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Cooperative Effects of Bacterial Mutations Affecting λ N Gene Expression

II. Isolation and Characterization of Mutations in the *rif* Region

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We report the isolation and initial characterization of a class of mutations, Snu, that map near the *rif* locus on the *Escherichia coli* chromosome. Snu mutations inhibit the growth of phage λ, an effect primarily seen when Snu mutations are combined with another class of mutations, *nus*. *Nus* mutations have previously been shown to inhibit the expression of the N gene product of λ, and the experiments reported here suggest that Snu mutations add to this inhibitory effect.

One Snu mutation, Snu-9, was shown to cause bacterial growth to be temperature-sensitive. This suggests that, at least, some Snu mutations may be in genes coding for a function essential for bacterial growth. Since genes coding for the β and β′ subunits of RNA polymerase map in this region, we recognize that Snu mutations might alter either of these subunits of RNA polymerase. Complementation studies demonstrate that Snu$^+$ is dominant to Snu$^-$, indicating that the mutant phenotype is due to the partial loss of a function necessary for full N expression.

Although the hosts carrying Snu and *nus* mutations (called Supernus) severely restrict the growth of phage which express the N function of λ, they do not show any increased inhibitory effect on the growth of λ*imm*P22 and λ*imm*21, phages which express N functions different from that of λ. However, Supernus hosts do restrict the growth of a λ variant that can grow well in bacteria carrying either component mutation, Snu$^-$ or *nus*$^-$. The restrictive effect of the Supernus strain is far greater than would be expected if the restriction was due to an additive effect of the two component mutations. This implies that there might be an interaction between Snu and *nus* products and that the Supernus phenotype results from an interference with this interaction.

INTRODUCTION

A number of mutants of *Escherichia coli* which inhibit the expression of the N function of phage λ have been isolated. The N function regulates λ development by permitting transcription complexes to transcend specific transcription termination signals (reviewed by Echols, 1971; Herskowitz, 1973). Variants of λ isolated as "N-independent" or "partially N-independent" mutants (i.e., able to grow in the absence of N function), will grow relatively well in these mutant bacterial hosts under conditions where "N-dependent" phage will not grow. Moreover, "N-like" functions have been identified in other temperate phages, coliphages 434 and 21 as well as *Salmonella* phage P22. For a detailed discussion of N-independent phages as well as the nature of the various N products, see the accompanying paper (Friedman, Baumann, and Baron, 1976).

Analysis of the bacterial mutants which inhibit N action reveals that there are at least three loci on the *E. coli* genome which appear to be directly involved in N expression: (1) *rif*, at minute 79; (2) *nus*A, at minute 61; (3) *nus*B, at minute 11. Only the product expressed by the *rif* locus has been identified, that being the β subunit of RNA polymerase (Heil and Zillig, 1970).

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TABLE 1
BACTERIAL STRAINS

Strain	Description	Pertinent markers	Reference or source
K-37	*E. coli* K12 F⁻	*nus*⁺ Rif^S Snu⁺	Friedman (1971)
K-95	(*nus*A-1) *E. coli* K12 F⁻	*nus*A⁻ Rif^S Snu⁺	Friedman (1971)
K-206	(*nus*A-1) *E. coli* K12 F⁻	*nus*A⁻ *arg*B⁻ Rif^S Snu⁺	L. S. Baron
K-388	(Supernus-1) *E. coli* K12 F⁻	*nus*A⁻ *arg*⁺ Rif^R Snu⁻ *pur*⁺	This paper
K-389	(Supernus-9) *E. coli* K12 F⁻	*nus*A⁻ *arg*⁺ Rif^R Snu⁻ *pur*⁺	This paper
K-425	*E. coli* K12 F⁻	*nus*A⁻ Rif^S Snu⁺ *pur*D⁻	This paper
K-450	(*nus*B-5) *E. coli* K12 F⁻	*nus*B⁻ Rif^S Snu⁺	This laboratory
K-476	(Snu-1) *E. coli* K12 F⁻	*nus*⁺ Rif^R Snu⁻ (K-388 derivative)	This paper
K-477	(Snu-9) *E. coli* K12 F⁻	*nus*⁺ Rif^R Snu⁻ (K-389 derivative)	This paper
K-478	*E. coli* K12 F⁻	*nus*B⁻ Rif^R Snu⁻	This paper
K-479	*E. coli* K12 F⁻	*nus*A⁻ Rif^R Snu⁻ *pur*D⁻	This paper
K-428	*E. coli* K12 F′	*arg*G⁻ *met*B⁻ *his*⁻ *trp*⁻ *leu*⁻ *mal*A⁻ F′112	L. S. Baron
K-359	*E. coli* K12 Hfr	*arg*G⁻ *thr*⁻ *leu*⁻ *thi*⁻ Str^R Spc^R Hfr (312 type)	L. S. Baron

A number of different studies have demonstrated that mutations in the *rif* locus affect N expression. Ghysen and Pironio (1972) showed that a bacterial mutation, *ron*, which shows 95% cotransduction with Rif^R, inhibits the growth of λ variants which carry various mutations, *mar*, that map in the λ N gene. Georgopoulos (1971) has shown that a mutation located in the *rif* region was necessary for a bacterial mutant, *gro*N, to inhibit N expression. The studies of Sternberg (1976) demonstrate that a *rif*^{R} mutation which has been shown to inhibit phage T4 growth (Montgomery and Snyder, 1973) also inhibits the growth of λ.

In studies previously reported from these laboratories (Friedman and Baron, 1974; Friedman, Baumann, and Baron, 1976), we have presented evidence that two mutations (*nus*A-1 and *nus*B-5) mapping outside of the *rif* region also inhibit N expression. Although each of these two mutations map in different loci, *nus*A and *nus*B, both cause similar phenotypic changes. At low temperature, λ growth is only slightly inhibited, while at higher temperatures, λ growth is drastically inhibited. Both the *nus*A and *nus*B loci appear to code for products needed for N expression since, in each case, the *nus*⁻ allele has been shown to be recessive to the *nus*⁺ allele. Studies in other laboratories (Franklin, personal communication; Adhya, personal communication, Szybalski, personal communication) have shown that the *nus*A-1 mutation interferes with N product action.

In the accompanying paper (Friedman, Baumann, and Baron, 1976), we present evidence of a cooperative effect of the *nus*A-1 and *nus*B-5 mutations. The restrictive effect of the double mutant is greater than either of the single mutants. In this paper, we present evidence of a similar cooperative effect of mutations mapping in the *rif* region and mutations in the *nus*A and *nus*B genes.

MATERIALS AND METHODS

Bacteria and bacteriophages. See Tables 1 and 2 for relevant information.

Media. All the variants of Tryptone broth (TB) and Luria broth (LB) used in these experiments have been described previously (Friedman and Yarmolinsky, 1972). EMBO agar is the EMB agar of

TABLE 2
PHAGE STRAINS

Phage	Genotype	Source
λ*c*I	λ*c*I60	M. Yarmolinsky
λ*c*Ic17	λ*c*I90c17	M. Yarmolinsky
λ*byp*	λ*c*I857*byp*	This laboratory
λ*nin*	λ*c*I60*nin*5	This laboratory
λ*imm*P22	λ*imm*P22-7	D. Botstein
λ*imm*434	λ*imm*434*int*⁻c⁻	M. Yarmolinsky
λ*imm*434*byp*	λ*imm*434c1ts*byp*	This laboratory
λ*imm*21	21hy5	M. Yarmolinsky
λ*pun*	λN*pun* *c*I857	This laboratory
T4	T4D	G. R. Greenberg
T4*β*gt⁻	T4*β*gt⁻	L. Synder

Campbell (1957) with sugar omitted (Gottesman and Yarmolinsky, 1968). M-9 medium described by Miller (1972) was used without $CaCl_2$ and when indicated the following were added: 1.1% agar (for plates) and amino acids at a final concentration of 20 μg/ml. When Rifampin (obtained from Calbiochem) was used, it was first suspended in 100% ethanol and then added to sterilized medium to a final concentration of 50 μg/ml.

Buffers. Citrate buffer: 1.0 *M* Sodium citrate adjusted to pH 6.8. Tris-maleate buffer (TM): 0.6% tris and 0.6% maleic acid adjusted to pH 6.0.

Transduction. P1 transduction was done according to the method of Lennox (1955).

Mutagenesis. Rifampin-resistant (Rif^R) mutants were selected following mutagenesis with nitrosoguanidine (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) (Aldrich Chemical Company) according to the method of Adelberg *et al.* (1965).

RESULTS

Isolation of Supernus Mutants

The observation that a number of mutations mapping in the *rif* region of the *E. coli* chromosome affect λ N gene expression, led us to consider whether mutations of this class might influence the phenotypic expression of the N-inhibitory mutations (Nus) mapping at other loci. A number of Rif^R mutants of the *nus*A-1 strain, K-95, was isolated following nitrosoguanidine mutagenesis. Since the *nus*A-1 mutant permits λ growth at 32° (a permissive temperature), we screened for an increased inhibitory effect by determining if the Rif^R *nus*A-1 double mutants excluded λ growth at the low temperature. Between 10 to 25% (depending on the experiment) of Rif^R mutants derived from the *nus*A-1 strain do not permit λ growth at 32°. In order to test if this added effect might be due to an increase in inhibition of N expression, we determined whether the Rif^R *nus*A-1 mutants permitted the growth of the partially N-independent phage λ*byp*. This phage has been shown to require much less N function than N-dependent wild-type phage (see the accompanying paper, Friedman, Baumann, and Baron, 1976). The ability of a λ-inhibiting bacterial mutant to plate λ*byp* under conditions where it does not plate N-dependent λ is taken as presumptive evidence that its restrictive effect is due to inhibition of N expression (Friedman, 1971). Since all of the Rif^R *nus*A-1 mutants which inhibited λ growth at low temperatures permitted relatively good growth of λ*byp* at this temperature, we conclude that the added restrictive effect is due to an increase in N inhibition and refer to this phenotype as "Supernus." The mutation(s) involved in converting Nus hosts to Supernus will be called Snu, *S*uperinhibiting *N-u*tilization. Two Supernus mutants, K-388 and K-389, were chosen for further characterization, referred to, respectively, as Supernus-1 and Supernus-9. The Supernus-9 strain exhibits a temperature-sensitive (*ts*) phenotype: it is able to form colonies at 32°, but not at 42°. Experiments demonstrating that this *ts* character is due to the Snu-9 mutation will be presented in a later section of this paper.

Mapping of Snu Mutations

The locations of the mutations (Snu) conferring Supernus activity were mapped by P1 mediated transduction. Since nitrosoguanidine is known to cause multiple mutations (Cerdá-Olmedo and Hanawalt, 1968), it was necessary to determine if the Snu mutations were identical to the Rif^R mutations selected. The genetic information in the *rif* region from the Supernus-1 and -9 donor strains was transferred to *nus*A-1 *arg*B (K-206) and *nus*A-1 *pur*D (K-425) recipients. The selective markers, *arg*B or *pur*D, map on either side of the *rif* locus (Fig. 1).

The mutations responsible for both Snu phenotypes cotransduce at a relatively high frequency with Rif^R (Table 3). However, in each case Rif^R can be separated from Snu: 10 to 20% of the Rif^R transduc-

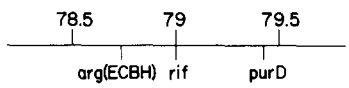

FIG. 1. Map showing the *rif* region of the *E. coli* chromosome.

TABLE 3

COTRANSDUCTION OF THE Rif^R AND Snu MUTATIONS[a]

Donor	Recipient	Selected marker	Distribution of unselected markers		Percentage cotransduction	
					rif^R/total	Snu/*rif*^{R}
			Rif^{R}Snu$^+$	41		
	K-206		Rif^{R}Snu$^-$	208		
		*arg*B$^+$	Rif^{S}Snu$^+$	458	35.2	83
	(*nus*A-1 *arg*B$^-$. . .)		Rif^{S}Snu$^-$	0		
K-388				707		
(*nus*A-1 Rif^{R}Snu$^-$)			Rif^{R}Snu$^+$	9		
Supernus-1			Rif^{R}Snu$^-$	32		
	K-425					
		*pur*D$^+$	Rif^{S}Snu$^+$	102	28.7	78
	(*nus*A-1 *pur*D$^-$. . .)					
			Rif^{S}Snu$^-$	0		
				143		
			Rif^{R}Snu$^+$	3		
	K-206		Rif^{R}Snu$^-$	60		
		*arg*B$^+$				
			Rif^{S}Snu$^+$	112	36.0	95
	(*nus*A-1 *arg*B$^-$. . .)					
			Rif^{S}Snu$^-$	0		
K-389						
				175		
(*nus*A-1 Rif^{R}Snu$^-$)						
			Rif^{R}Snu$^+$	5		
Supernus-9						
			Rif^{R}Snu$^-$	19		
	K-425					
		*pur*D$^+$	Rif^{S}Snu$^+$	25	48.9	79
	(*nus*A-1 *pur*D$^-$. . .)					
			Rif^{S}Snu$^-$	0		
				49		

[a] Transduction was done according to the method of Lennox (1955). When K-206 was used as the recipient, transductants were selected on M-9 medium containing all the necessary amino acids except arginine. When K-425 was the recipient, transductants were selected on M-9 medium with all requirements except adenosine. Rif^R was determined by streaking transductants onto TB plates containing 50 μg/ml of Rifampin. Since both recipients carried the *nus*A-1 mutation, the Snu phenotype of the transductants was determined by observing whether λ growth was permitted at 32°.

tants fail to exhibit the Supernus phenotype, when the recipient began as a *nus*A-1 derivative. However, no Rif^S-Snu$^-$ transductants were recovered, regardless of whether the selective marker lies to the left (*arg*B) or right (*pur*D) of the *rif* locus.

The most obvious difference between the Snu mutations revealed by these experiments is seen in the cotransduction frequencies of Rif^R and Snu (last column of Table 3). Although these results permit us to order the Snu-9 mutation in relationship to *rif* as well as to *arg*B and *pur*D, they permit the ordering of Snu-1 only in relation to *arg*B and *pur*D. The fact that both Snu-1 and Snu-9 cotransduce at a relatively high frequency with Rif^R, regardless of which selective marker is used, locates the Snu mutations between *arg*B and *pur*D. Since the frequency of cotransduction of Snu-9 and Rif^R is significantly higher when *arg*B is the selective marker (95%) than when *pur*D is the selective marker (83%), we infer a genetic order of *argB*, Snu-9, Rif^R, and *pur*D. The observation that the cotransduction frequencies of

TABLE 4

EFFICIENCY OF PLATING OF VARIOUS λ DERIVATIVES ON *nus*B-5 AND *nus*B-5 *rif*^{R} Snu$^-$ MUTANTS[a]

Temperature of incubation(°)	Bacterial strain		Phage tested							
			λcI	λcI*nin*	λcI857 *byp*	λ*imm*21	λ*imm* P22hy7	λ*imm* 434*int*$^-$	λ*imm* 434cIts *byp*	λ*pun*
32	*nus*A-1	K95	1.1	0.8	1.2	0.6	1.1	0.5	0.4	0.8
	*nus*A-1Rif^{R}Snu$^-$ (Supernus-1)	K388	6.3×10^{-6}	0.8	0.7	0.8	0.7	1.0×10^{-7}	0.2	0.3
	*nus*A-1Rif^{R}Snu$^-$ (Supernus-9)	K389	3.5×10^{-6}	0.4	0.1	0.5	0.6	4.6×10^{-8}	0.3	1×10^{-5}
	*nus*A$^+$Rif^{R}Snu$^-$ (Snu-1)	K476	0.8	ND	ND	ND	ND	ND	ND	0.6
	*nus*A$^+$Rif^{R}Snu$^-$ (Snu-9)	K477	0.8	ND	ND	ND	ND	ND	ND	0.6
42	*nus*A-1	K95	2.0×10^{-4}	1.4	0.7	2.2×10^{-4}	1.2	1.2×10^{-6}	2.1	0.7
	*nus*A-1Rif^{R}Snu$^-$ (Supernus-1)	K388	1.3×10^{-5}	1.3	0.8	8.3×10^{-6}	1.2	2.7×10^{-8}	2.7	$<10^{-7}$
	*nus*A-1Rif^{R}Snu$^-$ (Supernus-9)	K389	$<10^{-8}$	0.6	0.01	1.5×10^{-5}	1.0	$<10^{-7}$	1.8	$<10^{-7}$
	*nus*A$^+$Rif^{R}Snu$^-$ (Snu-1)	K476	1.1	ND	ND	ND	ND	ND	ND	1.2
	*nus*A$^+$Rif^{R}Snu$^-$ (Snu-9)	K477	0.8	ND	ND	ND	ND	ND	ND	0.9

[a] Bacteria were grown overnight in TB at 37° and used as lawns to titer the indicated phage strains. Titering was done in duplicate, one set of plates incubated at 32°, while the other set at 42°. The phage were also titered on the parental *nus*A$^+$ Rif^S Snu$^+$ strain (K37) as a control. The efficiency of plating was calculated as the (phage titer on the mutant)/(phage titer on the control). The efficiency of plating for each phage on the K37 control is 1.0. ND = Not done.

Snu-1 and Rif^R is almost the same when either outside marker is used for selection does not allow ordering of the Rif^R and Snu-1 mutations in relation to *arg*B and *pur*D.

Analysis of the Supernus Phenotype

In order to further characterize the Supernus phenotype, we determined how effectively Supernus hosts inhibit the growth of a number of λ variants. In these experiments, the growth of each phage in either the Nus$^-$ or Supernus hosts was compared with its growth in the parental Nus$^+$ host; these comparisons, determined in terms of efficiencies of plating (e.o.p.), are listed in Table 4.

We will first discuss the effects of Supernus on phage growth at 32°. Although most phages tested grow well on the *nus*A-1 host (K-95), phages carrying N_λ, λ, and λ*imm*434 do not plate on either Supernus host. In contrast, phages which express different N functions, λ*imm*21 (N_{21}) and λ*imm*P22 (N_{P22}), plate with high efficiency on both Supernus hosts. That the observed inhibitory effect of the Supernus hosts on phage carrying N_λ is due to inhibition of N expression is suggested by the observation that the e.o.p. of N_λ-carrying phages on Supernus hosts is significantly improved if these phages carry mutations which render them either partially or fully N-independent. Thus, λ*byp*, λ*nin*, and λ*imm*434-*byp* all plate well on the Supernus strains at 32°.

We next will discuss the effect of Supernus on phage growth at 42°. As shown in Table 4, the *nus*A-1 host significantly reduces λ growth at the high temperature. This reduction we attribute to N-inhibition, since *byp*- and *nin*-carrying phages will plate under these conditions. However, there is a significant difference observed between the two Supernus strains. Although the fully N-independent phage, λ*nin*, plates on both Supernus hosts, the partially N-independent phage, λ*byp*, plates relatively poorly on Supernus-9[2]

[2] Although bacteria carrying the Snu-9 mutation exhibit temperature-sensitive growth, it is still possible to use these bacteria in plating studies at 42°. If the bacterial suspension used for plating has been cultivated in TB broth at 32°, such mutant hosts will

(e.o.p. = 0.01) and well on Supernus-1 (e.o.p. = 0.5).

Since it has been shown that some Rif^R mutations which resemble Snu mutations inhibit T4 growth at low temperature, we determined whether the Supernus-1 and -9 strains inhibited T4 growth. We could find no effect on T4 growth in the Supernus hosts (data not shown). As expected, Supernus hosts had no effect on the growth of T4*βgt*, a mutant phage which plates on the Snu-like Rif^R mutant (Montgomery and Snyder, 1973) that does not plate T4.

Effect of Snu Mutations on λ Growth

In order to determine if Snu mutations, independent of the *nus*A-1 mutation, have any observable effect on λ growth, we removed the *nus*A-1 mutation from both Supernus-1 and -9. The *nus*A$^+$ allele was substituted for the *nus*A-1 allele by mating each of the Supernus strains with an Hfr donor (K-359) which transfers the *nus*A$^+$ allele as well as spectinomycin resistance (Spc^R) early in the mating process. Following the mating, putative Snu$^-$-Nus$^+$ bacteria were selected as Rif^R and Spc^R exconjugants. Spot tests with various λ derivatives demonstrated that by this criterion the putative Snu mutants had no effect on λ growth. The presence of the Snu mutation in two of these putative Snu$^-$ hosts, K-476 (derived from Supernus-1) and K-477 (derived from Supernus-9) was confirmed by transferring the *rif* region from each Snu mutant back into the *nus*A-1 bacterium using P1 transduction. Most of these transductants became Supernus, demonstrating that the donor strains carry the Snu mutations. The Snu-9 strain, K-477, like its Supernus progenitor, is temperature-sensitive.

As a more sensitive method for determining if Snu mutants affect λ growth, we examined the burst of λ*c*I in single-step growth experiments. In these experiments, the burst of λ*c*I in each of the Snu mutants was compared with the burst of λ*c*I in the Snu$^+$Nus$^+$ host K-37. As shown in Fig. 2, Snu mutations cause only a slight delay in the burst of λ. Thus, by themselves, Snu mutations appear to only marginally influence λ growth.

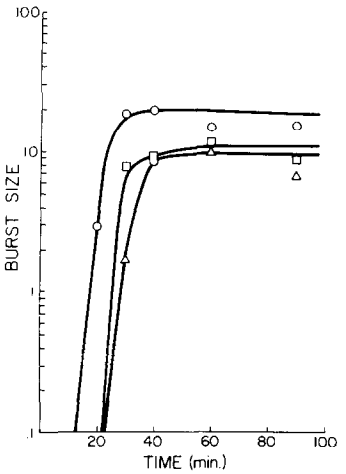

FIG. 2. Burst of λ*c*I in the Nus$^+$Rif^{R}Snu$^-$ strains at 42°. The Snu-1 and -9 strains (K-476 and K-477) and the Snu$^+$ strain (K-37), were grown in TB and diluted to a final concentration of 10^8/ml. The bacteria were infected with λ*c*I at a m.o.i. of 0.1. Adsorption took place at 42° and cells were then diluted into prewarmed TB and incubated at 42°. One-milliliter samples were removed at the indicated times and chloroform was added. The lysed samples were plated with a K-37 lawn on tryptone plates using top agar. Plates were incubated at 40° overnight. Burst size was computed as phage released per infected bacterium. ○——○, K-37 infected with λ*c*I; □——□, K-476 infected with λ*c*I; △——△, K-477 infected with λ*c*I.

Temperature-Sensitivity of the Snu-9 Mutation

We next determined whether the temperature-sensitivity associated with the Snu-9 mutation is due to a second mutation or is a direct consequence of the Snu-9 mutation. If the same mutation is responsible for both phenotypes, transductants receiving Snu-9 should always become temperature-sensitive and conversely transductants which receive genetic material mapping near Snu-9, but not Snu-9 itself, should remain temperature-resistant.

P1 transduction was used to transfer genetic material from the Snu-9 donor strain K-389 to either K-206 or K-425, the selective markers being, respectively, *arg*B

form lawns even when the plates are incubated at temperatures as high as 42°.

or *pur*D. Regardless of which selective marker was used, we were unable to separate the temperature-sensitive phenotype from the Snu⁻ phenotype. First, none of the 135 transductants which were Snu⁺ were temperature-sensitive. Second, all 89 transductants which became Snu⁻ also became temperature-sensitive. Thus, acquisition of temperature-sensitivity and the Snu⁻ phenotype is coincident. This leads us to conclude that the temperature-sensitive and Snu⁻ phenotypes of Snu-9 derivatives are either due to the same mutation or to two closely linked mutations.

Growth of λpun in Supernus and Snu Hosts

The preceding experiments have demonstrated that although the combination of *nus*A-1 and Snu mutations severely inhibits N expression, individually only the *nus*A-1 mutation exhibits any significant N-inhibitory effect and that is seen primarily at 42°. In order to explore the possibility that the combined effect of these two mutations on N expression is due to more than an additive process, we determined the nature of the inhibition by Supernus hosts of a λ variant whose growth is not demonstrably inhibited by the *nus*A-1 mutation. This phage mutant, λ*pun*, carries a mutation in the N gene which permits phage growth at 42° in the *nus*A-1 host (Friedman and Ponce-Campos, 1975). Thus, any significant inhibition of λ*pun* growth by Supernus strains at 32° would imply a strong synergistic action between *nus*A-1 and Snu mutations.

In these experiments we compared the growth of λ*pun* in the *nus*A-1 and Snu hosts with its growth in the two Supernus strains. As shown in Table 4, neither the *nus*A-1 nor Snu mutations individually have any significant effect on λ*pun* growth at 32 or 42°. However, at 42° both Supernus strains markedly interfere with λ*pun* growth, while even at 32° Supernus-9 significantly interferes with λ*pun* growth. This led us to look for subtle effects of the component Snu-9 and *nus*A mutations using a more sensitive assay, single-burst analysis. Figure 3 demonstrates that both the Snu-9 and *nus*A-1 mutations exhibit

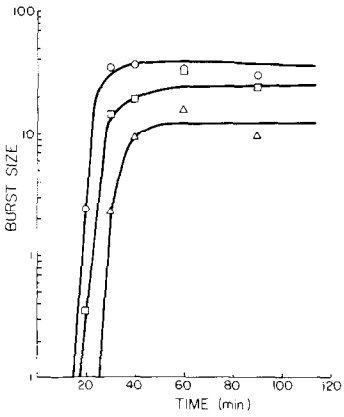

FIG. 3. Burst of λ*pun* in a *nus*⁺-Snu⁺ host (K-37), a *nus*A-1 host (K-95), and the Snu-9 host (K-477) at 42°. The experiment was carried out as described in the legend to Fig. 2. ○——○, K-37 infected with λ*pun*; □——□, K-95 infected with λ*pun*; △——△, K-477 infected with λ*pun*. A control of the Supernus-9 strain (K-389) was also infected and gave no detectable burst of the λ*pun* phage at 42°.

little effect on λ*pun* growth at 42°. In each case the burst is moderately reduced, and only slightly delayed. Therefore, in the case of Supernus-9 the component Snu⁻ and *nus*⁻ mutations alone have little effect on λ*pun* growth, while the combination of the two is very restrictive on the growth of this phage.

Effect of Snu Mutations on Expression of NusB Phenotype

The evidence presented thus far demonstrates that the combination of either Snu mutation with the *nus*A-1 mutation results in the expression of the Supernus phenotype. Evidence presented in this section will demonstrate that a Snu mutation also modifies the phenotype of bacteria carrying a mutation in the *nus*B locus. Like the *nus*A-1 mutant, the *nus*B-5 mutant limits λ growth at 42° by inhibiting N gene expression (Friedman, Baumann, and Baron, 1976). Double mutants, Snu *nus*B-5, were constructed by transducing the Rif^R marker with phage P1 from the Supernus-1 strain to the *nus*B-5 strain K-450. The resulting Rif^R *nus*B-5 transductants could be tested for additional λ-inhibitory activity since λ plates moderately well on the *nus*B-5 strain, K-450, at 32°.

We might expect, then, if the Snu mutation alters the *nus*B-5 host in a manner similar to the way that it alters the *nus*A-1 host, that a Snu *nus*B-5 double mutant would not plate λ at the low temperature. We find that 70% of the Rif^R transductants show the anticipated altered phenotype: failure to plate λ at 32°. The fact that 30% of the transductants showed no altered phenotype demonstrated that the Rif^R mutation of the Snu strain is not responsible for altering the phenotype of the *nus*B-5 recipient.

One of the Snu Rif^R *nus*B-5 transductants, K-478, was examined in more detail. Growth of phage was tested by measuring the burst of phage on the mutant host and comparing it with the burst of phage on the Snu$^+$ Nus$^+$ host, K-37. As shown in Table 5, the plating of λ*c*I, an N-dependent phage, is severely reduced in the Snu *nus*B-5 host (e.o.p. = 5×10^{-5}) when compared to its plating on the *nus*B-5 host (e.o.p. = 0.6). This effect is most likely due to an inhibition of N expression, since the partially N-independent phage, λ*byp*, plates well on the Snu *nus*B-5 host (e.o.p. = 1).

Dominance Patterns of Snu$^+$/Snu$^-$ Merodiploids

Bacteria diploid for the Snu region (Snu$^+$/Snu$^-$) were constructed using F '112, an episome which covers the *arg*B-*rif*-*pur*D region (Low, 1972). The *pur*D-Snu-1-*nus*A-1 strain K-479 was used as the recipient in a mating with the F '112 donor, K-478. Exconjugants were selected that could grow in the absence of exogenously supplied adenosine. The fact that these exconjugants yielded Pur$^-$ segregants at a high frequency was taken as evidence that these strains were true diploids.

The diploids were used to determine the dominance patterns for the Snu-1 mutation (carried originally by strain K-388). This could easily be determined using spot tests with the N-dependent phage λ*c*I, since at 32° *nus*A-1 strains plate this phage, while Snu$^-$ *nus*A-1 strains do not plate λ*c*I. These spot test experiments demonstrate that the diploid strains act like the *nus*A-1 single mutant; they regain the ability to plate λ*c*I at low temperature. Moreover, segregants which have lost the F′ will not plate λ*c*I at 32°. We therefore conclude that, at least in the case of the Snu-1 mutation carried by K-388, Snu$^+$ is dominant. Dominance tests were not carried out with a Snu-9 derivative.

DISCUSSION

The data presented in this paper demonstrate that some mutations mapping in the region of the *rif* locus enhance the N-inhibiting effect of Nus$^-$ mutants. Because we are not able to unambiguously map these *rif*-associated mutations, we have referred to them according to their phenotypic characteristic of Superinhibiting *N*-*u*tilization (Snu).

Mapping studies reveal that Snu and Rif^R mutations are linked. Although the Rif^R character can be separated from the Snu mutations, the Snu mutations cannot be isolated independent of Rif^R. This functional linkage between Snu and Rif^R can be explained in three ways: (1) The Snu phenotype results from a combination of different Rif^R mutations, each alone being Snu$^+$. (2) The Snu phenotype is only ex-

TABLE 5

EFFICIENCY OF PLATING OF VARIOUS λ DERIVATIVES ON *nus*B-5 AND *nus*B-5 *rif*^{R} Snu$^-$ MUTANTS[a]

Temperature of incubation(°)	Bacterial strain		Phage tested			
			λ*c*I	λ*c*I*nin*	λ*c*I857*byp*	λ*imm*21
32	*nus*B-5	K450	0.61	2.2	2.2	0.07
	*nus*B-5Rif^{R}Snu$^-$	K478	3.4×10^{-5}	1.3	1.7	0.3
42	*nus*B-5	K450	6.7×10^{-5}	1.0	1.7	8.7×10^{-8}
	*nus*B-5Rif^{R}Snu$^-$	K478	5.8×10^{-7}	1.2	1.5	1.8×10^{-5}

[a] The efficiency of plating was carried out as described in the legend to Table 4.

pressed in the presence of a second, Rif^R, mutation. (3) The Snu mutations are lethal in the absence of a Rif^R mutation. Although we cannot rule out any of these possible models, it is obvious that the first requires that Snu mutations map in the *rif* locus and the latter two are compatible with such a map location. It should be noted that Oeschger and Berlyn (1975) have isolated a temperature-sensitive mutation that affects the synthesis of the β and β' subunits of RNA polymerase and, like Snu mutations, cannot be isolated independent of the Rif^R character originally selected in the isolation procedure. Moreover, they find that Rif^R can be separated from the temperature-sensitive phenotype. Consistent with these experiments are studies showing that Rif^R is associated with the β subunit of RNA polymerase (Heil and Zillig, 1970) and that the genes coding for the β and β' subunits of RNA polymerase map close together (Kirschbaum and Scaife, 1974). Since mutations in the *rif* locus clearly affect N utilization (Ghysen and Pironio, 1972) and, even more in point, some Rif^R mutations exhibit the Snu phenotype (Sternberg, 1976), it seems likely that the Snu mutations are in genes coding for either the β or β' subunits.

Functional analysis of Snu mutations is difficult, because these mutations by themselves exhibit virtually no effect on λ growth. However, Snu mutations significantly add to the λ-inhibitory effects of both *nus*A-1 and *nus*B-5 mutations. The *nus*A-1 mutation is the more extensively studied and has been shown to inhibit λ growth by interfering with the action of the N gene product (Franklin, personal communication; Adhya, personal communication; Szybalski, personal communication). We will focus our discussion on the Supernus hosts carrying Snu and *nus*A-1 mutations.

Studies with two types of λ mutants suggest that Snu mutations increase inhibition of λ growth by amplifying the N-inhibitory action of the *nus*A-1 mutation. First, λ*pun* which carries a mutation that permits growth in the *nus*A-1 mutant without relieving the need for N function, cannot grow in either Supernus strain under restrictive conditions. This shows that merely being able to overcome the effect of the *nus*A mutation is not sufficient to permit λ growth in Supernus hosts. Second, λ*nin* which is able to grow in the absence of N function grows well in both Supernus hosts, even under restrictive conditions. Thus, λ*nin* must be able to grow in the Supernus hosts because N-independent growth obviates the combined effects of Snu and *nus*A mutations. This implies that Snu mutations potentiate the inhibitory effects of the *nus*A-1 mutation on N product action. Since the complementation studies demonstrate that Snu$^+$ is dominant, a plausible conclusion is that Snu is a product needed for full N expression.

The studies with λ*pun* and the Supernus-9 strain provide further information. Individually the Snu-9 and *nus*A-1 mutations exhibit little inhibition of λ*pun* growth, while together (in Supernus-9) they are strongly inhibitory. Such a synergistic effect suggests that there might be an inhibition of a normal interaction between the *nus*A, Snu, and N gene products. Since the Snu product appears to be a component of RNA polymerase, one inference of such an interaction is that Nus products might be components of RNA polymerase. Proof of such an interaction awaits the results of current studies on RNA polymerase isolated from the various bacterial mutants. The studies of Epp and Pearson (1975) give direct evidence that N and RNA polymerase may interact, since a λ-specific protein, which appears to be coded by the N gene, can be found associated with host RNA polymerase.

An interaction between N and polymerase is an integral facet of a model of N action which we have previously proposed (Friedman, Wilgus, and Mural, 1973). According to this model, N alters RNA polymerase at unique promoters (Friedman *et al.*, in press; Friedman and Ponce-Campos, 1975; Franklin, 1974; Adhya, Gottesman and de Crombrugghe, 1974; Friedman, Wilgus, and Mural, 1973) producing a modified polymerase capable of overcoming transcription termination at sites distal to the promoter.

We are not able to explain at this time why phages which express N functions different from that of λ, λ*imm*21 (Friedman,

Wilgus, and Mural, 1973), and λ*imm*P22 (Hilliker, 1975), show patterns of growth that differ from each other as well as from those of λ on the various N-inhibitory mutants. The fact that λ*imm*21 (21*hy*5) is inhibited by Nus mutants at 42° demonstrates that this phage requires Nus products for N activity. However, Snu mutations do not significantly influence N_{21} expression in Nus⁻ backgrounds. This could either mean that expression of N_{21} does not require the Snu product or that the Snu effect on N_{21} expression is not influenced by the Snu mutations we have been studying. The observations that λ*imm*P22 growth is not affected by Nus mutations suggests that N_{P22} expression either does not require the same host functions as N_λ or that the mutations we have been studying do not have a great effect on N_{P22} expression. The latter may be the case, since Hilliker and Botstein (personal communication) find that the growth of some phages which express N_{P22} is inhibited in *nus*A-1 mutants. This does not rule out the possibility that this N product requires other Nus functions for full expression.

Finally, it should be noted that the selection procedure outlined for obtaining Snu mutants might be useful in obtaining a great number of mutants in the *rif* region.

In conclusion, the isolation of mutations mapping in the *rif* region which influence the Nus phenotype suggests a possible multiple interaction between the N product, the β or β′ subunit of RNA polymerase, the *nus*A gene product, and, perhaps, the *nus*B gene product.

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