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Temperature-Inducible Amber Suppressor: Construction of Plasmids Containing the *Escherichia coli serU*$^-$ (*supD*$^-$) Gene Under Control of the Bacteriophage Lambda p_L Promoter

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An *Escherichia coli* DNA fragment containing the structural gene *serU132* for the nonsense suppressor $tRNA^{Ser}_{2am}$ was identified and purified by being cloned into a plasmid vector. Information obtained from DNA sequence analysis was used to select a *serU132* fragment for insertion downstream from the bacteriophage λ p_L promoter in two pBR322-λ derivatives. In nonsense mutant strains bearing the resulting *serU132* hybrid plasmids, the presence of the λ *c*I857 repressor gene carried on the same plasmid or in a prophage genome permits thermal regulation of suppressor synthesis.

The usefulness of mutations which confer thermal lability on a protein or RNA molecule and thereby set permissive and restrictive experimental conditions for gene activity is well established. Although it is not always possible to obtain temperature-sensitive mutants, nonsense mutations in a gene can effectively be converted to temperature-sensitive mutations in two ways. Thermolabile suppressor tRNAs (23, 24) allow completion of a polypeptide chain at low temperatures, but do not suppress nonsense mutations at high temperatures. The converse situation—no suppression at low temperature but rapid production of a suppressor at temperatures above 35°C—could be achieved if the gene specifying a nonsense suppressor were located downstream from the major leftward promoter of bacteriophage λ and its transcription were then controlled by the thermolabile *c*I857 repressor protein.

The well-characterized amber suppressor tRNA encoded by the *serU132* (*supD32*) gene of *Escherichia coli*, which was mapped at 43 min on the Taylor and Trotter map (8, 10), is well suited for use in the construction of a plasmid vector that gives temperature-inducible suppression. This tRNA ($tRNA^{Ser}_{2am}$) inserts the small neutral amino acid serine (22, 35, 38) and hence has a broad range of suppression activity. Our recent purification of the suppressor tRNA species made ^{32}P-labeled tRNA available for use as a hybridization probe, and the complete tRNA sequence (32) now provides a source of restriction endonuclease cleavage site information. This report describes the identification of a DNA fragment bearing the *serU132* sequence, its characterization, and the steps used to construct *serU132* derivatives of two pBR322-λ plasmids that show temperature-inducible amber suppression in nonsense mutant strains.

MATERIALS AND METHODS

Nomenclature. The *supD*$^-$ allele used in these studies was *supD32*, from the Garen strain S26rleλ$^-$ (8). Since this allele was identified as the gene encoding an amber-suppressing seryl tRNA, the wild-type locus is now designated *serU*$^+$ and the nonsense suppressor allele is designated *serU132*. The corresponding gene products are $tRNA^{Ser}_2$ and $tRNA^{Ser}_{2am}$. In accord with the format used for the *E. coli* map (2), Sup$^+$ and Sup$^-$ denote the suppressor-negative and suppressor-positive phenotypes, respectively.

Materials. Carrier-free $^{32}P_i$ was obtained from New England Nuclear Corp. and incorporated into [γ-^{32}P]ATP by the phosphate-ATP exchange reaction described by Glynn and Chapell (9) as given elsewhere (16). The sources of materials for nucleic acid extractions and polyacrylamide gel electrophoresis were those previously reported (3, 33). Agarose was obtained from Bio-Rad Laboratories, and nitrocellulose sheets (BA85) were from Schleicher & Schuell Co. Chloramphenicol, ampicillin trihydrate, *o*-nitrophenyl-β-D-galactoside, and isopropyl-β-D-thiogalactoside (IPTG) were purchased from Sigma Chemical Co. Restriction endonucleases were obtained commercially and used according to the suppliers' recommendations. Other enzymes were obtained as follows: T4 DNA ligase (P.L. Biochemicals, Inc.), *E. coli* DNA polymerase I Klenow fragment (Bethesda Research Laboratories), and egg white lysozyme (LYSF grade; Millipore Corp.).

Bacterial strains, bacteriophage, and plasmids. The *E. coli* strains used are listed in Table 1. The *recA1* allele was introduced into a spontaneous Thy$^-$ isolate of strain X7886, obtained via trimethoprim selection (18), by conjugation with the Hfr KL16 strain MA1079 (*recA1* Thy$^+$), obtained from K. B. Low (Yale University). The λ d*serU132* transducing phage (34) was maintained as a lysogen, with the helper prophage λ

TABLE 1. Bacterial strains

Strain no.	Description	Source/reference
LS289	F⁻ *pro-48 trpR55 trpA9605*(Am) *his-85*(Am) *ilv-632 tsx-84 serU*⁺	L. Soll
KL241(λ *c*I857 *S7*)	F⁻ *arg-47 trp-49*(Am) *lacZ53*(Am) *rpsL150 rel-1 serU*⁺	K. B. Low. Strain lysogenized with λ *c*I857 *S7*
DS122	F′ *lac pro*/W3110 Δ(*lac*) *ilv-632 argH trpR55 trpA9605*(Am) *his-85*(Am) *recA rpoB serU*⁺	L. Soll strain LS540, F′ *lac pro* from J. Miller strain GM1 (27)
DS68	F⁻ Δ(*att*λ-*bio*) *arg-47 trp-49*(Am) *lacZ53*(Am) *rpsL150 rel-1 serU132* (λ *c*I857)	34
S26rleλ⁻	Hfr (Cavalli) *phoA4 rel-1 tonA33 serU132*	A. Garen (8) via E. P. Hoffman
K38	Hfr (Cavalli) *phoA4 rel-1 tonA22 serU*⁺ (λ)	N. Zinder (14)
K37	Hfr (Cavalli) *phoA4 rel-1 tonA22 serU132* (λ)	N. Zinder (14)
K802	F⁻ *galK2 galT22 metB1 lacY1 supE44 hsdR2*	
DS125	F⁻ *ara recA1* Δ(*lac-proB*) *galE rpsL* Val^r (φ80 d*lac*⁺)	*recA1* derivative of strain X7886 (19), which carries a *tonB* deletion extending into the *I* gene (endpoint between amino acid positions 265 and 273) on the φ80 d*lac* phage. Strain is *lacI*⁻*Z*⁺
DS127	F⁻ *arg-47 trp-49*(Am) *lacZ53*(Am) *rpsL150 rel-1 serU132*	*serU132* derivative of strain KL241 via P1 transduction

*c*I857, in strain LS289. Wild-type phage f1 and amber mutants suppressible by *serU132* in gene IV (f1 R12) and gene VIII (M13 8H1) have been described previously (37). The amber mutant T4N58 (6) was used to score suppressor phenotypes. The pBR322-λ plasmid pKC30 (29), constructed by R. N. Rao, was obtained from M. Rosenberg (Smith Kline and Beckman) and propagated in strain N99(λ⁺). This plasmid contains the leftward promoter and the *N* gene of bacteriophage λ (Fig. 1). A similar derivative of pBR322, pGW7, includes additional sequences encoding the *c*I857 repressor protein (G. Wilson and W. Konigsberg, unpublished data). This plasmid was maintained in strain K802. Both the rich and minimal media used for bacterial growth and phage lysate preparations have been described previously (34). LG broth contained 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 5 g of glycerol per liter.

DNA preparation. Mixed lysates containing λ *c*I857 and λ d*serU132* were prepared by heat induction of the lysogenic strain LS289. Broth cultures grown at 30°C to a cell density of 2×10^8 to 3×10^8 per ml were heated at 43°C for 12 min in the presence of 0.01 M $MgSO_4$ and then shaken at 37°C until lysis was complete. After cell debris was removed by low-speed centrifugation, the lysates were treated with 15 μg of DNase I (D grade; Worthington Diagnostics) per ml for 30 min at 37°C and concentrated in 10% polyethylene glycol–0.5 M NaCl (40). Phage pellets were suspended in phage buffer (0.006 M Tris-hydrochloride pH 7.5, 0.01 M $MgSO_4$, 0.068 M NaCl, 0.5% gelatin [Sigma]) and banded initially in CsCl step gradients prepared by layering CsCl solutions made in phage buffer (ρ_0 = 1.3, 1.5, and 1.7 g/ml). Final purification was accomplished by equilibrium CsCl density gradient centrifugation (34). DNA was released from the purified phage particles by incubation at 65°C in 1% sodium dodecyl sulfate, separated from protein by potassium precipitation (26), and recovered by ethanol precipitation. Plasmid DNAs were isolated on a small scale by a sodium dodecyl sulfate lysis-phenol extraction procedure (K. McKenney, personal communication) and preparatively by CsCl-ethidium bromide centrifugation after chloramphenicol amplification (20). DNA was recovered from agarose gels by transfer to DEAE paper (DE-81; Whatman, Inc.) (39) and from polyacrylamide gels by electrophoretic elution (12).

Recombinant DNA methods. Standard methods were used for restriction mapping and cloning (15). For calcium chloride transformations, λ *c*I857 lysogens were grown at 30°C and, to minimize prophage induction, were heated for only 2 min at 42°C. ^{32}P-labeled $tRNA^{Ser}_{2am}$ was prepared for use as a hybridization probe as reported elsewhere (32). Restriction endonuclease fragments fractionated on 2% agarose slabs in 0.04 M Tris-acetate (pH 7.9)–0.001 M EDTA were stained with ethidium bromide and photographed, and then the gels were treated to successive 15-min washes with two changes of 1.5 M NaCl–0.5 M NaOH and two changes of 3.0 M NaCl–0.5 M Tris-hydrochloride (pH 7.0). Transfers to nitrocellulose in 20× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate) were done by the method of Southern (31), but the blots were rinsed briefly with 2× SSC before being dried and baked. Hybridizations to ^{32}P-labeled $tRNA^{Ser}_{2am}$ were carried out at 45°C for 24 h in 5× SSC–50% formamide. After removal of the hybridization solution, nitrocellulose sheets were washed successively for 10 min with 50 ml of 5× SSC–50% formamide, for 30 min with 200 ml of 5× SSC–50% formamide, and then for several hours with 250 ml of 2× SSC.

RESULTS

Purification of the *serU* gene from λ d*serU132* DNA. The strategy adopted for placing *serU*

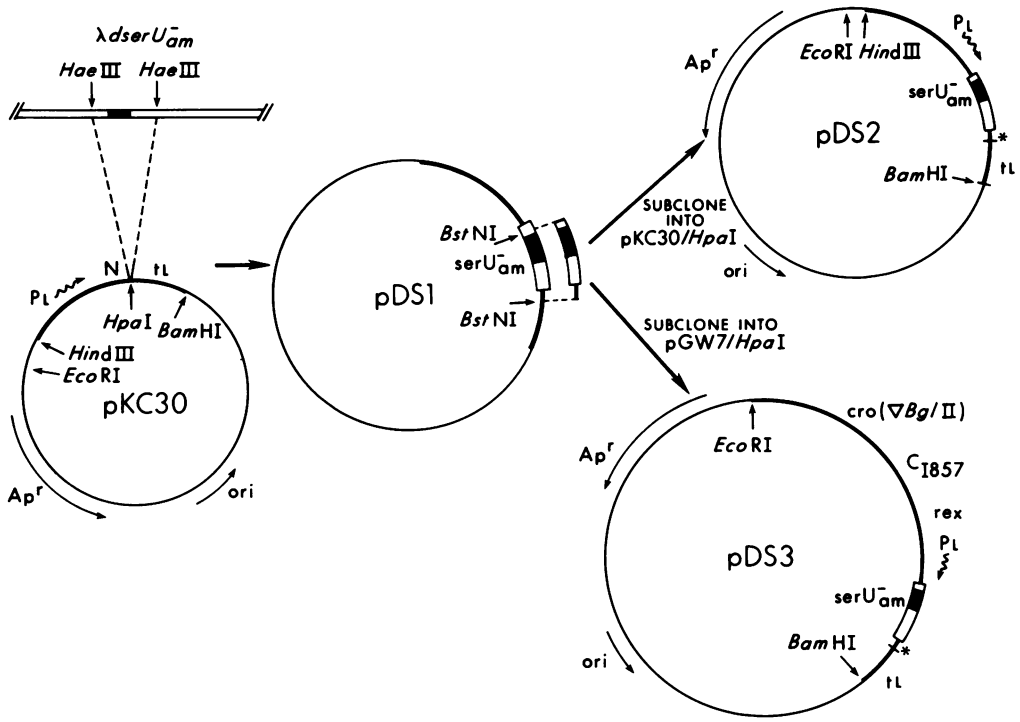

FIG. 1. Construction of temperature-inducible amber suppression vectors. The construction of plasmids pDS1, pDS2, and pDS3 is described in the text. When pDS2 and pDS3 DNAs isolated from independent clones were analyzed by digestion with *Hae*III, fragments of two sizes were found to contain the insert. One was the size expected for an insertion of a *Bst*NI fragment of about 650 bp in the plasmid, and the other was approximately 250 bp smaller. Since DNA sequence analysis showed that the junction between the λ *N* gene and the 5′ flanking region of *serU* was that expected, the smaller insert most likely contained a deletion in the region marked by asterisks (*), where there is a duplication of 158 bp in the vector DNA sequence and in the *Bst*NI fragment. Plasmids containing the shorter insert were used for all further work. Solid bars, Region of *serU132* corresponding to the 90-nucleotide mature tRNA sequence. Boldface lines distinguish the sequences in pKC30 and pGW7 of bacteriophage λ origin from pBR322 sequences. The locations of the ampicillin resistance (Ap^r) determinant and the origin of plasmid DNA replication (ori) are shown, as is the direction of transcription (⇝) from the λ p_L promoter and the position of the ρ-dependent termination site (tL).

gene expression under control of the bacteriophage λ p_L promoter was to clone a restriction fragment of only a few hundred base pairs (bp) that contained the sequences required for tRNA processing and post-transcriptional modifications but not a functional promoter. A search of the suppressor $tRNA^{Ser}_{2am}$ sequence identified a number of restriction enzymes that did not have sites in the region of the *serU* gene corresponding to the mature tRNA. Of these, three that give flush ends were selected for Southern blot analysis. A DNA mixture prepared from a lysate containing the defective transducing phage λ d*serU132* (34) and the helper phage λ *c*I857 was digested with *Alu*I, *Hinc*II, and *Hae*III. After being separated on a 2% agarose gel and transferred to sheets of nitrocellulose, the restriction fragments were hybridized with ^{32}P-labeled $tRNA^{Ser}_{2am}$. No hybridization was observed in control experiments with λ *c*I857 DNA. By constrast, two fragments that carried *serU* sequences were generated in this digest from the λ d*serU132*-λ *c*I857 DNA mixture by *Alu*I and one fragment of more than 1.5 kilobases was generated by *Hinc*II (Fig. 2). Based on other analyses, the larger *Alu*I fragment was likely the result of incomplete digestion. The sequence corresponding to $tRNA^{Ser}_{2am}$ was contained in a unique *Hae*III fragment of approximately 570 bp. This fragment was not further reduced in size when digested with *Hinc*II.

Construction of pDS1. Based on hybridization data, the λ d*serU132*-λ *c*I857 DNA preparations were digested with *Hae*III. DNA fragments in the appropriate size range were eluted from preparative 2% agarose gels and inserted into the unique *Hpa*I site of plasmid pKC30 (29), which is located in the λ *N* gene (Fig. 1). The

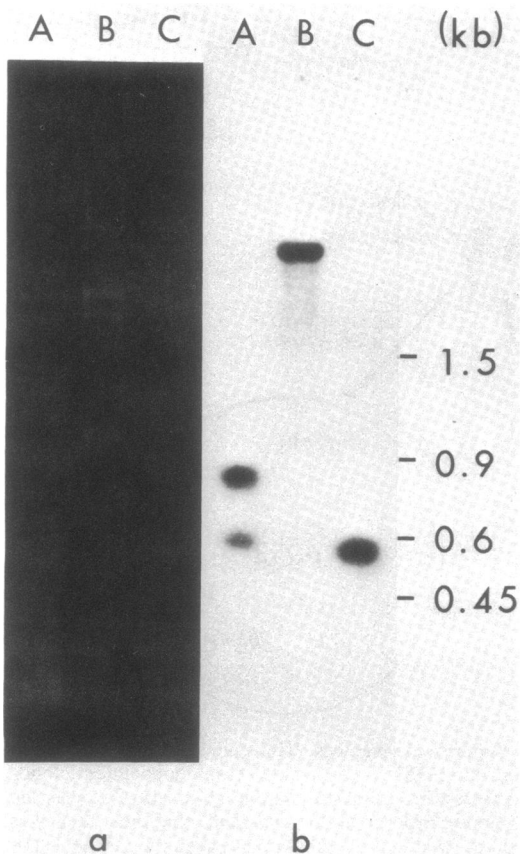

FIG. 2. Localization of *serU* DNA fragments in λ d*serU132* (a) Agarose gel separation. (b) Autoradiogram of nitrocellulose sheet. Lanes A, *Alu*I; B, *Hin*cII; C, *Hae*III. Numbers indicate the size of *Hae*III restriction fragments from plasmid pKG1800 (17) that were electrophoresed in the same gel.

ligation mixture was treated with *Hpa*I to linearize circular pKC30 DNA molecules that had not acquired an insert, and was then used to transform a λ *c*I857 *S7* lysogenic derivative of strain KL241, which has amber mutations in *lacZ* and *trpA*. From the ampicillin-resistant transformants with a Lac^+ Trp^+ Sup^- phenotype, one was selected for further analysis. This clone contained a plasmid (pDS1) that had an electrophoretic mobility in agarose gels consistent with its having an insertion of one DNA fragment into pKC30. The plasmid-bearing strain also had increased levels of ^{32}P-labeled $tRNA^{Ser}_{2am}$ after a temperature shift from 30 to 43°C. This suggested that pDS1 carried the *serU* gene in the correct orientation to the bacteriophage λ p_L promoter. The fact that the plasmid conferred a Sup^- phenotype under conditions in which expression from the lambda promoter is repressed (30°C), however, raised the possibility that the insert contained a functional promoter for the *serU* gene.

Primary structure of the *serU* gene. Nine nucleotides downstream from the position in the *serU132* gene corresponding to the tRNA 5′ end, a recognition sequence for *Bgl*I occurs. The presence of this restriction site made it possible for us to characterize the insert in pDS1 by sequencing from this position in both directions, using the chemical methodology of Maxam and Gilbert (16). The data obtained confirmed earlier results suggesting that the *Hae*III fragment in pDS1 had inserted in the correct orientation to the λ p_L promoter. The junction between the insert and the *Hpa*I site of the λ *N* gene (7) in the pKC30 vector was found 104 nucleotides upstream from the position of the tRNA 5′ end. A part of this region, and that corresponding to the mature suppressor tRNA, is shown in Fig. 3. In the sequence, appropriately spaced regions were found that had substantial homology with both the −10 region (5 of 7 nucleotides) and the −35 region (6 of 8 nucleotides) consensus sequences derived from analysis of *E. coli* promoters (25, 30). The *Bst*NI restriction site indicated between those regions was well positioned for cleavage of upstream DNA from that encoding the suppressor tRNA.

Construction of pDS2 and pDS3. The presence of *Bst*NI restriction sites in the 5′ flanking region of *serU* and in the plasmid vector 160 nucleotides beyond the *Hpa*I site in the λ *N* gene (7) made it possible to purify a fragment of about 650 bp from pDS1 DNA. This was subsequently repaired to give flush ends and then inserted into the *Hpa*I sites of plasmids pKC30 and pGW7. pGW7 is also a pBR322 derivative, but contains both the *c*I857 allele of the lambda gene encoding the *c*I repressor protein and the *rex* gene in addition to p_L and the *N* gene. Using the steps outlined for construction of pDS1, we generated plasmids pDS2 and pDS3 (Fig. 1). As expected, DNA sequence analysis revealed that now only 24 nucleotides separated upstream λ *N* gene sequences in pDS2 and pDS3 DNAs from the position of the tRNA 5′ end. More important, strains bearing these plasmids had the desired phenotype: they were Sup^+ at 30°C and Sup^- at 42°C. The *lacZ53* amber mutant strain KL241 (λ *c*I857 *S7*)(pDS2) formed white (Lac^-) colonies on lactose MacConkey agar when it was plated overnight at temperatures below 33°C, and pink (Lac^+) colonies at temperatures between 33 and 39°C; the induced lysogenic strain KL241 (λ *c*I857 *S7*) is not viable at higher temperatures. Likewise, strain KL241(pDS3) formed white colonies at or below 35°C and pink colonies at higher temperatures. Both strain KL241 and the pDS3-containing derivative showed reduced viability above 40°C.

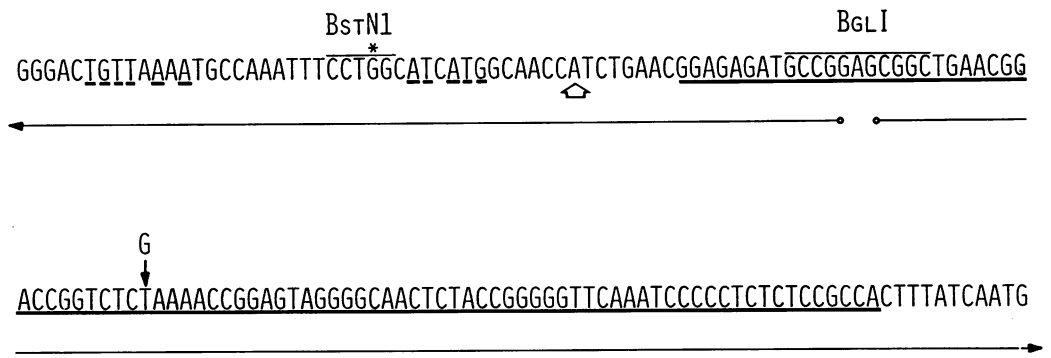

FIG. 3. Partial sequence of the DNA insert in pDS1 which contains the *serU132* structural gene. The polarity of 5'-end-labeled DNA strands analyzed by the method of Maxam and Gilbert (16) is shown by solid-head arrows under the sequence. Bold underlining indicates the region corresponding to mature $tRNA_{2am}^{Ser}$, with the only nucleotide that differs between the wild-type $tRNA_2^{Ser}$ and the suppressor tRNA species (32) shown (↓). This position corresponds to the middle nucleotide of the tRNA anticodon. In the region flanking the tRNA 5' end, nucleotides identical to the consensus sequences for the −10 (TATAATG) and −35 regions (TGTTGACA) of *E. coli* promoters (25, 30) are underlined, and an adenine residue appropriately positioned to serve as the RNA start point is indicated (⇑). The absence of a cleavage product in both pyrimidine ladders in the position corresponding to the G marked with an asterisk suggested that 5-methylcytosine was present in the complementary DNA strand. *Bst*NI and *Bgl*I restriction sites are shown.

Regulated synthesis of the *serU132* suppressor. Suppression of the *lacZ53* amber mutation to give active β-galactosidase was used to monitor *serU132* gene expression in strains bearing plasmid pDS2 or pDS3. We first asked how effectively expression was limited under growth conditions that permitted repression of transcription from p_L by the *c*I857 protein. β-Galactosidase activities were determined by the method of Miller (18) for several bacterial strains cultured for 12 h at 30°C with and without IPTG, an inducer of the *lac* operon (Table 2). In strain KL241 (λ *c*I857 *S7*)(pDS2), which carries *c*I857 on a prophage in the bacterial chromosome and *serU132* on the multicopy plasmid, β-galactosidase levels were 6.8 and 3.1% that in the constitutive (DS125) and induced (526r1eλ⁻) *lacZ*⁺ strains, respectively. The activity in strain KL241(pDS3), which carries both *c*I857 and *serU132* on the multicopy plasmid, was one-third that in the pDS2-containing strain. This suggests that transcription from the p_L promoter was more effectively repressed in the strain bearing pDS3, presumably due to overproduction of the *c*I857 product encoded on the multicopy plasmid. In both plasmid-containing strains, however, suppressor synthesis was limited sufficiently to give a Lac⁻ phenotype. As shown by the *lacI*⁺ *lacZ*⁻(Am) *serU132* strain (DS68), expression of the suppressor gene from its natural context in the chromosome resulted in induced β-galactosidase levels 25-fold higher than those observed in the pDS3-containing strain.

Thermal induction of *serU132* gene expression was then monitored in plasmid-bearing

TABLE 2. Suppression of the *lacZ53* amber mutation under conditions of *c*I857 repression[a]

Strain	Relevant genotype	β-Galactosidase activity[b]	
		Without IPTG	With IPTG
DS125	*lacI lacZ*⁺ *serU*⁺	1,260	1,220
S26rleλ⁻	*lacI*⁺ *lacZ*⁺ *serU132*	11.8	2,670
DS68	*lacI*⁺ *lacZ53 serU132*	7.8	703
KL241	*lacI*⁺ *lacZ53 serU*⁺	1.4	2.2
KL241(λ *c*I857 *S7*)(pDS2)[c]	*lacI*⁺ *lacZ53 serU*⁺ (*serU132*)	4.6	83.9
KL241(pDS3)[c]	*lacI*⁺ *lacZ53 serU*⁺ (*serU132*)	3.1	27.4

[a] Strains were cultured for 12 h at 30°C in LB broth with or without 5×10^{-4} M IPTG, an inducer of the *lac* operon.

[b] Units are expressed as 1,000 times the *o*-nitrophenol absorbance at A_{420} per A_{600} unit of cells per min.

[c] LB broth contained ampicillin (50 μg/ml).

strains and compared with the time course of β-galactosidase synthesis after IPTG induction of a *lacI*$^+$ *lacZ*$^+$ strain and two *lacI*$^+$ *lacZ*(Am) *serU132* strains. One of these (strain DS127) was simply a *serU132* derivative of the host strain for pDS2 and pDS3. The second (strain DS68), a λ *c*I857 lysogen, was used as a control for the pDS2-bearing strain, with the rationale that the prophages in both strains would be activated for lytic growth by the temperature shift from 30 to 42°C. A constitutive *lacI*$^-$ *lacZ*$^+$ strain was included to indicate any fluctuations in β-galactosidase levels in response to the change in temperature. Amber suppressor activity appeared in the plasmid-bearing strains after the temperature shift (Fig. 4). Although the β-galactosidase level attained 60 min after induction of a wild-type *lac* operon was higher than that in the *lacZ*(Am) strains, substantial levels developed at 42°C in the two strain KL241 derivatives carrying *serU132* on plasmid pDS3 or in the bacterial chromosome. Lower β-galactosidase activities were found in the two strains in which lytic growth of phage λ occurred.

Temperature-dependent suppression of filamentous phage amber mutants. As a first step in assessing the utility of the *serU132* plasmids, we asked whether the suppression of filamentous single-stranded DNA phage amber mutants could be regulated by temperature. Production of f1 phage with an amber mutation in a gene involved in morphogenesis (gene IV) by a derivative of strain DS122 bearing pDS3 was measured as a function of temperature over the range 33 to 40.5°C. Few progeny were obtained at 33°C (Fig. 5). This was not due to lack of infection, since wild-type f1 phage were produced in good yield by strain DS122(pDS3) at this temperature (Fig. 5). Phage production increased with temperature up to 38°C. The lower titer observed at 40.5°C was in keeping with the reduced viability of the strain at this temperature. At 38°C, the yield of the gene IV amber mutant phage obtained from DS122(pDS3) was nearly equivalent to that of wild-type phage f1 (Fig. 5). Fewer progeny were obtained from DS122(pDS3) for a phage with an amber mutation in the gene encoding the coat protein (VIII), which is required in large amounts to form a phage particle.

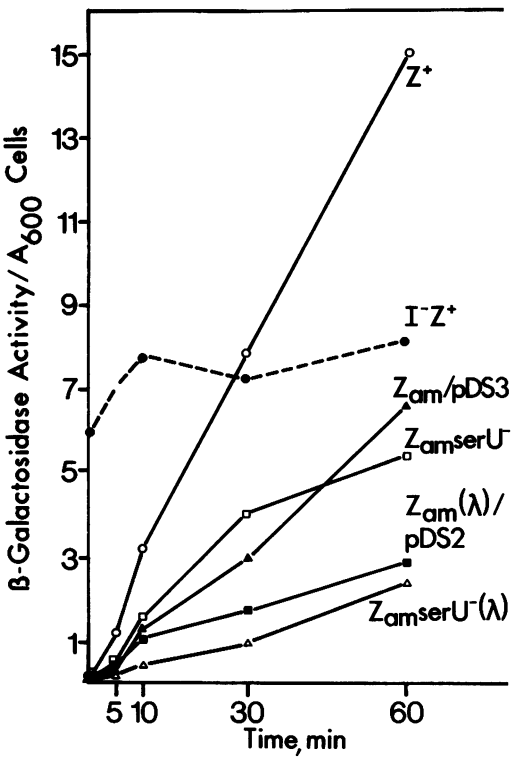

FIG. 4. Suppression of the *lacZ53* amber mutation after thermal induction of transcription from the λ p_L promoter. Bacterial strains were cultured overnight at 30°C in LG broth, which contained 0.5% glycerol and was supplemented with 50 μg of ampicillin per ml for strains bearing pDS2 or pDS3. After being diluted 1:50 with the same medium, cells were grown at 30°C to a density of approximately 2×10^8/ml. At zero time, the cultures were shifted to 42°C, and IPTG was added to $5 \times 10^{-}$ M to the cultures of *lacI*$^+$ strains to induce the *lac* operon. At the indicated times after the temperature shift, 0.5-ml samples were withdrawn for absorbancy determinations at 600 nm (A_{600}) and for assays of β-galactosidase activity. Activity is expressed as *o*-nitrophenol absorbancy units at A_{420} per 5-min reaction per A_{600} unit of cells. Strains: (○) S26rlλ$^-$ (*lacI*$^+$ *lacZ*$^+$), (●) DS125 (*lacI*$^-$ *lacZ*$^+$), (▲) KL241(pDS3) [*lacI*$^+$ *lacZ*(Am)], (□) DS127 [*lacI*$^+$ *lacZ*(Am) *serU132*], (■) KL241(λ *c*I857 *S7*)(pDS2) [*lacI*$^+$*lacZ* (Am)], and (△) DS68 [*lacI*$^+$ *lacZ*(Am) *serU132*(λ *c*I857)].

DISCUSSION

Current information about the seryl amber suppressor tRNA of *E. coli* was exploited to physically characterize the *serU132* gene that specifies the suppressor. Genes encoding tRNAs are located throughout the *E. coli* genome in several types of transcription units. They occur in rRNA operons and in clusters of the same or different tRNAs and are cotranscribed with genes that code for proteins (4). Several features of $tRNA_2^{Ser}$ biosynthesis can now be predicted from the primary sequence of *serU* and from the patterns of gene expression thus far examined. First, the precursor for this tRNA most likely forms the 5′-terminal portion of a primary transcript. Our finding that nonsense mutant strains bearing the primary

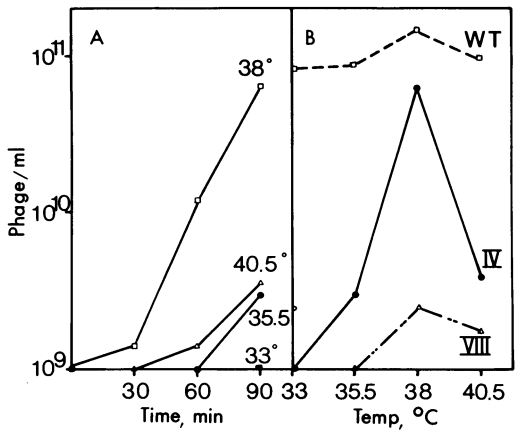

FIG. 5. Temperature-dependent suppression of filamentous phage amber mutants. Strain DS122(pDS3) was grown at 33°C to a density of 10^8 cells per ml. Samples (20 ml) were infected at a multiplicity of 10 with wild-type phage f1 or with gene IV (R12) or gene VIII (8H1) amber mutants. Immediately after infection, 5-ml portions of each culture were removed and incubated at 33, 35.5, 38, or 40.5°C. At 0, 30, 60, and 90 min after infection, samples were withdrawn and quickly chilled to 0°C, and the bacteria were pelleted by centrifugation. The supernatants were assayed for phage particles on strain K38 (Sup^+) and K37 (*serU132*). (A) Titer of gene IV amber mutant phage produced at 33 (■), 35.5 (●), 38 (□), and 40.5°C (△). Titers on the nonsuppressing strain K38 were below 10^7 phage per ml for all time points. (B) Titer of gene IV amber mutant phage (●), gene VIII amber mutant phage (△), and f1 wild-type phage (□) at 90 min after infection of strain DS122(pDS3) at the temperatures indicated.

serU132 hybrid plasmid pDS1 have a suppressor-positive phenotype at low temperatures initially suggested that the inserted DNA included a functional promoter for *serU*. Support for this possibility was provided in the *serU* DNA sequence by the presence, upstream from the tRNA coding region, of appropriately spaced nucleotides with strong homology to the −10 and −35 regions of *E. coli* promoters (25, 30). The 25 nucleotides that precede the −35 region of the proposed *serU* promoter are rich in adenine · thymine bp, as has been noted for the five other known *E. coli* tRNA promoters (1, 5, 13, 21, 28; B. Roe, personal communication) and many other promoters as well (25). The proposed *serU* promoter, however, does not contain an uninterrupted series of guanine·cytosine (G·C) bp between positions −6 and +1. The occurrence of a G+C-rich region that includes a nearly invariant CGCC sequence in the corresponding positions of the other tRNA promoters and in four rRNA promoters has been proposed as a characteristic of genes under stringent control (36). Further evidence that the region flanking the *serU* tRNA sequence functions as a promoter is that removal of DNA upstream from the −10 sequence appears to bring suppressor synthesis from pDS2 and pDS3 under transcriptional control of λ p_L. A second feature of $tRNA_2^{Ser}$ biosynthesis, indicated by functional suppressor tRNA production from plasmids pDS2 and pDS3, is that processing of two very different transcripts yields a mature tRNA. Were transcription to initiate at the proposed *serU* promoter, a tRNA precursor would be synthesized with only a few extra nucleotides that must be removed by RNase P to generate the mature 5′ end. By contrast, as part of the p_L-*serU* hybrid transcript, these extra nucleotides occur in a different context, more than 300 nucleotides from the RNA 5′ end. Finally, although there is no direct evidence to define the 3′ end of the *serU* transcription unit, it would not be surprising if further studies reveal that transcription terminates approximately 170 nucleotides beyond the CCA sequence encoding the tRNA 3′ terminus. This region has a 12-bp sequence (CTTTCGAGCGAA) with 83% homology to a portion of the ρ-dependent terminator in the *E. coli* gene for $tRNA_1^{Tyr}$ (13).

By inserting the appropriate DNA fragment downstream from the phage λ p_L promoter carried on two pBR322-λ plasmids, expression of the *serU132* gene is placed under thermal control. As shown by β-galactosidase synthesis and production of amber mutant filamentous bacteriophage, the plasmid constructions pDS2 and pDS3 conferred a temperature-sensitive suppression phenotype on the bacterial strains carrying them. Expression of the *serU* gene is repressed effectively at low temperature, particularly with pDS3, and is induced at temperatures above 34 to 35°C to provide efficient levels of suppression. Our finding that amber mutant phage yield increases with temperature between 33 and 38°C raises the possibility that the plasmids could be used to control the levels of a suppressed gene product. The regulated gene expression achieved by placing *serU132* under thermal control of the λ p_L promoter thus met initial expectations. Based on our experience and that of others (11), use of a promoter from an inducible *E. coli* operon, such as *lac*, would probably not produce the desired phenotypes. A single chromosomal copy of the $lacI^Q$ gene provides insufficient repressor to prevent β-galactosidase synthesis from a *lacZ* gene carried on pMB9 and pBR322 derivatives. If the genes specifying both repressor and β-galactosidase are placed on the plasmids, however, enzyme induction is not readily achieved after IPTG addition.

As assessed from growth curves and cell

viability determinations carried out with the nonsense mutant strains thus far examined and their corresponding derivatives bearing pDS2, pDS3, or the parent plasmids pKC30 or pGW7, the *serU132* plasmids have no discernible effect per se on cell growth in the temperature range from 30 to 39°C. At temperatures above 40°C, for reasons not yet clear, a general trend toward reduced viability appears to be more pronounced in some pDS3-containing strains. In view of this, 39°C is routinely used as a condition for the induction of suppressor synthesis that is compatible with long-term cell growth. The viable range for pDS2-containing strains, in which the *c*I857 repressor must be provided by a prophage, could presumably be extended beyond the range found in our studies by using phage λ strains that do not kill an induced host. Our motivation in undertaking this project was to develop a temperature-inducible nonsense suppression vector that could be useful in genetic selections, physiological studies of gene function, and efforts to identify the polypeptide products of genes which are marked genetically by nonsense mutations. pDS3, which encodes both the amber suppressor tRNA and the thermolabile phage λ *c*I857 repressor protein, is hopefully well suited for such applications.

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