operon on the *E. coli* chromosome, the order of these genes being *pheS-pheT-himA* (2). Since the M_r

TABLE 1. Bacteria, phages, and plasmids

Strain, phage, or plasmid	Relevant genotype or phenotype	Reference
E. coli strains		
JM101	Δ(*lac-pro*) *supE thi* (*F′traD36 proAB lacI*^{q} Δ*lacZM15*)	11
K37	*galK rpsL*	12
K936	*galK rpsL himA42*	12
Phages		
λp2	*thrS infC rplT pheS pheT himA cI857*	8
Mucts62	cts62	
M13mp8	M13 derivative carrying a polylinker inserted in *lacZ′*	11
M13mp9	Same as M13mp8 but opposite polarity of the polylinker	
Plasmids		
pB1	*thrS infC rplT pheS pheT* Amp^r Tet^r	17
pHL78	Derivative of pBR322 carrying a *ClaI* fragment of λ (*c*I857 P_R)	G. Hobom
pHIII-2	*himA* derivative of pHL78	This work
pHIII-2D	Derivative of pHIII-2	This work
pUC13	Amp^r derivative of pBR322, carrying the same polylinker as M13mp11	25
pUK2	*himA* derivative of pUC13	This work
pUK2D	Derivative of pUK2	This work

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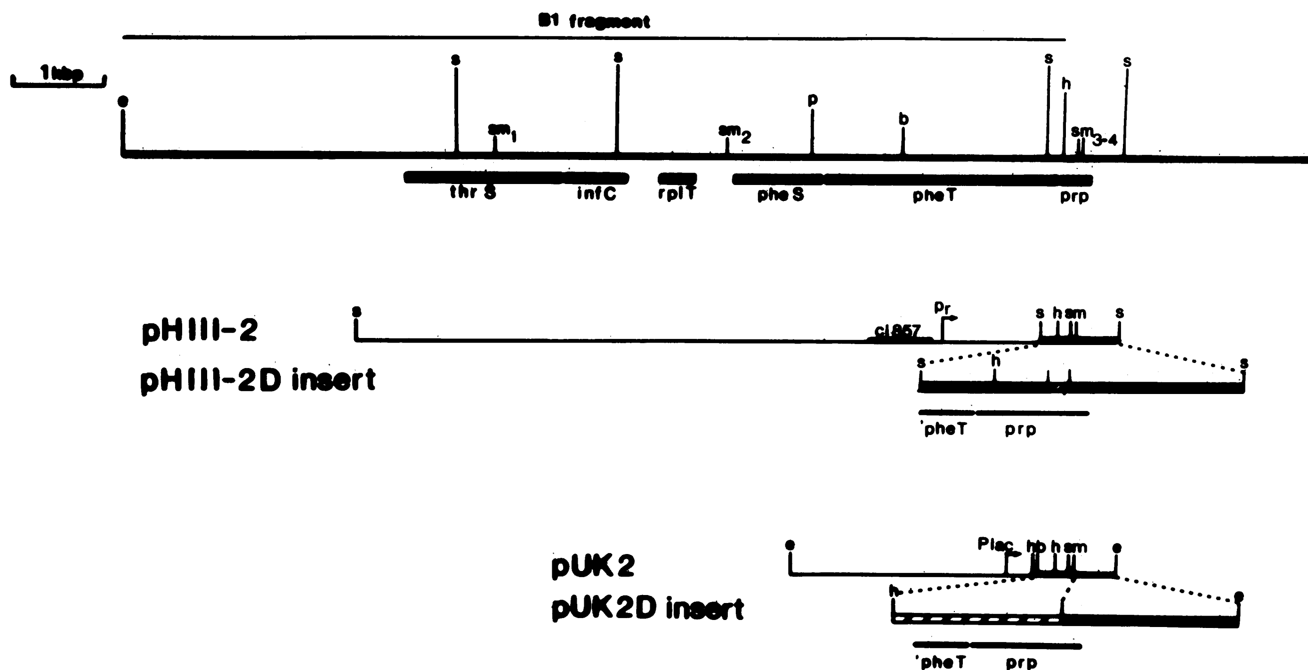

FIG. 1. Physical map of the *E. coli* chromosomal fragment carried by λp2. (Top) Relevant region of the λp2 insert. The *Eco*RI-*Hin*dIII fragment (B1 fragment) cloned in pBR322 to give plasmid pB1 is indicated. The locations of *thrS*, *infC*, *rplT*, *pheST*, and *prp* (*himA*), coding for threonyl-tRNA synthetase, initiation factor IF3, ribosomal protein L20, the β and α subunits of phenylalanyl-tRNA synthetase, and the α subunit of IHF, respectively, were deduced from DNA sequence data (6, 9, 19; this work). kbp, Kilobase pairs. (Center) Partial restriction map of pHIII-2, obtained by inserting the 850-bp *Sac*II-*Sac*II fragment of λp2 into the single *Sac*II site of plasmid pHL78. *prp* transcription on pHIII-2 is under the control of the strong inducible P_R promoter of phage λ (arrow). pHIII-2D was derived from pHIII-2 after deletion of a 67-bp *Sma*I-*Sma*I fragment internal to *prp* (hatched box). (Bottom) Partial restriction maps of pUK2 and its derivative pUK2D. pUK2 was obtained as follows: the *Sac*II insert of pHIII-2 was cloned in the *Sma*I site of M13mp8 after digestion of the *Sac*II 3'-protruding termini by T4 DNA polymerase. After transfection and characterization, the recombinant phage DNA was digested with *Eco*RI and *Bam*HI (both present on the polylinker of M13mp8), and the resulting fragment was cloned in the corresponding sites of the pUC13 vector. In this plasmid, *prp* transcription is under control of the *lac* promoter (arrow). By using the *Hin*dIII site in the polylinker of pUC plasmids, a 438-bp *Hin*dIII-*Sma*I fragment (hatched box) carrying the 3' end of *pheT* and most of *prp* was deleted, creating pUK2D. Restriction enzyme cleavage sites: e, *Eco*RI; s, *Sac*II; sm, *Sma*I; h, *Hin*dIII; b, *Bam*HI; p, *Pst*I.

of the putative *prp* product, 11,302, coincided with that of the *himA* protein, 11,000 (14), it was of interest to test the ability of *prp* to complement an *himA* mutant (*himA42*).

Two *prp*-bearing plasmids were used for complementation experiments: pHIII-2 and pUK2, a derivative of pUC13 carrying the *Sac*II fragment (Fig. 1). Complementation was

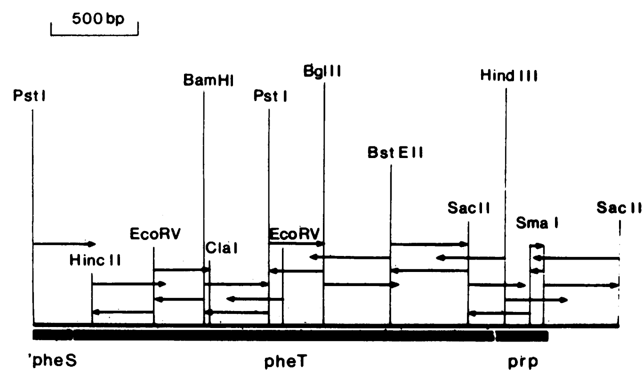

FIG. 2. Sequencing stategy for *pheT* and flanking regions. Arrows show the nucleotides sequenced by the dideoxy chain termination method. Recombinant M13mp8 or M13mp9 DNA was used as the template. Phage clones containing the *pheT* gene up to the *Hin*dIII site were constructed with pB1 as the source of DNA, whereas clones containing the *Sac*II-*Sac*II region were derived from pHIII-2. The DNA sequence of the first 300 nucleotides of the *pheT* structural gene had already been determined (6) by the chemical sequencing method.

monitored with bacteriophage Mu, which does not grow in *himA* strains (12). Strain K936 (*himA42*) transformed by either pHIII-2 or pUK2 recovered the ability to support Mu plaque formation (Table 2). To show that *himA* suppression by pHIII-2 or pUK2 depended on the integrity of the *prp* coding phase, the same experiments were performed with modified plasmids pHIII-2D and pUK2D. pHIII-2D is a derivative of pHIII-2 obtained by deleting the 67-bp *Sma*I fragment internal to *prp* (Fig. 1). This deletion removed 36 codons in the 3' end of *prp* and should result in a truncated protein of 64 amino acids. In plasmid pUK2D (Fig. 1), the DNA region corresponding to the 3' end of *pheT*, the intergenic *pheT*-*prp* region, and the first 83 codons of *prp* have been removed. Both of the truncated *prp* genes were unable to suppress the HimA$^-$ phenotype (Table 2). Finally, the burst size of phage Mu was used as a quantitative measurement of HimA$^-$ phenotype suppression. The results (Table 2) confirmed that *prp* carried by pUK2 suppressed the HimA$^-$ phenotype. The average burst size of the native HimA$^+$ phenotype (strain K37) recovered by 70% after

FIG. 3. Nucleotide sequence of *pheT* and flanking regions. Important restriction sites are indicated. Ribosomal binding sites (21) are underlined. The region of dyad symmetry resembling a rho-independent transcription terminator is shown by arrows. Two possible LexA protein-binding sites (1) are indicated by dashed lines. Sequences homologous to the procaryotic promoter consensus (18) are boxed. The deduced amino acid sequence is shown above the DNA sequence.

suppression of the mutation. These results strongly suggest that *prp* corresponds well to the *himA* structural gene.

Several studies have demonstrated that the *himA-himD* product complex (IHF) is able to bind DNA with high affinity (15). Therefore, it is worthwhile noticing that the *prp* product has extensive homology with several procaryotic, DNA-binding proteins, including NS1 and NS2 (HUα and HUβ) from *E. coli*, DNA-binding protein II from *Bacillus stearothermophilus*, and class II DNA-binding proteins from *Rhizobium meliloti*, *Clostridium pasteurianum*, and *Thermoplasma acidophilum*. For each of these proteins, the number of amino acids identical to those in the *B. stearothermophilus* sequence was between 29 and 54; for *prp*, comparison with the *B. stearothermophilus* protein showed that 39 amino acids were strictly conserved (Fig. 4). The three-dimensional crystal structure of the *B. stearothermophilus* DNA-binding protein II has been solved at a resolution of 3 Å (0.3 nm) (24). It is striking that the highly conserved amino acids responsible for its hydrophobic core and therefore for its dimer stability, i.e., Phe-29, -47, -50, and -79, Ile-32, and Leu-6, -36, and -44, are also found at the same positions in the *prp* product, except for the methionine at position 6 (Fig. 4). In addition, the arm region of the *B. stearothermophilus* protein (Fig. 4), thought to be involved in nucleic acid interactions, corresponded to the highly homologous region among the seven proteins, including the putative *prp* product (homologies between the *himA* product and HU were recently quoted in a personal communication from H. I. Miller in reference 7).

To conclude, the proximity of *pheST* and *himA*, as shown by the DNA sequence, suggests a link between the expression of these genes. The nature of the link and the possible role of *lexA* (13) in the expression of this cluster of genes are currently under study in our laboratory.

```
              1   2   3   4   5   6   7   8   9   10  11  12  13  14
PRP   Met Ala Leu Thr Lys Ala Glu Met Ser Glu Tyr Leu Phe Asp Lys Leu
Bst           Met Asn Lys Thr Glu Leu Ile Asn Ala Val Ala Glu Thr Ser
NS1           Met Asn Lys Ser Gln Leu Ile Asp Lys Ile Ala Ala Gly Ala
NS2           Met Asn Lys Thr Gln Leu Ile Asp Val Ile Ala Glu Lys Ala
Rhm           Met Asn Lys Asn Glu Leu Val Ala Ala Val Ala Asp Lys Ala
Tac       Met Val Gly Ile Ser Glu Leu Ser Lys Glu Val Ala Lys Lys Ala
Cpa           Met Asn Lys Ala Glu Leu Ile Thr Ser Met Ala Glu Lys Ser
              <------------------ALPHA 1--------------------->  <-

      15  16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
PRP   Gly Leu Ser Lys Arg Asp Ala Lys Glu Leu Val Glu Leu Phe Phe Glu
Bst   Gly Leu Ser Lys Lys Asp Ala Thr Lys Ala Val Asp Ala Val Phe Asp
NS1   Asp Ile Ser Lys Ala Ala Ala Gly Arg Ala Leu Asp Ala Ile Ile Ala
NS2   Glu Leu Ser Lys Thr Gln Ala Lys Ala Ala Leu Glu Ser Thr Leu Ala
Rhm   Gly Leu Ser Lys Ala Asp Ala Ser Ser Ala Val Asp Ala Val Phe Glu
Tac   Asn Thr Thr Gln Lys Val Ala Arg Thr Val Ile Lys Ser Phe Leu Asp
Cpa   Lys Leu Thr Lys Lys Asp Ala Glu Leu Ala Leu Lys Ala Leu Ile Glu
      ---------TURN----------> <-------------ALPHA 2-----------------

      31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46
PRP   Glu Ile Arg Arg Ala Leu Glu Asn Gly Glu Gln Val Lys Leu Ser Gly
Bst   Ser Ile Thr Glu Ala Leu Arg Lys Gly Asp Lys Val Gln Leu Ile Gly
NS1   Ser Val Thr Glu Ser Leu Lys Glu Gly Asp Asp Val Ala Leu Val Gly
NS2   Ala Ile Thr Glu Ser Leu Lys Glu Gly Asp Ala Val Gln Leu Val Gly
Rhm   Thr Ile Gln Gly Glu Leu Lys Asn Gly Gly Asp Ile Arg Leu Val Gly
Tac   Glu Ile Val Ser Glu Ala Asn Gly Gly Gln Lys Ile Asn Leu Ala Gly
Cpa   Ser Val Glu Glu Ala Leu Glu Lys Gly Glu Lys Val Gln Leu Val Gly
      --------ALPHA 2--------><βTURN> <---STRAND 1---> <-TURN-

      47  48  49  50  51  52  53  54  55  56  57  58  59  60  61  62
PRP   Phe Gly Asn Phe Asp Leu Arg Asp Lys Asn Gln Arg Pro Gly Arg Asn
Bst   Phe Gly Asn Phe Glu Val Arg Glu Arg Ala Ala Arg Lys Gly Arg Asn
NS1   Phe Gly Thr Phe Ala Val Lys Glu Arg Ala Ala Arg Thr Gly Arg Asn
NS2   Phe Gly Thr Phe Lys Val Asn His Arg Ala Glu Arg Thr Gly Arg Asn
Rhm   Phe Gly Asn Phe Ser Val Ser Arg Arg Glu Ala Ser Lys Gly Arg Asn
Tac   Phe Gly Ile Phe Glu Arg Arg Thr Gln Gly Pro Arg Lys Ala Arg Asn
Cpa   Phe Gly Thr Phe Glu Thr Arg Glu Arg Ala Ala Arg Glu Gly Arg Asn
      -> <--STRAND 2--> <--------------ARM REGION--------------------

      63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78
PRP   Pro Lys Thr Gly Glu Asp Ile Pro Ile Thr Ala Arg Arg Val Val Thr
Bst   Pro Gln Thr Gly Glu Glu Met Glu Ile Pro Ala Ser Lys Val Pro Ala
NS1   Pro Gln Thr Gly Lys Glu Ile Thr Ile Ala Ala Ala Lys Val Pro Ser
NS2   Pro Gln Thr Gly Lys Glu Ile Lys Ile Ala Ala Ala Asn Val Pro Ala
Rhm   Pro Ser Thr Gly Ala Glu Val Asp Ile Pro Ala Arg Asn Val Pro Lys
Tac   Pro Gln Thr Lys Lys Val Ile Glu Val Pro Ser Lys Lys Lys Phe Val
Cpa   Pro Arg Thr Lys Glu Val Ile Asn Ile Pro Ala Thr Thr Val Pro Val
      ---------------------ARM REGION-------------------------->  <-

      79  80  81  82  83  84  85  86  87  88  89  90
PRP   Phe Arg Pro Gly Gln Lys Leu Lys Ser Arg Val Glu Asn Ala Ser Pro
Bst   Phe Lys Pro Gly Lys Ala Leu Lys Asp Ala Val Lys
NS1   Phe Arg Ala Gly Lys Ala Leu Lys Asp Ala Val Asn
NS2   Phe Val Ser Gly Lys Ala Leu Lys Asp Ala Val Lys
Rhm   Phe Thr Ala Gly Lys Gly Leu Lys Asp Ala Val Asn
Tac   Phe Arg Ala Ser Ser Lys Ile Lys Tyr Gln Gln
Cpa   Phe Lys Ala Gly Lys Glu Phe Lys Asp Lys Val Asn Lys
      ----STRAND 3----> <--------ALPHA 3------->

PRP   Lys Asp Glu
```

FIG. 4. Amino acid sequence homologies between the putative *prp* product and some class II DNA-binding proteins. All homologies are with respect to the *B. stearothermophilus* protein sequence. Identical amino acids are underlined. The distribution of secondary structure deduced from the three-dimensional structure of the *B. stearothermophilus* protein (24) is indicated. PRP, *prp* (*himA*) product; Bst, DNA-binding protein II from *B. stearothermophilus*; NS1 and NS2, HUα and HUβ from *E. coli*; and class II DNA-binding proteins from *R. meliloti* (Rhm), *T. acidophilum* (Tac), and *C. pasteurianum* (Cpa).

TABLE 2. Suppression of the HimA⁻ phenotype[a]

Strain	Mu plaque formation	Avg burst value (%)
K37	+	100
K936	−	0.02
K936(pUK2)	+	70
K936(pUK2D)	−	0.02
K936(pHIII-2)	+	ND[b]
K936(pHIII-2D)	−	ND

[a] Burst values of Mucts62 were measured by the method of Miller and Friedman (12). It was verified that transforming strain K37 with the various plasmids did not significantly affect either Mucts62 plaque formation or Mu phage burst.

[b] ND, Not determined.

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