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Isolation of *Escherichia coli rpoB* Mutants Resistant to Killing by λ *c*II Protein and Altered in *pyrE* Gene Attenuation

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***Escherichia coli* mutants simultaneously resistant to rifampin and to the lethal effects of bacteriophage λ *c*II protein were isolated. The *sck* mutant strains carry alterations in *rpoB* that allow them to survive *c*II killing (thus the name *sck*), but that do not impair either the expression of *c*II or the activation by *c*II of the λ promoters p_E and p_I. The *sck-1*, *sck-2*, and *sck-3* mutations modify transcription termination. The growth of λ, but not of the *N*-independent λ variant, λ *nin-5*, is hindered by these mutations, which act either alone or in concert with the bacterial *nusA1* mutation. In contrast to their effect on λ growth, the three mutations reduce transcription termination in bacterial operons. The *E. coli pyrE* gene, which is normally regulated by attenuation, is expressed constitutively in the mutant strains. The *sck* mutations appear to prevent *pyrE* attenuation by slowing the rate of transcriptional elongation of the *pyrE* leader sequence. The *sck-6* mutation, unlike the other *sck* mutations, neither increases *pyrE* expression nor inhibits the ability of λ to suppress transcription termination. Instead, the *sck-6* mutation blocks the growth of the λ variants λ *nin-5* and λ *red-3*.**

The *c*II protein of bacteriophage lambda plays both positive and negative roles in viral development. It stimulates transcription initiation from the phage p_I, p_E, and p_Q promoters (9, 21, 22). The activation of p_E and p_Q inhibits the expression of early and late viral lytic genes; presumably, transcription initiating at these promoters converges with and inhibits transcription from the lytic λ p_R promoter.

Expression of *c*II from a multicopy plasmid is lethal to *Escherichia coli* (28). This lethality may result from a severe depression in host protein synthesis observed after *c*II induction. We assumed that the initial reaction in *c*II-induced killing was an interaction between RNA polymerase and the *c*II protein at certain bacterial promoters. Convergent transcription from these promoters might depress the expression of vital bacterial genes. By analogy with mutations in *rpoB* (the β subunit of RNA polymerase) which block the transcription antitermination activity of the λ *N* gene product (8), we sought *rpoB* mutants which survived *c*II killing (*sck* mutants). The properties of four such *sck* mutants are described below. Although we expected these *rpoB* mutations to block the action of the *c*II product, our results indicate that the mutant polymerases still, in fact, interact with the *c*II protein. Instead of affecting the action of the *c*II product, some of the mutations appear to affect transcription termination; they display or enhance the Nus$^-$ phenotype and derepress the bacterial *pyrE* gene, which is normally attenuation regulated.

MATERIALS AND METHODS

Media. LB medium has been described (19). The Tris-buffered minimal medium of Edlin and Maaløe (6), with the phosphate content reduced to 0.3 mM (03P), was used for growth when the level of the pyrimidine biosynthetic enzymes or of the nucleotide pools was determined. Glucose (0.2%) was used as the carbon source, and required amino acids were added.

Strains. (i) Bacteria. The bacterial strains used in these experiments and their constructions are listed in Table 1.

N6017 (21) carries a λ *c*I857 *N*am7 *N*am53 *int*2 *xis*1 prophage with λ DNA deleted from between the *Sal*I and *Xho*I sites at λ coordinates 32745 and 33498, respectively; it lacks *c*III, *kil*, *gam*, and *bet*. In addition, the H1 deletion removes all prophage genes from *cro* to *attR*.

Integrated to the left of the λ prophage in N6017 is λ imm^{21} *lacW205 bΔ53*. λ imm^{21} carries a promoterless *lacZ* gene in its *b* region (at λ coordinate 27479) and lacks t_I. *lacZ* can be expressed by transcription initiating at the *c*II-dependent p_I promoter of the neighboring λ. *c*II protein activity is determined by β-galactosidase measurements.

N6171 (21) carries a λ *c*I857 *N*am7 *N*am53 prophage with λ DNA deleted from between the two p_L operon *Bam*HI sites (λ coordinates 27972 and 34499); the deletion removes all p_L operon genes from *int* to *ral*, including p_I. The λ is also ΔHI. N6323 is N6171 carrying a λ imm^{21} 907 prophage integrated at *attL*; the prophage bears a *lacZ*-p_E transcription fusion in the *b* region (22). The expression of *lacZ* depends on active *c*II protein.

The source of *c*II protein in these strains was the pBR322-derived plasmid pOG7, which carries *c*II under p_L-o_L control. At 32°C, the λ *c*I857 repressor blocks *c*II expression; at 42°C, the repressor is inactivated and *c*II is transcribed from p_L.

Phage designated λ in Table 1 carry the *c*I857 mutant repressor which is inactivated at temperatures >38°C. Tet^r, Ap^r, and Rif^r indicate resistance to 15 μg of tetracycline per ml, 50 μg of ampicillin per ml, and 100 μg of rifampin per ml, respectively. The notation *hip-157*:Tn*10* signifies that transposon Tn*10* is P1 cotransducible with *hip-157* but not known to be located within that cistron. *cps-4*::Tn*10* is a Tn*10* insertion which is 85% P1 cotransducible with *rpoB*; it was a gift from Susan Gottesman (31). IR$^+$ indicates growth on inosine as the sole carbon source.

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TABLE 1. Bacterial strains

Strain	Structure	Parent, source, or reference[a]	Selection screening
N4903	F⁻ *su⁻ his ilvA relA1 strA gal⁺ Δ8*	NIH collection	
N6017	N4903 *lacZ21* (λ *int2 xis ΔS-X* Nam7 Nam53ΔH1) (λ *imm*^{21} *lacW205 bΔ53*)	(21)	
N6171	N4903 *lacZ21* (λ Nam7 Nam53 ΔBAMΔH1)	(21)	
N6188	N6017(pOG7)	N6017 + pOG7	Ap^r
N6214	N6017 *hip157*::Tn*10*(pOG7)	(21)	
N6226	N6017 *himA83*::Tn*10*(pOG7)	(21)	
N6323	N6171 (λ *imm*^{21} 907)	(22)	
KH23	N6323(pOG7)	N6323 + pOG7	Ap^r
KH29	N6323 *cps-4*::Tn*10*	N6323 + P1·SG20044	Tet^r
KH30	N6017 *cps-4*::Tn*10*	N6017 + P1·SG20044	Tet^r
KH31	KH23 *cps-4*::Tn*10 sck-1*	KH23 + P1·KH39 *sck-1*	Tet^r Rif^r
KH32	KH23 *cps-4*::Tn*10 sck-2*	KH23 + P1·KH39 *sck-2*	Tet^r Rif^r
KH39	KH29(pOG7)	KH29 + pOG7	Ap^r
KH40	KH30(pOG7)	KH30 + pOG7	Ap^r
KH41	N6188 *cps-4*::Tn*10 sck-3*	N6188 + P1·KH40 *sck-3*	Tet^r Rif^r
KH44	N6188 *cps-4*::Tn*10 sck-6*	N6188 + P1·KH40 *sck-6*	Tet^r Rif^r
KH49	N6323 *cps-4*::Tn*10 sck-1*	N6323 + P1·KH39 *sck-1*	Tet^r Rif^r
KH50	N6323 *cps-4*::Tn*10 sck-2*	N6323 + P1·KH39 *sck-2*	Tet^r Rif^r
KH51	N6017 *cps-4*::Tn*10 sck-3*	N6017 + P1·KH40 *sck-3*	Tet^r Rif^r
KH53	N6017 *cps-4*::Tn*10 sck-6*	N6017 + P1·KH40 *sck-6*	Tet^r Rif^r
KH110	N4903 *cps-4*::Tn*10 sck-1*	N4903 + P1·KH49	Tet^r Rif^r
KH111	N4903 *cps-4*::Tn*10 sck-2*	N4903 + P1·KH50	Tet^r Rif^r
KH112	N4903 *cps-4*::Tn*10 sck-3*	N4903 + P1·KH51	Tet^r Rif^r
KH114	N4903 *cps-4*::Tn*10 sck-6*	N4903 + P1·KH53	Tet^r Rif^r
S0853	*deo⁺ thr*	(4)	
S0931	HfrH *deoR7 cytR15 clmA Δdeo-11 Δlac thi udp upp ton*	(32)	
S01718	S0931 *deo⁺ thr*	S0931 + P1·S0053	*IR⁺ thr⁻*
S01748	S01718 *cps-4*::Tn*10*	S01718 + P1·KH111	Tet^r Rif^s
S01749	S01718 *cps-4*::Tn*10 sck-1*	S01718 + P1·KH110	Tet^r Rif^r
S01750	S01718 *cps-4*::Tn*10 sck-2*	S01718 + P1·KH111	Tet^r Rif^r
S01751	S01718 *cps-4*::Tn*10 sck-3*	S01718 + P1·KH112	Tet^r Rif^r
S01752	S01718 *cps-4*::Tn*10 sck-6*	S01718 + P1·KH114	Tet^r Rif^r
S01754	S0931 *deo⁺ thr nusA1*::Tn*10*	S01718 + P1·DB821	Tet^r
S01767	MC4100 Φ(*pyrE-lacZ⁺*)/λ p1(209)	As S01768 (13)	Mu dl *cts*
S01792	MC4100 Δ*carAB guaB galK*	(25)	
S03568	S0931 *deo⁺ thr nusA1 cps-4*::Tn*10 sck-1*	S03573 + P1·S01749	Tet^r Rif^r
S03569	S0931 *deo⁺ thr nusA1 cps-4*::Tn*10 sck-2*	S03573 + P1·S01750	Tet^r Rif^r
S03570	S0931 *deo⁺ thr nusA1 cps-4*::Tn*10 sck-3*	S03573 + P1·S01751	Tet^r Rif^r
S03571	S0931 *deo⁺ thr nusA1 cps-4*::Tn*10 sck-6*	S03573 + P1·S01752	Tet^r Rif^r
S03573	S0931 *deo⁺ thr nusA1*	S01754	Tet^s
S03597	MC4100 *carAB guaB galK cps-4*::Tn*10 sck-1*	S01792 + P1·S01749	Tet^r Rif^r
S03598	MC4100 *carAB guaB galK cps-4*::Tn*10 sck-2*	S01792 + P1·S01750	Tet^r Rif^r
S03599	MC4100 *carAB guaB galK cps-4*::Tn*10 sck-3*	S01792 + P1·S01751	Tet^r Rif^r
S03639	MC4100 *carAB guaB galK cps-4*::Tn*10 sck-6*	S01792 + P1·S01752	Tet^r Rif^r
S03682	MC4100 Φ(*pyrE-lacZ⁺*) *argE*::Tn*10*/λ p1(209)	S01767 + P1·TC540	Tet^r
DB821	MC4100 *nusA1*::Tn*10*	D. Boyd	
MC4100	F⁻ *araD139 lacU169 rpsL150 thi relA1 flB5301 deoC1*		
SG20044	MC4100 Δ*lon-100 cps-4*::Tn*10*	S. Gottesman (31)	
TC540	*argE*::Tn*10*	T. Atlung	

[a] NIH, National Institutes of Health. P1·SG20044, Transduction using P1 grown on SG20044.

(ii) Plasmids. The plasmids used in this study are Ap^r Tc^s derivatives of pBR322 (1). Plasmids pOG7, pJM1035, and pTA10-*lacZ* all carry the λ p_L promoter which is regulated by the *c*I857 temperature-sensitive repressor and which can be induced by heat. pOG7 carries a *Bgl*II-*Bgl*II DNA fragment from pKC30-*c*II (28) inserted into the *Bam*HI restriction site in pBR322 (21). In this plasmid, the p_L promoter directs clockwise the synthesis of *c*II. Plasmid pJM1035 was constructed by inserting the *Bgl*II-*Bgl*II DNA fragment containing *c*II into plasmid pMLB1034, creating a protein fusion between the *O* gene of phage λ and *lacZ*. This plasmid carries a synthetic operon in which p_L directs the synthesis of *c*II and the *O-lacZ* protein fusion. pMLB1034 has been described previously (29). p_L transcription in pMLB1034 is clockwise. Both pOG7 and pJM1035 carry the *cy-3048* mutation. pTA10-*lacZ* contains the same elements as pJM1035, except that *lacZ* is fused directly to the AUG start codon of *c*II (see Fig. 1). A detailed description of the construction of this plasmid will be published separately.

The *galK* plasmids pMZ105 and pMZ245 were obtained from D. Court. pMZ105 (see Fig. 3) contains the λ t_{R1} terminator fragment (*Hae*III at λ coordinate 38150 to *Hin*cII at λ coordinate 38549) inserted into the *Sma*I site of the termination cloning vector pKG100 (18). In pMZ245, the

*Eco*RI-*Hin*dIII fragment of pKG100 containing p*gal* and the beginning of *galE* is exchanged for p*lac* on a *Pvu*II-*Hin*dII fragment from pFW1 (33). In pMZ105, translation initiated at *galE* continues in frame into the C-terminal end of the *cro* gene contained in the λ t_{R1} insert. In pMZ245, no translation into the λ t_{R1} insert occurs (M. Zuber and D. Court, personal communication).

Plasmids pPP101, pPP102, and pPP112 are derivatives of the *galK* transcription-fusion vector pGD4 (5). In plasmid pPP101, transcription of *galK* is fused to the promoters of the *pyrE* operon, whereas pPP102 and pPP112 also contain the intercistronic attenuator (25). However, plasmid pPP112 contains translational stop codons at the end of *orfE* owing to insertion of oligonucleotides in the *Ava*I site (25) (see Fig. 5).

Plasmids pNF1492, pNF1931, and pCN3 are all pBR322 subclones from λ *drif18* (15) (see Fig. 6). pNF1931 and pNF1492 have been described previously (7). pCN3, which was a gift from Carsten Pedersen, Institute of Microbiology, Copenhagen, Denmark, expresses the ribosomal proteins L10 and L12 from the inserted DNA.

Mutagenesis and isolation of rifampin-resistant mutants. Two λ *c*I857 N^- lysogens, KH39 and KH40, carrying a multicopy plasmid with *c*II under λ p_L control, were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for 30 min at room temperature as described by Miller (19). After allowance of time for phenotypic expression, bacteria from separate cultures were plated at 42°C on MacConkey agar-lactose plates containing 100 μg of rifampin per ml. Mutants appeared with a frequency of 2×10^{-7} for KH40 and 3×10^{-8} for KH39. The mutants were screened for Ap^r at 32°C and for temperature resistance at 42°C on LB medium plates containing ampicillin (50 μg/ml). Only 10% of the original rifampin-resistant mutants had the desired Ap^r temperature resistance phenotype.

β-Galactosidase assays. Overnight cultures were diluted into fresh LB medium containing 30 μg of ampicillin per ml and were grown at 32°C. p_L transcription was induced by transferring the culture to 43°C at an optical density at 650 nm (OD_{650}) of 0.2 (after at least three generations of exponential growth). At various time intervals, cell density was determined. A culture sample (0.5 ml) was lysed in the presence of chloroform and sodium dodecyl sulfate and assayed for β-galactosidase as described by Miller (19). β-Galactosidase concentration is expressed as specific activity in Miller units (OD_{420}/OD_{650}).

Assays for pyrimidine biosynthetic enzymes. Cultures were grown exponentially, harvested, and assayed as described previously (14) for the following enzyme activities: carbamoylphosphate synthase (*carAB*), aspartate transcarbamylase (*pyrB*), dihydroorotase (*pyrC*), dihydroorotate oxidase (*pyrD*), orotate phosphoribosyltransferase (*pyrE*), and orotidine 5'-monophosphate decarboxylase (*pyrF*).

One unit of enzyme activity is defined as the amount of enzyme utilizing 1 nmol of substrate or producing 1 nmol of product under standard assay conditions.

Protein determinations were performed by the method of Lowry et al. (16) by using bovine serum albumin as a standard. The method of Bradford (3) was used when the buffer contained dithiothreitol (see Table 6).

Nucleotide pools. Exponential cultures in 03P medium were labeled for two generations with $^{32}P_i$ (specific activity, 25 μCi/μmol). The pool sizes were determined after separation of the nucleoside triphosphates by two-dimensional thin-layer chromatography (12).

Plasmid copy number. The relative amount of pBR322 plasmid DNA was estimated from measurement of the β-lactamase activity by using nitrocefin as the substrate (20).

TABLE 2. Growth inhibition by *c*II[a]

Plasmid[b]	*c*II expression	*hip* or *him*[c]	Growth at 42°C
pOG7	+	+	−
pOG7	−	−	+
pMJ1035	+	+	−
pTA10-*lacZ*	−	+	+

[a] All strains used bear a λ *c*I857 *N*am7 *N*am53ΔH1 prophage.
[b] pMJ1035 and pTA10-*lacZ* contain an *O-lacZ* and a *c*II-*lacZ* fusion, respectively; the plasmids are carried in strain N6171.
[c] The *hip-157* or *himA83* mutation was introduced into strain N6017 to yield N6214 or N6226 (22), respectively.

RESULTS

Lethality of lambda *c*II protein to *E. coli*. The λ *c*II gene cloned in a multicopy λ p_L expression vector can only be maintained in a λ lysogen under conditions of p_L repression (28). The lethality of *c*II protein is confirmed by the data presented in Table 2. Plasmid pOG7 is a pBR322-based expression vector in which *c*II is expressed from p_L (Fig. 1). The plasmid carries no other intact viral proteins, and the site of *c*II action, *pre*, is inactivated by mutation. The pOG7 plasmid can be maintained in cells lysogenic for a defective λ *c*I ts857 prophage at 32°C, at which temperature p_L is repressed. At 42°C, the lambda repressor is denatured, and *c*II is expressed from p_L. At 42°C, pOG7 transformants die. They do not lyse, but they stop growing and cannot form colonies. The *c*II protein is responsible for cell killing. Lysogens bearing a *c*II-deleted derivative of pOG7, pTA10-*lacZ*, survive thermal induction. Similarly, introduction of the *hip-157* or *himA83* mutation into the lysogens blocks *c*II synthesis from pOG7 and permits colony formation at 42°C (21). When *c*II is induced from pOG7 in the presence of λ *N* protein, growth of the cells stops within 10 min, and within 30 min almost all protein synthesis, except for synthesis of *c*II, also stops (26). At least 10-fold more *c*II protein is produced under N^+ conditions compared with the N^- lysogens used in the present study.

Isolation of *c*II-resistant rifampin-resistant mutants. The λ *c*II protein stimulates transcription initiation at the phage p_E,

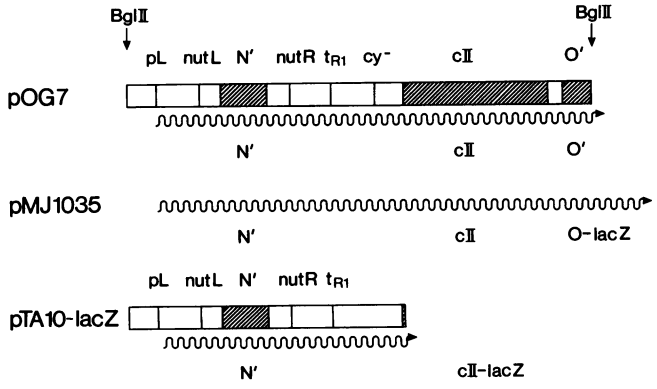

FIG. 1. DNA insertions containing p_L from plasmids pPG7, pMJ1035, and pTA10-*lacZ*. The *Bgl*II-*Bgl*II DNA fragment was isolated from pKC30-*c*II. The hatched area indicates the translated genes. The arrows show the p_L transcripts; below them, the protein products are given. pOG7 and pMJ1035 harbors the same *Bgl*II-*Bgl*II DNA fragment, whereas pTA10-*lacZ* only harbors the λ DNA until the AUG start codon of *c*II.

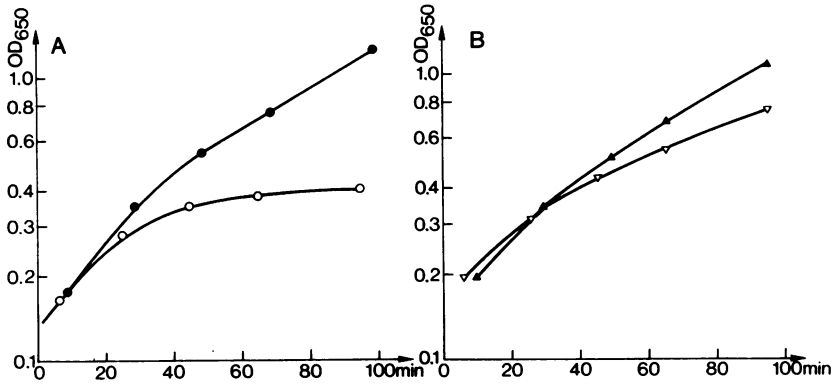

FIG. 2. Effect of *c*II expression on cell growth. The cells were grown in LB medium at 32°C; at time zero, the cultures were transferred to 43°C. Symbols: ○, wild-type KH23 carrying pOG7 (KH39 gave a similar result); ●, wild-type N6323 without a plasmid (KH29 gave a similar result); ▽, KH31 *sck-1* carrying pOG7; ▲, KH49 *sck-1* without a plasmid.

p_I, and p_Q promoters. We reasoned that *c*II protein might be lethal to *E. coli* because it interacted with RNA polymerase to activate inappropriate bacterial promoters. To isolate RNA polymerase mutants which could survive *c*II killing (*sck* mutants), we mutagenized, by using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, derivatives (KH39 and KH40) of our pOG7 lysogen carrying an *rpoB*-linked, Tn*10* insertion (*cps-4*::Tn*10*). A double selection was applied: resistance to rifampin (for *rpoB* mutations) and growth at 42°C (for resistance to *c*II). Mutants were isolated at a frequency of 2×10^{-9} to 8×10^{-9}. Eight independent clones were analyzed further by P1 transduction of *cps-4*::Tn*10* from the mutants into nonmutagenized KH23 and N6188 cells. In five mutants, rifampin resistance and survival of *c*II killing were 100% cotransducible (50 of 50 for each). The close linkage between the two phenotypes is consistent with *sck* being an allele of *rpoB*. These results suggest that our isolation protocol yields RNA polymerase mutants that are no longer inhibited by *c*II protein.

The growth of wild-type and *sck-1* lysogens with and without plasmid pOG7 is shown in Fig. 2A. Wild-type cells carrying pOG7 displayed growth inhibition starting at about 30 min after thermal induction. In contrast, the growth of the corresponding *sck-1* mutant lysogens was significantly less inhibited by a shift to 42°C (Fig. 2B).

Expression of *c*II in *sck* mutants. There are two possible explanations for the thermal resistance of the *sck* mutants; either *c*II is not expressed or *c*II is expressed but not lethal to the bacterium. Several lines of evidence support the latter explanation.

The *c*II gene of λ is located just promoter-distal to the Rho-dependent λ t_{R1} terminator. Termination at λ t_{R1} is partially suppressed by translation of the upstream λ *cro* gene (D. Court, personal communication). This effect of *cro* translation is demonstrated in Table 3, in which we compare two *galK* plasmids, both derivatives of pKG100. Plasmid pMZ105 carries the fusion p*gal-galE'-'cro-*λt_{R1}*-galK*, in which *galE* and *cro* form a protein fusion. Plasmid pMZ245 carries a p*lac-*λ t_{R1}*-galK* fusion in which there is no translation of RNA promoter-proximal to λ t_{R1} (Fig. 3). The expression of *galK* from pMZ105 is almost eightfold higher than from pMZ245 (Table 3, line 1). The *sck* mutations do not significantly affect *galK* expression from either plasmid relative to wild-type strains. Thus, an increased efficiency of termination at λ t_{R1}, with reduced transcription of *c*II, cannot explain the survival of *sck*(pOG7) mutant lysogens after thermal induction. The expression of a λ *O-lacZ* gene fusion located promoter-distal to *c*II is also unimpaired by the *sck* mutations (data not shown). The fusion is carried on

TABLE 3. Effect of *sck* mutations on termination efficiency at λ t_{R1}

Strain[a] (mutation)	Galactokinase activity[b] (U/OD_{436}) in strain bearing:	
	pMZ245	pMZ105
S01792 (wild type)	5.1 (1.0)	42 (1.0)
S03597 (*sck-1*)	6.8 (1.3)	26 (0.6)
S03598 (*sck-2*)	5.1 (1.1)	30 (0.7)
S03599 (*sck-3*)	5.6 (1.1)	33 (0.8)
S03639 (*sck-6*)	4.5 (0.9)	34 (0.8)

[a] The strains used were *sck* derivatives of S01792 into which the pMZ plasmids were introduced. The cells were grown at 37°C with glucose as the carbon source in phosphate minimal medium supplemented with thiamine, arginine, uracil, guanine, and ampicillin.

[b] The specific activity was corrected for small variations in copy numbers of the plasmids (see Materials and Methods). The numbers in parentheses indicate the enzyme level relative to that of the wild type for each plasmid.

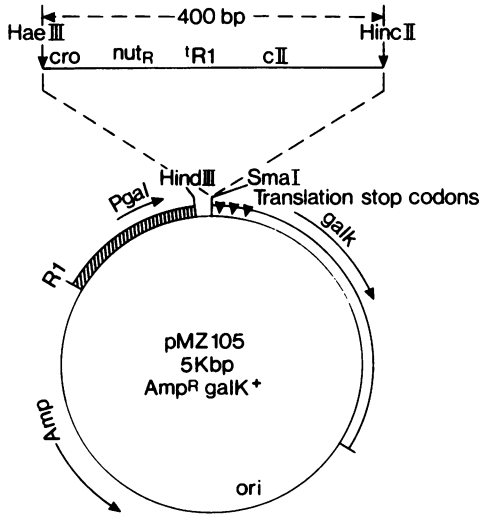

FIG. 3. pMZ105 carrying a 400-base-pair DNA fragment inserted into the *Sma*I site of pKG100. Translation starting at the *galE* N terminal contained on the p*gal* fragment continues in frame into the C terminal of the *cro* gene carried on the inserted DNA. Thus, the translation stops at the same site relative to t_{R1} as in phage λ.

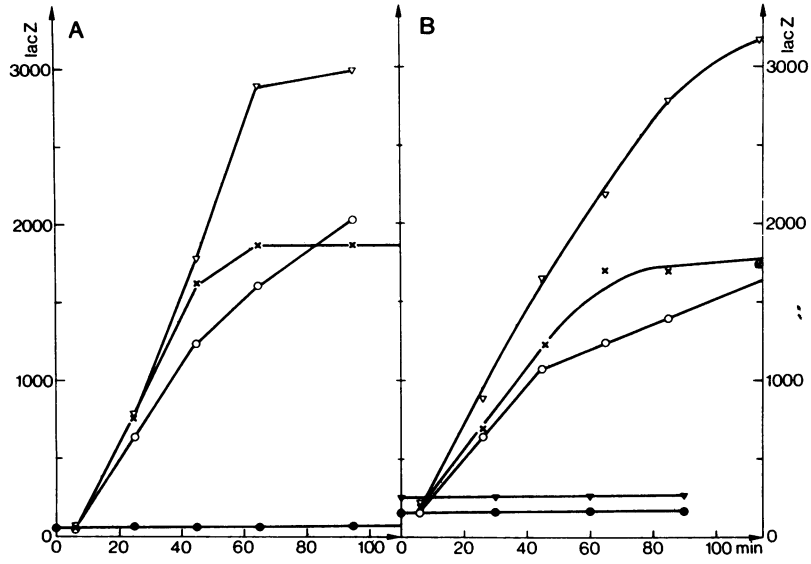

FIG. 4. Expression of β-galactosidase (*lacZ*) from the *c*II-activated promoter p_E (A) or p_I (B). Cultures were grown in LB medium at 32°C and then transferred to 43°C at time zero, and samples were withdrawn for determination of β-galactosidase activity. The specific activity is plotted in Miller units (19). All strains carry the plasmid pOG7 and are derivatives of N6323 (A) or N6017 (B). Symbols: (A) ○, wild-type KH23 and KH39; ▽, KH31 *sck-1*; ×, KH32 *sck-2*; ●, KH29 and N6323 (without plasmids) (KH31, KH32, and KH39 at 32°C); (B) ○, wild-type KH40; ×, KH41 *sck-3*; ▽, KH44 *sck-6*; ●, KH40 at 32°C; ▼, KH41 and KH44 at 32°C.

a multicopy plasmid, pMJ1035 (Fig. 1), and is analogous to pOG7. Thus, transcription through *c*II in *sck* mutants is equivalent to that in wild-type strains.

The synthesis of *c*II protein is not significantly different in wild-type and *sck* mutant cells. For this measurement, wild-type cells and mutant lysogens carrying pOG7 were thermally induced for 45 min and then labeled with [^{35}S]methionine for 1 min. Proteins were extracted and separated by electrophoresis in sodium dodecyl sulfate-polyacrylamide gradient gels, and the labeled *c*II protein was quantitated as described previously (17). No significant differences in *c*II levels between the wild-type cells and *sck* mutants were observed. Variations of twofold or less cannot be detected by this method.

The ability of *c*II protein to stimulate p_E and p_I in *sck* and wild-type lysogens is shown in Fig. 4A and B. All strains carry pOG7 and a chromosomal p_I-*lacZ* or p_E-*lacZ* transcription fusion. Thermal induction of *sck-1* and *sck-2* mutants leads to extensive *lacZ* expression from p_E. Similarly, induction of *sck-3* and *sck-6* mutants results in activation of p_I. β-Galactosidase levels in the mutant strains are, if anything, higher than in the wild-type cells. We conclude that *c*II protein is expressed in the *sck* mutants and that it can activate its target λ promoters. Therefore, the *rpoB* mutations in the *sck* strains appear to affect only the inhibitory activity of *c*II.

Growth of phage lambda on the *sck* mutants. The *sck* mutations were transferred to a nonlysogenic host, N4903, and their effects on the growth of phage lambda were tested. The growth of λ on *sck-1* mutants was normal at 32, 37, and 42°C (Table 4). The *sck-3* and *sck-6* mutations result in a clear-plaque morphology at 32°C, whereas *sck-2* mutants fail to plate λ at this temperature.

The growth of the λ variant, λ *nin5*, on the *sck* strains is also shown in Table 4. Unlike wild-type λ, λ *nin5* is capable of propagating in the absence of λ *N* gene function; the *nin5* mutation deletes the λ t_{R2} and λ t_{R3} terminators in the λ p_R operon that are normally suppressed by *N*. The *nin5* deletion restores λ plating and/or normal plaque morphology at 32°C on the *sck-2* and *sck-3* mutants. This restoration is consistent with the idea that transcription termination at certain terminators may be affected by these *sck* mutations, a point to which we shall return below. Surprisingly, the *sck-6* mutation, which does not restrict the growth of wild-type λ, completely blocks plaque formation by λ *nin5* at 32 and 42°C and partially inhibits phage growth at 37°C. Similarly, the failure of λ *nin5* to propagate on the *sck-6* strain may be related to the deletion of the λ p_R operon terminators. Alternatively, DNA encoding several open reading frames is removed by the *nin5* deletion; the products of these genes might be required specifically for phage development in an *sck-6* mutant.

The *sck-2* mutant displays a Nus$^-$ phenotype at 32°C; i.e., growth of λ is inhibited, whereas λ *nin5* develops normally (8). Therefore, we asked whether *sck* mutations, in combination with *nusA1*, might be less permissive for phage

TABLE 4. Plating of λ on *sck* and *nusA1 sck* derivatives

Strain	Relevant genotype	Plaque formation[a] of phage:					
		λ			λ *nin5*		
		32°C	37°C	42°C	32°C	37°C	42°C
N4903	Wild type	tb	tb	tb	tb	tb	tb
KH110	*sck-1*	tb	tb	tb	tb	tb	tb
KH111	*sck-2*	0	tb	tb	tb	tb	tb
KH112	*sck-3*	C/tb	tb	tb	tb	tb	tb
KH114	*sck-6*	C	tb	tb	0	min	0
S03573	*nusA1*	tb	tb	0	tb	tb	tb
S03568	*nusA1 sck-1*	0	0	0	tb	tb	tb
S03569	*nusA1 sck-2*	0	0	0	tb	tb	C
S03570	*nusA1 sck-3*	0	0	0	tb	tb	tb
S03571	*nusA1 sck-6*	C	C	0	ND	ND	ND

[a] tb, C, and 0 indicate turbid, clear, and no plaque, respectively; min, minute plaques; ND, not determined. Other phage which failed to form plaques on *sck-2* at 32°C were λ *c*I30, λ *c*II67, λ *imm*^{21}, and λ *imm*^{434}. The *sck-6* strain also failed to allow plating of λ *red*3.

TABLE 5. Enzyme levels and pool sizes in *sck* derivatives of S0931

Strain	Relevant genotype	Sp act[a] (U/mg of protein)				Pool size (μmol/g [dry wt])	
		pyrB		*pyrE*		UTP (−UR)	GTP (−UR)
		−UR[b]	+UR[b]	−UR	+UR		
S01748	Wild type	39	12 (1.0)	30	11 (1.0)	1.15 (1.0)	1.86 (1.0)
S01749	*sck-1*	77	25 (2.1)	156	143 (13)	1.62 (1.4)	2.57 (1.4)
S01750	*sck-2*	92	28 (2.4)	108	72 (6.5)	1.49 (1.3)	1.86 (1.0)
S01751	*sck-3*	88	29 (2.4)	146	132 (12)	1.77 (1.5)	2.77 (1.5)
S01752	*sck-6*	31	8 (0.7)	30	3 (0.3)	1.64 (1.4)	3.98 (2.1)

[a] *pyrB* and *pyrE* represent aspartate transcarbamylase and orotate phosphoribosyltransferase, respectively. Numbers in parentheses indicate fold of wild-type levels.

[b] −UR and +UR indicate that uridine (70 μg/ml) was not and was added to the growth medium, respectively.

growth than *nusA1* alone. One may recall that *nusA1* mutants restrict λ development only at 42°C. We found that the mutations *sck-1*, *sck-2*, and *sck-3*, when transferred into a *nusA1* background, exacerbated the Nus⁻ phenotype. Lambda no longer plated at 32°C; the growth of the control phage, λ *nin5*, was not affected. Unlike the other *sck* mutants, *sck-6* in combination with *nusA1* does not interfere further with λ plaque formation.

Increase of *pyrE* expression by the *sck-1*, *sck-2*, and *sck-3* mutations. The exaggerated Nus⁻ phenotype conferred by the *sck-1*, *sck-2*, and *sck-3* mutations suggested that they might affect transcription termination. Therefore, we studied the expression of several bacterial genes known to be regulated at the level of transcription termination. The genes of the pyrimidine biosynthetic pathway, *carAB*, *pyrB*, *pyrC*, *pyrD*, *pyrE*, and *pyrF*, are dispersed throughout the *E. coli* chromosome. They are repressed by high intracellular pyrimidine nucleotide pool levels. The expression of *pyrB* and *pyrE* is controlled by a transcription attenuation mechanism (10, 27); the role of attenuation in the regulation of the other *pyr* cistrons is not known. The *sck* mutations were transferred to SØ1718, a SØ931 derivative which grows well in minimal medium, and the levels of the enzymes coded for by *pyr* were determined.

We found that the *sck-1* and *sck-3* mutations do not affect the levels of the enzymes coded for by *carAB*, *pyrC*, *pyrD*, and *pyrF* (data not shown). However, the levels of aspartate transcarbamylase (*pyrB*) and especially of orotate phosphoribosyltransferase (*pyrE*) were significantly elevated in *sck-1*, *sck-2*, and *sck-3* mutants (Table 5). The expression of the latter enzyme was increased three- to fivefold for *sck* mutant cells grown in the absence of uridine, and, in contrast to the wild-type strain, the addition of uridine to the medium of mutant cells did not markedly reduce the levels of enzyme coded for by *pyrE*. Thus, *pyrE* expression is constitutive in the *sck* mutant background. The increase in *pyrB* expression was less marked (ca. twofold) and was repressible. The *sck-6* mutation had little effect on *pyrB* or *pyrE* expression in medium lacking uridine but appeared to increase the efficiency of uridine repression.

Since the *sck-1*, *sck-2*, and *sck-3* mutations stimulate the expression of only *pyrE* and *pyrB*, we did not expect to find gross abnormalities in the intracellular nucleotide pools of these mutants. Our results (Table 5) confirm this supposition; the levels of GTP, ATP, CTP, UTP, and ppGpp were slightly (20 to 60%) increased relative to those in the wild-type strain. The UTP pool was only marginally elevated in the *sck-6* mutant. However, the GTP and CTP pools in the *sck-6* mutant showed a more significant elevation, twofold (Table 5) and threefold (data not shown), respectively. At present we do not have an explanation for the abnormal nucleotide pools in this mutant.

To determine whether, in fact, the *sck* mutations increase the levels of enzyme coded for by *pyrE* by suppressing

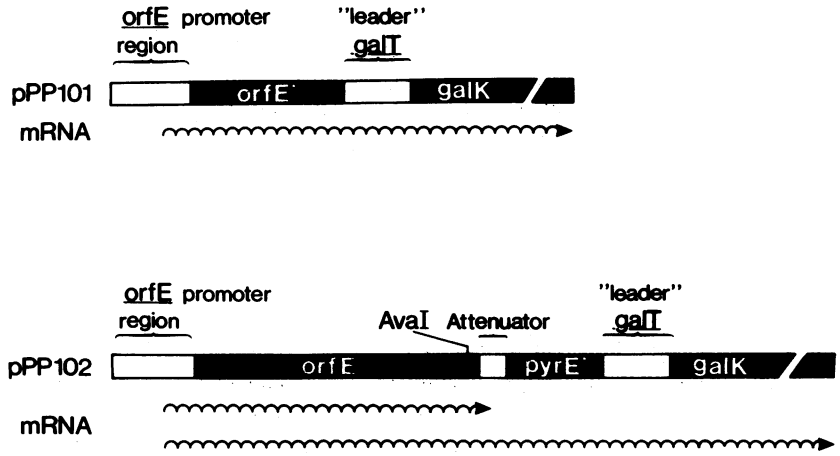

FIG. 5. Schematic representation of *orfE'-galK* and *pyrE'-galK* transcriptional fusions contained on plasmids pPP101, pPP102, and pPP112. In these plasmids, the *galK* gene is expressed from the *orfE* promoter region. In pPP102, *galK* transcription is subject to attenuation at the intercistronic *pyrE* attenuator. pPP112 is identical to pPP102 except that the coupled transcription and translation in the attenuator region of pPP112 are hindered due to insertion in the *Ava*I site of a linker that contains stop codons in all three translational reading frames (25).

TABLE 6. Enzyme levels in strains harboring *pyrE-galK* fusions[a]

Strain	Plasmid	Galactokinase (U/mg)	OPRTase[b] (U/mg)	% Readthrough[c]	Attenuation[c] (fold)
S01792 (wild type)	pPP101	450	54		1.0
	pPP102	54	63	12	8.3
	pPP112	40	54	6	17
S03597 *sck-1*	pPP101	416	375		1.0
	pPP102	230	371	58	1.7
	pPP112	26	386	6	17
S03598 *sck-2*	pPP101	572	210		1.0
	pPP102	139	237	30	3.3
	pPP112	19	233	5	20
S03599 *sck-3*	pPP101	434	350		1.0
	pPP102	203	349	55	1.8
	pPP112	20	352	4	24

[a] Cells were grown at 37°C in a 03P medium supplemented with glucose (0.2%), Casamino Acids (0.2%), thiamine (1 μg/ml), uracil (25 μg/ml), guanine (15 μg/ml), and ampicillin (50 μg/ml).
[b] OPRTase, Orotate phosphoribosyltransferase.
[c] Specific activities of galactokinase were normalized on the basis of the specific activities of β-lactamase before calculation of percent readthrough, defined as the galactokinase level in a strain bearing pPP102 (or pPP112) relative to the level in a strain bearing pPP101. The reciprocal ratio is the fold of attenuation and is a measure of the number of mRNA chains initiated before one chain reads past the attenuator.

attenuation, we introduced a set of *pyrE-galK* operon fusions into the mutant strains. The expression of *pyrE* is thought to depend upon the relative rates of transcription and translation of a 238-codon leader sequence, *orfE*. When the UTP levels are high, the transcription rate in the leader is increased relative to translation. This condition favors attenuation of transcription between *orfE* and *pyrE*. UTP scarcity decreases the rate of *orfE* transcription and suppresses attenuation. Failure to translate *orfE* results in efficient UTP-independent attenuation (2, 10, 24, 25).

The structures of three plasmid *galK* fusions are shown in Fig. 5. Plasmid pPP101 carries an *orfE-galK* fusion without an attenuator. Plasmid pPP102 bears a *pyrE-galK* fusion; *galK* expression in this plasmid is subject to attenuation (25). The efficiency of attenuation (reciprocal of percent readthrough) is defined as the ratio of the galactokinase level in a strain harboring pPP101 relative to that of a strain harboring pPP102. For strains grown in uracil-containing medium, the *sck* mutations reduced attenuation three- to fivefold (Table 6). The expression of *galK* from pPP101 is the same in wild-type and *sck* mutant strains, indicating that the *sck* mutation does not affect promoter strength. In SØ1792, the *sck* mutations increase the levels of enzyme coded for by chromosomal *pyrE* only four- to sixfold. This reflects a high basal level of *pyrE* expression in the parental strain under the growth conditions used.

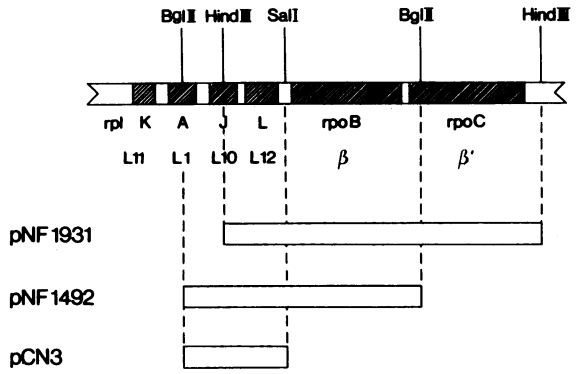

FIG. 6. Map of the chromosomal *rpoBC* operon (not drawn to scale); below it, the gene symbols and protein products are given. Open bars represent the chromosomal segment carried by each of the plasmids indicated. In pNF1931 and pNF1492, the chromosomal DNA is inserted into the *Hin*dIII site and the *Bam*HI site of pBR322, respectively. In pCN3, the DNA fragment was inserted into a *Bam*HI-*Sal*I-digested derivative of pBR322 containing a deletion of the *Eco*RI-*Hin*dIII region.

We next asked whether the *sck* mutations would suppress attenuation in the absence of *orfE* translation. Plasmid pPP112 is a derivative of pPP102 carrying translational stop signs in *orfE*, 67 base pairs upstream of the *orfE* terminator (see the legend to Fig. 5). The expression of *galK* from this plasmid is only 6% that from pPP101 in both wild-type and *sck* mutant strains (Table 6). We conclude that relief of attenuation in *sck* mutants still depends upon coupled transcription-translation.

Mapping of the *sck* mutations. We next verified that the phenotype of the *sck-1*, *sck-2*, and *sck-3* mutants, with respect to both λ growth and *pyrE* expression, results from a mutation in the β subunit of RNA polymerase. Plasmids pNF1931, pNF1492, and pCN3 are *Hin*dIII, *Bgl*II, and *Bgl*II-*Sal*I subclones, respectively, of λ *drif18*. The genetic contents of these plasmids are shown in Fig. 6 and Table 7; they carry and express all or some of the cistrons of the L10, L12, β, and β' gene group. The elevated levels of enzyme coded for by *pyrE* in strains *sck-1* and *sck-3* were restored to normal by plasmids pNF1931 and pNF1492 but not by pCN3. To show that pNF1492 suppresses the *sck* mutant phenotype by complementation rather than by recombination with the chromosome, we cured the mutant strains for the plasmid. The cured strains again displayed high *pyrE* expression (data not shown). The same complementation pattern was seen for the Nus⁻ phenotype of *sck-2* (Table 7). This analysis indicates that the *sck* mutants carry a recessive mutation in *rpoB* and that they can be complemented by a

TABLE 7. Plasmid complementation of *sck* mutations[a]

Plasmid	Protein expressed				Sp act of *pyrE* (U/mg)[b]			Plating of λ at 32°C[c]	
	L10	L12	β	β'	S01748 (wild type)	S01749 *sck-1*	S01751 *sck-3*	N4903 (wild type)	KH111 *sck-2*
None	−	−	−	−	37 (1.0)	382 (10)	423 (11)	tb	0
pNF1931	−	+	+	+	50 (1.0)	124 (2.5)	82 (1.6)	C	t
pNF1492	+	+	+	−	57 (1.0)	95 (1.7)	69 (1.2)	tb	C
pCN3	+	+	−	−	33 (1.0)	376 (11)	275 (8.3)	tb	0

[a] The strains were grown in LB medium plus uridine (70 μg/ml).
[b] *pyrE* represents orotate phosphoribosyltransferase. Numbers in parentheses indicate the enzyme level relative to that of the wild-type strain bearing the corresponding plasmid.
[c] tb, C, and 0 indicate turbid, clear, and no plaque, respectively.

plasmid expressing a wild-type β subunit. It was not ruled out, however, that the mutants carry a second, linked mutation affecting the Sck⁻ phenotype (see below).

We demonstrated above that Rif^r and survival of *c*II killing were 100% (50 of 50) linked by P1 transduction. We repeated the P1 transduction experiment, this time scoring for *pyrE* expression. P1 was grown on an *sck-2* mutant carrying *cps-4*::Tn*10*, and recipients bearing a chromosomal *pyrE-lacZ* fusion were transduced to Tet^r (SØ1767) or ArgE$^+$ (SØ3682). Selection for *cps-4* yielded 183 Rif^r recombinants (68% of the transductants), whereas selection for ArgE$^+$, located on the other side of *rpoB*, yielded 68 Rif^r transductants (23%). No segregation of Rif^r and *pyrE* overexpression, monitored on MacConkey agar-lactose-uridine (100 μg/ml), was observed among a total of 567 transductants. We conclude that the same mutational event in *rpoB* is responsible for the elevated transcription of *pyrE* and the Rif^r and most likely also for the resistance to *c*II protein of *sck-2*. A less-extensive analysis of *sck-1* and *sck-3* mutants (30 of 30 Rif^r mutants showed high levels of *pyrE* transcription) suggested that the same was true for these mutants.

DISCUSSION

We selected Rif^r *E. coli* mutants simultaneously resistant to lethal levels of the λ *c*II protein. These *sck* mutations (for survivors of *c*II killing) reside in the *rpoB* cistron, which encodes the β subunit of RNA polymerase. The mutant cistrons have been sequenced. (i) *sck-6* carries a TCC-to-TTC transition (Ser to Phe) at codon 531. (ii) *sck-1*, *sck-2*, and *sck-3* carry a CCT-to-CTT transition (Pro to Leu) at codon 564. Additional linked mutations have not been excluded (D. J. Jin and C. Gross, personal communication). Presumably, secondary mutations account for the phenotypic differences among the *sck-1*, *sck-2*, and *sck-3* strains. The mutations both in codon 531 and in codon 564 have been previously described as Rif^D mutations (23).

The *sck* mutations do not block the transcription or translation of the λ *c*II cistron. Furthermore, the stimulatory activity of *c*II protein at the λ p_E and λ p_I promoters is unimpaired in the *sck* strains. Therefore, the mutations appear to prevent specifically the lethal effects of the *c*II gene product.

Like other Rif^r mutations, the *sck-1*, *sck-2*, and *sck-3* mutations affect transcription termination. The growth of bacteriophage λ, which is dependent upon the suppression of transcription termination, is hindered by these *sck* mutations acting either alone or in concert with the bacterial *nusA1* mutation. The λ variant, λ *nin5*, does not require suppression of termination for plaque formation; it grows normally in the mutant strains.

The *sck-1*, *sck-2*, and *sck-3* mutations can also reduce transcription termination. The product of the bacterial *pyrE* gene, orotate phosphoribosyltransferase, is expressed constitutively in the mutant backgrounds. The *pyrE* gene is regulated by an attenuation mechanism which responds to the relative rates of transcription of a uridine-rich leader sequence and the translation of the leader transcript. When the transcription rate increases relative to the rate of translation, attenuation is favored (10). Our data show that attenuation at *pyrE* is suppressed in the *sck* mutants but only when leader translation is permitted. This suggests that the *sck* mutant polymerases are capable of termination at the *pyrE* attenuator but that the rate at which they transcribe the *pyrE* leader sequence is reduced.

Three RNA polymerase mutants have been isolated in *Salmonella typhimurium* that show increased *pyrE* expression. They are located in the distal end of *rpoB* or in *rpoC* (14). One of the mutants, KP1475, displays a 100-fold increase in *pyrB* expression; the other two show only moderately enhanced levels of enzyme coded for by *pyrB*. Purified KP1475 RNA polymerase shows a four- to sixfold increase in K_m for UTP and ATP during transcription elongation; the rate of elongation is also decreased in vivo (11). It is possible that the *E. coli sck* mutations produce similar changes in RNA polymerase; the differences in *pyrB* expression (2-fold elevation in *sck* strains versus 100-fold elevation in the *Salmonella* mutant KP1475) may reflect subtle variations in the regulation of *pyrB* and *pyrE* expression in response to changes in the mRNA chain elongation rate.

In contrast to the other *sck* mutations, *sck-6* does not increase *pyrE* expression or impair the ability of λ to suppress transcription termination. Instead, the growth of λ *nin5* (and of λ *red3*) is blocked. Failure of these phage to propagate on certain λ lysogens (Ren) has been reported (30). The phenotype of *sck-6* differs; a prophage is not required to block phage growth, and, in contrast to Ren strains, the ability of λ OP22 to propagate on the *sck* mutant strains is not affected (data not shown). The *nin5* mutation deletes, in addition to two p_R operon terminators, t_{R2} and t_{R3} (D. Leeson, D. I. Friedman, and M. Gottesman, manuscript in preparation), several open reading frames. The *nin* open reading frames may encode proteins required for λ growth on *sck-6* mutants. Alternatively, the program of λ gene expression in a *sck-6* background may entail transcription termination or pausing in the *nin* region. We succeeded in isolating pseudorevertants of λ *nin5* by plating a mutagenized stock of this phage on *sck-6* hosts. Some of these pseudorevertants do not form plaques on wild-type hosts. Further characterization of these phage, currently in progress, may help us choose among these possibilities.

These studies did not permit us to determine how λ *c*II protein kills *E. coli* or how the *sck* mutations protect against this lethality. Although it is conceivable that the *sck-1*, *sck-2*, and *sck-3* mutations protect by slowing the rate of transcription elongation, this explanation cannot extend to *sck-6*, which does not appear to produce this phenotype. Although our original goal remains elusive, we have obtained RNA polymerase mutations with regulatory effects. Analysis of the mutants should yield additional information on the role of the β subunit of the polymerase in the various steps of the transcription reaction.

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