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Simultaneous Gain and Loss of Functions Caused by a Single Amino Acid Substitution in the β Subunit of *Escherichia coli* RNA Polymerase: Suppression of *nusA* and *rho* Mutations and Conditional Lethality

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ABSTRACT

Transcript elongation and termination in *Escherichia coli* is modulated, in part, by the *nusA* gene product, an acidic protein that interacts not only with RNA polymerase itself but also with ancillary factors, namely the host termination protein Rho and phage λ antitermination protein, N. The *E. coli nusA1* mutant fails to support λ development due to a specific defect in N-mediated antitermination. Certain rifampicin-resistant (*rif*^{R}) variants of the *nusA1* host support λ growth. We report here the isolation and pleiotropic properties of one such *rif*^{R} mutant, *ts8*, resulting from a single amino acid substitution mutation in *rpoB*, the structural gene for polymerase β subunit. *ts8* is a recessive lethal mutation that blocks cell growth at 42°. Pulse-labeling and analysis of newly synthesized proteins indicate that the mutant cell is proficient in RNA synthesis at high temperature. Apparently, *ts8* causes a loss of some specialized function of RNA polymerase without a gross defect in general transcription activities. *ts8* is an allele-specific suppressor of *nusA1*. It does not suppress *nusAsal*, *nusB5* and *nusE71* mutations nor does it bypass the requirement for a functional *N* gene and the *nut* site for antitermination and λ growth. A mutation in the *N* gene, *punA1*, that restores λ growth in the *nusA1* mutant host but not in the *nusAsal* host, compensates for the *nusAsal* allele in the *ts8* mutant. This combined effect of two allele-specific suppressors suggests that they enhance some aspect of polymerase-NusA-N interaction and function. *ts8* suppresses the *rho15* mutation, but not the *rho112* mutation, indicating that it might render RNA polymerase susceptible to the action of a defective Rho protein. Marker rescue analysis has localized *ts8* to a 910-bp internal segment of *rpoB* that encodes the Rif domain. By amplification, cloning and sequencing of this segment of the mutant chromosome we have determined that *ts8* contains Phe in place of Ser522, caused by a C to T transition. By gene conversion, we have established that the simultaneous gain and loss of three functions of polymerase is caused by this single amino acid substitution. Clearly, a site in the β subunit critical for the functioning of both termination and antitermination factors is altered by *ts8*. The alteration, we imagine, might make this site on polymerase receptive to some factors but repulsive to others.

IN *Escherichia coli*, a single RNA polymerase carries out gene transcription that consists of several major steps: (a) recognition and binding of a promoter and melting of the DNA double helix, (b) initiation of RNA chain synthesis directed by the template strand, (c) chain elongation and progression of the transcription complex along the gene, (d) recognition of the stop signal at the end of the gene and cessation of RNA chain synthesis and, finally, (e) release of the RNA from the transcription complex and dissociation of RNA polymerase from the DNA template (McCLURE 1985; VON HIPPEL *et al.* 1984). RNA polymerase is a multisubunit enzyme constituted by two functional components (BURGESS 1969; BURGESS *et al.* 1969): (i) a core, $\alpha_2\beta\beta'$ (products of *rpoA*, *rpoB* and *rpoC*, respectively), which carries out all steps but promoter recognition and *de novo* initiation of RNA synthesis and (ii) one of several sigma subunits (the products of *rpoD*, *rpoH* and *rpoN*) that allows differential promoter recognition and specific initiation (BURGESS *et al.* 1987; HELMANN and CHAMBERLIN 1988). Productive initiation at certain promoters, however, requires specific regulatory proteins in addition to σ, which interact with both a nearby *cis*-acting site and the polymerase to promote initiation (McCLURE 1985).

Transcript elongation and termination is modulated by the interaction of RNA polymerase with a variety of ancillary factors (FRIEDMAN 1988; YEAGER and VON HIPPEL 1987). Early during elongation *in vitro*, the σ subunit dissociates from the transcription complex and the core enzyme then continues to elongate the RNA chain. Elongation by core polymerase proceeds at a reduced rate *in vitro*, as compared to *in vivo*. To date, a general transcription factor that might enhance the intrinsic elongation rate has not been reported. Although the core polymerase can stop RNA synthesis at some sites known as factor-independent

or intrinsic terminators, additional factors are required for accurate and efficient termination at many other sites. Rho and Tau are among these factors which promote termination at distinct classes of termination sites (ROBERTS 1969; BRIAT and CHAMBERLIN 1984). The other known factor to promote termination is NusA, a 55-kD acidic polypeptide encoded by the *nusA* gene essential for cell viability (FRIEDMAN and BARON 1974; GREENBLATT, MCLIMONT and HANLY 1981; KINGSTON and CHAMBERLIN 1981; WARD and GOTTESMAN 1981; NAKAMURA *et al.* 1987). NusA is a component of the elongating transcription apparatus (HORWITZ, LI and GREENBLATTL 1987), performing pleiotropic functions in transcript elongation and termination (see YEAGER and VON HIPPEL 1987). It reduces the overall rate of elongation, promotes transcriptional pause at distinct sites and modulates the efficiency with which polymerase stops RNA synthesis at different sites. Moreover, it modulates the activity of both termination and antitermination factors (LAU, ROBERTS and WU 1983; GREYHACK *et al.* 1985; WHALEN, GHOSH and DAS 1988). NusA binds not only the RNA polymerase itself (GREENBLATT and LI 1981a) but also the termination protein Rho (SCHMIDT and CHAMBERLIN 1984) encoded by the *rho* gene essential for viability of *E. coli* (DAS, COURT and ADHYA 1976; INOKO and IMAI 1976). It also binds directly to the antitermination protein N of phage λ (GREENBLATT and LI 1981b). Thus, NusA is believed to be an adaptor or a coupler which enables various ancillary factors to interact with RNA polymerase and modulate elongation (GREENBLATT 1981).

An understanding of RNA polymerase structure-function is central to the mechanism of gene regulation. The β subunit of RNA polymerase, encoded by the *rpoB* gene, has been implicated in most transcription steps (YURA and ISHIHAMA 1979; YEAGER and von Hippel 1987). Mutations in *rpoB*, readily selected by rifampicin-resistant phenotype, are known to affect gene expression and control. Certain *rif*^{R} alleles alter the susceptibility of RNA polymerase to the action of the N-antitermination protein (GEORGOPOULOS 1971; GHYSEN and PIRONIO 1972) and Rho termination protein (DAS, MERRIL and ADHYA 1978; GUARENTE and BECKWITH 1978), while others alter the intrinsic termination activity of polymerase both positively and negatively (NEFF and CHAMBERLIN 1980; YANOFSKY and HORN 1981).

To gain an understanding of the mechanism of polymerase-NusA interaction and the role of this interaction in transcript elongation and termination, we have sought suppressor mutations in *rpoB* that compensate for a mutant *nusA* allele. The particular allele chosen is *nusA1* which causes a specific defect in antitermination promoted by the λ *N* gene product without grossly affecting cellular transcription and viability (FRIEDMAN and BARON 1974). Here, we report one suppressor allele, *rpoBts8*, caused by a single amino acid substitution in the β subunit residue 522, phenylalanine in place of Serine. We further demonstrate that this mutation is pleiotropic: First, in addition to allowing N-dependent antitermination with the *nusA1* allele specifically, the *ts8* mutation (*F522*) also suppresses the termination defect of a mutant *rho* allele, *rho15*. Second, in contrast to causing this gain of two opposite functions, the mutation also results in the conditional lethality. The mutant cell is unable to grow at temperatures above 40°; however, it does not reveal any gross defect in transcription at the nonpermissive temperature. This raises the possibility that the conditional growth arrest might result from the loss of some specialized function, for instance, interaction with a transcription factor and thereby a failure to express some essential genes. Consistent with this hypothesis, the growth defect is suppressed by a dominant mutation in a gene, we named *greC*, located at 90.5 min on the *E. coli* chromosome (SPARKOWSKI 1990). The growth defect is also suppressed by an elevated expression of yet another gene of *E. coli*, *greA*, that may encode a transcription factor (SPARKOWSKI and DAS 1990, 1991).

MATERIALS AND METHODS

Bacterial strains, phages and plasmids: *E. coli* K12 strains used in this study are listed in Table 1. λ*NpunA1* was provided by D. FRIEDMAN (University of Michigan, Ann Arbor). λ*imm*21c, λ*imm*21*D69*, λ*imm*434*r32*, λ*b*$_2$*c*, λ*cI857Nam53*, λ*virNam500* and λ*imm*434*Nam213* were obtained from the collection of S. ADHYA and R. WEISBERG (National Institutes of Health, Bethesda). Plasmid pDJJ1 containing the *rpoB* gene was a gift from D. JIN (University of Wisconsin, Madison). Plasmid pDL19 used to engineer *rpoB* fragments is a derivative of pUC19 containing a *Bgl*II site within the multilinker (DAS 1990). Plasmid pDL34 (LAZINSKI, GRZADZIELSKA and DAS 1989) was used to supply λN protein in *trans*.

Transformation, conjugation and other genetic manipulations and tests have followed the procedures compiled by SILHAVY, BERMAN and ENQUIST (1984). Transduction of *nusA*, *nusB*, *nusE*, *rho* and *rpoB* alleles was done with phage P1*vir*, employing linked markers *Zgi*::Tn*10*, *proC*::Tn*10*, *Zhb*::Tn*10*, *ilv* and *argE*::Tn*5*, respectively. Prototrophs were selected by plating the transduction mixture directly on selective minimal-agar plates and incubation for 2 days at the appropriate temperature. For the selection of antibiotic-resistant transductants, P1-infected cells were diluted and grown for 1 hr in Luria broth at 32° (temperature-sensitive strains and λ*cIts* prophage strains) or 37° (all other strains) prior to plating on selective antibiotic plates. Tetracycline, kanamycin and ampicillin were used at 12.5, 25 and 50 μg/ml, respectively. Plates were incubated at 32° or 37° for 20–40 hr. Transductants obtained by lowest multiplicity of infection were purified on selective plates to single colonies and saved.

Isolation of *nusA1* suppressor mutants: Aliquots of 2 × 10^9 cells of SP1 were spread on LB plates containing 20 μg/ml rifampicin and incubated at 32° for 2 days. rif^R colonies (small, medium and large) were purified on the LB-Rif plates

TABLE 1

List of strains

Strain	Genotype	Source/Construction
N99	*sup° strA galK2*	NIH collection
SA500	*relA1 strA sup° his*	DAS, COURT and ADHYA (1976)
SA1615	SA500 *ilv galP3*	GULLETTA, DAS and ADHYA (1983)
SA1934	SA500 *ilv bio galP3*(λ *del-Bam cI857 del-H1)*	ADHYA and GOTTESMAN (1982)
AD1600	SA500 *galP3 rho15*	DAS, COURT and ADHYA (1976)
AD1919	SA500 *galP3 rho112*	DAS, MERRIL and ADHYA (1978)
N4831	SA500 *ilv bio*(λ *del-Bam Nam7Nam53* λ*cI857 del-H1)*	GOTTESMAN, ADHYA and DAS (1980)
N5261	SA1934 cI^-	WARD, DELONG and GOTTESMAN (1983)
N6441	SA1934 *nutL44*	ADHYA and GOTTESMAN (1982)
OR1265	*sup° strA ilv*(λ*Nam7Nam53 cI857 pR-cII::*IS2 *galETK)*	REYES *et al.* (1979)
K1102	$nusA_{ad}$*his leu thr malA*$^+$ *argG thi mtl xyl ara gal-6 lac*	SCHAUER *et al.* (1987)
CAG3002	Wild-type K12 *btuB::*Tn*10*	JIN and GROSS (1988)
C600	*thi-1 thr-1 leu-6 lacY1 tonA21 supE44*	NIH collection
AD7071	SA500 *del-galETK Zgi:*Tn*10 nusA1*	DAS and WOLSKA (1984)
AD7216	SA1934 *proC::*Tn*10*	SA1934 × P1 *proC::*Tn*10*
SP1	SA1934 *Zgi:*Tn*10 nusA1*	SA1934 × P1 *Zgi:*Tn*10 nusA1*
SP2	SA1934 *nusB5*	AD7216 × P1 *ProC*$^+$ *nusB5*
SP3	SA1934 *Zhb:*Tn*10 nusE71*	SA1934 × P1 *Zhb:*Tn*10 nusE71*
SP124	SA1934 *Zgi:*Tn*10 nusA1 rpoBts8*	SP1 rif^R isolate #8
SP166	SA1934 *argE::*Tn*5*	SA1934 × P1 *argE::*Tn*5*
SP168	SA1934 *Zgi:*Tn*10 nusA1 argE::*Tn*5*	SP1 × P1 *argE::*Tn*5*
SP172	SA1934 *rpoBts8 argG::*Tn*5*	SP124 × P1 *argG::*Tn*5 nusA*$^+$
SP180	SA1934 *Zgi:*Tn*10 nusA1 rpoBts8*	SP168 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP186	AD7071 *argE::*Tn*5*	AD7071 × P1 *argE::*Tn*5*
SP190	SA1934 *rpoBts8*	SP166 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP194	AD7071 *rpoBts8*	SP186 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP201	SA1615 *argE::*Tn*5*	SA1615 × P1 *argE::*Tn*5*
SP209	SA1615 *rpoBts8*	SP201 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP215	SA1615 *rpoBts8 Ilv*$^+$ *rho112*	SP209 × P1 *Ilv*$^+$ *rho112*
SP230	N5261 *argE::*Tn*5*	N5261 × P1 *argE::*Tn*5*
SP232	N6441 *argE::*Tn*5*	N6441 × P1 *argE::*Tn*5*
SP236	N5261 *argE::*Tn*5 Zgi:*Tn*10 nusA1*	SP230 P1 × *Zgi:*Tn*10 nusA1*
SP240	N5261 *Zgi:*Tn*10 nusA1 rpoBts8*	SP236 × P1 *ArgE*$^+$*rpoBts8* (SP124)
SP250	N6441 *rpoBts8*	SP232 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP261	OR1265 *argE::*Tn*5*	OR1265 × P1 *argE::*Tn*5*
SP272	OR1265 *rpoBts8*	SP261 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP336	SA1934 *rpoBts8 argG::*Tn*5 Zhb:*Tn*10 nusE71*	SP172 × P1 *Zhb:*Tn*10 nusE71*
SP342	SA1934 *nusB5 argE::*Tn*5*	SP2 × P1 *argE::*Tn*5*
SP364	SA1934 *nusB5 rpoBts8*	SP342 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP385	K1102 *rpoBts8*	K1102 × P1 *ThiA*$^+$ *rpoBts8* (SP124)
SP448	SA1615 *Ilv*$^+$ *rho112*	SP201 × P1 *Ilv*$^+$ *rho112* (AD1919)
SP490	SA1615 *Ilv*$^+$ *rho15*	SP201 × P1 *Ilv*$^+$ *rho15* (AD1600)
SP492	SA1615 *rpoBts8 Ilv*$^+$ *rho15*	SP209 × P1 *Ilv*$^+$ *rho15* (AD1600)
SP494	SA1615 *Ilv*$^+$ *rho15 argE::*Tn*5*	SP492 × P1 *argE::*Tn*5 rpoB*$^+$
SP583	N4831 *argE::*Tn*5*	N4831 × P1 *argE::*Tn*5*
SP587	N4831 *rpoBts8*	SP583 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP706	SA1934 *Zgi:*Tn*10 nusA1 rpoB[F522]*(pJS26)	SP1(pJS26) *F522* isolate
SP716	SA1934 *Zgi:*Tn*10 nusA1 rpoB[F522]*	SP168 × P1 *ArgE*$^+$ *rpoB[F522]* (SP706)
SP717	SA1615 *rpoB[F522]*	SP201 × P1 *ArgE*$^+$ *rpoB[F522]* (SP706)
SP719	SA1615 *rpoB[F522] Ilv*$^+$ *rho112*	SP717 × P1 *Ilv*$^+$ *rho112* (AD1919)
SP728	SA1615 *rpoB[F522] Ilv*$^+$ *rho15*	SP717 × P1 *Ilv*$^+$ *rho15* (AD1600)
SP729	SA1615 *Ilv*$^+$ *rho15 argE::*Tn*5*	SP728 × P1 *argE::*Tn*5 rpoB*$^+$
SP741	SA1615 *rpoBts8 Ilv*$^+$ *rho15 argE::*Tn*5*	SP492 × P1 *argE::*Tn*5*
SP746	SA1615 *rpoBts8 Ilv*$^+$ *rho15 argE::*Tn*5*(*F' ArgE*$^+$ *rpoB*$^+$)	SP741(*F' ArgE*$^+$ *rpoB*$^+$)
SP750	CAG3002 *argE::*Tn*5*	CAG3002 × P1 *argE::*Tn*5*
SP756	CAG3002 *rpoBts8*	SP750 × P1 *ArgE*$^+$ *rpoBts8* (SP124)
SP782	N4831 *rpoB[F522]*	SP583 × P1 *ArgE*$^+$ *rpoB[F522]* (SP706)
SP790	OR1265 *rpoB[F522]*	SP261 × P1 *ArgE*$^+$ *rpoB[F522]* (SP706)

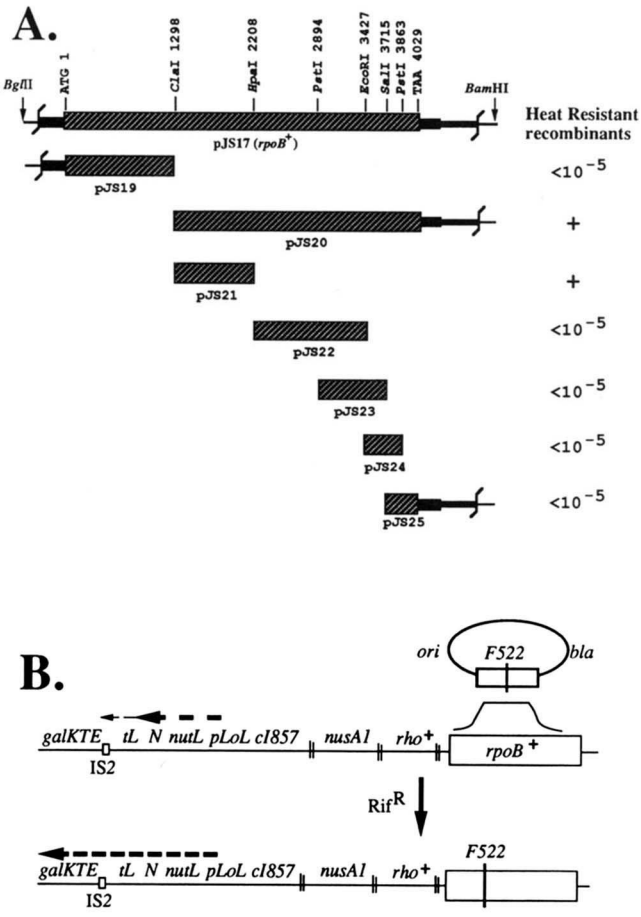

FIGURE 1.—Mapping of *ts8* by marker rescue and reconstruction of the *rpoB* (*F522*) allele by gene conversion. A, Schematic map of plasmids and their ability to rescue *ts8* are shown. The start and stop codons of *rpoB* along with relevent sites of cleavage by restriction enzymes are shown. Position 1 corresponds to 2969 of *rplrpo* DNA sequence listed in GenBank. B, Diagram shows the conversion of *rpoB*$^+$ to *rpoBF522* by homologous recombination between the resident plasmid and chromosome.

and subsequently screened for their galactose phenotype by patch tests using MacConkey galactose agar plates and their ability to plate λ*imm434r32* phage by a cross-streak test using EMBO agar plates (GOTTESMAN and YARMOLINSKY 1968).

Construction of recombinant phages and plasmids: All recombinant DNA methods followed the procedures compiled by MANIATIS, FRITSCH and SAMBROOK (1982). Plasmid pDJJ1 contains the entire *rpoB* gene and the N-terminal portion of *rpoC* constructed by cloning of the 5.7-kb *Hin*dIII-*Bgl*II fragment into the pBR322 vector. The *rpoB* gene in pDJJ1 is bracketed by *Nar*I cleavage sites, one located upstream of *rpoB* (GenBank designation) and another downstream within the vector. The 5123-bp *Nar*I fragment of pDJJ1 was cloned into pDL19 in its *Acc*I site such that *rpoB* is expressed from the *lac* promoter (Figure 1). The resultant plasmid, pJS17, complements the *ts8* mutant, allowing growth at high temperature with a 100% effeciency of plating and confers rifampicin-sensitivity to the mutant. Next, the *Bgl*II-*rpoB-Bam*HI fragment of pJS17 was inserted into the unique *Bam*HI site of the λ*D69* vector located within the *int* gene (MIZUSAWA and WARD 1982). The resultant recombinant phage contains *rpoB* in the opposite orientation with respect to the *int* gene. Upon lysogeny, it complements *ts8* and allows growth at 42°. The *ts8* (λD69*rpoB*$^+$) lysogens, selected by their immunity against infection by λ*imm*21c phage (GOTTESMAN and YARMOLINSKY 1968), are rif^R at 32° but Rif^S at 42°, indicating that these merodiploids express both mutant and wild-type *rpoB* alleles.

Several fragments of *rpoB* were subcloned from pJS17 into pDL19 (Figure 1A). pJS19 contains the *Bgl*II-*rpoB-Cla*I fragment joined to the *Bgl*II-*Acc*I vector backbone and pJS20 contains the *Cla*I-*rpoB-Bam*HI fragment joined to the *Acc*I-*Bam*HI vector backbone. Since pJS20, but not pJS19, was able to rescue the *ts8* mutation, further subcloning of the 3′ portion was performed. pJS21, pJS22, pJS23, pJS24 and pJS25 contain the *Cla*I-*Hpa*I, *Hpa*I-*Pst*I-*Eco*RI, *Pst*I-*Eco*RI-*Sal*I, *Eco*RI-*Sal*I-*Pst*I and *Sal*I-*Pst*I-*Bam*HI fragments, respectively. These *rpoB* fragments substituted the *Acc*I-*Sma*I, *Hin*cII-*Eco*RI, *Pst*I-*Sal*I, *Eco*RI-*Pst*I and *Sal*I-*Bam*HI polylinker fragments of pDL19, respectively.

Mapping the *ts8* mutation: *ts8* was localized to the *rpoB* region by cotransduction with *argE* and *thiA*. Fine structure mapping, done by marker rescue, was performed by transforming SP194 with Amp^R plasmids containing various *rpoB* segments and measuring the production of heat-resistant progenies. Plasmids pJS20 and pJS21 produced heat-resistant, rifampicin-sensitive recombinants at a frequency several orders of magnitude higher than pDL19 and plasmids containing the other segments of *rpoB* (Figure 1A).

Amplification and cloning of chromosomal DNA segments: Amplification of both the wild-type and mutant DNA segments corresponding to the *Cla*I-*Hpa*I region was performed by the DNA polymerase chain reaction method (SAIKI *et al.* 1985) utilizing a Perkin Elmer Cetus instrument. Three samples each of chromosomal DNA from strains SP1(*rpoB*$^+$) and SP209(*ts8*) were amplified using two olionucleotides as primers: oligo 1 (5′-GGGGGGATCCGACGACATCATTGATGT) is partially complementary to the noncoding strand of *rpoB* 32 bp upstream of *Cla*I at position 1298; oligo 2 (5′-GGGGAAGCTTCCGGATACATCTCGTCT) is partially complementary to the coding strand of *rpoB* 22 bp downstream of *Hpa*I at position 2208. Twenty-five cycles each were carried out under conditions of denaturation at 94°, annealing at 50° and polymerization at 64°. The resulting DNA samples were extracted with phenol-chloroform, precipitated with ethanol and aliquots were digested with *Cla*I-*Hpa*I. The cleaved fragments were analyzed by gel electrophoresis to confirm the succesful synthesis of desired DNA and then an appropriate amount was ligated to the *Acc*I-*Sma*I vector backbone of pUC19. Upon transformation, plasmid libraries were isolated and each library was subjected to DNA sequence analysis. Plasmids pJS16 (WT) and pJS26/27/28 (*ts8*) represent these libraries. The sequence of both strands of the cloned DNA was determined by the dideoxy method of SANGER using Sequenase from U.S. Biochemical. The following oligonucleotides were used as primers: universal primer: 5′-GTTTTCCCAGTCACGAC; reverse primer: 5′-AGCGGATAACAATTTCACACAGGA; oligo 3: 5′-GAGATTACGCACAAACG; oligo 4: 5′-AGGCCACTTCGTAGAAG; oligo 5: 5′-AAGCTGGATTCGCCTTT; oligo 6: 5′-ACGGGTCAGACCGCCTG. Oligonucleotides were synthesized in a Biosearch Cyclone DNA synthesizer.

Reconstruction of the *rpoBts8* (*F522*) allele by gene conversion: The *Rif*^{S} strain SP1 (Table 1) was transformed with pJS16, pJS26 and pJS27, tested for rifampicin sensitivity and then tested for the ability to produce rif^R recombinants (Figure 1B). Plasmids pJS26 and pJS27, both carrying *F522* mutant DNA, led to the production of rif^R colonies at about 10^3-fold higher frequency, as compared to plasmid pJS16 with the wild-type *rpoB* DNA segment. To isolate the

F522 recombinant, a rifampicin-sensitive colony from each of the strains SP1(pJS26) and SP1(pJS27) was streaked on an LB-Rif plate and incubated for 48 hr at 32°. Two rif^R isolates from both SP1(pJS26) and SP1(pJS27) were saved and P1 lysates were made using these rif^R strains as donors. The rif^R allele from each of the two donors was then transduced to a number of recipient strains listed in Table 1.

Determination of phage plating efficiency and yield: Measurements of phage plating efficiency were done by mixing serial dilutions of phage stocks with 0.2 ml overnight cultures, incubation of the mixture at 34° for 15 min, plating with 2.5 ml soft agar on LB plates and incubation at the appropriate temperature for 12–16 hr (SILHAVY, BERMAN and ENQUIST 1984). Phage yield was measured as follows: strains were grown overnight at 32° in L broth containing 0.2% maltose and 10 mM $MgSO_4$ (LBMM). Cultures were diluted in 25 ml LBMM and grown at 32° to $A_{600} = 0.200(1 \times 10^8$ cells/ml). Ten milliliters of cells were pelleted and resuspended in 10 mM $MgSO_4$ to a density of approximately 10^9 cells/ml. For each infection, 0.2 ml cells (2×10^8) were used. Phages were added at about 0.1 multiplicity of infection (m.o.i.) and allowed to absorb at room temperature for 20 min. Upon absorption, 120 μl of the infection mixture were added to 12 ml prewarmed LB containing 10 mM $MgSO_4$ and shaken at 42°. One-milliliter aliquots were removed at various intervals, treated with chloroform, diluted appropriately and then titered using the amber suppressor host C600. Phage dilutions used to infect were retitered to determine the number of infecting phage particles.

Quantitation of galactokinase expression: The relative rates of galactokinase synthesized were measured at various times after induction of *pL* or *pR* by a temperature shift to 42° and of *Pgal* by treatment with 5 mM D fucose at 32°, essentially as described by WARREN and DAS (1984), using toluene lysates of cultures grown in M9 salts medium containing 2% caseamino acids, 0.4% fructose and 0.00003% biotin. Aliquots of cultures were pelleted, resuspended in 40 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol (DTT) to A_{600} = 1.0, treated with 50 μl toluene/ml in cold and incubated at 37° for 10 min. The amount of extracts added in galactokinase reactions varied to obtain a linear response to extract concentration, allowing the determination of the rate of *galK* expression. Values presented in Results correspond to periods of linear increase in the rate of synthesis.

Pulse-labeling of proteins: Cultures were grown at 32° to 0.2 A_{600} in M9 medium containing 0.2% glucose, 0.4% histidine and 0.00003% biotin. Cultures were then divided and grown at 32° and 42°. At time 0, 30, 60 and 90 min of incubation, 100 μCi [^{35}S]Met (Amersham-10 μCi/μl) were added to 1-ml aliquots of cells grown at 42° and were incubated for a 10-min period at the same temperature. In addition, a labeling at 32° was performed with the culture grown at 32° for 90 min. Cells were pelleted, washed in saline and finally resuspended in 60 μl sample buffer (0.0625 M Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol, 5% β-mercaptoethanol and 0.001% bromophenol blue). Ten-microliter samples, boiled at 100° for 10 min, were electrophoresed on a 10% polyacrylamide gel containing 0.1% SDS. The gel was fixed in 30% methanol, 10% acetic acid for 15 min and 1 M Na-salicylate for 30 min followed by drying and autoradiography using X-ray films (Fuji).

RESULTS

Rationale: The *N* gene product of λ, an essential early gene activator, acts by suppressing premature termination of transcription within the *pL* and *pR* operons of the phage (FRIEDMAN 1988). N causes antitermination in these operons by modifying the host RNA polymerase at a specific promoter-proximal locus, *nut*, recognized by N (FRIEDMAN, WILGUS and MURAL 1973; LAZINSKI, GRZADZIELSKA and DAS 1989; SALSTROM and SZYBALSKI 1978). To modify polymerase, N engages the transcription complex at *nut*, thereafter remaining as an operon-specific subunit of the elongating transcription apparatus (BARIK *et al.* 1987; HORWITZ, LI and GREENBLATT 1987). Among the host factors (NusA, NusB, S10 and Rho) whose alteration by missense mutations affects antitermination (FRIEDMAN 1988), NusA is sufficient for N to modify RNA polymerase at the *nut* site *in vitro* (WHALEN, GHOSH and DAS 1988). Due to a specific defect in N-mediated antitermination, a *nusA1* mutant host fails to support λ development.

The *in vitro* studies of GREENBLATT and LI (1981b) and HORWITZ, LI and GREENBLATT (1987) suggest that the N-NusA interaction is affected by the *nusA1* mutation. However, it remains to be determined whether this is the sole basis of the antitermination defect. Several suppressor mutations have been isolated which can compensate for *nusA1* and permit λ growth in the *nusA1* mutant host. These include mutations in the *N* gene called *punA* (FRIEDMAN *et al.* 1981), in host genes, *nusB* and *nusG* (WARD and GOTTESMAN 1982; WARD, DELONG and GOTTESMAN 1983; DOWNING *et al.* 1990) and in *rplP* encoding the L16 ribosomal protein (SCHAUER and FRIEDMAN 1985). In addition, the *nusA1* mutation can be compensated for by an optimal *boxA* sequence (SCHAUER *et al.* 1987; D. FRIEDMAN, personal communication), an element of the *nut* site conserved among the various lambdoid phages (FRANKLIN 1985; TANAKA and MATSUSHIRO 1985). Initially, these seemingly indirect suppression mechanisms have prompted us to test whether the defect of *nusA1* can be compensated for by an appropriate alteration of *rpoB*, the presumed primary actor upon which both N and NusA are thought to act in affecting transcript elongation.

Isolation of *ts8*: RNA polymerase suppressor mutations were sought specifically by isolating spontaneous rifampicin-resistant mutations. Wild-type *E. coli* is sensitive to rifampicin which blocks transcription initiation by inhibiting RNA polymerase. Mutations causing resistance to rifampicin map in the *rpoB* gene and, as mentioned above, rif^R alleles cause an alteration in the termination phenotype. To isolate and identify *nusA1* suppressor alleles, we employed the strain SP1, which contains a λ*cI857* prophage with extensive deletions (Figure 2). In this strain and its $nusA^+$ parent, SA1934, the *pL* operon of the prophage with an intact *N* gene is fused to the neighboring bacterial *gal* operon carrying an IS2 insertion element

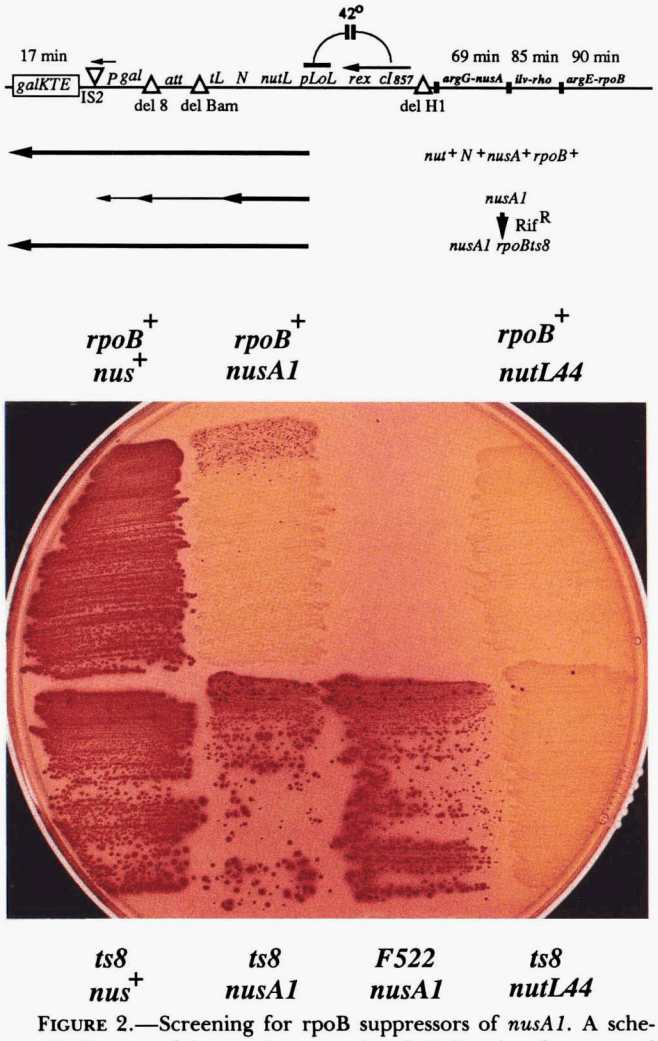

FIGURE 2.—Screening for rpoB suppressors of *nusA1*. A schematic diagram of the prophage, *nusA* and *rpoB* regions is presented at top. Galactose phenotype of the following strains are shown at the bottom: SA1934 (*rpoB*$^+$ *nusA*$^+$), SP1 (*rpoB*$^+$ *nusA1*), N6441 (*rpoB*$^+$ *nutL44*), SP190 (*ts8 nusA*$^+$), SP180 (*ts8 nusA1*), SP716 (*F522 nusA1*) and SP250 (*ts8 nutL44*). Strains were streaked on MacConkey galactose agar plates, incubated at 32° to allow for growth of colonies, shifted to 42° and incubated 3–5 hr.

between the promoter and the first structural gene, *galE* (GOTTESMAN, ADHYA and DAS 1980). Although transcription of *gal* from its own promoter is virtually abolished by efficient Rho-dependent termination within the DNA insertion element IS*2* (DE-CROMBRUGGHE *et al.* 1973; DAS, COURT and ADHYA 1976), the *gal* operon could be expressed by transcription initiating at the prophage *pL* promoter provided that N is functional to cause antitermination (ADHYA, GOTTESMAN and CROMBRUGGHE 1974).

Transcription from λ*pL*, repressed at 32°, is induced by inactivation of the *cI857* mutant repressor upon shifting the growth temperature to 42°. Thus, the wild-type *nusA*$^+$ parent, which is gal$^-$ at 32°, displays a Gal$^+$ phenotype (*i.e.*, red on MacConkey-galactose indicator plates) upon 3–5-hr incubation at 42° (Figure 2). As demonstrated by WARD, DELONG and GOTTESMAN (1983), the *nusA1* mutant strain SP1 is defective in allowing N-dependent antitermination and, hence, displays a gal$^-$ phenotype at 42° (Figure 2). Another striking phenotype of strain SP1 is its inability to plate, at 32°, a variant of λ, λ*imm434r32*. This mutant phage, due to the presence of an IS*2* insertion sequence between the ribosome binding site and the initiation codon of the *cII* gene in the *pR* operon (ROSENBERG *et al.* 1978), shows a severe dependence on N function for the expression of essential downstream genes (FRIEDMAN 1988). Unlike wild-type λ, the mutant λ*imm434r32* phage fails to form visible plaques in any one of the several *nus* mutants isolated to date ($<10^{-6}$ efficiency of plating compared to the wild-type host at 32°).

We have tested the *rif*^{R} derivatives of SP1, isolated at 32°, for their ability to plate λ*imm434r32* at 32° and their Gal$^+$ phenotype upon incubation at 42°. Only a few (12/236) of the *rif*^{R} mutants supported λ*imm434r32* growth. These were all Gal$^+$ at 42° but not at 32°. The gal$^-$ phenotype at low temperature indicated that (a) the mutants contained the *gal::*IS*2* allele and (b) the mutants are not defective in termination within IS*2* at low temperature. Interestingly, two of the mutants failed to form colonies at temperatures above 40°, in the presence or in the absence of rifampicin. We have characterized one of these two heat-sensitive mutant isolates, SP124, hereafter called *ts8*.

Properties of heat-resistant revertants: The relationship among the three phenotypes, rifampicin-resistance, suppression of *nusA1* and temperature sensitivity was examined by subjecting the *ts8* mutant strain to further genetic analysis. Revertants of SP124 were isolated and analyzed to test whether all three phenotypes of *ts8* were due to the same mutation. Heat-resistant revertants arose at a frequency of 2×10^{-8} at 42°. Of 437 heat-resistant revertants tested, 35 appeared to be true revertants in that they were sensitive to rifampicin, gal$^-$ at 42°, and unable to support the growth of λ*imm434r32*. The other revertants (72% rif^R gal$^-$ and 20% rif^R Gal$^+$) arose due to second site mutations. While many of these second site mutations are intragenic, a fraction of the rif^R Gal$^+$ revertants contain extragenic suppressor mutations mapping at 90.5 min of the *E. coli* chromosome (SPARKOWSKI 1990).

Linkage to *argE-rpoB* region: Transduction analyses showed that the responsible mutation in SP124 is linked to the *rpoB* region of the *E. coli* chromosome. An *argE::*Tn*5* derivative of SP1 was transduced to *Arg*$^+$ using phage P1*vir*, propagated in the SP124 strain, as the donor. As expected from the linkage between the *argE* and *rpoB* loci (BACHMAN 1990), roughly 50% of the *Arg*$^+$ transductants were resistant to rifampicin. All of the Rif^S transductants tested were able to grow at 42° and displayed a gal$^-$ phenotype.

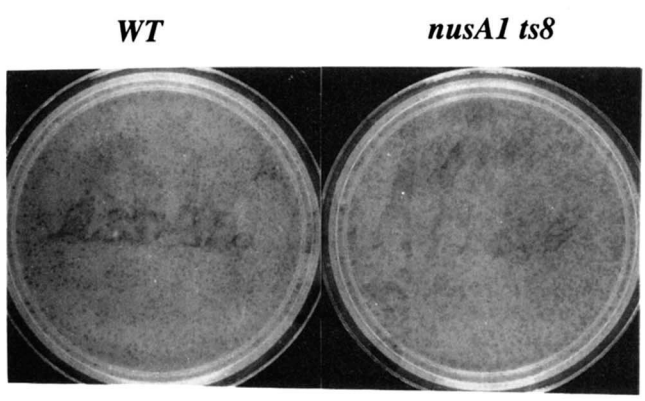

FIGURE 3.—Efficient growth of λ*imm*434*r32* phage in a *ts8 nusA1* double mutant. Phage plaques formed at 32° on the lawns of SA1934 (WT) and SP180 (*ts8 nusA1*) are shown.

In contrast, all of the rif^R transductants (several hundred tested) were temperature sensitive for growth. These transductants displayed a Gal$^+$ phenotype upon shifting growth temperature from 32° to 42°. Moreover, these transductants, like the wild-type *nusA*$^+$ strain, supported the growth of λ*imm*434*r32* phage at an efficiency of plating (EOP) = 1.0 (Figure 3). Similar transduction was performed with the *nusA*$^+$ parental strain SA1615 and a second *nusA1* strain, AD7071, both devoid of the λ prophage (Table 1). The rif^R transductants of these strains (SP209 and SP194, respectively) were unable to grow at high temperature indicating that the temperature sensitivity of *ts8* is independent of not only the *nusA1* allele but also the λ prophage. Finally, SP194 also supported the growth of λ*imm*434*r32* phage at EOP = 1 demonstrating that the suppression of *nusA1* was independent of the λ prophage in SP124.

Recessive-lethal, dominant suppressor phenotype: The relationship of the suppressor mutation with *rpoB* was investigated further by complementation studies performed with a recombinant plasmid and phage each encoding the wild-type rifampicin-sensitive RNA polymerase β subunit. First, the complementation ability of multiple copies of *rpoB*$^+$ was tested by transformation of the *ts8* strain SP209 with the plasmid pJS17. All Amp^R transformants isolated at 32° also grew at 42°. The same was also observed with a *recA* mutant derivative of SP124. Next, the complementation ability of a single copy of *rpoB*$^+$ was tested by lysogenization of the *nusA1 ts8* strain (SP194) with a λ*imm*21*rpoB*$^+$ recombinant phage. The lysogens were selected at 32° on the basis of immunity against further infection by λ*imm*21c phage. The resultant merodiploid strains were resistant to rifampicin at 32° and were able to grow at 42° in the absence of rifampicin but not in its presence (4/4 tested). These results demonstrate the recessive lethal nature of the *ts8* mutation.

On the other hand, the merodiploid strains supported the growth of λ*imm*434*r32* mutant phage at

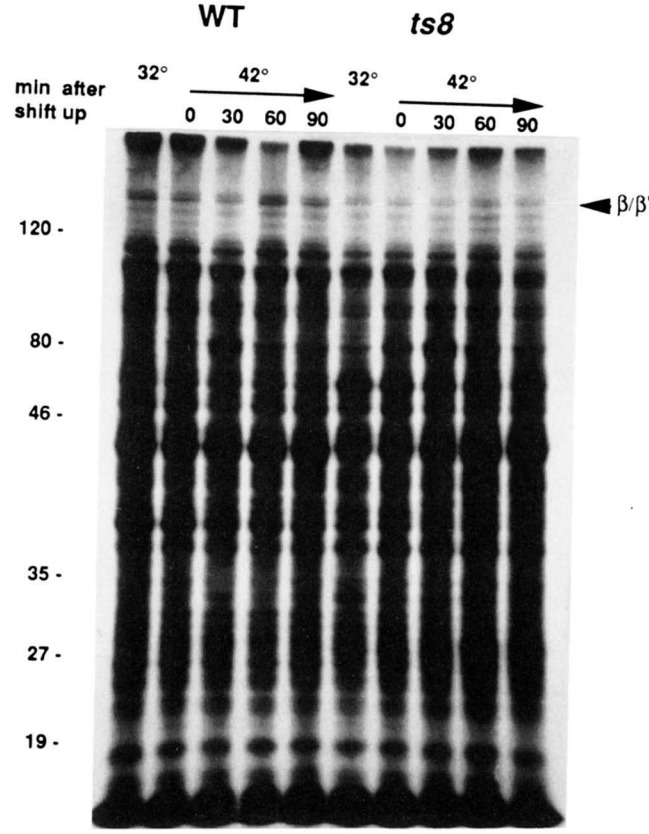

FIGURE 4.—*De novo* synthesis of proteins in the *ts8* mutant is relatively unaffected at high temperature. SA1615 (WT) and SP209 (*ts8*) were labeled with [^{35}S]methionine and labeled proteins were analyzed on polyacrylamide gels as detailed in the MATERIALS AND METHODS. Numbers (in kD) on the left indicate the positions of standard protein markers.

32° at the same efficiency as SP124. This demonstrates that the suppressor phenotype of *ts8* is dominant. It should be noted, however, that the *ts8* allele is unable to suppress *nusA1* in the presence of the multicopy plasmid pJS17 and also displays a Rif^S phenotype indicating that the dominance is dependent on the relative expression of the two alleles of *rpoB*.

Transcription proficiency of the mutant polymerase: The *ts8* mutant cells can grow for 1.5 generations after shift to 42°; however, they fail to form colonies at temperatures above 40°. To examine the basis of the growth arrest, we labeled mutant cells with [^{35}S]methionine with a 10-min pulse at various times of incubation at 42°. Gel electrophoretic analysis of *de novo* synthesized proteins did not reveal any gross defect in overall protein synthesis at high temperature relative to the wild-type strain (Figure 4). Since *de novo* protein synthesis in *E. coli* is largely dependent on *de novo* synthesis of mRNA, there is apparently no gross change in the general transcription activity of the mutant polymerase. This notion is confirmed by quantifying the relative rates of expression of galactokinase at various times upon induction of the prophage *pL* promoter at 42°. In the *nusA*$^+$

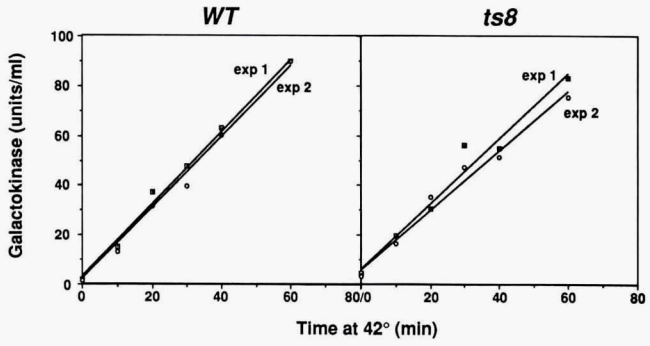

FIGURE 5.—Galactokinase synthesis upon heat induction of the prophage *pL* promoter is not grossly affected by *ts8*. Rate of galactokinase synthesis in SA1934 (WT) and SP190 (*ts8 nusA*$^+$)was measured at indicated times after prophage induction at 42° as detailed in the MATERIALS AND METHODS.

background, the *ts8* mutant polymerase is virtually as efficient as the wild-type polymerase in transcribing *galK* from the *pL* promoter (Figure 5). Thus, the initiation and elongation rates of the mutant polymerase is relatively unaffected by the *ts8* mutation. In experiments described below we have not detected any termination defect associated with *ts8*. Taken together, these results suggest that the growth defect of *ts8* is not likely due to temperature sensitivity of the mutant polymerase. Rather, the growth arrest at high temperature might be due to the lack of expression of one or more essential gene(s).

The basis of suppression of *nusA1*: We have tested the termination property of the *ts8* mutant by two general criteria: by measuring N independence for λ growth and by measuring readthrough in the absence of *N*.

First, phages with one of three different *Nam* alleles were unable to grow in the *ts8* mutant (Table 2, compare SP209 with SA1615) as determined by the poor efficiency of plating ($<10^{-6}$ compared to that in the amber suppressor host) and also the low yield of phage after 90 min of infection (consistently lower compared to the nonsuppressor host). By comparison, two of these amber alleles of N are partially suppressed by the *rho15* mutation (Table 2, compare SP490 with

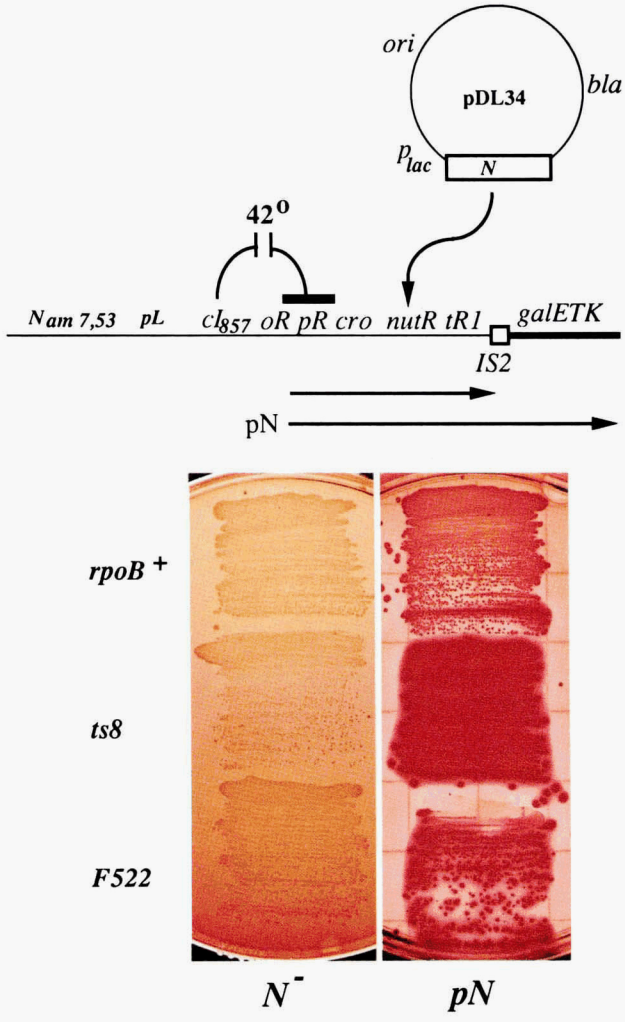

FIGURE 6.—Expression of galactokinase from λ *pR-cro-nutR-tR1-IS2-gal* fusion in *ts8/F522* mutant strains requires N-mediated antitermination. Galactose phenotypes of strains with or without the N-containing plasmid pDL34 were tested as described in Figure 3. OR1265 (*rpoB*$^+$), SP272 (*ts8*) and SP790 (*F522*) and their respective Amp^R derivatives of each containing pDL34 plasmid were used.

SA1615) as has been reported previously (DAS, COURT and Adhya 1976).

Second, transcription readthrough beyond Rho-dependent terminators in the *pR* operon was assessed by

TABLE 2

λ growth in the *ts8/F522* mutant host requires the *N* gene product

Strain	*sup*	*rpoB*	*rho*	Plating efficiency			Phage yield	
				Nam53	*Nam213*	*Nam500*	*Nam213*	*Nam500*
C600	+	+	+	1.0	1.0	1.0	500	6750[a]
SA1615	−	+	+	$<10^{-7}$	1.5×10^{-5}	1.4×10^{-5}	0.345	0.285
SP490	−	+	*15*	1.4×10^{-3}	1.0	1.0	2.25	5.50
SP209	−	*ts8*	+	$<10^{-7}$	2.8×10^{-5}	3.3×10^{-5}	0.162	0.155
SP717	−	*F522*	+	4.3×10^{-5}	2×10^{-5}	2.5×10^{-5}	0.250	0.225

Phages used were λ*cI857Nam53*, λ*imm434 Nam213*, and λ*vir Nam500*. Efficiency of plating is expressed relative to the suppressor strain C600. Phage yields (multiples of 10^8/ml culture) at 90 min after infection of 2×10^8 cells (moi = 0.1) are presented.

[a] At 90 min after infection of C600, λ*virNam500* appeared to be undergoing a second round of infective cycle causing the high phage yield.

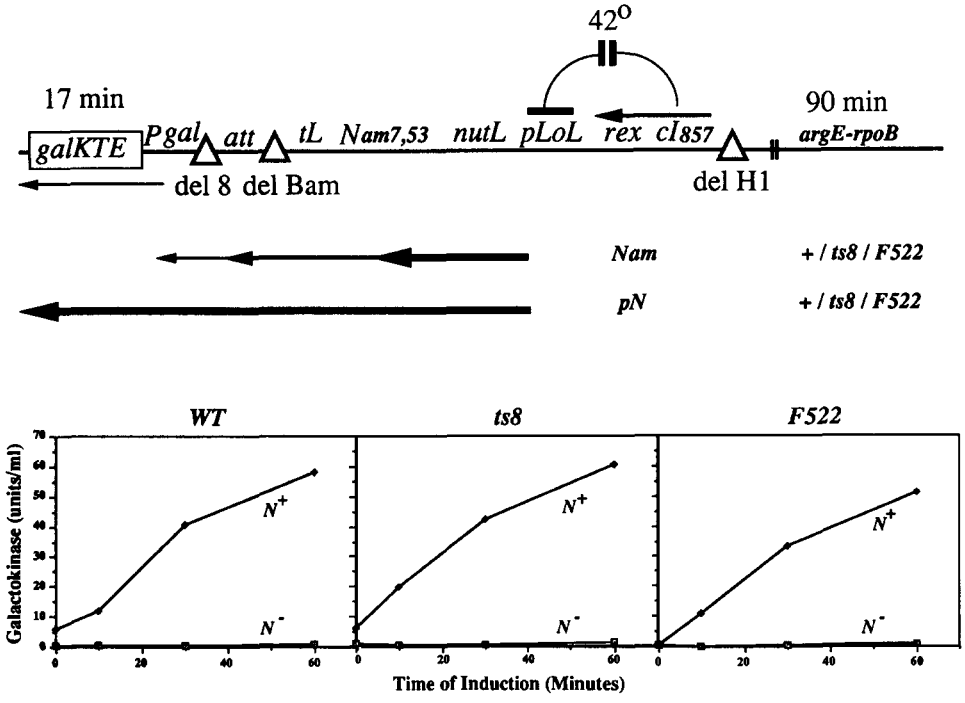

FIGURE 7.—Rho-dependent termination in the λ*pL* operon is not affected by the *ts8* and *F522* alleles. Rates of galactokinase synthesis upon prophage induction at 42° were measured as detailed in the MATERIALS AND METHODS. Strains SP583 (WT), SP587 (*ts8*), SP782 (*F522*) and their respective Amp^R derivatives of each containing plasmid pDL34 were used.

employing a strain constructed by REYES, GOTTESMAN and ADHYA (1979) that contains a *cI857 Nam* prophage with a *pR-gal::*IS2 fusion. Both OR1265 (*rpoB*$^+$) and its *ts8* derivative, SP272, displayed the gal$^-$ phenotype upon incubation at 42°; compared to the *rpoB*$^+$ parent, there was no indication of even a small increase in readthrough caused by *ts8* (Figure 6). The expression of the *gal* operon was restored by transformation of the strain with plasmid pDL34 that supplies functional N protein in *trans* (LAZINSKI, GRZADZIELSKA and DAS 1989). Note that the same phenotypes were also observed when respective *nusA1* derivatives were tested (data not shown).

Finally, we employed a second *Nam* prophage strain (N4831), devoid of the IS2 insertion sequence within *gal*, to examine readthrough at other Rho-dependent terminators (Figure 7). Due to efficient termination, transcription from *pL* fails to read through the *gal* operon in this strain in the absence of N, producing very little galactokinase. Most, if not all, of the terminators present between *pL* and the *gal* operon in this strain are Rho-dependent since a variety of *rho* mutations, including *rho15* and *rho112*, overcome the barrier to read through (GOTTESMAN, ADHYA and DAS 1980). When N protein was supplied in trans by transforming the strain with pDL34, significant synthesis of galactokinase was achieved; there was >50-fold stimulation of galactokinase synthesis (Figure 7). The *ts8* derivative of the strain, SP587, behaved similarly. It produced a significant amount of galactokinase only in the presence of N and there was no detectable difference in the amount of N-independent synthesis of galactokinase, as compared to the *rpoB*$^+$ parent (Figure 7).

TABLE 3

Effects of *rpoBts8* mutation on cell growth, phage development and antitermination in *nusA*, *nusB* and *nusE* mutant strains

	Genotype		Transcription readthrough			
Strain	*nus*	*rpoB*	A. Colony formation at 42°C	B. Growth of λ*r32* mutant at 32°C	C. Galactose phenotype	D. Galactokinase [% wild type]
1. SA1934	+	+	+	1.0	+	100
2. SP190	+	*ts8*	−	1.0	+	81
3. SP1	*A1*	+	+	3.0×10^{-8}	−	1.3
4. SP180	*A1*	*ts8*	−	1.0	+	12
5. SP2	*B5*	+	+	2.2×10^{-8}	−	<1
6. SP364	*B5*	*ts8*	−	2.2×10^{-8}	−	<1
7. SP3	*E71*	+	+	2.0×10^{-8}	−	<1
8. SP336	*E71*	*ts8*	−	2.2×10^{-8}	−	<1

Column A: (+) denotes colony formation at 100% efficiency, and (−) represents that at $<10^{-7}$; note that the *ts8* strains form small colonies at 32°. Column C: (+) means bright red color of the colonies on MacConkey-galactose plates at 3–5 hr upon shift up to 42°. Column D: Rates of galactokinase synthesis upon prophage induction at 42° is presented; 100% corresponds to 1.87 ± 0.28 units of galactokinase/minute.

TABLE 4

Compensation for the *nusAsal* allele by a combination of two suppressor mutations, *NpunA1* and *rpoBts8*

Strain	Genotype		Phage growth	
	nusA	*rpoB*	λ*cN*$^+$	λ*cNpunA1*
1. N99	+	+	1.0	1.0
2. K1102	*sal*	+	3.9×10^{-8}	3.5×10^{-6}
3. SP385	*sal*	*ts8*	4.9×10^{-7}	1.2

NpunA1 is a suppressor of *nusA1* and *nusE71* mutations (FRIEDMAN *et al.* 1981). Efficiency of plating was measured at 37°.

Taken together, these results provide three important pieces of information. (i) Since both λ development and the suppression of the IS2 terminator in the λ *pR* operon is N-dependent, the *ts8* mutation allows active antitermination by N in the *nusA1* host; (ii) since no change in readthrough is observed beyond several Rho-dependent terminators in the absence of N, the *ts8* mutation does not cause a defect in Rho-dependent termination; (iii) since *ts8* does not confer N-independence for phage growth, it most likely does not cause a defect in termination within the NusA-dependent terminator *tR2* of λ.

We then tested whether antitermination in the *ts8* mutant is dependent on a functional *nut* site by employing a *pL-nutL44-N-gal*::IS2 fusion strain, N6441. As shown by ADHYA and GOTTESMAN (1982), this strain is unable to express *gal* at 42° because the *nutL44* mutation abolishes the suppression of IS2 by N. The *ts8* derivative of this strain, SP250, also remained gal$^-$ at 42° (see Figure 2). The *nutL* mutant prophage expressed the same low level of galactokinase (less than 1% compared to the *nutL*$^+$ parent) irrespective of whether the strain was *rpoB*$^+$ or *ts8*. The requirement for a functional *nut* site in antitermination indicates that the mutant polymerase does not interact with N nonspecifically.

Efficiency of *nusA1* suppression: Since the *nusA1 ts8* double mutant supports development of the *r32* phage at the same efficiency as the *nusA*$^+$ parent, the level of suppression at 32° is efficient and significant biologically. This notion was confirmed by quantitative measurements of the steady state level of galactokinase expressed from a *cI*$^-$ prophage constructed by WARD and GOTTESMAN (1982) that constitutively transcribes the λ*pL* and *pR* operons at all temperatures. At 32°, the level of galK expression from the *cI*$^-$ prophage is reduced by *nusA1* to about 50% compared to *nusA*$^+$. The *ts8 nusA1* strain produce approximately the same amount of galactokinase as in the *nusA*$^+$ parent. Thus, at low temperature, the IS2 element is unable to pose a significant barrier to *pL* transcription in the *nusA1 ts8* double mutant. This, however, is not the case at 42°. Though *ts8* increases transcription readthrough in the *nusA1* mutant about

TABLE 5

The effects of *ts8* mutation on the suppression of polarity in *rho* mutant strains

Strain	Genotype			Galactose phenotype	Galactokinase [% wild type]
	rpoB	*rho*	*gal*		
1. SA500	+	+	+	+	100
2. SA1615	+	+	IS2	−	<1
3. SP209	*ts8*	+	IS2	−	<1
4. SP448	+	*112*	IS2	+	32
5. SP215	*ts8*	*112*	IS2	+	44
6. SP490	+	*15*	IS2	+	34
7. SP492	*ts8*	*15*	IS2	−	<1

Rate of galactokinase synthesis was measured in cultures grown at 32° upon induction of the *gal* promoter with 5 mM D-fucose; 100% corresponds to 0.475 ± 0.035 galactokinase units/minute.

10-fold (Table 3, compare lines 3 and 4), the rate of galactokinase expression is at most 15% compared to the respective *nusA*$^+$ strain (compare lines 2 and 4). The lower level of suppressor activity might reflect the severe defect of *nusA1* in antitermination at 42° compared to 32° (FRIEDMAN 1988; this study). Alternatively, or in addition, the suppressor activity of the mutant polymerase might be compromised at high temperature.

Allele specificity: Specificity of suppression by *ts8* was examined by testing whether the suppressor can compensate for a *nusB* and a *nusE* mutation. Like the *nusA1* mutant, both *nusB5* and *nusE71* mutants do not support antitermination by *N in vivo* and *in vitro* (DAS and WOLSKA 1984; DAS *et al.* 1985; GHOSH and DAS 1984; FRIEDMAN 1988). As shown in Table 3 (lines 5 and 7), either of these mutations severely reduced the expression of *gal* from the *pL-nutL*$^+$*-N*$^+$*-gal*::IS2 fusion at 42°. The introduction of *ts8* in these strains did not produce a Gal$^+$ phenotype and, also, did not elevate the expression of galactokinase to any significant level (Table 3, lines 6 and 8). Consistent with this, *ts8* did not allow the growth of λ*r32* phage in the *nusB* and *nusE* mutant strains. Thus, by both quantitative and biological criteria, the *ts8* suppressor compensates for *nusA1* specifically. This is in apparent contrast to the suppressor mutations in *N*, *nusB*, *nusG* and *rplP* which can suppress the *nusE71* mutation as well as *nusA1* (FRIEDMAN 1988).

To examine whether *ts8* can compensate for another allele of *nusA* or not, we have compared the antitermination proficiency of both wild-type and *ts8* polymerase in a strain of *E. coli* whose *nusA* gene is substituted by a hybrid *E. coli-Salmonella typhimurium nusA* gene (FRIEDMAN and OLSON 1983). As shown previously, the *nusAsal* allele is defective in supporting λ growth at any temperature (Table 4, line 2). The *ts8* suppressor could not overcome the defect of *nusAsal* (line 3) suggesting that the *ts8* mutation does

not cause NusA-independent antitermination by N. Curiously, phage λ carrying a mutation in the *N* gene that can compensate for *nusA1*, but not *nusAsal* (FRIEDMAN and OLSON 1983), grew normally in the *ts8 nusAsal* double mutant (line 3). Thus, the defect of the *nusAsal* allele in antitermination by N can be compensated by combining two independent suppressors of *nusA1*. Similar synergistic effects have been noted by Friedman and coworkers when an optimal *boxA* is combined with the *punA* alleles (FRIEDMAN and OLSON 1983).

Suppression of *rho15*: Previously, a number of rif^R alleles have been found to restore transcription termination in certain *rho* mutant strains (DAS, MERRIL and ADHYA 1978; GUARENTE and BECKWITH 1978). To assess whether the *ts8* allele might have a similar capacity, we examined the suppression of two *rho* mutations, *rho15* and *rho112*, which were previously shown to respond differently to a suppressor RNA polymerase mutant (*rpoB101*) both *in vivo* and *in vitro* (DAS, MERRIL and ADHYA 1978). These *rho* mutants were selected as conditional lethal suppressors of transcriptional polarity in the *gal* operon caused by the insertion sequence IS2; *rho15* was selected using the $rpoB^+$ strain, and *rho112* using the *rpoB101* strain (DAS, COURT and ADHYA 1976; DAS, MERRIL and ADHYA 1978). We have constructed a *ts8* derivative of the rho^+ strain SA1615 which carries the *gal::*IS2 mutation and an *ilv* marker that is about 70% cotransducible with *rho*. Like the $rpoB^+$ parent, the *ts8* mutant strain SP209 displayed a gal^- phenotype and produced about 1% galactokinase as compared to the Gal^+ parent (Table 5, lines 1–3) confirming that *ts8* does not affect the susceptibility of RNA polymerase to wild-type Rho. We then introduced the *rho15* and *rho112* alleles in SP209 by P1 transduction.

About 75% of the transductants obtained from the *rho112* cross (29 of 39) were Gal^+. One of the *rho112* transductants examined further, SP215, produced roughly 40–50% galactokinase compared to the wild-type Gal^+ strain (Table 5, compare lines 1 and 5). The level of readthrough in the *ts8 rho112* strain was consistently slightly higher than that in the $rpoB^+rho112$ strain, SP448 (Table 5, compare lines 4 and 5). Since the *ts8* mutation did not suppress the defect of *Rho112* mutant protein, the mutant RNA polymerase is dependent on Rho to terminate transcription within IS2.

In contrast, none of the Ilv^+ transductants of *ts8* isolated from the *rho15* cross (several hundred tested) were Gal^+. Two reasons could account for this result. The *ts8 rho15* double mutant might not be viable. Alternatively, *ts8* might obscure the phenotype of *rho15*, *i.e.*, suppression of IS2 polarity. To test whether a fraction of these transductants carry the *rho15* mutation, we have transduced four of these to *argE::*Tn*5* $rpoB^+$ (Figure 8, section I). In each case, the Rif^S transductants were obtained at the expected 50% frequency. However, two of the parental strains (SP492 and SP493) gave rise to Rif^S transductants that retained the ts phenotype and displayed the Gal^+ phenotype expected of a $rpoB^+rho15$ strain [Figure 8, section II (E and I)]. The presence of *rho15* in SP492 was also evident from the fact that the Gal^+ phenotype was restored by the introduction of an $F'rpoB^+$ episome [Figure 8, section II (K)]. The notion was further confirmed by back-crosses.

In the $rpoB^+$ background, *rho15* shows a significant defect in termination within IS2, displaying the Gal^+ phenotype [Figure 8, section II (E)] and allowing galactokinase synthesis as much as 30–40% compared to the wild-type Gal^+ parent (Table 5, compare lines 1 and 6). By comparison, in the *ts8* background, termination within IS2 was virtually unaffected by *rho15*. The double mutant displayed a gal^- phenotype [Figure 8, section II (J)] and produced markedly low amounts of galactokinase *i.e.*, less than 1% compared to the wild-type Gal^+ parent (Table 5, compare lines 1 and 7). Thus, the *ts8* mutation has caused the gain of a second function: susceptibility of RNA polymerase to the action of one Rho mutant protein but not another.

Molecular nature of the *ts8* mutation: As mentioned earlier, plasmid pJS17, which encodes the wild-type β subunit, complements the *ts8* mutant enabling growth at high temperature. To map the responsible mutation(s), we constructed a nested set of plasmids in which various segments of the *rpoB* gene was inserted (Figure 1, MATERIALS AND METHODS). Upon transformation of the *nusA1 ts8* strain SP194, we examined the ability of these plasmids to rescue *ts8* by homologous recombination and, thereby, produce heat-resistant recombinants. Only two plasmids, pJS20 and pJS21 which have a 910-bp internal segment of *rpoB* in common, passed this test. These plasmids produced about 10^5-fold more heat resistant survivors as compared to the others which do not contain the *Cla*I-*Hpa*I segment of *rpoB*. One hundred of the survivors produced by pJS21 displayed a rifampicin-sensitive phenotype, demonstrating that they were wild-type recombinants.

We then proceeded to determine the mutational change(s) in *ts8* by amplification, cloning and sequencing of the mutant DNA segment. Chromosomal DNA from both wild type and the *ts8* mutant were isolated and subjected to a DNA polymerase chain reaction with two oligonucleotide primers designed to amplify the 910-bp internal segment of *rpoB* within which *ts8* had been mapped (MATERIALS AND METHODS). Each DNA sample was amplified in triplicate and the amplified DNA segments were cloned to produce libraries containing wild-type and mutant DNA. The se-

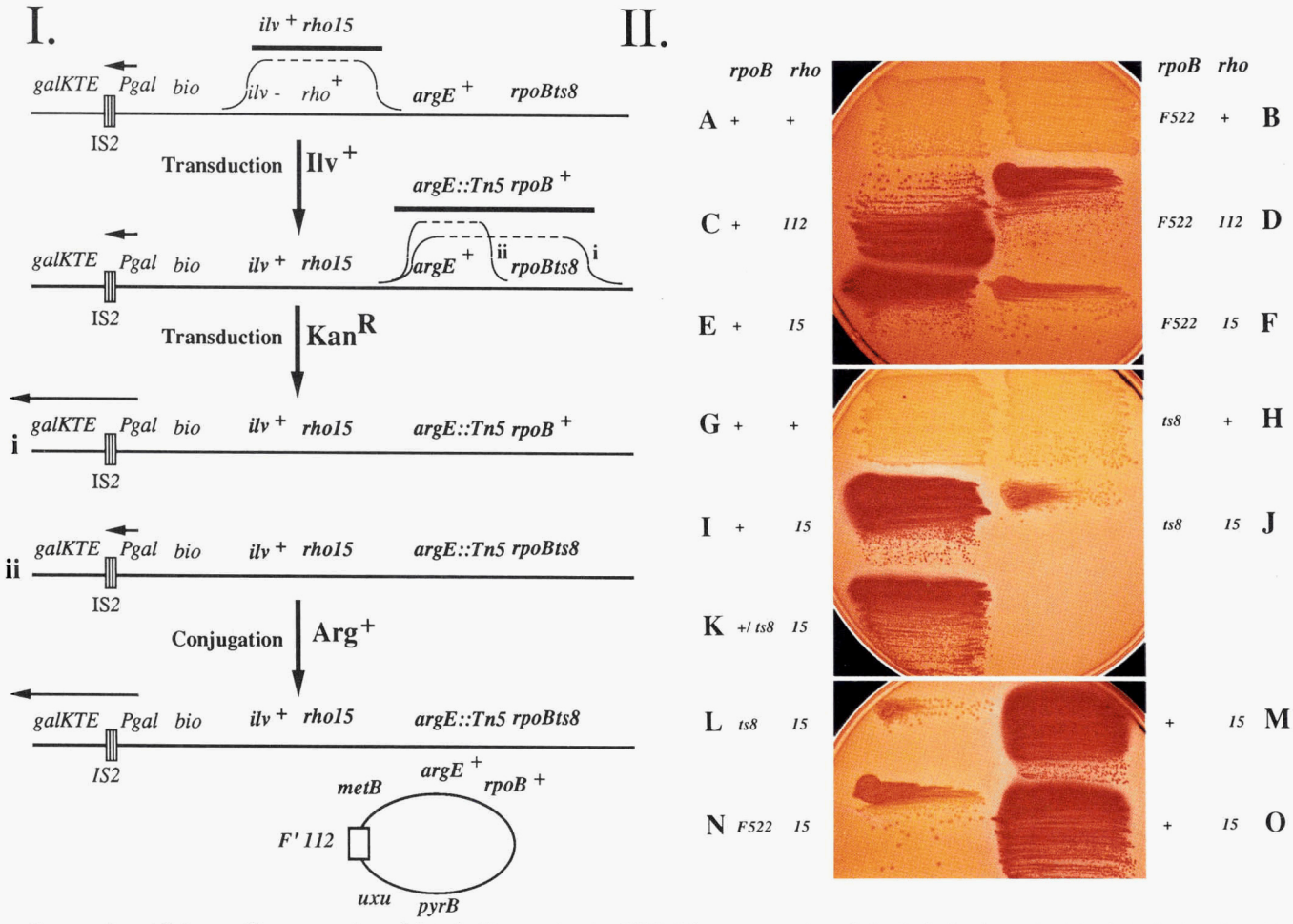

FIGURE 8.—Allele-specific suppression of the *rho15* mutation by *F522*. The construction of *rho15* derivatives of *ts8* (and similarly, of *F522*) strains and their galactose phenotypes are shown. Strains were streaked on McConkey galactose plates and incubated for 24–36 hr at 32°. Section I: *ts8* recipient (SP209), *rho15 ts8* recipient (SP492), *rho15 argE::Tn5 rpoB*$^+$ transductant (SP494), *rho15 argE::Tn5 ts8* transductant (SP741) and F'112 exconjugant (SP746). The F' donor was counterselected by streptomycin. Note that transductants of *rho15* strains are obtained at 32° at a reasonable frequency despite their reduced recombination proficiency (DAS, COURT and ADHYA 1976). Section II: A, SA1615; B, SP717; C, SP448; D, SP719; E, SP490; F, SP728; G, SA1615; H, SP209; I, SP490; J, SP492; K, SP746; L, SP492; M, SP494; N, SP728; O, SP729.

quence of one of the libraries containing the wild-type *rpoB* segment was determined and found to be in perfect agreement with that listed in GenBank. The sequences of all three libraries containing *ts8* mutant DNA revealed the presence of a single C to T transition mutation leading to the substitution of serine (TCT) by phenylalanine (TTT) at amino acid 522 of β.

Relationship of the *F522* substitution and the pleiotropic phenotype: To determine whether the various phenotypes of the *ts8* mutant described above is caused by the *F522* substitution itself, we reconstructed the *F522* allele of *rpoB* by gene conversion. The *F522* mutation was transferred from the cloned mutant DNA back to the chromosome by homologous recombination (Figure 1, MATERIALS AND METHODS). The approach relied on the assumption that *F522* was responsible for resistance to rifampicin. The *rpoB*$^+$ *nusA1* strain SP1, that contains the *pL*-IS2-*gal* fusion as the antitermination reporter, is rifampicin-sensitive and remained so upon transformation by pJS26 having the 910 bp *rpoB F522* segment. We asked if the mutant segment could recombine with homologous chromosomal DNA to produce rifampicin-resistant derivatives. Indeed, pJS26 caused the production of rif^R progenies at a significantly higher frequency, about 10^3 fold higher compared to the plasmid pJS16 which contains the wild-type *rpoB* segment. More significantly, the rif^R progenies were also heat sensitive for growth and displayed a Gal$^+$ phenotype upon incubation at 42° (14/14 tested). This suggested on the one hand our attempt to reconstruct the *F522* allele was successful and on the other hand the suppressor phenotype and lethality of *ts8* mutation was due to the *F522* substitution in β.

To investigate this relationship rigorously, we first transferred the rifampicin-resistant *F522* allele from one of the recombinants into several strains by phage

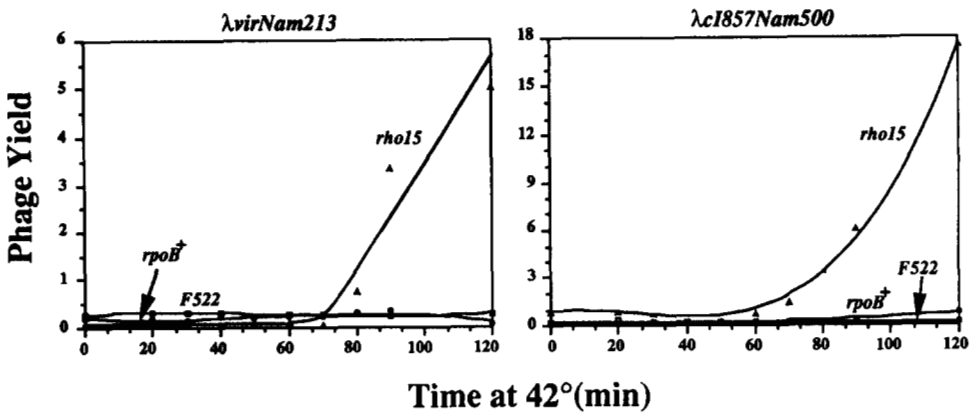

FIGURE 9.—The *ts8* and *F522* alleles do not cause N independence for λ growth. Relative yields of *Nam* mutants were measured in SA1615 (*rpoB*$^+$), SP490 (*rho15*) and SP717 (*F522*) as detailed in methods. Values are presented as multiples of 10^8 and reflect phages/ml of infected culture at indicated times.

P1 mediated cotransduction with *argE* (Table 1). Further genetic analyses and both phenotypic and quantitative tests performed with these reconstructed strains demonstrate that the *F522* allele is indistinguishable from its *ts8* counterpart:

1. *F522* causes temperature sensitivity for cell growth but it does not show an appreciable defect in transcription at 42° (see Figure 7).

2. *F522* is a suppressor of the *nusA1* allele as judged by two independent criteria. It allows *galK* expression from both the *pL-nutL-N-tL*-IS*2-gal* fusion (Figure 2, bottom picture) and is able to permit the growth of λ*r32* phage at 32° at EOP = 1.

3. *F522* does not cause termination suppression within IS*2* in *gal* [Figure 8, section II (B)] or in the *pR* and *pL* operons in the absence of N (Figures 6 and 7).

4. *F522* does not permit λ development in the absence of N, as measured by the efficiency of plating (Table 2), and phage yield upon infection in liquid culture (Figure 9).

5. *F522* is a suppressor of the *rho15* mutation but not of the *rho112* mutation, causing the restoration of transcription polarity in the *gal* operon imparted by the IS*2* insertion [Figure 8, section II (C, D, N and O)].

We conclude that the simultaneous loss and gain of three distinct functions of RNA polymerase is caused by the *F522* substitution.

DISCUSSION

The rifampicin-resistant RNA polymerase mutant *rpoBts8* reported in this study displays a combination of three interesting properties: conditional lethality, proficiency in antitermination by the λ*N* gene product with a defective *nusA* allele (*nusA1*) and proficiency in termination with a defective *rho* allele (*rho15*). We have demonstrated that all three phenotypes of *ts8* are due to the single amino acid substitution, *F522*, in the β subunit of RNA polymerase.

Prior to our study, several classes of *rpoB* mutants have been selected on the basis of their effects on factor-dependent termination and antitermination: (i) *groN* and *nusC* mutants, failing to support antitermination by N (GEORGOPOULOS 1971) (see FRIEDMAN 1988); (ii) *ron* mutants, affecting antitermination with certain alleles of *N* (called *mar*) which function normally in wild-type *E. coli* (GHYSEN and PIRONIO 1972); (iii) *Rif501* and *nitB*, causing *N*-independent expression of λ genes (LECOCQ and DAMBLY 1976; INOKO and IMAI 1976); and (v) *rpoB101* and *rpoB203*, restoring termination in various *rho* mutant strains (DAS, MERRIL and ADHYA 1978; GUARENTE and BECKWITH 1978). During the course of our study, JIN, GROSS and their colleagues have performed a DNA sequence analysis of many of these and other mutants (JIN and GROSS 1988). Through a rigorous and systematic phenotypic characterization of many of these mutants, they discovered that several *rif*^{R} alleles compensate for *nusA1* (JIN, WALTER and GROSS 1988; JIN *et al.* 1988). These include the mutation *rpoB101* having the L513 substitution, *rpoB3370* having the P563 substitution and *rpoB3595* and *Rif501*, both having the F522 substitution present in *ts8* (Table 6).

The *rpoB3370* allele isolated by JIN and GROSS shows a cold sensitive phenotype for cell growth. Whether the activity of the mutant polymerase is cold sensitive or not is not known. *RpoB3370* has other properties that are either dissimilar or similar to *ts8*. In contrast to *ts8*, it is defective in Rho-dependent termination (JIN, WALTER and GROSS 1988). However, it does not cause N independence as is observed with *ts8*. Like *ts8*, *rpoB3370* is a suppressor of *rho15*. Though the allelic specificity of this mutation toward other *rho* mutations has not been reported, it is affected in Rho-dependent termination when the cell has the wild-type Rho allele. Thus, *rpoB3370* appears to have unique allele specificity.

The *rpoB3595* allele, a spontaneous *rif*^{R} mutant selected by J. GARDNER on the basis of its termination defect at the *thr* attenuator, causes N independence for λ growth, is defective in Rho-dependent termination and is not a suppressor of *rho15*. All of these properties are different from the *ts8* allele despite the

TABLE 6

Properties of *nusA1* suppressor alleles of *rpoB*

Allele	Altered residue	Growth phenotype	Suppression of *nusA1*: λ growth at 38°	Suppression of *nusA1*: λ*r32* growth at 32°	Termination	Suppression of *rho15*	N requirement for λ growth
ts8	F522	ts	+	+	Proficient	+	+
101	L513	None	+	−	Proficient	+	+
3370	P563	cs	+	NR	Defective	+	+
3595	F522[a]	ts	+	NR	Defective	−	−
501	F522[a]	ts	+	NR	Defective	−	−

Properties of *ts8* are compared with those of other *rpoB* suppressor alleles as described by JIN, WALTER and GROSS (1988) and JIN *et al.* (1988). NR, not reported.

[a] The *rpoB3595* and *501* alleles are likely to contain additional mutation(s) as discussed in text.

presence of the *F522* substitution. The *Rif501* mutant of LECOCQ and DAMBLY, selected by its ability to allow N-independent λ gene expression, was induced by nitroso-guanidine mutagenesis. It shows close resemblence to the *rpoB3595* allele in all criteria except for the level of suppression of *nusA1*, a basis on which *Rif501* was suspected to contain multiple mutations (JIN *et al.* 1988).

Our data argue quite strongly that *F522* does not cause a defect in Rho-dependent termination in either the *pL* or the *pR* operon of λ and also does not cause N independence for λ growth. *F522* does not cause any detectable stimulation in readthrough beyond a variety of Rho-dependent terminators in either the *pL* or *pR* operon when N is absent. These same set of terminators are suppressed to significant levels in *nusA*$^+$ and *nusA1* strains when N is present. *F522* does not permit the growth of several λ*N* mutants including the *Nam53* mutant which has been reported to grow in the *rpoB3595* and *Rif501*mutants (JIN *et al.* 1988; LECOCQ and DAMBLY 1976). *In vitro*, the *Rif501* mutant RNA polymerase does not respond to NusA-mediated pausing at *tR2* and this failure to respond to NusA had been hypothesized to result in N independence (GREENBLATT, MCLIMONT and HANLY 1981). However, our results demonstrate that the *F522* allele is responsive to both *nusA*$^+$ and *nusA1* alleles; in fact it is at least fivefold more active in antitermination with *nusA*$^+$ as it is with *nusA1*. To see if some of these differences might be attributed to extraneous mutations in the different *E. coli* strains, we transferred the *ts8* allele into the wild-type strain CAG3002 used by Jin and Gross. The *ts8* derivative of CAG3002, SP756 (Table 1), is indistinguishable from SP209 in that it is both temperature sensitive for growth and unable to support the growth of λ*N* mutants. Based on all of these results, it seems likely that both *rpoB3595* and *Rif501* alleles have multiple lesions responsible for their varied behavior. Additional mutation(s) present in these alleles could cause a termination defect and N independence and also might be the basis why *rho15* suppression by *F522* is not revealed in these strains. The varied efficiency of *nusA1* suppression might result from a differential termination defect between the two alleles.

Mechanism of *nusA1* suppression: The *F522* substitution can compensate for *nusA1* by a number of different mechanisms. Since the NusA function as a termination/antitermination protein is vital to the cell and the *nusA1* mutant does not show a gross alteration in termination (WARD and GOTTESMAN 1981), the mutation has specifically compromised some aspect of N-dependent antitermination. Operationally, antitermination by N is divided into three basic steps: (1) recognition of the *nut* site and capture of polymerase (*i.e.*, formation of the N-antitermination complex); (2) elongation of the complex to the downstream terminator; and (3) suppression of the terminator and continued transcription through additional downstream terminators, *i.e.*, processive antitermination. NusA could be required for any one or more of these processes.

In vitro reconstitution of N antitermination with purified protein components by WHALEN, Ghosh and DAS (1988) suggested that NusA is sufficient for all three events to occur with the exception of processivity of antitermination (W. WHALEN and A. DAS, unpublished data). On the basis of the evidence that N binds NusA and NusA binds RNA polymerase, GREENBLATT (1981) had proposed that NusA couples N to polymerase. However, NusA is unable to couple N to polymerase without the participation of the nut site (BARIK *et al.* 1987; HORWITZ, LI and GREENBLATT 1987; WHALEN, GHOSH and DAS 1988). Recent studies favor the idea that N binds a unique sequence of *nut* RNA, known as *boxB*, and that this binding allows N to capture RNA polymerase in the presence of NusA (LAZINSKI, GRZADZIELSKA and DAS 1989; WHALEN and DAS 1990; S. CHATTOPADHYAY, J. DEVITO, J. GARCIA-MENA and A. DAS, unpublished data). Previously, NusA has been thought to bind *boxA* (FRIEDMAN and OLSON 1983), a sequence conserved

among the *nut* sites of various lambdoid phages (FRANKLIN 1985), and in the *rrn* operons of *E. coli* (BERG, Squires and SQUIRES 1989). More recent studies suggest that *boxA* might be recognized by some other component of the transcription machinery (FRIEDMAN 1988; HORWITZ, LI and GREENBLATT 1987; LAZINSKI 1989; FRIEDMAN *et al.* 1990; T. PATTERSON and D. COURT, personal communication). Since the defect of *nusA1* is compensated by altering not only N and β but also *boxA*, NusB, NusG and L16 (FRIEDMAN *et al.* 1981; WARD, DELONG and GOTTESMAN 1983; SCHAUER and FRIEDMAN 1985; SCHAUER *et al.* 1987), it seems unlikely that *nusA1* prevents N to capture polymerase. Indeed, *in vitro* immunochemical studies with the *nusA1* mutant extract show that N can capture RNA polymerase upon transcription through *nut* nearly as efficiently as with the wild-type extract (BARIK *et al.* 1987; S. BARIK and A. DAS, unpublished data). Nevertheless, the antitermination complex might be unstable and also function poorly due to the lack of proper polymerase-NusA-N contacts.

Conceivably, RNA polymerase mutants with altered pausing and elongation properties might form the complex more rapidly and efficiently and remain stable over a large distance. Alternatively, if the N-NusA interaction causes a conformational change to elicit antitermination, the altered polymerase mutant might gain this conformation with defective NusA. Since, the stability of the polymerase-NusA-N complex might be bestowed by additional interactions involving *boxA* and the other Nus factors (WHALEN, GHOSH and DAS 1988; LAZINSKI 1989), it is conceivable that some of the suppressor mutations could enhance one or more of these individual interactions to indirectly compensate for a weak interaction. It is tempting to speculate that N binds to a site ("cleft") on polymerase that is constituted by both NusA and the β subunit. The *nusA1* and *nusAsal* alleles might distort this cleft in addition to compromising individual aspects of the tripartite interaction. The synergistic effects of the *punA1* mutation in *N* and the *ts8* mutation in *rpoB* on the *nusAsal* allele might suggest that these suppressor mutations might, on the one hand, compensate for the distorted cleft and, on the other hand, increase individual binding forces.

Basis of *rho15* suppression: One can imagine that the interaction of RNA polymerase with a number of cellular factors might utilize a cleft constituted by NusA and the β subunit. For instance, Rho could bind to this site to exert its effect in termination. Rho is an RNA-binding protein and an RNA-dependent ATPase (CARMICHAEL 1975; LOWERY-GOLDHAMMER and RICHARDSON 1974) and also possesses a DNA-RNA helicase activity (BRENNEN, DROMBROSKI and PLATT 1987). Rho is thought to bind at preferred sites on nascent mRNA and extract the transcript from a paused ternary complex through its helicase activity (CHEN, GALLUPPI and Richardson 1986; BRENNEN, DOMBROSKI and PLATT 1987; YEAGER and VON HIPPEL 1987; BEAR and PEABODY 1988). Conceivably, this extraction process could be facilitated if Rho physically contacts polymerase either to disengage the polymerase from the transcription bubble or to use the polymerase as an anchor.

Since we do not know the basis of the termination defect of the mutant proteins, we can only speculate how a mutant polymerase might be able to suppress the defect of one *rho* allele but not another. First of all, *rpoB* mutations might cause unknown effects in gene expression enabling the polymerase to terminate by new mechanism(s) effective with one *rho* allele but not another. Second, a polymerase mutant elongating at a reduced rate or pausing more frequently and more extensively might overcome one *rho* allele but not another depending on the relative defect of the alleles. Recent *in vitro* studies by D. J. JIN and C. A. GROSS (personal communication) suggest that this is indeed true for certain *rpoB* mutants. Third, the mutant RNA polymerase might restore the productive interaction with one Rho mutant but not another. The studies of SCHMIDT and CHAMBERLIN (1984) demonstrate that Rho forms a complex with RNA polymerase when NusA is present. The extension of this study to some of the mutant RNA polymerases, Rho factors and NusA proteins might provide insights to whether Rho directly contacts RNA polymerase and if this interaction is important in termination.

Basis of lethality: The transcription proficiency of the *ts8* mutant polymerase for a significant period at high temperature indicates that the conditional lethality might be due to a loss of some specialized function, possibly the interaction of RNA polymerase with some vital transcription factor(s). If *ts8* (*F522*) affects a domain of RNA polymerase that interacts with a number of regulatory molecules, it may not be surprising that the mutation causes both a gain and loss of function. While the *F522* substitution optimizes the receptivity of the domain to one regulatory molecule, it might simultaneously hinder the binding of another. Consistent with this hypothesis, we have isolated and characterized a dominant, extragenic suppressor mutation, we named *greC*, that enables the *ts8* mutant to grow at high temperature. This suppressor of *ts8* that defines a new locus at approximately 90.5 min on the *E. coli* chromosome might be required for some aspect of gene expression at high temperature (SPARKOWSKI 1990). Moreover, we identified another gene, *greA* mapping at 69.2 min on the *E. coli* chromosome, whose elevated expression suppresses the growth defect of *ts8* (SPARKOWSKI and DAS 1991). The predicted sequence of the protein product of

greA suggests that it might be a transcription factor (SPARKOWSKI and DAS 1990). We do not yet know whether the *ts8* mutation affects heat shock response and how *greA* and *greC* control growth regulation at high temperature.

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