JOURNAL OF BACTERIOLOGY, Dec. 1991, p. 7903–7910
0021-9193/91/247903-08$02.00/0

Vol. 173, No. 24

Control of *Escherichia coli* Lysyl-tRNA Synthetase Expression by Anaerobiosis

FRANÇOISE LÉVÊQUE,[1] MYRIAM GAZEAU,[1,2] MICHEL FROMANT,[1] SYLVAIN BLANQUET,[1,2] AND PIERRE PLATEAU[1]*

Laboratoire de Biochimie, Unité de Recherche Associée 240 du Centre National de la Recherche Scientifique, Ecole Polytechnique, 91128 Palaiseau Cedex,[1] and Service de Biologie Macromoléculaire, Institut de Biologie Physico-chimique, 75005 Paris,[2] France

Received 11 February 1991/Accepted 12 October 1991

Escherichia coli **lysyl-tRNA synthetase was previously shown to occur as two distinct species encoded by either the *lysS* or the *lysU* gene. The expression of one of these genes, *lysU*, is under the control of cell growth conditions. To study the regulation of *lysU*, Δ*lysU* and Δ*lysS* strains were constructed. During aerobic growth at 37°C or below, the amount of the *lysU* product in the cell is so reduced that Δ*lysS* bacteria grow only poorly. The reduced expression of *lysU* is not related to the steady-state lysyl-tRNA synthetase concentration in the cell, since the expression of a *lysU*::*lacZ* fusion is insensitive to the absence of either *lysS* or *lysU* or to the addition of a multi-copy plasmid carrying either *lysU* or *lysS*. During anaerobic growth in rich medium, the *lysU* gene becomes strongly expressed and, in cell extracts, the amount of lysyl-tRNA synthetase activity originating from *lysU* may become seven times greater than the activity originating from *lysS*. In minimal medium, *lysU* expression is only slightly induced. Evidence that the sensitivity of *lysU* expression to anaerobiosis, as well as to low external pH conditions (E. W. Hickey and I. N. Hirshfield, Appl. Environ. Microbiol. 56:1038–1045, 1990), is governed at the level of transcription is provided.**

Lysyl-tRNA synthetase (LysRS) from *Escherichia coli* has the pecularity to occur as two species encoded by two distinct genes (16, 18). The corresponding genes, *lysS* and *lysU*, have been cloned and sequenced (22). They code for proteins differing only by 59 of 505 amino acids. The two genes have been mapped on the *E. coli* chromosome at kbp 3050 and 4430, respectively. *lysS* is cotranscribed with *prfB*, the gene encoding the peptide chain release factor 2 (20, 22).

The regulation of *lysU* and that of *lysS* appear to be very different. While the *lysS* gene seems to be constitutively expressed (16, 18), the *lysU* gene is usually almost silent. However, it can be induced in strains deficient for *metK* or *cadR* (8, 17, 23) or under certain physiological conditions such as low external pH or the presence of L-alanine, L-leucine, L-glycyl-leucine, or D-fructose (7, 16). *lysU* was also shown to belong to the heat shock regulon (29). However, its expression is not modified by the overexpression of the heat shock factor σ^{32} from a multicopy plasmid (41).

To study the regulation of the *lysU* gene, we constructed *lysU* or *lysS* null mutants, as well as operon and protein fusions between *lysU* and *lacZ*, the gene for β-galactosidase. Lysyl-tRNA synthetase and β-galactosidase activities were measured in crude extracts of these strains grown under various conditions. The data obtained reveal that the *lysU* product markedly accumulates when bacteria are grown anaerobically in rich medium. In addition, the regulation of *lysU* is shown to occur at the transcriptional level.

MATERIALS AND METHODS

Enzymes and substrates. DNA restriction and modification enzymes were purchased from Boehringer (Mannheim, Germany), Bethesda Research Laboratory (Rockville, Md.), or Pharmacia (Uppsala, Sweden). [γ-^{32}P]ATP (111 TBq/mmol) was from NEN (Cambridge, Mass.). [^{14}C]lysine (12 GBq/mmol) was from the Commissariat à l'Energie Atomique (Saclay, France). Pure unfractionated *E. coli* tRNA was purchased from Boehringer.

Strains were grown either in LB medium (26) or in MOPS (morpholinepropanesulfonic acid) minimal medium (28) supplemented with 0.4% glucose, 40 μg of proline per ml, and 40 μg of methionine per ml. Unless otherwise stated, anaerobic conditions were obtained by the use of GasPaks (from BioMérieux, Craponne, France) in a hermetically closed jar. In some cases, bacteria were grown in a 2-liter fermentor from Biolaffitte (Saint-Germain-en-Laye, France). Antibiotics in the growth media were used at the following concentrations (in micrograms per milliliter): ampicillin, 60; kanamycin, 80; chloramphenicol, 5; and tetracycline, 10.

tRNA aminoacylation and β-galactosidase activities were measured in crude cell extracts obtained by sonication, as already described (5, 26). The total amount of protein in the extract was estimated by using the Bio-Rad protein assay. One unit of enzymatic activity is defined as the amount of enzyme capable of producing 1 nmol of aminoacyl-tRNA or of *o*-nitrophenol, respectively, per min.

Strains, plasmids, and phages. The strains used in this study are listed in Table 1. Plasmid pMAK705 was a gift from S. R. Kushner (12). The generalized transducing phage P1vir was used as described by Miller (26). Phages λXP817 and λXD22 have been previously described (24). They carry mutated *pheST-lacZ* fusions. In the first one, three point mutations in the attenuator of the *pheST* promoter result in superattenuation of *pheST* transcription. In the second one, a deletion in the attenuator region results in constitutive derepression of *pheST* transcription.

Plasmids pXLysCla3 and pXLys5 carried the *lysU* gene inserted into vector pBluescript(+)KS (22). Plasmid pXUK was derived from pXLysCla3 by (i) removing the *Pst*I site in the polylinker by a *Bam*HI-*Hin*dIII deletion and (ii) inserting the *kan* gene cassette from pUC4K (42) between the *Pst*I[1] and the *Pst*I[4] sites (Fig. 1).

* Corresponding author.

TABLE 1. *E. coli* strains used in this study

Strain	Relevant genotype	Reference
XA103	F⁻ Δ(*lac-pro*) *gyrA rpoB metB argE*(Am) *ara supF*	9
K37	*galK rpsL*	25
JC7623	F⁻ *thr-1 leuB6* Δ(*gpt-proA*)*62 hisG4 argE3 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 rpsL31 supE44 tsx-33 recB21 recC22 sbcB15*	33
JC10240	Hfr(PO45) *thr-3000 recA56 srl-300*::Tn*10 relA1 ilv-318 spoT1 thi-1 rpsE2300*	11
JM101TR	*supE thi* Δ(*lac-pro*) *recA56 srl-300*::Tn*10* F′ (*traD36 lacI*^{q} *proAB lacZ*Δ*M15*)	15
PAL2103UK	XA103 Δ*lysU*::*kan*	This work
PAL2103UKTR	XA103 Δ*lysU*::*kan srl-300*::Tn*10 recA56*	This work
PAL3103SΔK	XA103 *lysS*::*kan*	This work
PAL3103SK	XA103 Δ*lysS*::*kan*	This work
PAL3103SΔKTR	XA103 *lysS*::*kan srl-300*::Tn*10 recA56*	This work
LCB261	F⁻ *thi thr-1 leuB6 lacY1 ana-1 fnr*(*nirR22*) *zcj261*::Tn*10 rpsL*	31

Plasmids pXLysSK2 and pXLysKS1 contained the *lysS* gene region, inserted into pBluescript(+)SK and pBluescript(−)KS, respectively (22). Plasmid pXLysSK2 was restricted by *Bam*HI and *Hpa*I, filled in, and recircularized. The *kan* gene was inserted into the *Pst*I site of the resulting plasmid to make pXΔBH. Plasmid pXSK was constructed by replacing the *Eco*RI[1]-*Eco*RI[2] fragment of plasmid pXLysSK2 by the *kan* gene. Plasmids pMSK and pMΔBH

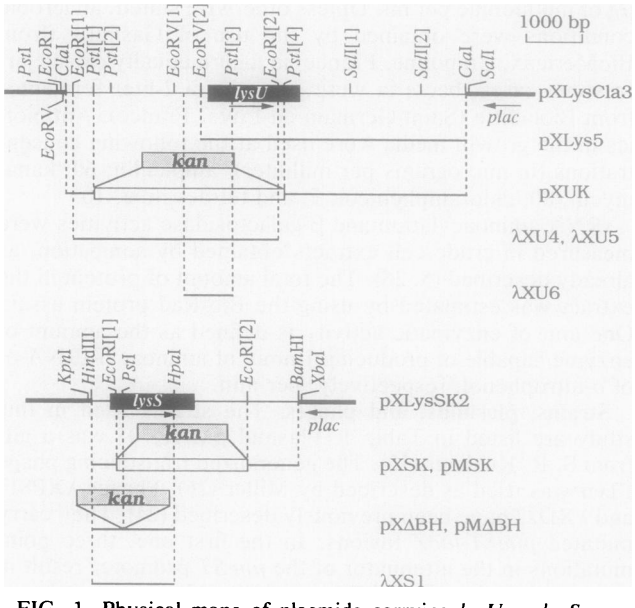

FIG. 1. Physical maps of plasmids carrying *lysU* or *lysS* gene regions. pBluescript vector is indicated by a thick line. Black boxes symbolize the *lysU* or *lysS* genes. Open boxes correspond to deletions and grey boxes to the kanamycin resistance gene cassette. Arrows indicate the direction of transcription from the *lysU* or *lysS* promoter and from the *lacZ* promoter carried by pBluescript. Plasmid pXLysKS1 was identical to pXLysSK2, except that the *Hin*dIII-*Bam*HI fragment was cloned into pBluescript(−)KS instead of pBluescript(+)SK.

were obtained by inserting the *Kpn*I-*Xba*I fragment of pXSK and pXΔBH, respectively, into the corresponding restriction sites of pMAK705 (Fig. 1).

For the construction of *lysU-lacZ* operon or protein fusions carried by λXU5 or λXU4, the *Eco*RI[1]-*Eco*RI[2] fragment of pXLysCla3 (Fig. 1) was cloned into the *Eco*RI site of plasmid pRS415 (36) or into the *Eco*RI site of a plasmid derived from pRS414 (36) by filling in the *Bam*HI site. For the construction of λXS1, the *Hin*dIII-*Hpa*I fragment of pXLysKS1 was filled in and inserted in the correct orientation into the *Sma*I site of pRS415. When introduced in the strain JM101TR, the recombinant plasmids gave blue colonies in the presence of 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-Gal). The *lysU-lacZ* or *lysS-lacZ* fusions carried by these plasmids were transferred to λRS45 (36) by homologous recombination.

For the construction of the *lysU-lacZ* operon fusion carried by λXU6, the oligonucleotides 5′-AATGAATTCGGCT GGCAGGCGTCGTGGCACAGC-3′ and 5′-AATGGATCC CAGACCATGGAATTTATCTGGTAA-3′ were used to amplify by the polymerase chain reaction a 910-bp fragment encompassing the 5′ end of the *lysU* structural gene (Fig. 1). After digestion by *Eco*RI and *Bam*HI, the amplified fragment was cloned into the plasmid pRS415 previously digested by the same enzymes. As described above, transfer to λRS45 of the *lysU-lacZ* fusion was performed by homologous recombination. Two lysogens were derived from independent amplification experiments. It was verified that the two clones yielded the same results.

Construction of *lysU* null mutants. Plasmid pXUK (Fig. 1) was used to transform the *recBC sbcB* strain JC7623. Since the replication of ColE1 plasmids is not possible in this strain (4), recombination events between the plasmid and the chromosome could be selected by plating bacteria on a medium containing kanamycin. To avoid the special genetic background of JC7623, the kanamycin resistance gene was then transferred into strain XA103 by P1 transduction, to give PAL2103UK (Table 1). Disruption of the *lysU* gene in strain PAL2103UK was confirmed by Southern blot analysis of chromosomal DNA. PAL2103UK was made deficient for recombination by transduction of the *recA56* allele of the Hfr strain JC10240, to give the strain PAL2103UKTR (Table 1).

Construction of *lysS* null mutants. Strain K37 was transformed by plasmids pMSK and pMΔBH. Since the replication of these plasmids is thermosensitive, their integration into the chromosome could be followed at 43°C in the presence of chloramphenicol (12). After subsequent growth at 30°C, cells that no longer carried the plasmid into their chromosome were identified as chloramphenicol-sensitive colonies at 43°C. Among them, about 2% were also kanamycin resistant at 43°C, suggesting that the *lysS*::*kan* mutations carried by pMSK or pMΔBH were now located on the chromosome. The kanamycin resistance marker was transferred from these strains into XA103 by P1 transduction, to give PAL3103SK and PAL3103SΔK, respectively. Southern blot analysis confirmed the disruption of the *lysS* gene in these strains. A *recA* derivative of the strain PAL3103SΔK was obtained by transduction of the *recA56* allele and was named PAL3103SΔKTR (Table 1).

Mapping of the *lysU* promoters. Total cellular RNAs were isolated from strains XA103 and PAL2103UK by phenol extraction, as described previously (1). For primer extension analysis, the 30-mer oligonucleotide 5′-TCTCAGTTCATC GTTAAAATCAATAGCCTC-3′, corresponding to the bases 31 to 60 within the *lysU* structural gene sequence (22), was chemically synthesized. Two picomoles of this oligonucleo-

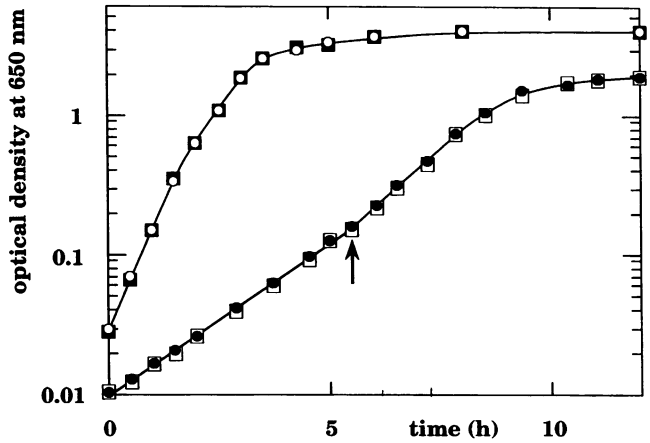

FIG. 2. Growth curves of strains XA103 (*lysU*$^+$) and PAL2103UK (*lysU*). Bacteria were grown aerobically in 500-ml flasks containing 40 ml of medium. Growth in LB medium at 44°C of strain XA103 (■) or PAL2103UK (○) and growth in glucose-supplemented MOPS medium at 28°C and after a shift at 44°C (at the time indicated by the arrow) of strain XA103 (□) or PAL2103UK (●) are shown. The latter condition was used by Clarke and Neidhardt when they studied the behavior of another *lysU* mutant (8).

tide labeled with [γ-^{32}P]ATP (34) and about 150 μg of total RNA were mixed in 30 μl of a solution containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 100 mM KCl containing 5 U of AMV reverse transcriptase (from Pharmacia), and 1 mM (each) dATP, dCTP, dGTP, and dTTP. After a 30-min incubation at 42°C, the sample was diluted 10-fold in a solution containing 10 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM EDTA containing 25 μg of boiled RNase A (from Boehringer), left to incubate for 30 min at 37°C, and ethanol precipitated. The labeled extension products were electrophoresed on a 6% polyacrylamide-urea gel.

Nucleotide sequence accession number. The DNA sequence of the *cadA* gene region has been assigned GenBank/EMBL accession number M67452.

RESULTS AND DISCUSSION

Growth of *lysU* or *lysS* null mutants. To determine whether any of the two isospecies of LysRS was able to ensure cell growth, chromosomal *lysS* or *lysU* genes were inactivated by insertion of a kanamycin resistance gene, as described in Materials and Methods. The disrupted genes could be easily transferred by P1 transduction. Secondary mutations not closely linked to the inactivated gene could therefore be excluded.

The growth of *lysU* mutant strains (PAL2103UK and PAL2103UKTR) was studied at 30, 37, 42, or 44°C either in LB medium or in MOPS-glucose medium. Whatever the medium or the temperature, growth curves were indistinguishable from those of the parental *lysU*$^+$ strain. Typical experiments are shown in Fig. 2. These results contrast with the behavior of another *lysU* mutant, reported to grow poorly at 44°C (8), whereas they are in agreement with the behavior of recently characterized *lysU* mutants with different genetic backgrounds (13).

lysS cells (PAL3103SΔK, PAL3103SK, and PAL3103 SΔKTR) grew more slowly than the control strain, especially at low temperatures. In LB medium, at 30, 37, and 42°C, the *lysS* cells displayed growth rates equal to 40, 70, and 90% of the control rate, respectively. The phenotypic cold sensitivity of a *lysS* mutant has been already noted by Kawakami et al. (21) under the nomenclature of *herC*.

TABLE 2. LysRS activity in various *E. coli* mutant strains[a]

Strain	LysRS activity (U/mg)			
	30°C		42°C	
	Exponential growth	Stationary phase	Exponential growth	Stationary phase
XA103	11	13	12	33
PAL2103UK (*lysU*)	10	11	10	11
PAL3103SΔK (*lysS*)	1.1	1.3	2.7	23

[a] Bacteria were grown aerobically in 18-mm test tubes containing 5 ml of LB medium. The tubes were incubated with shaking at the indicated temperatures. An aliquot of the culture was first withdrawn when the optical density of the culture reached 0.5 at 650 nm. The remaining part of the culture was further grown until the stationary phase was reached, and then a second aliquot was withdrawn. LysRS activity (in units per milligram of total proteins) was measured in crude extracts obtained by sonication.

The cold-sensitive phenotype of the *lysS* cells was accompanied by a significant decrease in LysRS activity (Table 2). At 30°C, the activity of strain PAL3103SΔK (*lysS*) was about 10 times smaller than that of parental strain XA103. The direct relationship between a lowered growth rate of the *lysS* strain at 30°C and a lowered cellular LysRS activity was further established as follows. Strain PAL3103SΔKTR was plasmid transformed with either pXLys5 (carrying *lysU*), pXLysKS1 (carrying *lysS*), or pBluescript (control). At 30°C in LB medium, pXLys5 and pXLysKS1 increased cellular LysRS activity in exponentially growing cells, to values of 55 and 280 U/mg of protein, respectively. Concomitantly, these plasmids restored a 100% growth rate at 30°C. pBluescript had no effect on both growth and LysRS activity. This shows that, despite the low temperature, the *lysU* gene product alone is able to sustain a normal growth rate, provided its concentration is made high enough in the cell.

Studies of *lysU* gene expression using *lysU-lacZ* fusions. Surprisingly, when bacteria were grown at 42°C, LysRS activity in the *lysS* extracts strongly depended on the stage of the culture. In several experiments, cells were grown in 18-mm test tubes containing 5 ml of LB medium. In these conditions, LysRS activity in the lysS mutant strain was 8.5-fold higher during the stationary phase than during exponential growth (Table 2). Remarkably, the increase in extracts of the control *lysS*$^+$ *lysU*$^+$ strain (from 12 to 33 U/mg) was identical to that observed in extracts of the *lysS* *lysU*$^+$ strain (from ~3 to 23 U/mg), while activity remained independent of the growth stage in the *lysS*$^+$ *lysU* strain extracts. This suggested that the increase in LysRS activity in stationary cells originated from the presence of *lysU* and that *lysU* expression was insensitive to the presence or absence of *lysS*.

To further probe this conclusion, operon and protein fusions between *lysU* and *lacZ* were constructed. In the operon fusion carried by λXU5, *lacZ* was expressed from its own translational regulatory signals, whereas these signals were lacking in the protein fusion carried by λXU4. Each of the phages λXU4 and λXU5 was used to lysogenize the *lac* mutant strain XA103 and its *lysU* or *lysS* derivatives. β-Galactosidase activity measurements in extracts from these strains are summarized in Table 3. In the case of each fusion, the same activity was found in the *lysS* mutant, the *lysU* mutant, or the parental strain, whatever the growth conditions assayed. In particular, a strong increase in β-galactosi-

TABLE 3. β-Galactosidase activity in various mutant strains[a]

Strain	β-Galactosidase activity (U/mg)			
	30°C		42°C	
	Exponential growth	Stationary phase	Exponential growth	Stationary phase
XA103(λXU5)	80	130	120	850
PAL2103UK(λXU5)	60	160	100	730
PAL3103SΔK(λXU5)	70	110	90	780
XA103(λXU4)		40		200
PAL2103UK(λXU4)		50		230
PAL3103SΔK(λXU4)	20	30	40	250
XA103TR(pBluescript)(λXU5)		140		670
XA103TR(pXLys5)(λXU5)[b]		130		760
XA103TR(pXLysKS1)(λXU5)[c]		120		830

[a] Bacteria were grown aerobically in 18-mm test tubes containing 5 ml of LB medium. In the case of cells harboring a plasmid, ampicillin was added to the growth medium at a final concentration of 60 μg/ml. The tubes were incubated with shaking at the indicated temperatures. An aliquot of the culture was first withdrawn when the optical density of the culture reached 0.5 at 650 nm. The remaining part of the culture was further grown until the stationary phase was reached, and then a second aliquot was withdrawn. β-Galactosidase activity (in units per milligram of total proteins) was measured from crude extracts obtained by sonication.

[b] In this strain, LysRS activity during stationary phase was equal to 50 and 470 U/mg at 30 and 42°C, respectively.

[c] In this strain, LysRS activity during stationary phase was equal to 640 U/mg, whatever the temperature (30 or 42°C).

dase activity was observed at the end of exponential growth at 42°C in all three of the strains described above. In addition, the amount of β-galactosidase activity in strain XA103TR (*lysS*$^+$ *lysU*$^+$) was independent of the *trans* overexpression of *lysU* or *lysS* (Table 3). From these results, it could be concluded that (i) *lysU* expression is not under the control of the concentration of the *lysS* gene product, (ii) *lysU* expression is not directly or indirectly autoregulated, and (iii) the observed stimulation of *lysU* expression is governed at the transcriptional level.

Induction of *lysU* expression at the end of exponential growth at 42°C. To determine the cause of the induction of *lysU* observed at the end of the exponential growth at 42°C in LB medium, LysRS and β-galactosidase activities in the strain PAL3103SΔK(λXU5) (*lysS lysU*$^+$ *lysU*::*lacZ*) were measured under various growth conditions. A culture was performed in a fermentor in which the concentration of dissolved oxygen during growth at 42°C was maintained equal to the oxygen concentration normally dissolved in LB medium at 42°C under atmospheric pressure in the presence of air and in the absence of bacteria. Moreover, the LB medium was buffered at pH 7.0 with 100 mM MOPS to avoid any modification of *lysU* expression through a pH variation of the culture medium (see below). In these conditions, LysRS- and β-galactosidase-specific activities each increased two- to threefold when bacteria reached the stationary phase (Fig. 3). This factor of increase was significantly smaller than that observed during cultures in test tubes (compare Table 2 with Fig. 3). Consequently, it was expected that *lysU* expression could be influenced by the degree of oxygenation of the growth medium.

***lysU* expression is increased by anaerobiosis.** To verify the above idea, bacteria were grown anaerobically in test tubes and stopped at the stationary phase. The absence of oxygen caused a marked increase in the LysRS content of PAL3103SΔK(λXU5) (*lysS lysU*$^+$) compared with the same cells grown at saturation in aerobic conditions (Table 4). It is

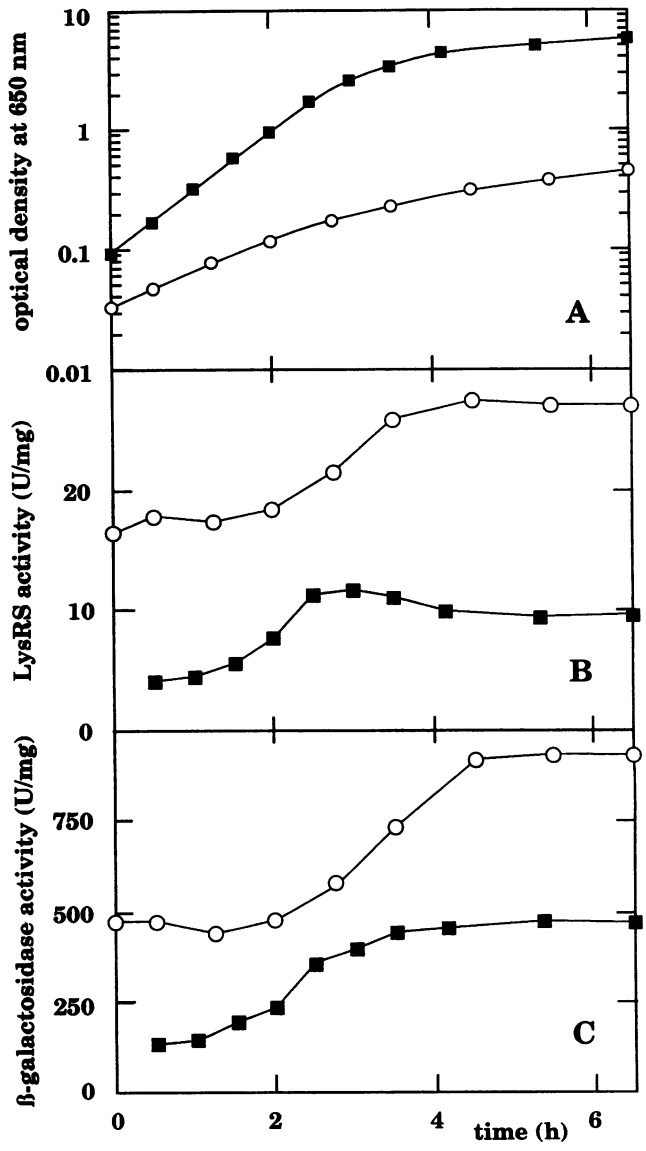

FIG. 3. LysRS and β-galactosidase activities of strain PAL3103SΔK(λXU5) grown in LB medium under aerobic (closed symbols) or anaerobic conditions (open symbols). Aerobic growth was performed at 42°C in a fermentor in which the concentration of dissolved oxygen was maintained constant during growth by adjusting the flow rate and/or the total pressure inside the fermentor. Anaerobic growth was performed at 37°C in the same fermentor, in which the medium was continuously flushed with argon. In both cultures, the LB medium was buffered at pH 7.0 with 100 mM MOPS. The optical densities of the cultures were measured at 650 nm (A). Aliquots of the cultures were withdrawn at the times indicated, and LysRS (B) and β-galactosidase (C) activities were measured in crude extracts obtained by sonication.

noteworthy that this increase was observed whatever the temperature of the culture (30, 37, or 42°C). The LysRS activity in the control *lysS*$^+$ *lysU*$^+$ strain was also enhanced by anaerobic conditions. The observed increases were likely to originate from *lysU* since (i) the LysRS activity in a *lysS*$^+$ *lysU* strain was indifferent to the presence or absence of oxygen and (ii) the amount of LysRS activity in the *lysS*$^+$ *lysU*$^+$ strain was exactly equal to the sum of the LysRS

TABLE 4. LysRS and β-galactosidase activities in different *E. coli* mutant strains grown under anaerobic conditions[a]

Strain	LysRS activity (U/mg)			β-Galactosidase activity (U/mg)		
	30°C	37°C	42°C	30°C	37°C	42°C
XA103(λXU5)	37	51	73	1,500	1,600	1,750
PAL2103UK(λXU5)	8	8	9	1,350	1,850	1,850
PAL3103SΔK(λXU5)	27	44	66	1,300	1,950	1,800

[a] Bacteria were inoculated at a final density of 0.005 at 650 nm and grown with shaking in test tubes at the indicated temperatures. Anaerobiosis was achieved in a hermetically closed jar by the use of GasPaks. Cultures were arrested when the stationary phase was reached (optical density at 650 nm of about 0.3). LysRS and β-galactosidase activities were measured from crude extracts obtained by sonication.

activities measured in the *lysS lysU*$^+$ and *lysS*$^+$ *lysU* strains. This conclusion was confirmed by measurements of β-galactosidase activity. Under the conditions of anaerobiosis, the β-galactosidase activity expressed from the *lysU*::*lacZ* operon fusion was increased by the same manner in the *lysS*$^+$ *lysU*$^+$, *lysS lysU*$^+$, and *lysS*$^+$ *lysU* strains (Table 4).

To exclude that this increase could originate from an enhanced stability of β-galactosidase in the absence of oxygen, control experiments were performed with lysogenized strain PAL3103SΔK carrying fusions between *lacZ* and genes other than *lysU*. *pheST-lacZ* fusions which were no longer regulated by transcriptional attenuation were carried by phages λXP817 and λXD22 (24). A fusion between *lacZ* and a 1,445-bp DNA fragment including the 5′ end of the *lysS* structural gene was carried by phage λXS1. After aerobic growth in LB medium at 37°C until the stationary phase, β-galactosidase activities in the lysogens corresponding to the three phages described above were equal to 52, 1,000, and 28 U/mg, respectively. After anaerobic growth in the same conditions, β-galactosidase activity from the phages amounted to 48, 810, and 16 U/mg, respectively. These experiments show that almost identical amounts of β-galactosidase activity were expressed from the *pheST* promoter, whatever the oxygenation of the culture. With the *lysS-lacZ* fusion, anaerobic conditions caused a twofold decrease in the β-galactosidase activity. Therefore, the stimulation of *lysU* in the absence of oxygen can be safely attributed to an effect on the *lysU* transcription.

To determine the effect of anaerobiosis during exponential growth, PAL3103SΔK(λXU5) cells were grown at 37°C in a fermentor in which the medium was continuously flushed with argon. As judged from LysRS and β-galactosidase measurements before the onset of the stationary phase (Fig. 3), *lysU* was strongly expressed. It is noteworthy that further increase in the LysRS and β-galactosidase activities occurred when cells entered the stationary phase. The latter induction of *lysU* expression resembles the effect of the onset of the stationary phase observed in aerobic cultures at 42°C. However, it might be simply caused by a decrease in the residual oxygen concentration in the fermentor due to oxygen consumption by bacteria.

Many genes induced by anaerobiosis have been reported to be controlled by *fnr* (40). Therefore, the *fnr* mutant allele from the strain LCB261 was transduced into the strain PAL3103SΔK. After anaerobic growth at 37°C until the stationary phase, LysRS activities in *lysS fnr* and *lysS fnr*$^+$ backgrounds were equal to 44 and 42 U/mg, respectively. This result makes it unlikely that, in these growth conditions, *lysU* regulation could depend on the *fnr* allele.

TABLE 5. LysRS and β-galactosidase activities in the strain PAL3103SΔK(λXU5) grown aerobically at 37°C under various pH conditions[a]

Growth additive[b]	pH	Generation time (min)	LysRS activity (U/mg)	β-galactosidase activity (U/mg)
None	6.9	37	1.6	66
100 mM MOPS	7.0	37	1.5	63
100 mM MES	4.8	61	3.8	155
100 mM MES	4.4	105	5.8	215
100 mM MES + 40 mM acetic acid	6.9	35	2.4	95
100 mM MES + 40 mM acetic acid	6.0	105	7.2	260
100 mM MES + 40 mM acetic acid	5.8	192	13.4	395

[a] Aerobic conditions were achieved with vigorous shaking of a 500-ml flask containing 40 ml of medium. Bacteria were withdrawn when the optical density of the culture reached 0.2 at 650 nm. It was systematically verified that the pH of the medium culture had not significantly changed at the time of the withdrawal. LysRS and β-galactosidase activities (in units per milligram of total proteins) were measured in crude extracts obtained by sonication.

[b] MES, morpholineethanesulfonic acid.

***lysU* expression is increased by acidic external pH.** Since *lysU* expression was recently reported to be stimulated by a downshift of external pH (14), we measured LysRS and β-galactosidase activities in PAL3103SΔK(λXU5) exponentially grown at 37°C in LB medium buffered at various pHs. LysRS activities were 3.6-fold higher at pH 4.4 than at pH 6.9 (Table 5). The effect of pH on *lysU* expression was reinforced by the presence of acetate in the culture medium. With 40 mM acetic acid, LysRS activity was 5.6-fold higher at pH 5.8 than at pH 6.9 (Table 5). Such an effect of acetic acid is possibly related to its known capacity to depress internal pH (32). The observed increases in LysRS were paralleled by increases in β-galactosidase activity (Table 5).

The effect of pH was controlled by using PAL3103SΔK lysogens carrying either *pheST-lacZ* or *lysS-lacZ* operon fusions. β-Galactosidase activities in the λXP817, λXD22, and λXS1 lysogens were measured from cultures grown aerobically (37°C) at pH 4.4 or 6.9 up to an optical density at 650 nm of 0.2. Activities at pH 4.4 were 2.3-, 2.0-, and 2.6-fold lower than those at pH 6.9.

Altogether, these experiments establish that the effect of pH on *lysU* expression occurs at the level of transcription.

Influence of the growth medium on *lysU* expression. All the experiments described above were performed with LB medium. When PAL3103SΔK(λXU5) cells were grown aerobically at 42°C in minimal MOPS-glucose medium, the LysRS and β-galactosidase activities remained relatively small during the exponential phase of growth (1.2 and 40 U/mg, respectively), as well as during the stationary phase (1.3 and 40 U/mg, respectively).

In MOPS-glucose medium, anaerobiosis did not markedly stimulate *lysU* expression. After anaerobic growth at 37°C in this minimal medium until the stationary phase, LysRS and β-galactosidase activities were only about twofold higher (2.3 and 65 U/mg, respectively) than after aerobic growth in the same conditions (1.0 and 35 U/mg, respectively).

A dependence on the growth medium of the pH-induced accumulation of the *lysU* product and of the heat shock protein C62.5 has already been noted by Hickey and Hirshfield (14). Another example of a gene inducible by either anaerobiosis or a low expernal pH is provided by the *Salmonella typhimurium aniG* gene which, as in the case of

lysU, is markedly more expressed in rich medium than in minimal medium (2).

Mapping of *lysU* promoters. The above results indicate that *lysU* expression is governed, at least partly, by an anaerobiosis-sensitive promoter cloned into the phage λXU5. To localize this promoter, a new *lysU-lacZ* fusion containing only 430 bp upstream to the *lysU* structural gene was constructed. A lambda phage carrying this fusion, λXU6, was used to lysogenize PAL3103SΔK. No difference in the β-galactosidase activities of strains PAL3103SΔK(λXU5) and PAL3103SΔK(λXU6) were observed after growth in LB medium under various conditions, including anaerobic growth at 30 or 42°C (exponential or stationary phase), anaerobic growth at 37°C (stationary phase), and aerobic growth at 37°C and pH 4.4 (exponential phase). Therefore, it could be concluded that the promoter was present on the DNA fragments cloned into λXU6.

The initiation sites of *lysU* transcription were searched for in this DNA region by primer extension with reverse transcriptase and a 5′-end-labeled oligonucleotide as primer. Extension products were analyzed by polyacrylamide gel electrophoresis (PAGE), in parallel with the products of a dideoxy sequencing reaction performed with the same labeled primer. Several extension products appeared on the electrophoretograms of the extension reactions performed with the RNAs extracted from strain XA103 (*lysU*$^+$) grown at 30°C in LB medium (Fig. 4, lane 1). Of these products, two were absent within the products made from strain PAL2103UK (*lysU*) (lane 2) and present or reinforced under conditions which stimulate *lysU* expression: exponential growth at 42°C, anaerobic exponential growth at 37°C, exponential growth at pH 4.4 and at 37°C, and last stage of exponential growth at 42°C (lanes 3, 4, 5, and 6, respectively). The sizes of these two products indicated initiation sites of transcription located 80 and 88 bp upstream from the *lysU* start codon AUG (Fig. 4). Upstream from these transcriptional starts, two −10 consensus boxes and one −35 consensus box could be recognized (Fig. 4). Their sequence composition resemble standard *E. coli* promoters rather than sequences recognized by σ^{32} (10) or σ^{54} (19). The other extension products made visible with the set of RNAs expressed from strain XA103 (*lysU*$^+$) were also present in lane 2. Therefore, we concluded that these products reflected other genes whose RNAs were capable of hybridizing with the probe used. In particular, a strong band of size ~125 bp vanished under conditions which stimulate *lysU* expression (lanes 3 to 6).

Initiation of *lysU* transcription from the same two start sites during induction by either temperature, pH, or anaerobiosis may indicate that, whatever the physiological condition applied, a common regulatory signal is involved. In this context, it is interesting to note that genetic controls responding both to anaerobiosis and pH have already been described in *S. typhimurium* (2). This suggests that the intracellular pH itself is an important regulatory signal. When aerobically grown cells are properly energized, they are able to maintain an internal pH of 7.5, whatever the external pH between 6.0 and 8.0 (27). On the other hand, when cells are grown anaerobically, the magnitude of the ΔpH maintained by the bacteria may be insufficient to keep the intracellular pH value at pH 7.5 (39). The reduction of the oxygenation rate of aerated cultures may also be accompanied by a decay of the intracellular pH (30, 38). Therefore, it is conceivable that the induction of *lysU* by anaerobiosis or by a low external pH is triggered by an acidification of the cytoplasm.

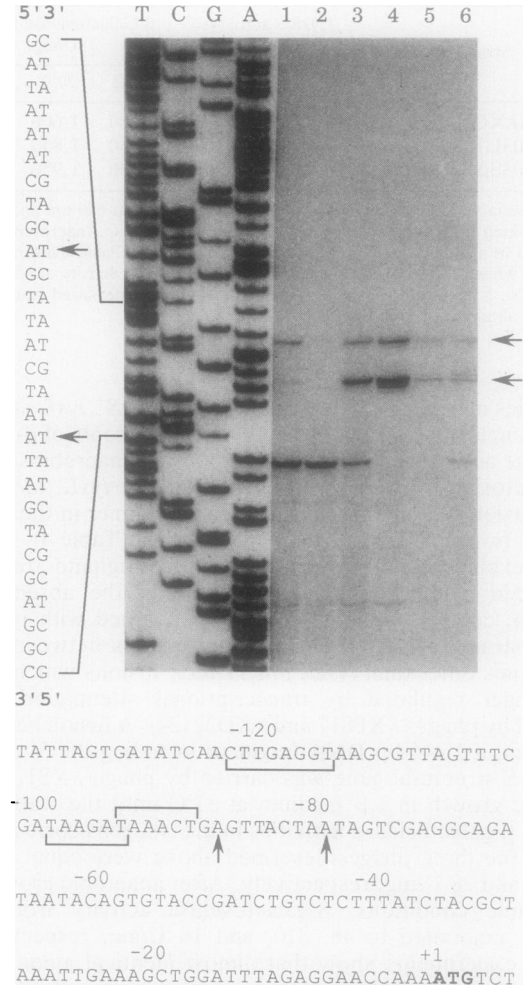

FIG. 4. Mapping of the 5′ terminus of *lysU* mRNAs. Total RNAs from strains XA103 (*lysU*$^+$) or PAL2103UK (*lysU*) were hybridized with a labeled single-stranded oligonucleotide probe corresponding to the bases 31 to 60 of the *lysU* gene sequence (8, 22). After extension of the probe with avian myeloblastosis virus reverse transcriptase, the products were electrophoresed on a 6% polyacrylamide-urea gel. Their sizes were determined by comparison to the products of a sequencing reaction performed with the same oligonucleotide probe. Lanes T, C, G, and A correspond to the sequencing pattern of *lysU* obtained through the dideoxy chain termination method (35) with a single-stranded DNA template. Lanes 1 to 6, various extension products obtained from RNAs of strain XA103 or PAL2103UK grown in LB medium; lane 1, strain XA103 grown aerobically at 30°C up to an optical density of 0.7 at 650 nm; lane 2, strain PAL2103UK grown aerobically at 30°C (up to an optical density of 0.7 at 650 nm); lane 3, strain XA103 grown aerobically at 42°C (up to an optical density of 0.3 at 650 nm); lane 4, strain XA103 grown anaerobically at 37°C (up to an optical density of 0.18 at 650 nm). In this case, growth was performed in a fermentor flushed with argon, as described in the legend to Fig. 3; lane 5, strain XA103 grown aerobically at pH 4.4 (up to an optical density of 0.14 at 650 nm) in LB medium supplemented with 100 mM MES; lane 6, strain XA103 grown aerobically at 42°C (up to an optical density of 3.2 at 650 nm). In this case, growth was performed in a fermentor in which the concentration of dissolved oxygen was maintained constant, as described in the legend to Fig. 3. During printing of the photograph, lanes 1 to 6 were exposed two times as long as lanes T, C, G, and A.

Concluding remarks. This work establishes that *lysU* expression is strongly coupled to the oxygenation of the growth medium. The effect of the pH of the medium, as previously reported by Hickey and Hirshfield (14), is confirmed.

These two conditions, anaerobiosis and acidic external pH stimulate the expression of another *E. coli* gene, *cadA* (sometimes called *exa*) (3, 37). The *cadA* gene encodes lysine decarboxylase, an enzyme producing cadaverin, and it is reasonable to suspect some functional link between the cationic character of cadaverin and the stimulation of *cadA* by conditions which might acidify cellular pH. The DNA sequence of the 5′ part of *cadA* was recently determined (E. R. Olson, E. B. Watson, and D. S. Dunyak). *cadA* appears to form an operon with an upstream gene, *cadB*, whose function is unknown. We noted that a sequence (ATGTTTATCTTTTCATGATATCAACTTG), located from 185 to 212 bp upstream of the putative initiator codon of *cadB*, is almost identical to a sequence (ATGGTTATTT ATTAGTGATATCAACTTG) located from 120 to 147 bp upstream of the *lysU* initiator codon. This similitude indicates that common regulatory elements might be shared by *cadBA* and *lysU* when responding to culture conditions.

Under the anaerobiosis conditions used in this study, the *lysU* gene does not appear essential for the growth of *E. coli* in the absence of oxygen since the *lysU* mutant strain grows anaerobically with a rate roughly identical to that of the isogenic *lysU*$^+$ strain. Whatever the functional role of the *lysU* product, it may be assumed, however, that it provides some advantage to the strain capable of expressing it. Under physiological conditions not yet discovered, the *lysU* product might be useful to adapt the cell to the new metabolic context caused by the accumulation of a lysine derivative produced by the *cadA* decarboxylase. In addition, the possibility that the *lysU* gene could be expressed to recognize tRNAs specifically produced or maturated during anaerobiosis must be underlined. It is noteworthy that several *E. coli* tRNAs, including $tRNA^{Lys}$, are known to incorporate selenium in response to anaerobic growth conditions (43). In *S. typhimurium*, the hydroxylation of the isopentenyl side chain of the modified base 2-methylthioisopentenyl-adenosine does not occur in the absence of oxygen (6). It was suggested that this modification could participate in the switch between aerobic and anaerobic pathways (6).

ACKNOWLEDGMENTS

We are indebted to M. A. Mandrand for the gift of bacterial strains.

F.L. is a member of the Direction des Recherches, Etudes et Techniques de la Délégation Générale pour l'Armement, Ministère Français de la Défense. This work was supported in part by a grant from the Fondation pour la Recherche Médicale.

REFERENCES

1. **Aiba, H., S. Adhya, and B. de Crombrugghe.** 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **256**:11905–11910.
2. **Aliabadi, Z., Y. K. Park, J. L. Slonczewski, and J. W. Foster.** 1988. Novel regulatory loci controlling oxygen- and pH-regulated gene expression in *Salmonella typhimurium*. J. Bacteriol. **170**:842–851.
3. **Auger, E. A., K. E. Redding, T. Plumb, L. C. Childs, S. Y. Meng, and G. N. Bennett.** 1989. Construction of *lac* fusions to the inducible argininie- and lysine decarboxylase genes of *Escherichia coli* K12. Mol. Microbiol. **3**:609–620.
4. **Bassett, C., and S. R. Kushner.** 1984. Exonucleases I, III, and V are required for stability of ColE1-related plasmids in *Escherichia coli*. J. Bacteriol. **157**:661–664.
5. **Brevet, A., J. Chen, F. Lévêque, P. Plateau, and S. Blanquet.** 1989. *In vivo* synthesis of adenylylated bis(5′-nucleosidyl) tetraphosphates (Ap_4N) by *Escherichia coli* aminoacyl-tRNA synthetases. Proc. Natl. Acad. Sci. USA **86**:8275–8279.
6. **Buck, M., and B. N. Ames.** 1984. A modified nucleotide in tRNA as a possible regulator of aerobiosis: synthesis of *cis*-2-methylthioribosylzeatin in the tRNA of Salmonella. Cell **36**:523–531.
7. **Buklad, N. E., D. Sanborn, and I. N. Hirshfield.** 1973. Particular influence of leucine peptides on lysyl-transfer ribonucleic acid ligase formation in a mutant of *Escherichia coli* K-12. J. Bacteriol. **116**:1477–1478.
8. **Clark, R. L., and F. C. Neidhardt.** 1990. Roles of the two lysyl-tRNA synthetases of *Escherichia coli*: analysis of nucleotide sequences and mutant behavior. J. Bacteriol. **172**:3237–3243.
9. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. J. Mol. Biol. **117**:525–575.
10. **Cowing, D. W., J. A. Bardwell, E. A. Craig, C. Woolford, R. W. Hendrix, and C. A. Gross.** 1985. Consensus sequence for *Escherichia coli* heat shock gene promoters. Proc. Natl. Acad. Sci. USA **82**:2679–2683.
11. **Csonka, L. N., and A. J. Clark.** 1980. Construction of an Hfr strain useful for transferring *recA* mutations between *Escherichia coli* strains. J. Bacteriol. **143**:529–530.
12. **Hamilton, C. M., M. Aldea, B. K. Washburn, P. Babitzke, and S. R. Kushner.** 1989. New method for generating deletions and gene replacements in *Escherichia coli*. J. Bacteriol. **171**:4617–4622.
13. **Hassani, M., M. V. Saluta, G. N. Bennett, and I. N. Hirshfield.** 1991. Partial characterization of a *lysU* mutant of *Escherichia coli* K-12. J. Bacteriol. **173**:1965–1970.
14. **Hickey, E. W., and I. N. Hirshfield.** 1990. Low-pH-induced effects on patterns of protein synthesis and on internal pH in *Escherichia coli* and *Salmonella typhimurium*. Appl. Environ. Microbiol. **56**:1038–1045.
15. **Hirel, P. H., F. Lévêque, P. Mellot, F. Dardel, M. Panvert, Y. Mechulam, and G. Fayat.** 1988. Genetic engineering of methionyl-tRNA synthetase: *in vitro* regeneration of an active synthetase by proteolytic cleavage of a methionyl-tRNA synthetase-β-galactosidase chimeric protein. Biochimie **70**:773–782.
16. **Hirshfield, I. N., P. L. Bloch, R. A. Van Bogelen, and F. C. Neidhardt.** 1981. Multiple forms of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli*. J. Bacteriol. **146**:345–351.
17. **Hirshfield, I. N., C. Liu, and F. M. Yeh.** 1977. Two modes of metabolic regulation of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli* K-12. J. Bacteriol. **131**:589–591.
18. **Hirshfield, I. N., R. Tenreiro, R. A. VanBogelen, and F. C. Neidhardt.** 1984. *Escherichia coli* K-12 lysyl-tRNA synthetase mutant with a novel reversion pattern. J. Bacteriol. **158**:615–620.
19. **Hunt, T. P., and B. Magasanik.** Transcription of *glnA* by purified *Escherichia coli* components: core RNA polymerase and the products of *glnF*, *glnG*, and *glnL*. Proc. Natl. Acad. Sci. USA **82**:8453–8457.
20. **Kawakami, K., Y. H. Jönsson, G. R. Björk, H. Ikeda, and Y. Nakamura.** 1988. Chromosomal location and structure of the operon encoding peptide-chain-release factor 2 of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85**:5620–5624.
21. **Kawakami, K., S. Naito, N. Inoue, Y. Nakamura, H. Ikeda, and H. Uchida.** 1989. Isolation and characterization of *herC*, a mutation of *Escherichia coli* affecting maintenance of ColE1. Mol. Gen. Genet. **219**:333–340.
22. **Lévêque, F., P. Plateau, P. Dessen, and S. Blanquet.** 1990. Homology of *lysS* and *lysU*, the two *Escherichia coli* genes encoding distinct lysyl-tRNA synthetase species. Nucleic Acids Res. **18**:305–312.
23. **Matthews, R. G., and F. C. Neidhardt.** 1988. Abnormal induction of heat shock proteins in an *Escherichia coli* mutant deficient in adenosylmethionine synthetase activity. J. Bacteriol. **170**:1582–1588.
24. **Mayaux, J. F., G. Fayat, M. Panvert, M. Springer, M. Grun-**

berg-Manago, and S. Blanquet. 1985. Control of phenylalanyl-tRNA synthetase genetic expression. Site-directed mutagenesis of the *pheS*, *T* operon regulatory region *in vitro*. J. Mol. Biol. **184:**31–44.

25. **Mechulam, Y., M. Fromant, P. Mellot, P. Plateau, S. Blanchin-Roland, G. Fayat, and S. Blanquet.** 1985. Molecular cloning of the *Escherichia coli* gene for diadenosine-5′,5‴-P^1,P^4-tetraphosphate pyrophosphohydrolase. J. Bacteriol. **164:**63–69.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Navon, G., S. Ogawa, R. G. Shulman, and T. Yamane.** 1977. High-resolution ^{31}P nuclear magnetic resonance studies of metabolism in aerobic *Escherichia coli* cells. Proc. Natl. Acad. Sci. USA **74:**888–891.
28. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
29. **Neidhardt, F. C., and R. A. VanBogelen.** 1981. Positive regulatory gene for temperature controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100:**894–900.
30. **Ogawa, S., R. G. Shulman, P. Glynn, T. Yamane, and G. Navon.** 1978. On the measurement of pH in *Escherichia coli* by ^{31}P nuclear magnetic resonance. Biochim. Biophys. Acta **502:**45–50.
31. **Pascal, M. C., J. F. Burini, and M. Chippaux.** 1984. Regulation of the trimethylamine N-oxide (TMAO) reductase in *Escherichia coli*: analysis of *tor*::Mud1 operon fusion. Mol. Gen. Genet. **195:**351–355.
32. **Repaske, D. R., and J. Adler.** 1981. Change in intracellular pH of *Escherichia coli* mediates the chemotactic response to certain attractants and repellents. J. Bacteriol. **145:**1196–1208.
33. **Richaud, C., W. Higgins, D. Mengin-Lecreux, and P. Stragier.** 1987. Molecular cloning, characterization, and chromosomal localization of *dapF*, the *Escherichia coli* gene for diaminopimelate epimerase. J. Bacteriol. **169:**1454–1459.
34. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
35. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
36. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
37. **Slonczewski, J. L., T. N. Gonzalez, F. M. Bartholomew, and N. J. Holt.** 1987. Mu d-directed *lacZ* fusions regulated by low pH in *Escherichia coli*. J. Bacteriol. **169:**3001–3006.
38. **Slonczewski, J. L., B. R. Rosen, J. R. Alger, and R. M. Macnab.** 1981. pH homeostasis in *Escherichia coli*: measurement by ^{31}P nuclear magnetic resonance of methylphosphonate and phosphate. Proc. Natl. Acad. Sci. USA **78:**6271–6275.
39. **Ugurbil, K., H. Rottenberg, P. Glynn, and R. G. Shulman.** 1978. ^{31}P nuclear magnetic resonance studies of bioenergetics and glycolysis in anaerobic *Escherichia coli* cells. Proc. Natl. Acad. Sci. USA **75:**2244–2248.
40. **Unden, G., and J. R. Guest.** 1985. Isolation and characterization of the *Fnr* protein, the transcriptional regulator of anaerobic electron transport in *Escherichia coli*. Eur. J. Biochem. **146:** 193–199.
41. **VanBogelen, R. A., M. A. Acton, and F. C. Neidhardt.** 1987. Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev. **1:**525–531.
42. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
43. **Wittwer, A. J.** 1983. Specific incorporation of selenium into lysine- and glutamate-accepting tRNAs from *Escherichia coli*. J. Biol. Chem. **258:**8637–8641.