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Interference with the Expression of the N Gene Function of Phage λ in a Mutant of *Escherichia coli*

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An *Escherichia coli* mutant *nus*, which inhibits the growth of λ by inhibiting the expression of the λ N gene product, has been further characterized. The *N* protein regulates phage development by stimulating transcription of the λ genome in three ways: directly, by stimulating transcription initiating at the two promoters, P_R and P_L and indirectly by stimulating the synthesis of Q protein, which in turn activates transcription of late functions. We report in this study that all three types of λ transcription are affected by the *nus* mutation. However, the inhibitory effect of *nus* on N stimulation of transcription initiating at P_R is shown to be qualitatively different from its effect on N stimulation of the synthesis of Q product.

Evidence is also presented showing that some N function is expressed by λ in the *nus* mutant, but that the level of expression of N function is reduced.

INTRODUCTION

The isolation of an *Escherichia coli* mutant which inhibits the growth of phage λ was reported in a previous study (Friedman, 1971). This mutant was isolated using a selection procedure designed to yield host mutants which interfere with the N gene function of phage λ. Preliminary studies supported the conclusion that in this mutant the expression of N function by λ was reduced or "undersupplied." The mutation was called *nus* for *N* *u*ndersupplied.

Two other *E. coli* mutants which affect the expression of N function have been reported. Both of these mutations map near the gene coding for rifamycin resistance (*rif*^{R}), minute 77 on the *E. coli* chromosome (Pironio and Ghysen, 1970; Georgopoulos, 1971). Baron and co-workers (1970) have shown that a *Salmonella typhosa–Escherichia coli* hybrid bacterium, which carries sufficient *E. coli* genetic material to permit λ adsorption, limits λ growth by inhibiting N function. The N gene product can function in the hybrid bacterium, if an additional small region of the *E. coli* chromosome is introduced into the hybrid bacterium (Baron *et al.*, 1972). This region lies somewhere between minutes 66 and 72. The *nus* mutation also has been shown to map in this same region and to be unlinked to the *rif*^{R} locus by P1 transduction (Friedman, manuscript in preparation). Moreover, these studies show that in a bacterium diploid for the *nus* region, the Nus$^+$ phenotype is dominant to the Nus phenotype.

The N function of phage λ, which is expressed early after infection or induction, subsequently regulates the expression of other phage functions by stimulating transcription of the λ genome (reviewed by Echols, 1971). The transcription of three regions of the λ genome is stimulated in the following way: transcription of the regions coding for "early" phage functions initiating from P_R (the *c*II-O-P operon) and P_L (the N-*c*III-*int* operon) (see Fig. 1) is directly stimulated by N product. Transcription of a third region, coding for "late" phage functions is stimulated by Q product, a function whose expression is under N control.

Vegetative growth of phage λ requires only the expression of functions under P_R

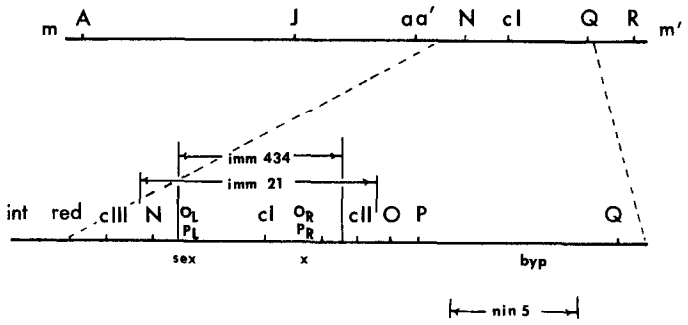

FIG. 1. Genetic map of λ with the early region expanded.

and Q control (Echols, 1971). Functions under P_L control distal to N are involved in lysogeny and recombination and are not essential for normal phage growth. This accounts for the fact that phage carrying mutations which allow N-independent expression of both the *c*II-O-P operon and Q product permit phage growth.

Two types of λ mutants which grow in the absence of N product (N-independent) have been isolated. The first type, λN⁻*c*17*byp* (Butler and Echols, 1970; Hopkins, 1970), as expected from the above considerations, carries two mutations; *c*17 (mapping in the y region) which frees transcription of the *c*II-O-P operon from N control, and *byp* (mapping in the P-Q region) which permits Q synthesis in the absence of N product. A second type, λ *nin* (Court and Sato, 1969), is genetically altered in only one region. It carries a 5% deletion in the P-Q region (Fiandt *et al.*, 1971). Since the functions coded for in the *c*II-O-P operon are expressed, albeit at a lower level, in the absence of N product (Ogawa and Tomizawa, 1968; Signer, 1969; Lieb, 1970), the *nin* deletion must augment the normal low levels of N-independent transcription initiating at P_R (Court, 1970).

Bacterial mutants in which N product is "undersupplied," were obtained by screening for bacteria which had the ability to plate the N-independent phage λN⁻*c*17*byp* under conditions where an N-dependent phage would not plate; *nus*-1 was characterized as such a mutant. Although the *nus*-1 mutant (K-95) showed the Nus phenotype at 42°, it appeared to be Nus⁺ at 34°. We assumed that the *nus* mutation resulted in the synthesis of a temperature-sensitive product (Friedman, 1971).

If, in fact, the *nus* mutation interfered with the expression of N function, we would expect that each component of λ growth regulated by N product would be affected by the *nus* mutation. That is, for vegetative growth of λ to occur in the bacterial mutant both *c*17 as well as the *byp* mutations would be necessary. However, this was not the case. Whereas at 42° the *c*17 mutation alone was not sufficient to allow λ to plate on K-95, the *byp* mutation alone was sufficient to allow λ to plate on this host (Friedman, 1971). This result suggested that the *nus* mutation might affect only one of the areas under N regulation, N stimulated turn-on of Q product. However, in this paper we present evidence showing that the *nus* mutation does, in fact, affect all three components of N activity.

MATERIALS AND METHODS

Bacterial strains. The *nus* mutant (Friedman, 1971) was selected without mutagenesis using a λ lysogen of K-37 (strain 28 of Meselson's). K-37 (strain 28 of Meselson) is a *str*^{R} derivative of W3102 (Lederberg, 1960). In the experiments reported in this paper the *nus* mutant used is K-95, a derivative of *Nus*-1 which was cured of the λ prophage. C600 (Appleyard, 1954) is a strain permissive for growth of phage carrying amber mutations.

Phage stocks. Obtained from M. Gottesman, λ*c*I857, λ*c*I857*sex*1, λ*c*I60, P2, and P2*vir*; from M. Yarmolinsky, λ*imm*434cIts; from I. Herskowitz, λ*c*I857*sus*N7N53. A series of phage were constructed in this labo-

ratory: λcI857*byp* and λcI857*sus*N7N53*byp* using a λ*sus*N7N53c17*byp* supplied by H. Echols; λcI60 *nin* using a λ*bio*10*nin*5 supplied by F. Blattner.

Lysogenization. λ lysogens were constructed according to the method of Gottesman and Yarmolinsky (1968). P2 lysogens were obtained by plating P2 phage on a lawn of the bacteria which was to be lysogenized. Bacteria were picked from the center of the plaque and purified by streaking on a tryptone plate. Single colonies were tested for lysogeny by determining whether they would grow P2*vir* but not P2.

Exonuclease determination. λ exonuclease was assayed using Pero's (1970) modification of Radding's method (1966). However, cells were resuspended in 0.01 *M* Tris, pH 7.1, instead of a glycylglycine buffer. Protein content of lysates was measured using the method of Lowry *et al.* (1951). One unit of activity is defined as that amount of enzyme solubilizing 10 nmoles of nucleotide in 30 min at 37°.

Media. Adsorption broth: 1% tryptone, 0.5% NaCl, 1.0 μg/ml thiamine hydrochloride, 10^{-2} *M* $MgSO_4$ and 0.2% maltose. Tryptone agar: 1% tryptone, 0.25% NaCl, 1.0 μg/ml thiamine hydrochloride, and 1.1% agar. Tryptone top agar: 1% tryptone, 0.5% NaCl, and 0.7% agar.

EXPERIMENTAL

Effect of the nus Mutation on N Stimulation of Q Expression

The synthesis of late λ gene products is stimulated by the Q gene product, the expression of Q, in turn, is regulated by N product. In the absence of N protein, Q function is not expressed (reviewed by Echols, 1971). The most obvious effect of the *nus* mutation on λ development is its effect on the expression of Q gene product. This is demonstrated by experiments showing that the restriction of λ growth imposed by the *nus* mutation is overcome if the phage carries either of two mutations, *nin* or *byp*, mapping in the P-Q region. These mutations permit λ to express Q function in the absence of N product.

Single-step growth experiments at 42° show that although the final bursts of λ*nin* and λ*byp* produced in the *nus* mutant, K-95, are similar, the development of the two phages in the *nus* bacterium are quite different. A comparison of Figs. 2 and 3 shows that whereas the onset of viable phage production, at 42°, of λ*nin* is nearly the same in the *nus* and *nus*$^+$ bacteria, the onset of phage production of λ*byp* is delayed by over 20 min in the *nus* bacterium.

The effect of the two mutations, *nin* and *byp*, on λ physiology are clearly quite different (reviewed by Echols, 1971). This is evident from the fact that while the *nin*

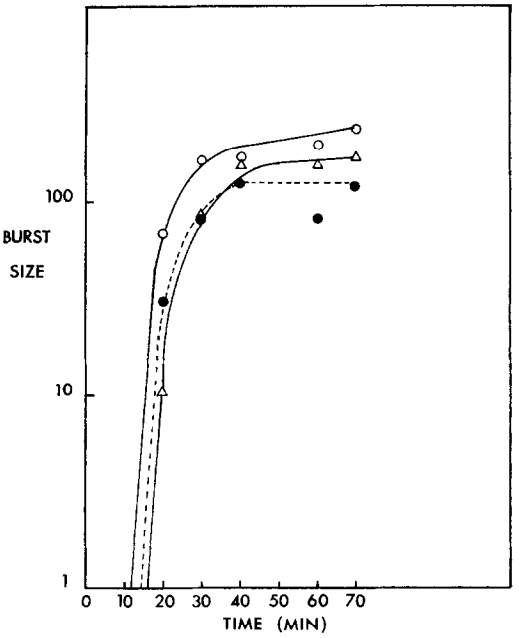

FIG. 2. Burst of λ*nin* in *nus* at 42°. Bacterial strains, *nus* (K-95) and *nus*$^+$ (K-37), were grown in adsorption broth and diluted to a final concentration of 10^8/ml. The bacteria were infected with either λcI60 or λ*nin*5 at a multiplicity of infection (m.o.i.) of 0.1. Adsorption was effected by incubating the infected cell at 42°. Cells were then diluted into adsorption broth prewarmed at 42°. One-milliliter aliquots were removed at the indicated times, and a few drops of chloroform were added. The infected cells were incubated at 40° for 30 min. The lysed cultures were plated using top agar on tryptone plates. Plates were incubated at 40° overnight. Burst size was computed as phage released per infected bacterium. ○——○, K-37 infected with λcI60; ●----●, K-37 infected with λ*nin*; △——△, K-95 infected with λ*nin*.

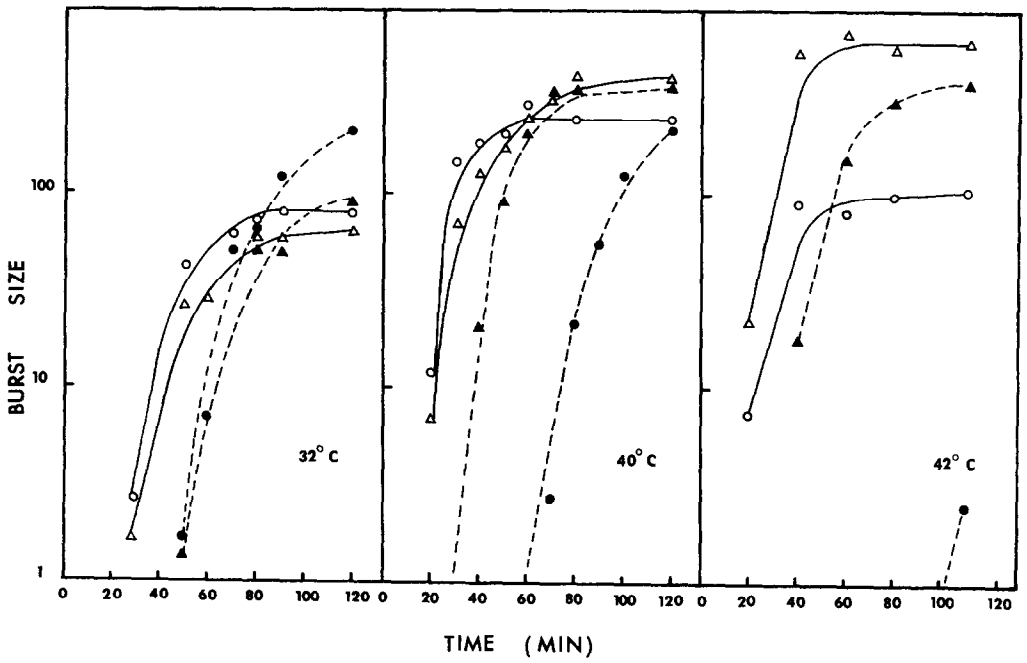

FIG. 3. Burst of λ*byp* in *nus* at various temperatures. The methods of phage growth and determination of burst time were the same as outlined for Fig. 2. The temperature at which each experiment was run is recorded in the lower right-hand corner of each panel. ○——○, K-37 infected with λ*c*I60; ●-----●, K-95 infected with λ*c*I60; △——△, K-37 infected with λ*c*I857*byp*; ▲-----▲, K-95 infected with λ*c*1857*byp*.

mutation is sufficient to permit N-independent growth, the *byp* mutation alone does not permit N-independent growth of λ. In order to plaque, a λN⁻ phage requires, in addition to *byp*, a second mutation, such as *c*17, which frees the *c*II-O-P operon from N control. The degree of N-independence of a λ mutant, therefore, correlates with its growth characteristics in the *nus* bacterium. It is not clear from these experiments why the appearance of λ*byp*, in contrast to the appearance of λ*nin*, is delayed in K-95. Since N function acts in two ways to stimulate vegetative growth, this delay could either reflect (1) an interference with N-stimulated transcription from P_R or (2) an inadequate expression of Q. In either case λ*nin* must overcome the block. What is clear from these experiments is that the *nus* mutation inhibits the expression of Q function. Evidence consistent with the idea that the delay in the appearance of λ*byp* in K-95 is due to an inhibition of N-stimulated expression of the *c*II-O-P operon will be presented in the next section.

Effect of the nus Mutation on N Stimulation of the Expression of the cII-O-P Operon

The N protein stimulates transcription of the *c*II-O-P operon which initiates at P_R (reviewed by Echols, 1971). We have studied the effect of the *nus* mutation on expression of this operon by measuring the level of O and P gene product. The O and P genes code for replication functions (reviewed by Kaiser, 1971). The expression of these two functions by a λ prophage is sufficient to result in the death of the cell it lysogenizes (Pereira da Silva, *et al.*, 1968). The induced λ prophage expresses an additional function lethal to the host which maps in the N-*c*III-*int* operon and is directly under N control (Herskowitz, personal communication). The expression of this lethal function can be eliminated by a number of different phage mutations. One of these mutations is the *sex* mutation (Gottesman and Weisberg, 1971), which is located in the region of P_L (Blattner *et al.*, 1972) and results in a lowering of the transcription initiated at P_L

(Roberts, 1969). A λ*sex* phage does express sufficient N function to permit vegetative growth. Induction of a λ*sex* prophage is lethal to the host and this lethality then must be due to the expression of O and P function. That this is the case is indicated by the fact that induction of lysogens carrying either a λ*sexO*$^-$ or λ*sexP*$^-$ prophage is not lethal (Gottesman, personal communication).

Thus, by measuring the loss of viable bacteria following the induction of a λ*sex* prophage it is possible to determine the level of expression of λ replication functions O and P. This, in turn, should give an estimate of the level of expression of the *c*II-O-P operon. Induction of a λ*c*I857*sex*1 prophage is lethal both to the *nus* mutant as well as the *nus*$^+$ parent. But, significantly, when the kinetics of lethality are studied (Fig. 4), there is a 20-min delay in the killing by the λ*sex* prophage in the *nus* mutant as compared to the *nus*$^+$ parent. This reflects a 20-min delay in the expression

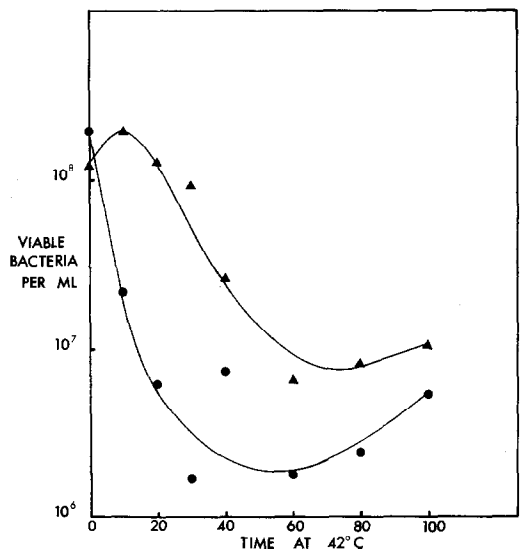

FIG. 4. Lethal effect of induced λ*sex* prophage on *nus* and *nus*$^+$ bacteria. Lysogens, carrying λ*c*I857*sex*1 prophage, were grown to a concentration of ~10^8/ml at 34°. Cultures were shifted to 42°, and aliquots were removed at the indicated times and immediately plated for viable cells on tryptone agar plates. Plates were incubated at 34°. ●——●, K-37·λ*c*I857*sex*1; ▲——▲, K-95·λ*c*I857*sex*1.

of the lethal functions, O and P. Unreported studies by Friedman and Yarmolinsky, using a λ*c*I857*sus*N7N53 prophage, have shown that in the absence of the N function there is a 30- to 35-min delay in the occurrence of prophage induced killing. Therefore, we conclude that the 20-min delay in the onset of killing seen in the case of the λ*sex* lysogen of K-95 reflects a 20-min delay in the N-stimulated expression of the *c*II-O-P operon. It is interesting to note that this delay is similar to the delay in the appearance of λ*byp* following its infection of K-95 at 42°.

Additional experiments demonstrated that the delay in the appearance of viable phage following infection of K-95 with λ*byp* was relatively constant over temperatures ranging from 32° to 42°. As shown in Fig. 3, the appearance of λ*byp* following infection of the *nus* mutant always lagged about 20 min behind its appearance following infection of the *nus*$^+$ strain. A similar delay was observed using a *byp*$^+$ phage, λ*c*I60 (Fig. 3A). However, this effect was only observed at temperatures at which the phage can plate on K-95. Comparison of the growth of λ*c*I60 in the *nus* and *nus*$^+$ hosts shows that the burst of phage decreases and the delay in appearance of phage increases as the temperature of incubation increases (Fig. 3).

The inhibition of λ growth related to increases in temperature we ascribe to the effect of the *nus* mutation on Q expression, since it is overcome by introducing the *byp* mutation. The relatively constant 20-min delay in the appearance of phage we ascribe to the effect of the *nus* mutation on transcription from P_R, since this delay occurs even when the *byp* mutation is present.

Effect of the nus Mutation on Expression of Functions in the N-cIII-int Operon

The operon located immediately to the left of the immunity region, the N-*c*III-*int* operon (see Fig. 1), includes the genetic information for the essential function N as well as the information for a number of nonessential functions (Szybalski and Herskowitz, 1971). Most of the latter functions appear to be involved in lysogeny and recombination (Manly *et al.*, 1969). Trans-

cription of this operon initiates in the immunity region adjacent to the N gene at P_L. Although the N gene can be transcribed in the absence of the N protein, the rest of the operon is not transcribed to any extent in the absence of the N protein (reviewed by Echols, 1971). Since the N product regulates the expression of functions in the N-*c*III-*int* operon, we studied the effect of the *nus* mutation on the expression of some of the functions located in this operon.

The expression of three cistrons (*red* α, δ, or γ) which map in the N-*c*III-*int* operon, inhibit the growth of λ on a host carrying a P2 prophage, the so-called Spi phenotype (Zissler *et al.*, 1971; Lindahl *et al.*, 1970). A λ mutant which is unable to express these functions will plate on such a P2 lysogen. An N-independent phage, λN⁻*nin*, which cannot express N function and consequently does not synthesize *red* α, δ, or γ products, can plate on a P2 lysogen (Court and Campbell, 1972). Therefore, it would be expected that a host mutant which inhibits the expression of N, might allow an N-independent, but N^+, λ to plate even if such a mutant bacterium carried a P2 prophage. We have constructed P2 lysogens of both the *nus* bacterium and its *nus*⁺ parent. The N^+ phage used in this experiment was λ*c*I857*byp*, a phage able to plate on K-95 even at 42°. As shown in Table 1,

TABLE 1

EFFECT OF *nus* MUTATION ON P2 RESTRICTION OF λ GROWTH[a]

Temp (°C)	Phage	Bacteria			
		K-37	K-37·P2	K-95	K-95·P2
32	λ*c*I857*byp*	+	−	+	+
43		+	−	+	+
32	λ*bio*10*nin*5	+	+	+	+
43		+	+	+	+

[a] The *nus* and *nus*⁺ bacteria carrying P2 prophage were grown overnight in absorption broth. Lawns of each lysogen were poured onto tryptone plates using top agar. The top agar was allowed to harden. Plates were divided in half, and a fine wire loop was used to streak a large amount of either λ*c*I857*byp* or λ*bio*10*nin* on the seeded lawn. Plates were incubated at either 32° or 43°. If obvious phage growth was observed it was recorded as +; if no phage growth occurred it was recorded as −.

the control phage, λ*bio*10*nin*, which is deleted for the N-*c*III-*int* operon and is *nin* grows on P2 lysogens of both the *nus* and *nus*⁺ bacterium. The N^+ phage λ*c*I857*byp* which carries the N-*c*III-*int* region is also able to plate on the K-95 (P2) lysogen. However, this phage is unable to plate on the P2 lysogen of the parental *nus*⁺ strain. This experiment suggests that, in the *nus* mutant, λ is unable to express at least three of the functions coded for in the N-*c*III-*int* operon.

The λ *red* α gene codes for the phage exonuclease (Shulman *et al.*, 1970; Signer, 1971) an enzymatic activity which can easily be measured. In order to get some quantitative measure of the expression of functions lying in the N-*c*III-*int* operon, we have measured the levels of exonuclease synthesized following induction of a λ prophage in both the *nus* mutant and its *nus*⁺ parent. The λ prophage carried the temperature-sensitive repressor mutation *c*I857, which permits prophage induction by shifting the lysogen to temperatures above 39° (Sussman and Jacob, 1962). The level of exonuclease product in the *nus* mutant at 42° was determined using lysogens carrying λ*c*I857 prophage. As shown in Fig. 5, induction of λ*c*I857 in the *nus*⁺ bacterium results in the synthesis of λ exonuclease, but induction of the same phage in the *nus* bacterium does not result in the synthesis of λ exonuclease. No λ exonuclease was observed in the latter case even when assayed in extracts prepared 60 min after induction.

It can therefore be concluded that the *nus* mutation acts to inhibit expression of the N-*c*III-*int* operon. Since the N gene also lies within this operon, it is important to determine whether the N gene is expressed to any extent in the *nus* mutant.

Expression of the N Gene Product

Experiments with λ and λ*byp* show that the N function is expressed in the *nus* mutant. Both these phages require N product for growth, and each plates on K-95 at 34°, with λ*byp* plating on K-95 even at high temperatures. Further, neither λ*c*I857*sus* N7N53 nor λ*c*I857*sus*N7N53*byp* plates on K-95 at any temperature—demonstrating that N product is required for λ growth in

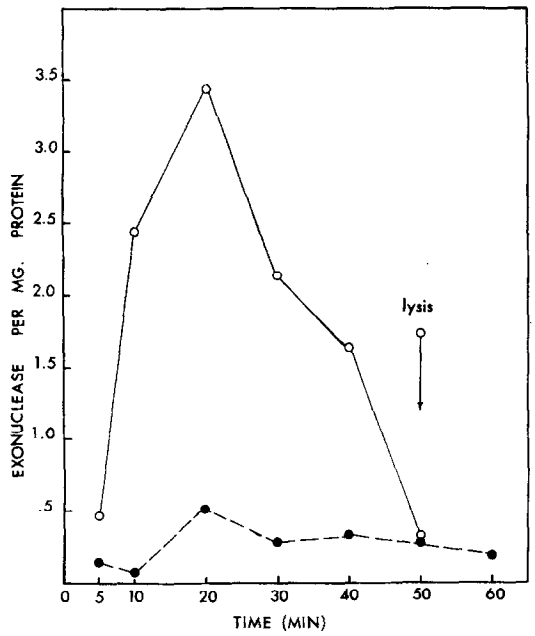

FIG. 5. Synthesis of λ exonuclease in the *nus* bacterium at 42°. *Nus*$^+$ (K-37) and *nus* (K-95) lysogens each carrying a λ*c*I857 prophage were grown in adsorption broth to a concentration of $\sim 10^8$/ml at 34°. Cultures were shifted to 42° and aliquots of 2 ml were removed at the indicated times, immediately cooled in an alcohol ice bath, sedimented by centrifugation, resuspended in 0.01 *M* Tris, pH 7.1, sonicated, cleared of debris by centrifugation, and the supernatants were assayed for exonuclease activity according to the method of Pero (1970). One unit of exonuclease activity solubilized 10 nmoles of ^{3}H-labeled P22 DNA per milligram of protein in 30 min at 37°. The specific activity of the labeled DNA was 25,000 cpm/nmole. ○——○, K-37·λ*c*I857; ●-----●, K-95·λ*c*I857.

K-95. Thus, in the *nus* mutant, the N function is expressed under conditions where other functions in the N-*c*III-*int* operon are not expressed. The fact that N is expressed in the *nus* mutant was demonstrated in another way. A λ mutant, λ*c*I857*sus* N7N53, which is unable to express N function when it infects a nonpermissive host does not kill that host, but does express sufficient replication functions to permit the phage to persist as a plasmid (Signer, 1969; Lieb, 1970). If an infecting λ cannot express N function in *nus*, we would expect it to act like the infecting N$^-$ phage. However, it does not; an infecting λ fails to replicate as a plasmid in K-95 (Friedman, 1971). This observation is consistent with the idea that λ expresses N function in the *nus* bacterium.

If the *nus* mutation acts to lower the expression of N function, then we would expect that a phage which normally expresses lower levels of N function in the *nus*$^+$ bacterium would express even lower levels in the *nus* mutant. λ*c*I857*sex*1 appears to express reduced levels of N function in the *nus*$^+$ host. The *sex* mutation results in a reduced expression of the N-*c*III-*int* operon (Gottesman and Weisberg, 1971). Specifically, the N-mRNA synthesized by this phage is 10-fold lower than that synthesized by the *sex*$^+$ phage (Nijkamp *et al.*, 1970). The single-step growth experiments presented in Fig. 6 show that the growth of λ*c*I857*sex*1 is severely inhibited in the *nus*

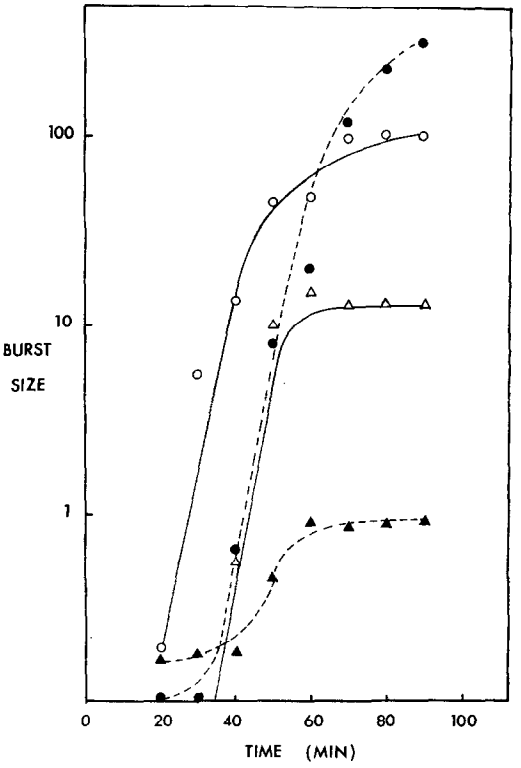

FIG. 6. Growth of λ*sex* in *nus*. The methods of phage growth and determination of burst were the same as outlined for Fig. 2. The experiment was run at 34°. ○——○ K-37 infected with λ*c*I60; ●-----●, K-95 infected with λ*c*I60; △——△, K-37 infected with λ*c*I857*sex*1; ▲-----▲, K-95 infected with λ*c*I857*sex*1.

mutant. These experiments were run at 34°, a temperature at which λ is able to grow in K-95 (see control in Fig. 6).

Studies discussed in the beginning of this section showed that λN⁻ replicates as a plasmid; this implies that if the level of N function expressed by λ*sex* in the *nus* mutant is extremely low, then λ*sex* may replicate as a plasmid in the *nus* host. We have exploited a unique characteristic of the λ plasmid (Friedman and Yarmolinsky, 1972) to determine whether λ*sex* grows as a plasmid. Induction of a λ*c*I857*sus*N7N53 prophage in K-37, a nonpermissive host, is lethal to the lysogen. However, if the lysogen is infected prior to induction with a λ*c*I857*sus*N7N53 phage this lethality is prevented. The lysogen now survives heat induction. This sparing effect was shown to be due to the presence of the infecting λN⁻ phage as a replicating plasmid.

If λ*sex* is unable to express N function in the *nus* host then we would expect that it would replicate as a plasmid in that host. Further, the infecting λ*sex* phage should prevent the lethality due to induction of the λ*sex* prophage. Experiments presented in Table 2 demonstrate that in the *nus* host λ*sex* acts precisely as a λN⁻ has been shown to act in the *nus*⁺ host. An induced λ*sex* lysogen is spared both by infecting λ*sex* and λN⁻ phages. Control experiments with the *nus*⁺·λ*sex* lysogen show that it is not spared by either infecting λ*sex* or λN⁻ phages. We conclude that λ*sex* persists as a plasmid in the *nus* host. Thus the effects of the *nus* and *sex* mutations appear to be synergistic in their effect on λ growth. This is consistent with our contention that the *nus* mutation acts by inhibiting the expression of N function.

It is also interesting to note that although λ*sex* grows in the *nus*⁺ parent, the single-step growth experiment shows that there is about a 20-min delay in the appearance of this phage. This delay roughly corresponds to the 20-min delay seen in the case of infection with λ in the *nus* bacterium. Thus, if the expression of N function is interfered with in either of two ways, by a phage mutation or by a bacterial mutation, there appears to be a mild inhibitory effect on phage development manifested by a delay in the appearance of phage.

TABLE 2

SAVING OF INDUCED LYSOGEN BY INFECTING λ MUTANT[a]

Lysogen	Infecting phage			
	λ*c*I857	λ*c*I857-*sex*1	λ*c*I857-*sus* N7N53	None
K37·λ*c*I857*sex*1	−	−	−	−
K95·λ*c*I857*sex*1	−	+	+	−

[a] Lysogens were streaked onto tryptone plates and allowed to grow overnight at 34°. In each case, a fresh colony was picked and suspended in either a drop of each phage (titer of 5×10^8) or buffer and allowed to stand at room temperature for 20 min. Each isolate was then streaked onto tryptone plates. The plates were incubated overnight at either 34° or 40°. All isolates survived equally well at 34°. The table represents the survival pattern at 40°. Symbols: −, no growth; +, confluent growth.

DISCUSSION

The experiments presented here extend previous studies and show that the *nus* mutation affects all the components of λ growth known to be regulated by the N product.

The observation that the N-independent phage λ*nin* plates on the *nus* mutant under conditions where N-dependent phage do not, suggests that the *nus* mutation inhibits λ growth by interfering with the expression of N function. But two lines of evidence show that some N function must be expressed: (1) A phage which is only partially N-independent, λ*byp*, plates on the *nus* mutant at the restrictive temperature. (2) A phage unable to express N function, the N-defective phage λ*c*I857*sus*N7N53*byp*, cannot grow in *nus*. However, these observations are consistent with the idea that *nus* affects expression of N function, if we assume that *nus* causes lower levels of N function to be expressed. Experiments with λ*sex*, a phage which expresses lower levels of N, in the *nus*⁺ host indicate that this is the case. In the *nus* host the expression of N function by λ*sex* is sharply reduced and the

phage acts as though it were N-defective. In addition, our studies indicate that *nus* has varying effects on the regulation of various components of λ growth which are under N control.

Although a formal model explaining *nus* action has been proposed (Friedman, 1971), it is not presently possible to define the precise mechanism of action of the *nus* mutation. Clearly, the dominance of the Nus^+ phenotype rules out the possibility that the inhibitory effect is due to the synthesis of a product which inactivates the N protein. But what still remains to be determined is at what level *nus* acts; as an inhibitor of N gene transcription or translation or as an inhibitor of N protein action. We have therefore discussed *nus* action in as general a way as possible, as an interference with the expression of N function without any implication of any specific mechanism.

Functions which map distal to N in the N-*c*III-*int* operon do not seem to be expressed in the *nus* bacterium. This was shown by the observation that three genes coded for in this operon, *red* α, δ, and γ, are not expressed in *nus*. Quantitative measurements of the *red* α gene product, exonuclease, showed that this enzyme is not synthesized at any time during the infection at 42°. This complete turn-off of exonuclease synthesis can be explained in either of two ways. The stimulation of synthesis of exonuclease by N function might be totally inhibited by *nus*. Alternatively, *nus* might delay N expression for sufficient time to allow a second regulatory function to act. This function is coded for by the *tof* (or *cro*) gene. The *tof* gene product is known to shut off expression of genes in the N-*c*III-*int* operon during the later part of the phage growth cycle (Pero, 1971). Studies in this laboratory are in progress to distinguish between the alternative explanations.

The observation that N function is expressed under conditions where other functions coded for in the N-*c*III-*int* operon are not, is not surprising. In the absence of N function only the N gene of this operon is transcribed (Kumar *et al.*, 1968; Kourilsky *et al.*, 1968). And as would be expected, the site where N acts to stimulate transcription of the N-*c*III-*int* operon has been mapped to the left of the N gene, downstream from both P_L and N (Franklin, 1971). Therefore, if *nus* inhibits N stimulation of the N-*c*III-*int* operon, we would expect it to affect only the expression of functions downstream from the site of action of N product.

In the case of vegetative functions, the action of the *nus* mutation on N stimulation is not clear. Obviously, *nus* inhibits the expression of sufficient functions to block λ phage production at 42°. The fact that the *byp* mutation relieves part of the block in λ growth in *nus* is taken as strong evidence that N stimulation of Q is inhibited by the *nus* mutation. In addition, the *nin* mutation which also frees Q synthesis from N control also permits λ to grow on *nus* at 42°. However, single-step growth experiments revealed a significant difference between the growth of the two phages in *nus* compared to their growth in the nus^+ host. The appearance of viable phage following infection with λ*nin* occurs at the same time in the two hosts. The appearance of λ*byp* delays about 20 min in *nus*.

The difference in the nature of N-independent growth of λ*nin* and λ*byp* suggests a reason for their differing growth patterns on K-95. While both the *nin* and *byp* mutations free Q expression from N control, only *nin* appears to free expression of functions regulated by P_R from N control (Court, 1970). The *byp* mutation appears only to remove Q synthesis from N control, suggesting that the effect of *nus* on λ*byp* growth must be on N stimulation of transcription initiating at P_R. This conclusion is consistent with other studies presented which show a delay in the expression of the *c*II-O-P operon in *nus*. We have shown that in *nus* there is a 20-min lag in the onset of killing when prophage-induced lethality depends on the expression of functions under P_R control. Since there is evidence that in the absence of N function, the *nin* mutation causes an increased expression of functions under P_R control (Court, 1970), it is consistent, then, to explain the difference in the growth of λ*byp* and λ*nin* in K-95 as a difference in the expression of functions controlled by that promoter.

Thus we suggest that the delay in the appearance of viable phage as well as the delay in prophage-induced killing is due to the same cause, an inhibitory action of the *nus* mutation on the expression of functions under P_R control. The temperature-sensitive step in λ growth in *nus*, we then conclude, is the N stimulated turn-on of Q synthesis. If, as we suspect, N function in *nus* is expressed at a reduced level, it would then imply that the observed delay in the appearance of viable phage reflects the time it takes for λ to express sufficient N function to stimulate transcription from P_R. The additional fact that λ*sex* expresses little or no N function in *nus* leads to the conclusion that the *nus* mutation causes *N* product to be "undersupplied."

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