Homology of *lysS* and *lysU*, the two *Escherichia coli* genes encoding distinct lysyl-tRNA synthetase species

Françoise Lévêque, Pierre Plateau*, Philippe Dessen and Sylvain Blanquet
Laboratoire de Biochimie de l'Ecole Polytechnique, Unité associée 240 du Centre National de la Recherche Scientifique, 91128 Palaiseau Cedex, France

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ABSTRACT

In *Escherichia coli*, two distinct lysyl-tRNA synthetase species are encoded by two genes: the constitutive *lysS* gene and the thermoinducible *lysU* gene. These two genes have been isolated and sequenced. Their nucleotide and deduced amino acid sequences show 79% and 88% identity, respectively. Codon usage analysis indicates the *lysS* product being more efficiently translated than the *lysU* one. In addition, the *lysS* sequence exactly coincides with the sequence of *herC*, a gene which is part of the *prfB-herC* operon. In contrast to the recent proposal of Gampel and Tzagoloff (1989, Proc. Natl. Acad. Sci. USA 86, 6023 – 6027), the *lysU* sequence is distinct from the open reading frame located adjacent to *frdA*, although large homologies are shared by these two genes.

INTRODUCTION

In *Escherichia coli*, lysine is an exceptional amino acid since it is activated and transfered to $tRNA^{Lys}$ by two distinct forms of lysyl-tRNA synthetase (LysRS) (1,2). The reason for the occurrence of two LysRS isospecies is presently unsolved. One LysRS species is expressed from *lysU*, a gene which appears to be inducible under some extreme physiological conditions. In particular, the *lysU* gene is assigned as a member of the heat shock regulon (3). In addition, *lysU* can be induced under certain nutritional conditions like the presence of dipeptides (1, 4, 5), while it remains almost silent during normal growth conditions (1). Also, *lysU* was shown to be induced in various mutants like, for instance, those deficient in S-adenosylmethionine synthetase activity (1, 6). The other LysRS species, encoded by *lysS*, appears to be not submitted to the above regulations (1,2).

In this study, as a first step in the deciphering of the *lysS* and *lysU* gene functions, we report the cloning, sequencing and comparisons of these two genes. This work sheds some light on recent speculations by Gampel and Tzagoloff (7), based on the search of homologies between yeast aminoacyl-tRNA synthetase sequences and *E. coli* DNA sequences. In particular, it firmly establishes that the *lysS* gene coincides with the *herC* sequence (8) and denies that the reading frame adjacent to the *frdA* gene (9) could be the structural gene of the heat-inducible LysRS.

MATERIALS AND METHODS

Strains, phages and plasmids

Plasmids pBluescript KS and SK were from Stratagene (San Diego, USA). Phages M13mp18 and M13mp19 (10) and plasmids pBluescript were produced in strain JM101TR (Δ(*lac,pro*) *supE thi-1 recA*56 *srl*300::*Tn*10 F'(*traD*36 *proAB lacI*^{q} *lacZ*ΔM15))(11).

Enzymes and substrates

DNA restriction and modification enzymes were purchased from either Boehringer (Mannheim, West Germany), Bethesda Research Laboratory (Rockville, Maryland, USA), Pharmacia Fine Chemicals (Uppsala, Sweden) or Appligène (Strasbourg, France). [γ-^{32}P]ATP (29.6 TBq/mmol) and [α-^{35}S]dATP (37 TBq/mmol) were from Amersham (U.K.), [^{14}C]lysine (12 GBq/mmol) was from the Commissariat à l'Energie Atomique (Saclay, France). Pure unfractionated *E. coli* tRNA was from the pilot facilities of the Institut des Substances Naturelles (Centre National de la Recherche Scientifique, Gif-sur-Yvette, France). Initial velocities of tRNA aminoacylation were measured according to Brevet et al. (12).

Recombinant DNA techniques

General genetic and cloning techniques were as previously described (13 – 16). Southern blot analysis was performed according to Maniatis et al. (14), or by using the unblot method of Wallace and Miyada (17). DNA probes were labeled by phosphorylating oligonucleotides in the presence of [γ-^{32}P]ATP (14).

LysRS purification and N-terminal sequencing of purified LysRS

The purifications of the LysRS samples used for the N-terminal sequencing have been already described. The LysRS encoded by *lysU* was purified from strain JM101TR transformed by the plasmid pXLysCla3 harboring *lysU*. This strain overproduces LysRS activity 25-fold (12). The obtained LysRS sample unambiguously provided a unique N-terminal sequence. This excluded a contamination by the *lysS* gene product greater than 5%, a value in agreement with the above overproduction factor. The *lysS*-encoded LysRS was purified from strain EM20031 (*rpsL*, *dsdC*3, F32(*dsdC*$^+$)) (18). The peak of LysRS activity

* To whom correspondence should be addressed

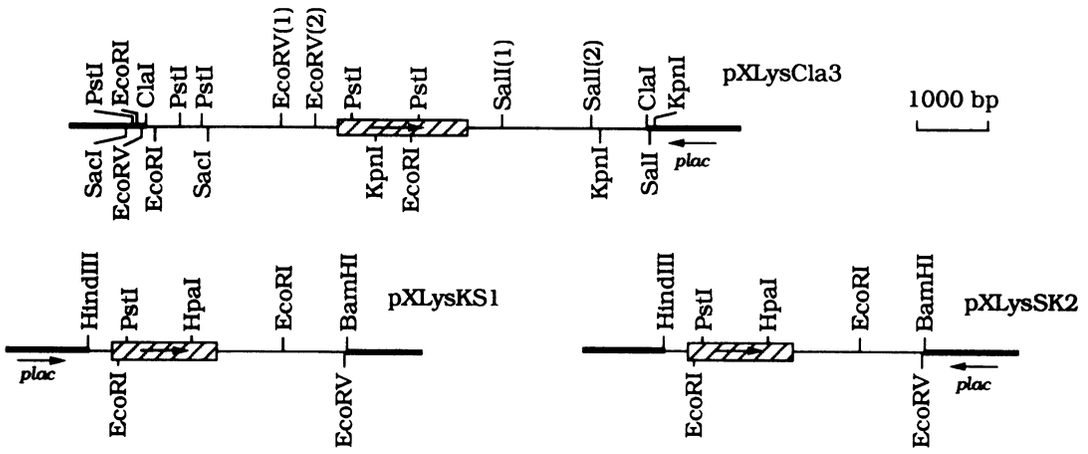

Figure 1: Restriction map of plasmid pXLysCla3 containing the *lysU* gene, and of plasmids pXLysKS1 and pXLysSK2 containing the *lysS* gene. Hatched boxes indicate the locations of the two genes. Arrows indicate transcription directions from the cloned genes and from the lactose promoter carried by pBluescript.

eluting at the highest phosphate concentration through hydroxylapatite chromatography was chosen for further purification and N-terminal sequencing. The identity between the chemically determined N-terminal sequence and that deduced from the *lysS* sequence, as shown under Results, *a posteriori* valids the choice made on the hydroxylapatite profile.

For N-terminal sequencing, one nanomole of purified LysRS was submitted to automated gas phase sequencing in an Applied Biosystems model 470 A sequencer (19).

Construction and screening of M13 libraries

Chromosomal DNA from *E. coli* strain JM101TR was digested by *Bam*HI and *Hind*III enzymes. The resulting DNA fragments were separated by high performance size exclusion chromatography (20). Aliquots of the different fractions were electrophoresed on a 1% agarose gel and hybridized to a radioactive oligonucleotide probe. The DNA fragments from the fraction that gave the strongest hybridization signal were ligated to M13mp18 or M13mp19 DNAs and used to transform strain JM101TR. For each construction, 500 lysates were prepared as follows: strain JM101TR was infected with isolated M13 plaques and grown overnight in 200 µl of 2×TY medium, in microtitration plates. Plates were centrifuged and the supernatants filtered through nitrocellulose filters. Filters were dried at 80°C under vacuum for two hours and, then, hybridized overnight with the radioactive oligonucleotide probe. Filters were washed with 2×SSC (14) at room temperature, and autoradiographed for two hours.

DNA sequencing

The dideoxy chain termination method was used to determine the nucleotide sequence of both strands of the *lysS* and *lysU* genes. Sequencing was performed on single- or double-stranded DNA (21, 22).

Computer analyses of amino acid and nucleic acid sequences were carried out using the DNAid program on a Macintosh computer (23) and the data bases and facilities of the Centre Inter-Universitaire de Traitement de l'Information (CITI2, Paris).

RESULTS

Subcloning of the *lysU* gene

Plasmid pLC4-5 of the Clarke and Carbon library (24), known to carry *lysU* (25), was used to subclone this gene. For this purpose, pLC4-5 was digested by *Cla*I and the resulting fragments were ligated with pBluescript(+)KS DNA. After transformation of strain JM101TR with the ligation mixture, clones overproducing $tRNA^{Lys}$ aminoacylation activity were analysed. Each of them carried a plasmid with a common 7 kbp *Cla*I insert.

One of the above plasmids, pXLysCla3, was selected for further studies. Its restriction sites were mapped (Figure 1), and various deletions were made, in order to precisely localize the *lysU* gene. After *Eco*RV, *Eco*RI, *Pst*I, *Sal*I or *Sac*I complete digestion and recircularization, plasmids were assayed for LysRS overproduction. Only the plasmids with *Sac*I, *Eco*RV or *Sal*I deletions, still caused an increased LysRS activity. Therefore, it could be concluded that *lysU* was located between the *Eco*RV(2) and *Sal*I(1) sites of pXLysCla3.

By comparison to the complete restriction map of the *E. coli* chromosome (26), the restriction pattern of pXLysCla3 was found to correspond to a unique region of the *E. coli* physical map, at the kbp 4430. This position is close to the previously reported location of *lysU*, at 92 min on the *E. coli* linkage map (25).

Cloning of the *lysS* gene

Firstly, the N-terminal sequence of purified LysRS (see Materials and Methods) was used to design an oligonucleotide probe. Sequential Edman degradation indicated the sequence SEQHAQGADAVVDLNNELKTRREKLANL, different from that of the *lysU* product (which is shown further below). Using all the possible codon combinations for the six consecutive amino acids EQHAQG, a 64-fold degenerated 17-bases long oligonucleotide was synthesized.

Then, Southern blot analyses were performed by hybridizing this oligonucleotide probe to *E. coli* chromosomal DNA previously digested by combinations of *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, *Kpn*I, *Pvu*II and *Sal*I enzymes. From the lengths of the hybridizing DNA fragments, the N-terminus of *lysS* could be

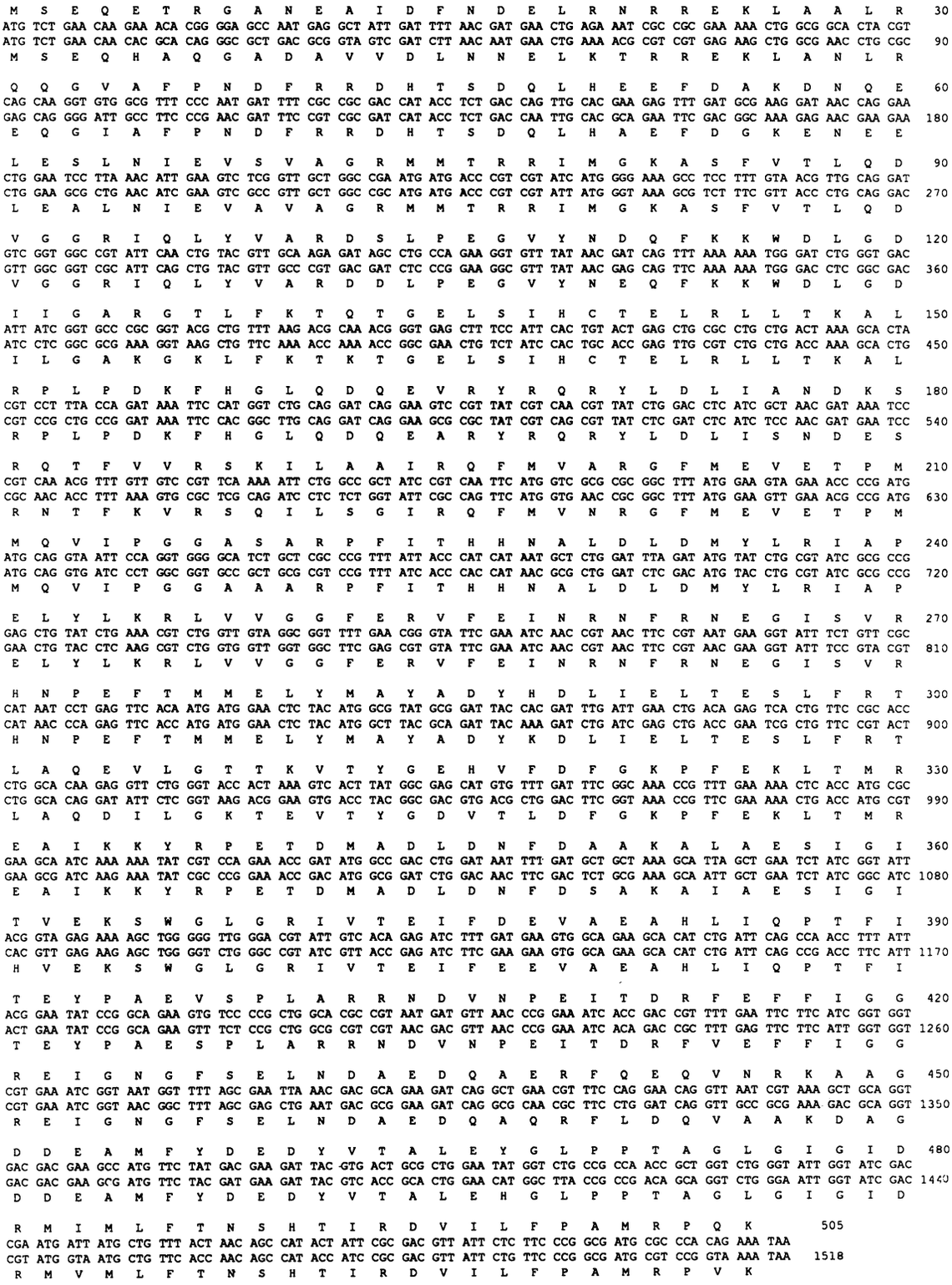

Figure 2: Nucleotide and deduced amino acid sequences of the *lysU* (upper lines) and *lysS* (lower lines) structural genes.

located on the *E. coli* physical map at the kbp 3050 (26). This position is in good agreement with the genetic mapping of *lysS* at 62.1 min on the linkage map (27).

The hybridizing *Hind*III-*Bam*HI fragment (3.5 kbp) was further isolated from *E. coli* chromosomal DNA and inserted into M13mp19, using the strategy described in Materials and Methods. Strong hybridization signals were obtained with three out of 500 recombinants. One positive clone served to prepare

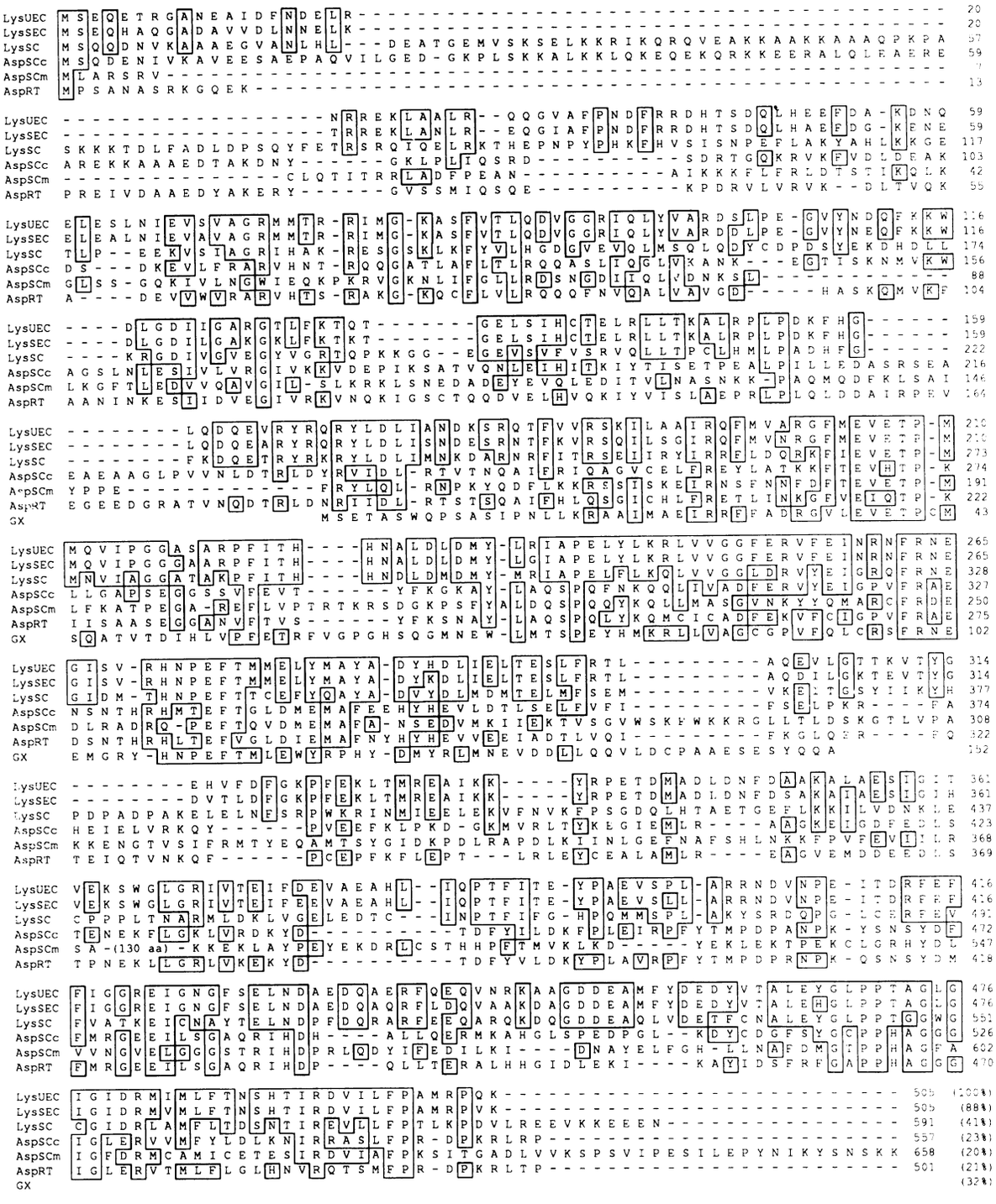

Figure 3: Protein sequence alignment of the *lysU* gene product (LysUEC), the *lysS* gene product (LysSEC), the LysRS of *S. cerevisiae* (LysSC) (40), the cytoplasmic AspRS of *S. cerevisiae* (AspSCc) (62), the mitochondrial AspRS of *S. cerevisiae* (AspSCm) (7), the cytoplasmic AspRS of rat (AspRT) (38) and the putative protein GX, an open reading frame located beside the *E. coli frdA* gene (9). Only the 152 amino-terminal residues of the putative protein GX are known. For clarity, a stretch of 130 amino acids from *S. cerevisiae* mitochondrial AspRS sequence has been deleted in the figure (amino acids 371 – 500). The residues which are conserved in at least three sequences including one *E. coli* LysRS sequence are boxed. The numbers on the right of the figure indicate the distances from the N-termini. Identity rates of each examined aminoacyl-tRNA synthetase with the *lysU* encoded LysRS are given as percentages at the end of each sequence. Sequences have been aligned with the CLUSTAL program (63) and further adjusted by hand to maximize the number of conserved residues among the 7 compared sequences.

the *Hind*III-*Bam*HI fragment for insertion between the corresponding sites of pBluescript(−)KS and pBluescript(+)SK. Resulting plasmids were quoted pXLysKS1 and pXLysSK2, respectively (Figure 1). LysRS activity was measured in extracts of strain JM101TR transformed by either each of these plasmids or the corresponding control pBluescript plasmids. These measurements were performed at the stationary stage of growth, in the presence or absence of IPTG. Independent of IPTG, plasmid pXLysSK2 caused a 4-fold overproduction of LysRS, if compared to the control plasmid. In contrast, plasmid

Table 1: Codon usage in the *lysS* and *lysU* genes.
The codon usages in the *lysS* and *lysU* genes are given as fractions of each codon within its redundancy family. They are compared with the average codon usage in either strongly expressed genes (SEG) or weakly expressed genes (WEG) (30). Numbers are boxed when the usage of the corresponding codon coincides in (a) both *lysS* and the strongly expressed genes (SEG), and (b) both *lysU* and the weakly expressed genes (WEG).

	U					C					A					G				
		lysS	*lysU*	SEG	WEG		*lysS*	*lysU*	SEG	WEG		*lysS*	*lysU*	SEG	WEG		*lysS*	*lysU*	SEG	WEG	
U	Phe	0.17	0.61	0.26	0.60	Ser	0.50	0.29	0.36	0.12	Tyr	0.36	0.73	0.26	0.60	Cys	0.00	1.00	0.36	0.46	U
		0.83	0.38	0.74	0.40		0.19	0.29	0.34	0.16		0.64	0.27	0.74	0.40		1.00	0.00	0.64	0.54	C
	Leu	0.02	0.11	0.03	0.12		0.00	0.12	0.02	0.12		ochre					opal				A
		0.06	0.09	0.04	0.11		0.12	0.06	0.04	0.21		amber				Trp	1.00	1.00	1.00	1.00	G
C		0.02	0.02	0.06	0.12		0.05	0.10	0.10	0.13	His	0.50	0.73	0.20	0.62		0.66	0.52	0.67	0.29	U
	Leu	0.21	0.09	0.08	0.12	Pro	0.00	0.05	0.01	0.21		0.50	0.27	0.80	0.38	Arg	0.34	0.33	0.30	0.38	C
		0.00	0.04	0.01	0.04		0.05	0.37	0.12	0.20	Gln	0.17	0.38	0.18	0.35		0.00	0.05	0.01	0.08	A
		0.69	0.64	0.79	0.49		0.89	0.47	0.77	0.46		0.83	0.62	0.82	0.65		0.00	0.05	0.00	0.12	G
A		0.39	0.58	0.20	0.52		0.07	0.24	0.36	0.17	Asn	0.09	0.50	0.08	0.51	Ser	0.00	0.00	0.04	0.19	U
	Ile	0.61	0.42	0.79	0.39	Thr	0.70	0.38	0.48	0.43		0.90	0.50	0.92	0.49		0.19	0.23	0.19	0.20	C
		0.00	0.00	0.01	0.09		0.07	0.14	0.05	0.42	Lys	0.74	0.90	0.71	0.79	Arg	0.00	0.05	0.01	0.08	A
	Met	1.00	1.00	1.00	1.00		0.15	0.24	0.10	0.28		0.26	0.10	0.29	0.21		0.00	0.00	0.00	0.05	G
G		0.48	0.55	0.44	0.31		0.12	0.30	0.37	0.17	Asp	0.45	0.73	0.36	0.63		0.45	0.67	0.54	0.33	U
	Val	0.11	0.25	0.09	0.19	Ala	0.12	0.15	0.10	0.34		0.55	0.33	0.64	0.37	Gly	0.49	0.18	0.41	0.38	C
		0.15	0.21	0.27	0.14		0.30	0.32	0.25	0.20	Glu	0.74	0.76	0.76	0.68		0.03	0.06	0.01	0.11	A
		0.26	0.18	0.19	0.36		0.45	0.22	0.28	0.29		0.26	0.24	0.24	0.32		0.03	0.09	0.03	0.18	G

pXLysKS1 caused 50-fold and 100-fold overproduction, in the absence or presence of IPTG, respectively. These values clearly established the presence of an open reading frame coding for an active LysRS on the cloned 3.5 kbp *Hind*III-*Bam*HI fragment.

Sequencing of the *lysU* and *lysS* genes

The nucleotide sequences of *lysS* and *lysU* genes, along with the corresponding deduced amino acid sequences are shown in Figure 2.

The 20 N-terminal residues of the *lysU* product, as predicted by the DNA sequence, could be confirmed by chemical sequencing of a homogeneous LysRS sample purified from a *lysU* overexpressing strain. The N-terminal sequence SEQETRGANEAIDFNDELRN starts with a serine, as in the case of the *lysS* product. The lack in the two mature products of the N-terminal methionine was not surprising in view of recent data on the rules of N-methionine excision (28 and references therein).

Translations of the DNA sequences of *lysS* and *lysU* reveal that each LysRS contains only one cysteine and two tryptophan residues. Such very low amounts of cysteine and tryptophan agree with previous amino acid analysis of *E. coli* LysRS (29). For the other amino acids, the agreement is also rather good, since the difference between chemically determined and DNA predicted compositions (from *lysS* or *lysU* sequences) never exceeds 12%.

The *lysS* DNA sequence is found identical to *herC*, a gene defined by a suppressor mutation that restores replication of a ColE1 plasmid mutant (8). In fact, the *herC* gene was already suspected by Gampel and Tzagoloff to be *lysS*, on the basis of homologies between its product and yeast cytoplasmic LysRS (7). Based on the same type of sequence similarities, the above authors also proposed that another *E. coli* locus, close to *frdA* (9), could be *lysU*. The *lysU* primary structure solved in the present work excludes such a hypothesis, although 32 % identity can be recognized between *lysU* as well as *lysS* products and the sequenced part of the putative product of the unidentified gene (Figure 3).

Comparison of *lysS* and *lysU* genes and of their products

The *lysU* and *lysS* gene products share 88% homology, i.e. only differ by 59 amino acids out of 505. There is no change in number or place for tryptophan, methionine, cysteine and proline residues. The molecular weights and the isoelectric points, as calculated from the sequences, are 57.8 kDa and 5.39, respectively, for the *lysU* gene product, and 57.4 kDa and 5.31, respectively, for the *lysS* gene product. These values agree with the migration positions of these two proteins on 2D-electrophoresis gels (1).

When comparing the *lysS* and *lysU* structural genes, 268 codons (among 505) are different. Such a difference contrasts with the high level of identity between the protein primary structures. The codon usages in the *lysU* and *lysS* genes were compared to those for highly and weakly expressed *E. coli* genes (30). For proline, asparagine, aspartic acid, tyrosine, phenylalanine, glutamine and isoleucine, the codons preferentially used in *lysS* indicate an abundant protein (Table 1). On the contrary, the codons used for the same set of amino acids in *lysU* indicate a weakly translated protein.

A quantitative measurement of the bias in codon usage can be given by the average number of tRNA discriminations per elongation cycle (31). In the case of *lysS*, this number is equal to 28, a value which corresponds to a protein of medium abundance, whereas, in the case of *lysU*, it reaches 32, a value corresponding to a weakly expressed protein. Another independent criterium of gene translation is the C/U index, a ratio which describes the choice between C and U among codons beginning with AA, AU, UA, UU, GG, GC, CG and CC (32−35). This index is normally high for abundant proteins. The C/U index for *lysS* (67%) is greater than that for *lysU* (53%), suggesting well a higher translation efficiency of the former, in agreement with *in vivo* studies (1,36).

Homology with other genes

As shown in the present study, *herC* exactly coincides with *lysS*, and *lysS* is strongly identical to *lysU*. Recently, primary structure homologies were noted between the *herC* gene product (i.e. *lysS* product, from our work) and yeast cytoplasmic LysRS (7). When *E. coli* and yeast LysRS are further compared, it may be noted that the yeast LysRS mainly differs from the two *E. coli* enzyme species by the occurrence of an N-terminal extension of about 55 amino acids. This observation remarkably supports the suggestion made by Cirakoglu and Waller (37), on the basis of limited proteolysis studies, that the yeast LysRS was composed of two domains: one C-domain of Mr ≈ 65 kDa, homologous to the prokaryotic enzymes, and one additional positively charged N-domain of Mr ≈ 8 kDa, capable of causing the association of the yeast enzyme to polyanionic carriers.

Homology between yeast cytoplasmic LysRS and yeast cytoplasmic and mitochondrial AspRSs has been noted by Gampel and Tzagoloff (7). The presently determined *lysU* structure enlarges the validity of this homology to both the *E. coli* species of LysRS (Figure 3). The homology between the two families of AspRSs and LysRSs, specific of an acid and a basic amino acid, respectively, is further reinforced by the examination of the rat and the human cytoplasmic AspRS sequences (38, 39). These sequences are also strongly identical to the *E. coli* and yeast LysRS sequences (40) (Figure 3). More precisely, 21%, 21% and 19% identity can be calculated between the rat (or human) cytoplasmic AspRS sequence and the *lysS* gene product sequence, the *lysU* gene product sequence and the yeast LysRS sequence, respectively, while the identity rates between rat (or human) AspRS and the cytoplasmic and mitochondrial forms of yeast AspRS are 58% and 20%, respectively.

In addition to the above extended identity between LysRS and AspRS families, restricted regions of similitude could also be noted between *E. coli* LysRSs, yeast cytoplasmic and mitochondrial PheRS (41, 42), *E. coli* PheRS (43), *E. coli* AlaRS (44) and *E. coli* HisRS (45) (Figure 4). The functional unicity of the reactions catalyzed by all aminoacyl-tRNA synthetases may explain the presence in the above proteins of similar amino acid

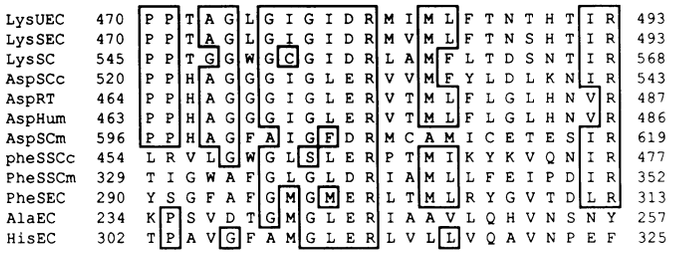

Figure 4: Amino acid sequence identity between *E. coli* LysRSs and other available sequenced aminoacyl-tRNA synthetases. The numbers on the left and the right of the sequences refer to the first and the last listed residues relative to the N-terminus of the corresponding synthetase. Solid boxes indicate both identical amino acids and conservative substitutions. Abbreviations and references: LysUEC, *E. coli lysU* gene product; LysSEC, *E. coli lysS* gene product; LysSC, *S. cerevisiae* cytoplasmic LysRS (40); AspSCc, *S. cerevisiae* cytoplasmic AspRS (62); AspSCm, *S. cerevisiae* mitochondrial AspRS (7); AspRT, rat cytoplasmic AspRS (38); AspHum, human cytoplasmic AspRS (39), PheSSC, small subunit of the *S. cerevisiae* cytoplasmic PheRS (41); PheSSCm, small subunit of the *S. cerevisiae* mitochondrial PheRS (42); PheSEC, small subunit of the *E. coli* PheRS (43); AlaEC *E. coli* AlaRS (44), HisEC, *E. coli* HisRS (45).

stretches. Noteworthy, the peptide of human AspRS shown in Figure 4, includes a motif proposed to indicate the ATP site on the synthetase sequence (39).

CONCLUSION

The *lysS* sequence presented in this work unambiguously establishes its identity to *herC*. The latter gene was defined through the mutation *herC*180, isolated as a host suppressor of a *cer-114* replication-deficient ColE1 plasmid (8). The *cer-114* mutation is a 1 bp substitution in the primer RNA II gene abolishing initiation of plasmid DNA synthesis, probably by an alteration of primer RNA II conformation (8). The fact that *herC* is *lysS* points to a possible involvement of LysRS in some reaction that affects RNA primer stability or conformation. The clover-leaf structures that can be adopted by the primer RNA II in the region of the *cer-114* mutation (46, 47) could make this RNA a possible ligand of LysRS.

herC (thus *lysS*) is cotranscribed downstream of *prfB*, the gene for peptide chain release factor 2 (RF2) (8). The role of this factor is to promote polypeptide chain termination at UGA and UAA codons (48). The expression of RF2 is particular since its complete translation depends on a frameshift at a UGA codon located at amino acid 26 from the amino terminus (49). It has been suggested that this mechanism might be the basis of an autotranslational control: excess of RF2 would favor termination over frameshifting at the UGA codon, and, therefore, would decrease RF2 expression. The location of *lysS*, 9 bp downstream of the end of *prfB*, the absence of a promoter specific for *herC* transcription (8) and the translation polarity usually observed in polycistronic operons, contribute all to suggest a linked regulation of *lysS* and *prfB* expression.

The *lysU* product sequence is similar (32% identity) but not identical to the amino acid sequence deduced from the open reading frame GX located beside the *E. coli frdA* gene (9). Several possibilities can be envisaged to explain the presence of this open reading frame: GX may encode (a) an uncharacterized protein capable of interacting with either lysine, aspartic acid or tRNA,

or (b) a not yet identified *E. coli* aminoacyl-tRNA synthetase. In this context, it is noticeable that, to our knowledge, *proS*, *cysS*, and *asnS* genes have not yet been isolated. However, their locations on the *E. coli* linkage map appear markedly different from that of the *frdA* locus (50).

The present work was undertaken to compare the two *E. coli* genes encoding two different species of LysRS: *lysS* and *lysU*. The protein primary structures deduced from the DNA sequences are 88% identical. Such a high degree of similarity was not *a priori* expected, although common features had already been observed in the tryptic maps of the two LysRS species (1).

The high identity between *lysS* and *lysU* genes strongly indicates a common origin, possibly through duplication of an ancestral gene or translocation from a related prokaryotic species. In *E. coli*, sequence similarities between genes have already been recognized. Usually, they correspond to proteins whose functions are only slightly different, or whose substrates are identical: chemoreceptors (*tar*, *tap*, *tsr*, *trg*) (51, 52), outer membrane proteins (*ompC*, *ompF*, *phoE*) (53), cystathionine-γ-synthase and β-cystathionase (*metB*, *metC*) (54), peptide chain releasing factors 1 and 2 (*prfA*, *prfB*) (49). In a few cases, however, the proteins encoded by the homologous genes appear to have exactly the same function: EF-Tu is encoded by the *tufA* and *tufB* genes (55, 56), aspartokinase-homoserine dehydrogenase, by the *thrA* and *metL* genes (57), and ornithine carbamoyltransferase, by the *argF* and *argI* genes (58). The degree of identity and the differences in codon usage in the latter example resemble the differences observed here between the two LysRS genes. Indeed, the two ornithine carbamoyl-transferase genes are 78% identical at the nucleotide level and 86% identical at the amino acid sequence level (58). The presence of two IS1 elements flanking *argF* suggested that this gene could result from the translocation of the *argI* gene from a related species into *E. coli* (58). Such an idea, however, leaves unsolved the functional advantage conferred by the presence in the bacterium of two genes, instead of one.

In the case of the *lysS* and *lysU* genes, a striking feature is the unusual regulation of the *lysU* gene. As mentioned in the introduction, the expression of this gene can be induced under exceptional physiological conditions. This special behaviour of *lysU* expression may reflect the need of the cell to accumulate more LysRS activity to respond to extreme conditions, like a temperature upshift. Another possibility is that the *lysU* product, although very similar to the *lysS* one, has acquired specific, not yet revealed, catalytic properties necessary to cell adaptation. In this context, it may be useful to recall that aminoacyl-tRNA synthetases are capable of sustaining unusual functions, beyond the normal reaction of aminoacylation of the tRNAs involved in translation. In particular, a recent work from this laboratory has established that, *in vivo*, aminoacyl-tRNA synthetases contribute to the synthesis of bis(5'-nucleosidyl) tetraphosphates (12). In eukaryotes, aminoacyl-tRNA synthetases are suspected to participate in the regulation of the transport of certain amino acids (59) and in the post-translational aminoacylation of proteins (60, 61).

In conclusion, the present work opens the way to further studies on (a) the regulation of the *lysU* expression versus the *lysS* one and, (b), the physiological consequences of the inactivation of each of these genes. Such comparisons will possibly help to solve the question raised by the occurrence in *E. coli* of two apparently very similar LysRS species.

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