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Cooperative Effects of Bacterial Mutations Affecting λ N Gene Expression

I. Isolation and Characterization of a *nus*B Mutant

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We report the isolation and characterization of an *Escherichia coli* mutant which limits the growth of phage λ by inhibiting the expression of the N gene regulatory function. The mutation involved maps near minute 11 of the *E. coli* chromosome and dominance tests show that the mutant allele is recessive to the wild one. Therefore, we conclude that the locus involved normally codes for a function necessary for N expression. Another mutant which exhibits a similar phenotype has previously been reported and the mutation involved, in that case, maps at minute 61. This mutant is called Nus (*N utilization substance*); we have named the locus at minute 61 *nus*A, and the locus at minute 11, *nus*B. Although the *nus*A allele is not found in *Salmonella typhosa*, our studies demonstrate that the *nus*B allele is found in this closely related enterobacteriaciae.

A *nus*A-1 *nus*B-5 double mutant was constructed and exhibited a far more restrictive effect on λ growth than either of the single *nus* mutants. Further, we have constructed a λ variant which carries the *nus*B$^+$ allele. This phage plates on *nus*B-5 mutants under restrictive conditions, but not on the *nus*A-1 mutants.

INTRODUCTION

The development of bacteriophage λ is an orderly process, regulated by a number of phage-coded functions (reviewed by: Echols, 1970; Herskowitz, 1973). One of these regulatory functions, the N gene product, is directly required for expression of "early" phage functions and indirectly required for expression of "late" phage functions. The N product appears to act by permitting transcription to proceed through specific termination sequences (see Fig. 1; Roberts, 1969; Echols, 1971; Herskowitz, 1973). The effects of these termination sequences can be obviated by phage mutations; e.g., termination signals t_{R1} by *c*17 (Packman and Sly, 1968) and t_{R2} by *byp* (Hopkins, 1970; Butler and Echols, 1970) or *nin* (Court and Sato, 1969). Variants of λ carrying both *c*17 and *byp*, or *nin* alone, can grow, albeit poorly, in the absence of N and therefore can be considered to be "N-independent." Variants of λ carrying the *byp* mutation alone require some N function for growth, but much less than that required by wild-type λ and can be considered to be "partially-N-independent" (Friedman, Jolly, and Mural, 1973).

Regulation by functions analogous to N has been demonstrated for other temperate phages. The N function of coliphage 434 is the same as that of λ (Thomas, 1966). In the cases of coliphage 21 and *Salmo-*

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nella phage P22, the N products differ functionally from each other as well as from the N function of λ (Couturier and Dambly, 1970; Friedman, Wilgus, and Mural, 1973; Friedman and Ponce-Campos, 1975; Hilliker, 1974). Genetic homology between these temperate phages has permitted the construction of a number of hybrid phages; λ*imm*21 (Liedke-Kulke and Kaiser, 1967), λ*imm*434 (Kaiser and Jacob, 1957), λ*imm*P22 (Gemski, Baron, and Yamamoto, 1972; Botstein and Herskowitz, 1974). These various hybrid phages which primarily carry genetic material of λ have the immunity region of the non-λ phage and express the N function corresponding to that immunity region (see Fig. 1).

The isolation of bacterial mutants, as well as the construction of hybrid bacteria, which limit λ growth by inhibiting the expression of N function indicates that bacterial products are involved in N expression. In some cases this inhibition has been shown to be at the level of N product action, but in other cases it is not clear whether the inhibition is at that level or at the level of N product synthesis. Therefore, the term N expression will be used in the most inclusive sense covering both synthesis and action of N gene product. Three bacterial loci appear to influence N expression. (1) Mutations in the *rif* locus have been shown in a number of ways to affect N expression (Pironio and Ghysen, 1970; Georgopoulos, 1971; Sternberg, 1976; Baumann and Friedman, 1976). These findings are not surprising since regulation by N is effected at the level of transcription and *rif* mutations (mapping at minute 79) are known to alter the β subunit of RNA polymerase (Heil and Zillig, 1970). (2) Two lines of evidence have shown that another locus, mapping at minute 61, is involved in N expression. First, a mutation affecting N expression (Friedman, 1971) at the level of gene product action (Franklin, personal communication; Adhya, personal communication; Szybalski, personal communication) maps in this region. Second, *Escherichia coli–Salmonella typhosa* hybrid bacteria only permit N expression if the genetic information covering this region is of *E. coli*

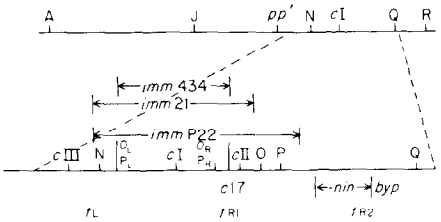

FIG. 1. Genetic map of λ with "immunity" region expanded. The extents of the nonhomology in the immunity region for the various hybrid phages used are shown.

origin (Friedman and Baron, 1974). Since diploid studies demonstrate that the effect of the mutation is recessive, we have concluded that the locus involved codes for a product needed for N action and have called the locus *nus, N utilization substance*. (3) Studies reported in this paper, as well as those reported by Keppel *et al.* (1974), define a third locus affecting N expression which maps at minute 11. Since mutants in this locus phenotypically resemble the mutant mapping at minute 61, we have called the minute-11 locus *nus*B and the minute-61 locus *nus*A.

In this paper, we report the genetic and initial physiological characterization of a mutant carrying a *nus*B mutation. Moreover, we have studied the joint effect of mutations in these two loci on λ growth using hosts carrying both the *nus*A-1 and *nus*B-5 mutations.

MATERIALS AND METHODS

Selection of nus mutants. E. coli mutants which inhibit N expression were isolated using a procedure outlined in detail in a previous communication (Friedman, 1971). In short, the method is based on the observation that induction of a λ prophage defective in P gene expression is lethal to the host, a lethality dependent on N expression (Sly *et al.*, 1968). This lethality can be prevented if N expression is reduced either by phage mutations or bacterial mutations (Eisen *et al.*, 1968; Friedman, 1971). Prophage mutations can be eliminated as a factor in survival by using lysogens carrying multiple copies of the prophage. We therefore select for N-inhibitory mutants starting with a lysogen (K-

386) carrying multiple copies of a *int*⁻λ*c*I-tsP⁻ prophage. The *int*⁻ mutation reduces excision of the prophage (Gottesman and Yarmolinsky, 1968) and insures that surviving bacteria are not "cured" of the prophages; the *c*I-ts mutation results in the expression of a temperature-sensitive repressor protein and permits lysogen induction by shifts to higher temperature.

Operationally, the bacterial lysogen K-386 is grown overnight in TB adsorption broth and ~10^8 cells are seeded on a TB plate using TB top agar. Plates are then incubated at 42° overnight. Surviving bacteria, which are found at a frequency of 10^{-6}–10^{-7}, are then screened for possible N-inhibitory activity by determining if they restrict growth of N-dependent phage (λ*c*I), but permit growth of N-independent phage (N⁻λ*c*17*byp*) at 42°.

Curing of lysogens. Those lysogens initially determined to be N-inhibitory are cured of their resident prophages by transferring in the *gal-att*λ region from a nonlysogenic and *str*ˢ HfrH donor which transfers this genetic region early. Since the recipient is *gal*⁻ and *str*ᴿ, recombinants carrying the nonlysogenic *gal-att*λ region are easily obtained by using galactose utilization and streptomycin resistance for selection and counterselection, respectively. The cured state is confirmed by showing that λ markers cannot be "rescued" from the putative cured cells.

Media. See Friedman *et al.* (1973) and Baron *et al.* (1970).

Bacteriophage and bacteria. See Tables 1 and 2.

Construction of nusA-1 nusB-5 double mutant. The *nus*A-1 and *spc*Aᴿ Hfr strain

TABLE 1
PHAGE STRAINS

Phage	Genotype	Source
λ*c*I	λ*c*I60	M. Yarmolinsky
N⁻λ*c*17*byp*	N*am*7N*am*53λ*c*17*byp*	H. Echols
λ*imm*21	21*hy*5	M. Yarmolinsky
λ*imm*P22	λ*imm*P22-7	D. Botstein
λ*c*I*c*17	λ*c*I90*c*17	M. Yarmolinsky
λ*byp*	λ*c*I857*byp*	These laboratories
N⁻λ*byp*	N*am*7N*am*53λ*c*I857*byp*	These laboratories
λ*imm*434*c*17		M. Gottesman

TABLE 2
CHARACTERISTICS OF BACTERIAL STRAINS[a]

Strain	Description	Pertinent markers	Reference or source
K-37	*E. coli* K12 F⁻	*nus*⁺ *str*ᴿ *gal*⁻	Friedman (1971)
K-95	*E. coli* K12 F⁻	*nus*A-1 *str*ᴿ	Friedman (1971)
K-386	*E. coli* K12 F⁻	K-37(*int*29λ*c*I857P*am*3)$_n$	Friedman (1971)
K-450	*E. coli* K12 F⁻	*nus*B-5 *str*ᴿ	This paper
K-461	*E. coli* K12 F⁻	*nus*A-1 *nus*B-5 *spc*Aᴿ	This paper
N1849	*E. coli* K12 F⁻	λ*c*I857 in *tsx*	S. Adhya
SA1384	*E. coli* K12 F⁻	*lon*⁻	S. Adhya
WR2099	*E. coli* K12 F⁻	*lac*⁻ *proC*⁻ *purE*⁻	These laboratories
K-358	*E. coli* K12 Hfr	*nus*A-1 *spc*Aᴿ *thi*⁻ *leu*⁻ *thr*⁻	These laboratories
HfrH	*E. coli* K12 Hfr	*str*ˢ	W. Hayes
W1895	*E. coli* K12 Hfr	*met*B⁻ *str*ˢ	J. Lederberg
P4X6	*E. coli* K12 Hfr	*met*B⁻ *str*ˢ	E. A. Adelberg
AB2297	*E. coli* K12 Hfr	*thi*⁻ *leu*⁻ *thr*⁻	E. A. Adelberg
WR2075	*E. coli* K12 Hfr	*nus*B-5 *met*B⁻	These laboratories
WR4204	*S. typhosa* F⁻	*lac*⁻ *cys*⁻ *trp*⁻ *str*ˢ	These laboratories
WR4292	*S. typhosa* hybrid	*lac*⁻ *nus*A⁺ *mal*A⁺ *mal*B⁺ *trp*⁻	These laboratories

[a] Abbreviations: *cys*, cysteine; *gal*, galactose; *lac*, lactose; *leu*, leucine; *lon*, long form; *mal*, maltose; *met*, methionine; *nus*, N utilization substance; *pro*, proline; *pur*, purine; *spc*, spectinomycin; *str*, streptomycin; *thi*, thiamine; *thr*, threonine; *trp*, tryptophan; *tsx*, T-six.

K-358, a derivative of Hfr AB2297 (see Fig. 2), which transfers the *nus*A$^-$ region early, was mated with the *nus*B-5 recipient strain K-450. Since the spectinomycin (*spc*A) locus is linked to the *nus*A locus, selection of *spc*A^R recombinants of K-450 (*nus*B-5) assured receipt of the *nus*A-1 marker by a majority of hybrids.

Conjugation and transduction. Standard methods previously outlined were used (Friedman and Baron, 1974). The Lon$^-$ phenotype is observed by the formation of mucoid colonies on minimal medium at 32° (Markovitz and Rosenbaum, 1965).

RESULTS

Preliminary Characterization of nus Mutants

Initial experiments using conjugation and P1 transduction showed that none of the 12 newly selected mutants exhibiting the Nus$^-$ phenotype carried mutations which mapped at minute 61 of the K12 chromosome, the location of the *nus*$^-$ mutation previously studied (Friedman and Baron, 1974). It is not clear why the selection did not yield mutations at this locus. All of the 10 mutations which we were able to map were located near the *lac* operon (minute 11). We have named the locus at minute 61 *nus*A and the locus near *lac*, *nus*B. One *nus*B mutant, *nus*B-5 was chosen for intensive study.

Conjugal Mapping of nusB Locus

Derivatives of the *nus*B-5 mutant, K-450, were constructed carrying mutations in various selective markers distributed around the K12 chromosomal map. Crosses were then performed between different Hfr strains and suitably marked strains of K-450 as recipients. The results established that the *nus*B locus was closely linked to the locus for lactose (*lac*$^+$) utilization, the *nus*B$^+$ marker replacing the *nus*B$^-$ allele of K-450 in 75–95% of the *lac*$^+$ recombinants when HfrH or W1895 were employed as the donors (see Fig. 2). When P4X6 was used as the donor, replacement of the *nus*B$^-$ allele by the *nus*B$^+$ allele of the donor dropped to less than 5%. This result established the location of *nus*B between the origin of W1895 and the origin of P4X6 as seen in Fig. 2.

Transductional Mapping of nusB Locus

P1*vir* was used to map the location of *nus*B within the 9- to 12-minute region of the K12 chromosome. P1 lysates grown on K-450 (*nus*B-5) were used to infect a Nus$^+$ recipient, WR2099 (*lac*$^-$ *pro*C$^-$ *pur*E$^-$) with subsequent selection for *lac*$^+$, *pro*C$^+$, and *pur*E$^+$ single marker transductants. The results show that *nus*B-5 was not cotransduced with either *lac*$^+$ (0/380) or *pur*E$^+$ (0/402), but was cotransduced with *pro*C$^+$ at a frequency of approximately 2% (6/304). Cotransduction of *lac*$^+$ with *pro*C$^+$ was 10% (18/182); none of the *lac*$^+$ *pro*C$^+$ cotransductants were *nus*B$^-$, nor did 56 doubly-selected *lac*$^+$ *pro*C$^+$ cotransductants acquire the *nus*B$^-$ locus. An experiment was also performed, using a P1 lysate grown on SA1384 (*pro*C$^+$ *lon*$^-$), to select for *pro*C$^+$ transductants of a *pro*C$^-$ derivative of K-450 (*nus*B-5). The purpose was to determine the order of the *nus*B locus with respect to *pro*C and *lon*. The results show that the order of the loci is as indicated in Fig. 3, since cotransduction of *pro*C$^+$ and *lon*$^-$ was 25% (42/166), while

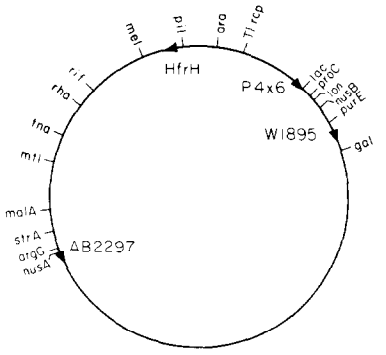

FIG. 2. Chromosome map of *Escherichia coli* K12 with locations of pertinent genes and origins and directions of transfer of Hfr strains based on Taylor and Trotter (1972).

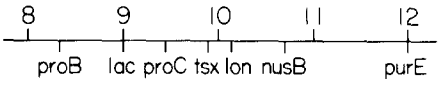

FIG. 3. Chromosomal region showing map location of the *nus*B locus.

the cotransduction of *pro*C$^+$ and *nus*B$^+$ was 2% (3/166) as expected.

Effect of nusB-5 Mutation on λ Growth

We have found that even though the *nus*A and *nus*B loci map at distant sites on the *E. coli* chromosome, the *nus*A-1 mutant K-95 and the *nus*B-5 mutant K-450 exhibit strikingly similar effects on λ growth. A number of these similarities are shown in the experiments outlined in Table 3. First, growth of λ in both hosts is severely restricted at higher temperature, 43°, but only moderately at lower temperature, 32°. This is shown by the observation that the N-dependent phage, λ*c*I, which plates on the two *nus*$^-$ mutants at 32°, does not plate on these hosts at 43°. Since the partially "N-independent" phage λ*byp* plates on the *nus*A-1 and *nus*B-5 hosts at the high temperature, we conclude that the observed inhibition is due to a reduction in N expression. Second, although the *c*17 mutation obviates the effect of the t_{RI} termination signal, both mutant hosts restrict the growth of λ*c*I*c*17 at low, "permissive" temperature. Third, λ*imm*P22, which expresses an N function different from λ, grows on both mutants at the higher temperature. Fourth, λ*imm*21, which expresses the N_{21} function, shows inhibited growth on both *nus*$^-$ mutants at high temperature. However, we have observed one difference between the *nus*A-1 and *nus*B-5 mutants; at low temperature the burst of phages carrying the immunity region of 21 is somewhat inhibited in the *nus*B-5 mutant, but not in the *nus*A-1 mutant (Table 4).

Cooperative Effect of nusA-1 and nusB-5 Mutations

In order to determine the effect of the combination of the *nus*A-1 and *nus*B-5 mu-

TABLE 3

COMPARISON OF THE GROWTH OF VARIOUS LAMBDOID DERIVATIVES ON *nus*$^+$ AND *nus*$^-$ HOSTS[a]

Temperature of growth	Bacterial strain		Phage tested				
			λ*c*I	λ*byp*	λ*c*I*c*17	λ*imm*21	λ*imm*P22
32°	*nus*$^+$	K-37	+	+	+	+	+
	*nus*A-1	K-95	+	+	−	+	+
	*nus*B-5	K-450	+	+	−	+	+
43°	*nus*$^+$	K-37	+	+	+	+	+
	*nus*A-1	K-95	−	+	−	−	+
	*nus*B-5	K-450	−	+	−	−	+

[a] Bacteria were grown overnight at 32° in TB adsorption broth. TB plates were seeded with indicated bacterial lawns using TB top agar. Dilutions of the listed phages were "spotted" on the lawns. Plates were then incubated overnight at the indicated temperatures. +, Good growth; −, poor growth, even when a concentrated suspension of phage was used (10^4/ml).

TABLE 4

COMPARISON OF BURST SIZES OF VARIOUS λ DERIVATIVES ON *nus* MUTANTS AT 32°[a]

Bacterial strain		Phage tested			
		λ*c*I	λ*byp*	λ*imm*21	N$^-$λ*byp*
nus$^+$	K-37	27	108	137	10
*nus*A-1	K-95	78	142	173	−
*nus*B-5	K-450	45	122	15	−
*nus*A-1 *nus*B-5	K-461	<0.01	10	<.01	−

[a] Bacteria were grown at 32° in TB adsorption broth to a final concentration of 10^8/ml. Bacteria were infected at a multiplicity of 0.1 phage per bacterium, and infected cells were incubated at 32° for 20 min to facilitate adsorption. Infected cultures were then diluted into prewarmed TB adsorption broth at 32°. An aliquot of each sample was removed immediately, treated with chloroform, and titered to measure unadsorbed phage. After 2 hr of incubation, diluted samples were treated with chloroform and titered for resulting phage burst.

tations on λ growth, we constructed the double *nus*⁻ mutant, K-461 (see Materials and Methods section). Similar to the single *nus*⁻ mutants, the double *nus*⁻ mutant shows no grossly observable variation in growth rate from that of the *nus*⁺ parent strain (data not shown). On the other hand, the double *nus*⁻ mutant is far more restrictive on λ growth than either of the single *nus*⁻ mutants. As shown in Table 4, there is no detectable burst of the N-dependent phage λ*c*I in the double mutant at 32°. In contrast, there is a larger burst of λ*c*I in each of the *nus*⁻ single mutants than occurs even in the *nus*⁺ host.

The effectiveness of the double mutant in inhibiting N expression is shown even more dramatically by the observation (Table 4) that growth of the partially N-independent phage λ*byp* is inhibited in the *nus*A-1 *nus*B-5 host. The level of phage production in this case is very close to that of N⁻λ*byp* in the *sup*⁻ *nus*⁺ parental strain. This suggests that the phage production by λ*byp* in the double *nus*⁻ host results from either extremely low levels of N expression or from N-independent growth.

The double *nus*⁻ mutant also shows a strong inhibitory effect on the growth of phage carrying the immunity region of 21 (Table 4). At 32° there is no detectable burst of λ*imm*21 in the double mutant. In comparison, λ*imm*21 gives a normal burst in the *nus*A-1 host and a significant, but reduced burst in the *nus*B-5 host.

Isolation of a λ Transducing Phage Carrying nusB

Using the technique developed by Shimada *et al.* (1973), we have isolated a λ specialized transducing phage, λ*nus*B, which carries the *nus*B⁺ region. In brief, a λ temperature inducible phage (λ*c*I857) is inserted near the gene which is to be transduced. Following heat induction of the lysogen, the resulting lysate usually contains phage particles which transduce genes located near the site of prophage attachment. In the case of *nus*B, a lysogen (N1849) carrying λ*c*I857 inserted in the *tsx* locus, located near the *nus*B locus, was induced and a low titer lysate of about 10^{-5} phage per induced bacterium was obtained. Approximately 30% of these phages plated on the *nus*B-5 host at 43°. This frequency is much too high to be explained on the basis of a mutation and leads us to conclude that we were, in fact, isolating transducing phage carrying the *nus*B locus. Two observations are consistent with this conclusion: First, as would be expected for phage carrying *nus*B⁺, they only plate on the *nus*B-5 mutant and not on the *nus*A-1 mutant. Second, lysogens constructed from the putative λ*nus*B phage and from the *nus*B-5 bacterium now exhibit the Nus⁺ phenotype. This is shown by the observation that λ*imm*434*c*17, which plates on a λ lysogen because it carries another "immunity" region and does not plate on a *nus* mutant because it carries the *c*17 mutation (Friedman *et al.*, 1976), now plates on the lysogenized *nus*B-5 host. The observation that λ*nus*B phage can render a *nus*B-5 host Nus⁺ leads us to conclude that the *nus*B⁻ allele is recessive to the *nus*B⁺ allele.

Presence of nusB⁺ Allele in Salmonella

We have previously demonstrated that the closely related enteric bacterium *S. typhosa* does not carry the *nus*A allele (Baron *et al.*, 1970; Baron *et al.*, 1972; Friedman and Baron, 1974). Furthermore, *S. typhimurium* strains were also observed to behave in the same way (Gemski *et al.*, 1972).

To determine if *Salmonella* carry the *nus*B⁺ allele, we constructed intergeneric hybrids by crossing Hfr P4X6 with the *S. typhosa* F⁻ recipient WR4204 and selecting for *ara*⁺ recombinants. As described in Baron *et al.* (1972), diploid hybrids can be isolated which contain extensive segments of the K12 chromosome. Stable haploid segments can be obtained from these diploids. One such *E. coli*-*S. typhosa* hybrid segregant was examined which acquired the *nus*A⁺ as well as the *mal*B⁺ and *mal*A⁺ loci (required for λ adsorption by *Salmonella*), but retained the *Salmonella* region encompassing the *nus*B locus. Since this strain permits λ growth, we conclude that *S. typhosa* carries the *nus*B⁺ allele or some substitute.

We were also able to transfer the *nus*B⁻

allele from *E. coli* Hfr WR2075, a *nus*B-5 donor with the transfer orientation of W1895 (see Fig. 2), to *S. typhosa* hybrid WR4292 by selecting for *lac*$^+$ recombinants. The recombinants were either unstable *lac*$^+$/*lac*$^-$ diploids expressing the *nus*B$^+$ trait or stable *lac*$^+$ hybrids which had integrated the *lac*$^+$ K12 chromosomal region (Johnson *et al.*, 1972). Such stable *lac*$^+$ *S. typhosa* hybrids were found to express the Nus$^-$ phenotype, indicating that they had integrated the *nus*B$^-$ allele transferred from the K12 donor.

DISCUSSION

Although the mode of action of the N gene product has been studied in a number of ways, little is known about the nature of N protein interaction with host functions. We have addressed this question by selecting bacterial mutants which inhibit N expression, assuming that at least some will be mutant in functions essential for N activity. Analysis of such bacterial mutants has identified three loci on the *E. coli* chromosome involved directly in N expression. Evidence presented in this paper, as well as that presented by Keppel *et al.* (1974), has identified a locus involved in N expression which maps at minute 11 on the *E. coli* chromosome.

Although Keppel and co-workers were unable to transduce the mutation involved to another strain and thus could not determine if that mutation alone sufficed to limit N expression, we have been able to transduce the *nus*B-5 mutation to another strain. This result unambiguously demonstrates that a mutation in the *nus*B locus is sufficient to produce the Nus phenotype. Two other loci have also been implicated in N expression: *rif*^{R} mapping at minute 79 and *nus*A mapping at minute 61. While the function coded by the *rif*^{R} locus has been identified as the β subunit of RNA polymerase, that coded by the *nus*A locus has not been identified. However, the fact that the *nus*A$^+$ allele is dominant led us to conclude that this allele normally expresses a function necessary for N expression (Friedman and Baron, 1974). The experiments presented in this paper lead to the similar conclusion that the *nus*B gene codes for another function necessary for N expression. The dominance of the *nus*B$^+$ allele is shown in three ways: First, a phage carrying the *nus*B$^+$ region plates on a *nus*B$^-$ host. Second, when a *nus*B$^-$ host is lysogenized by the λ*nus*B$^+$ phage, it becomes Nus$^+$. Third, the *Salmonella* diploid carrying the *nus*B$^-$ allele of *E. coli* and the *nus*B$^+$ allele of *Salmonella* expresses the Nus$^+$ phenotype.

The isolation of the *nus*A-1 *nus*B-5 double mutant permitted us to study the joint action of these two mutations on N expression. The observation that the double bacterial mutant exhibits a greater effect than either of the single mutants can be interpreted in one of two ways: (1) Each *nus* product acts in a similar way to facilitate N expression; removal of the products of both loci reduces activity even further. (2) Each *nus* product acts in a different manner to facilitate N expression. Experiments with the λ*nus*B$^+$ phage rule out the first alternative. The observation that λ*nus*B$^+$ does not plate on the *nus*A-1 host demonstrated that even when the gene dosage of *nus*B$^+$ is increased, the *nus*A-1 bacterium still exhibits the Nus$^-$ phenotype. Therefore, it cannot be the case that the *nus*A and *nus*B loci code for gene products which act similarly in promoting N expression, but rather that these genes code for products which act in different ways to promote N expression.

The utilization of Nus functions is not unique to λ, since a phage expressing N_{21} shows similar inhibited patterns of growth in both the *nus*A-1 and *nus*B-5 mutants. On the other hand, λ*imm*P22, a phage which expresses N_{P22}, is not inhibited by either *nus* mutant. In the case of the *nus*A-1 mutant, this is not a surprising observation since *Salmonella*, the normal host for phage P22, does not carry the *nus*A$^+$ allele (Friedman and Baron, 1974). However, the ability of λ*imm*P22 to grow on the *nus*B-5 mutant is a little surprising since *Salmonella* does carry the *nus*B$^+$ allele. Thus, either expression of P22-N does not require the *nus*A and/or *nus*B gene products or it requires one or both, but in much lower effective amounts.

In conclusion, we have presented evi-

dence locating another locus involved in λ N gene expression. This locus, *nus*B, maps in the *pro*C region of the *E. coli* K12 chromosome, and defines another function necessary for the expression of some *N* proteins.

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