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# Gene N Regulator Function of Phage $\lambda imm21$: Evidence that a Site of N Action Differs from a Site of N Recognition

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We report the isolation and characterization of a new mutation in the hybrid phage $\lambda imm21$. Both genetic and physiological studies demonstrate that this new mutation, $N_{21-1}$, is similar to N mutations of phage $\lambda$. As in the case of the N gene of $\lambda$ ($N_\lambda$), the $N_{21-1}$ mutation maps immediately to the left of the *c*I gene and has a pleiotropic effect on the expression of phage functions. Although these studies strongly suggest that phage 21 has an N function, they do not definitely locate the $N_{21-1}$ mutation within the $N_{21}$ structural gene.

Reported here are studies demonstrating that $N_{21}$ acts in *trans*, similar to $N_\lambda$, to stimulate the expression of phage functions. N products show an immunity specificity; $N_{21}$ being only active on phage carrying the immunity region of phage 21, while the $N_\lambda$ is only active on phage carrying the immunity region of $\lambda$ or phage 434. However, one site of action for $N_\lambda$ can be rescued from phage 21. We propose that the specificity of an N function is determined by its sites of recognition and that these sites may be different from the sites of N action.

## 1. Introduction

The development of the temperate coliphage $\lambda$ is an orderly process controlled by phage-specified regulator functions (reviewed by Echols, 1971). Negative control is exerted by the repressor, specified by the *c*I gene, which binds to two operators in the vicinity of the *c*I gene (Ptashne, 1971). Since this small region of the $\lambda$ genome, involved in both repressor synthesis and binding, determines the immunity characteristics of the phage, it is referred to as the "immunity region". Hybrid phages which carry genetic material from both $\lambda$ and other related temperate phages have the immunity specificity of the phage from which they inherited their *c*I region. The hybrid phages $\lambda imm434$ and $\lambda imm21$, which are composed primarily of genetic material inherited from $\lambda$ but carry the *c*I regions from either phages 434 or 21, show the immunity specificities of the latter phages (Kaiser & Jacob, 1957; Liedke-Kulke & Kaiser, 1967). The genetic non-homologies observed in this region extend beyond the *c*I gene and the term immunity (*imm*) region is used to include the total length of non-homology between $\lambda$ and any given hybrid phage within and surrounding the *c*I gene (see Fig. 1).

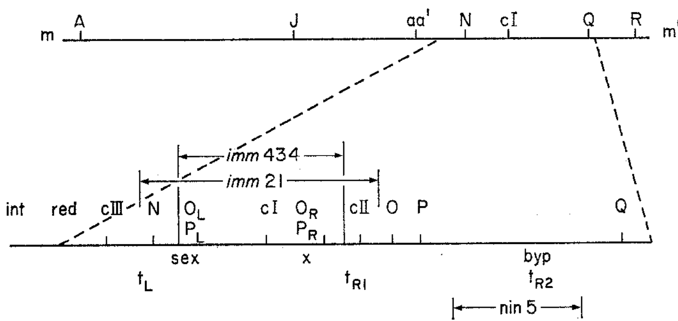


FIG. 1. Genetic map of phage λ showing the immunity region of 21 and 434. The symbol t locates putative N-sensitive transcription termination signals.

Positive control of λ development is exerted, in part, by the N gene product which promotes λ development by stimulating transcription of the phage genome. In the absence of the N function, λ transcription is drastically reduced (Skalka *et al.*, 1967; Kumar *et al.*, 1969; Kourilsky *et al.*, 1968) and λ development is aborted (see Echols, 1971). The N gene maps to the left of the *c*I gene (Fig. 1) and its product can act in *trans* to stimulate expression of λ functions (Thomas, 1966; Dambly *et al.*, 1968; Luzzati, 1970; Couturier & Dambly, 1970). The transcription of three sets of genes is regulated in the following ways (see Echols, 1971): N directly stimulates transcription of the early genes *c*II-P and *c*III-*int* and indirectly stimulates transcription of late genes by turning on synthesis of Q function which, in turn, regulates transcription of late genes. One model for N action, which is consistent with all the experimental data, proposes that N protein acts to stimulate λ transcription by inhibiting transcription termination at specific sites on the λ genome (Roberts, 1969). Genetic and physiological studies have been interpreted as identifying three termination signals on the λ genome (see Fig. 1; Szybalski & Herskowitz, 1971). Accordingly, mutations which permit λ to be N-independent would eliminate or reduce the effect of these terminators. Formally, then, such terminators can be viewed as defining sites of N action.

In the case of hybrid phages, genetic studies show that the N gene is carried by λ*imm*434, but not by λ*imm*21 (Eisen *et al.*, 1966). Functional studies show that N function can be supplied to λ by λ*imm*434 (Dambly & Couturier, 1971; Herskowitz & Signer, 1970*b*; Luzzati, 1970), but not by λ*imm*21 (Couturier & Dambly, 1970; Herskowitz & Signer, 1970b). This suggests that if λ*imm*21 synthesizes an N protein, it differs both structurally and functionally from the N protein of λ.

Experiments with a bacterial mutant, *nus*, which limits λ growth by inhibiting the action of the N gene product, indicated that λ*imm*21 does have an N-like function (Friedman, 1971; Friedman *et al.*, 1973). At 42°C, λ will not plate on the *nus* mutant unless the phage carries a mutation, such as *byp* (see Fig. 1; Butler & Echols, 1970; Hopkins, 1970), which renders λ partially N-independent. Since under the same conditions, the λ*imm*21 phage, 21*hy*5 (Liedke-Kulke & Kaiser, 1967) will plate on *nus* only if it carries the *byp* mutation, it was suggested that λ*imm*21 might have an N-like function (Friedman, 1971). According to the anti-termination model of N action, the *byp* mutation might result in partial N-independence because a termination signal between P and Q is either eliminated or altered.

## 2. Materials and Methods

### (a) *Bacterial and phage strains*

See Table 1.

TABLE 1

*Bacteria*

| Strain | Characteristics | Source and/or reference |
|---|---|---|
| N99 | $sup^0$ | Gottesman & Yarmolinsky (1968) |
| K95 | $nus^-$ | Friedman (1971) |
| C600 | $sup^+2$ | Appleyard (1954) |
| SA462 | $sup^0$ carries λcI857 ∇P-ChlA† | S. Adhya |
| N100 | *rec*A | M. Gottesman |
| N1456 | $sup^+2$ Gro P | M. Gottesman (Georgopoulos & Herskowitz, 1971) |
| K267 | Derived from N99, prophage: λ*imm*21*int*6cIts100*P*am3 | This work |
| K268 | Derived from K267, prophage: λ*imm*21*int*6$N_{21-1}$cIts100*P*am3 | This work |
| K305 | Derived from N99, prophage: 21gp | This work |
| | *Phage* | |
| λ*int*6cI857*P*am3 | | M. Yarmolinsky |
| 21*hy*5 | Carries right-hand region from phage 21 | M. Yarmolinsky; Liedke-Kulke & Kaiser (1967) |
| λcI60 | | M. Gottesman |
| λcI60*nin*5 | | This laboratory; Court & Sato (1969) |
| λcI857*byp* | | This laboratory |
| 21gp | | M. Yarmolinsky |
| λ*imm*434cI⁻ *Q*am117*R*am60 | | M. Gottesman |
| λ*imm*21cI⁻ *Q*am117*R*am60 | | This laboratory |

† ∇P-ChlA is a prophage deletion extending from phage gene P through the bacterial gene ChlA and thus the prophage is deleted for the P–Q region (S. Adhya, personal communication).

### (b) *Media*

Tryptone broth: 1% Tryptone, 0·5% NaCl, 1·0 μg thiamine hydrochloride/ml, $10^{-2}$ M-$MgSO_4$ and 0·2% maltose. Tryptone agar: 1% Tryptone, 0·25% NaCl, 1·0 μg thiamine hydrochloride/ml, and 1·1% agar. Tryptone top agar: 1% Tryptone, 0·5% NaCl, and 0·7% agar.

### (c) *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 nmol of nucleotide in 30 min at 37°C.

### (d) *Endolysin determination*

The method of Butler & Echols (1970) was used.

(e) *Recombination*

Phage recombination was stimulated using u.v. irradiation of infected cultures, 9000 ergs/cm$^2$.

## 3. Results

### (a) *Selection of $N_{21}$ mutants*

In order to determine conclusively whether $\lambda$*imm*21 does have an N-like function, we began a search for mutants of $\lambda$*imm*21 that have properties similar to N mutants of $\lambda$. Fortunately, a simple selection procedure for isolating N mutants of $\lambda$ has already been demonstrated (Gottesman, personal communication). The selection procedure is based on two observations: (1) induction of a $\lambda$ prophage deficient in either of the two phage replication functions, O or P, is lethal to the host (Sly *et al.*, 1968) and (2) this prophage-induced killing depends on the re-action of the N function, since if in addition to the O or P mutation a prophage carries a mutation in the N gene, induction is no longer a lethal event (Eisen *et al.*, 1968; Calef & Neubauer, 1968). The lethality in this case is due to the expression of a function encoded in the N-*c*III*int* operon (Greer, unpublished data). Thus, by starting with lysogens that carry either a $\lambda P^-$ or $\lambda O^-$ prophage, it is possible to obtain bacteria which survive induction because the prophage now has an additional mutation in the N gene. The efficiency of this selection is substantially increased if the prophage carries two additional mutations (Gottesman, personal communication): one in the *int* gene, which reduces prophage excision and therefore eliminates cured cells as a class of survivors (Gottesman & Yarmolinsky, 1968), and a second in the *c*I gene, which results in the synthesis of a thermolabile repressor thereby permitting efficient prophage induction upon shifting to a higher temperature (Sussman & Jacob, 1962).

In order to adapt this procedure for use in the selection of N-like (hereafter referred to as $N_{21}$) mutants of $\lambda$*imm*21, we constructed the following phage, $\lambda$*imm*21*int*6*c*Its100-*P*am3. The *c*Its100 mutant isolated in this laboratory by the method of Rosner (1972) plates turbid at 32°C and clear at 40°C. Further, lysogens carrying this phage are stable at 32°C, but induce at 40°C. A derivative of $\lambda$*imm*21*c*Its100 carrying both the *int*6 and *Pam*3 mutations was constructed by crossing that phage with $\lambda$*int*6-*c*I857*P*am3 and selecting, in the following way, for $\lambda$*imm*21 phage which carried the *P*am3 mutation. The number of phage with the immunity of $\lambda$ in the lysate was reduced by treating with 0·1 M-EDTA (pH 8·1) for ten minutes at 37°C (Parkinson & Huskey, 1971). This procedure selectively destroys phage particles containing relatively large amounts of DNA and since the immunity region of phage 21 is significantly smaller than that of $\lambda$ (Simon *et al.*, 1971), the EDTA procedure can be used to isolate selectively phage with the immunity of 21 from a mixture of $\lambda$ and $\lambda$*imm*21 phages. *P*am3 phage were selected by plating on a lawn of a *sup*$^+$2Gro-P mutant (Georgopoulos & Herskowitz, 1971). *P*am phages will plate on this lawn while $P^+$ phages will not plate. The $\lambda$*imm*21*P*am3 phages thus obtained were then screened to determine if they carried the *int*6 mutation (Gottesman & Yarmolinsky, 1968). A $\lambda$*imm*21*int*6*c*Its100*P*am3 phage was found and was used to lysogenize a *sup*$^0$ strain, N99, using a helper $\lambda$ phage (Gottesman & Yarmolinsky, 1968). One of these lysogens, K267, was shown to carry only a phage with the immunity of 21 and the *P*am3 marker.

Thermoinduction of K267 resulted in lysogen death, with survivors occurring at a frequency of $\sim 10^{-4}$. All of the survivors still carried some prophage genes, since the

$\lambda O^+$ marker could be rescued from these bacteria. In order to determine whether the prophages now carried a mutation in the $N_{21}$ gene, the following screening procedure was used on the assumption that an $N_{21}$ mutation would have characteristics similar to those of N mutants of $\lambda$. We reasoned that a mutation which frees $\lambda$ from N-dependence should permit a $\lambda imm21N_{21}^-$ to grow. The *nin*5 mutation, which maps between the P and Q genes, permits N-independent growth of $\lambda$ (Court & Sato, 1969; Fiandt *et al.*, 1971). *nin*5 can be crossed to $\lambda imm21$, and therefore it seemed likely that viable phage with the immunity of 21 should only be formed when a $\lambda imm21N_{21}^-$ is crossed with a $\lambda nin$ and not when it is crossed with an N-dependent $\lambda$.

Independently isolated lysogens of K267 that survived thermoinduction were screened for the presence of a prophage with an $N_{21}$ mutation by testing their ability to form viable $\lambda imm21$ phage after infection with either $\lambda c$I60 or $\lambda c$I60*nin*5. Of 30 lysogens screened 29 yielded viable phage with the immunity of 21 following infection with either $\lambda c$I60 or $\lambda c$I60*nin*. The reason why these lysogens survived is not known, but the question is currently under investigation. One surviving lysogen, K268, yielded viable phage with the immunity of 21 only when it was infected with $\lambda c$I60*nin*5. No viable phage with the immunity of 21 were obtained when this lysogen was infected with $\lambda c$I60 and the resulting lysate plated on either $sup^+$ or $sup^0$ bacterial hosts. Thus, $\lambda$ can not supply the genetic information missing in this mutant, but the *nin*5 mutation can render this mutant $\lambda imm21$ viable; these are both characteristics that would be expected for an $N_{21}$ mutant. We therefore proceeded to characterize the new prophage mutation ($N_{21-1}$) in order to determine whether the phenotype it exhibited was analogous to that of $N^-$ mutants of $\lambda$.

### (b) *Pleiotropic effects of the* $N_{21-1}$ *mutation*

Genes located in the N-*c*III-*int* operon (Fig. 1) excluding N, are not expressed in the absence of N product (see Echols, 1971). The expression of three genes located in this operon affect the ability of $\lambda$ to grow on a *rec*A host; for such growth to occur, either the $\gamma$ gene or both the *red*$\alpha$ and *red*$\beta$ genes must be expressed (Zissler *et al.*, 1971). Court & Campbell (1972) have shown that a $N^-nin$ does not grow on a *rec*A host, presumably because neither *red* nor $\gamma$ are expressed. We would therefore expect that a $\lambda imm21N_{21}^-nin$ phage would not grow on a *rec*A host. The $\lambda imm21nin$ phage that was obtained by crossing $\lambda c$I60*nin*5 with K268 does not plate on a *rec*A host.

Marker rescue experiments showed that the prophage in K268 does carry the *red*$\alpha$ gene. Since the *red*$\alpha$ gene product, exonuclease, can be assayed directly (Radding, 1966; Pero, 1970), we compared the amount of exonuclease synthesized by the K268 lysogen with the amount synthesized by the K267 lysogen. In order to make the experiments comparable we used a derivative of K268 which had regained immunity after prolonged growth at 32°C. The return of immunity, in this case, was a physiological event and not due to a prophage mutation (Eisen *et al.*, 1970; Eisen & Ptashne, 1971) since, upon induction, this lysogen again loses immunity and only infrequently regains it when grown at low temperatures. Both lysogens were grown at 32°C and induced by shifting to 40°C. As shown in Table 2, following induction K267 does synthesize exonuclease. But K268, the lysogen carrying $\lambda imm21$ with the $N_{21-1}$ mutation does not synthesize detectable levels of exonuclease.

Another $\lambda$ function that can be directly assayed is the R gene product, endolysin (Jacob *et al.*, 1957). The R gene maps to the right of the immunity region (Fig. 1) in an operon that is regulated by the Q function, a function whose synthesis is regulated

TABLE 2

*Biochemical characteristics of induced lysogens carrying* λimm21 *defective prophages*

| Lysogen | Prophage | Time after induction (min) | Exonuclease† (units/mg protein) | Endolysin‡ (Δ0·001 O.D. unit/min/$10^8$ cells) |
|---|---|---|---|---|
| K267 | λ*imm*21 cIts100 *P*am3 *int*6 | 0 | 0 | <3 |
| | | 20 | 0·92 | |
| | | 30 | | 18 |
| | | 40 | 2·0 | |
| | | 60 | 4·8 | 48 |
| | | 90 | | 62 |
| K268 | λ*imm*21 cIts100 *P*am3 *int*6 $N_{21-1}$ | 0 | 0 | 0 |
| | | 20 | <0·1 | |
| | | 30 | | 0 |
| | | 40 | <0·1 | |
| | | 60 | <0·1 | 0 |
| | | 90 | | 0·75 |

Lysogens were grown with vigorous aeration in Tryptone broth to a concn of $10^8$/ml. Induction was effected by shifting cultures to 40°C and continuing the aeration. Samples were removed at the indicated times.

† The methods of preparing cell extracts and assaying for exonuclease have been reported in detail by Friedman *et al.* (1973). One unit of exonuclease activity solubilizes 10 nmol of $^3$H-labeled P22 DNA per mg of protein in 30 min at 37°C. The specific activity of the labeled DNA was 40,000 cts/min/nmol.

‡ The methods used for preparing cell extracts and assaying for endolysin are essentially those described by Butler & Echols (1970). However, lysogens were grown in Tryptone broth and the cell extracts were not dialyzed. One unit of endolysin activity is that amount of enzyme which causes a drop in optical density of 0·001 units/min/$10^8$ cells.

by N protein (Herskowitz & Signer, 1970*a*). The fact that N mutants do not synthesize either exonuclease or endolysin clearly demonstrates the pleiotropic nature of N mutations (Eisen *et al.*, 1966). The $N_{21}$ mutant of λ*imm*21 shows a similar pleiotropy. As shown in Table 2, following induction, K268 does not synthesize detectable levels of endolysin, while under the same conditions, K267 does. Marker rescue experiments showed that the prophage in K268 carries the R gene. Thus, functions located in two different operons are not synthesized by an induced prophage carrying the $N_{21-1}$ mutation.

### (c) *Mapping of the* $N_{21-1}$ *mutation*

In addition to having similar phenotypes, $N_\lambda$ and $N_{21}$ mutants map in the same location, to the left of the *c*I gene. Since the original screening experiments showed that viable λ*imm*21 phage could not be obtained in a cross between the λ*imm*21$N_{21-1}$ prophage and an infecting λ, we conclude that the $N_{21-1}$ mutation lies within the immunity region of 21 (Fig. 1). A three-factor cross was used in locating the position of the $N_{21-1}$ mutation within the immunity region. The two other markers used in

this mapping were the *c*Its100 and *P*am3 mutations. K268 was infected with $\lambda imm21$ and phage which formed clear plaques at 40°C on a $sup^+$ strain (C600) were isolated. These recombinants were then tested to determine if they carried the *P*am3 marker by testing whether these phage could grow on a $sup^0$ strain (N99). Depending on which side of the *c*I gene the $N_{21-1}$ mutation is located, there are two alternative ways by which a single recombination event could form a viable phage carrying the *c*Its100 marker (Fig. 2). If $N_{21}$ lies to the left of immunity, then the majority of

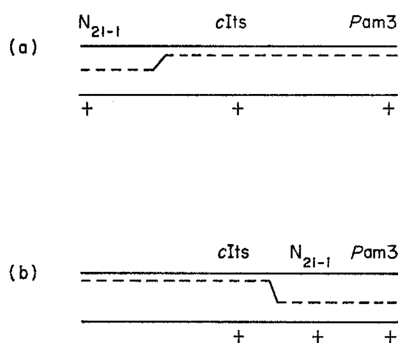


FIG. 2. Mapping of $N_{21-1}$ mutation.

The two theoretical alternative locations for the $N_{21-1}$ mutation are shown in relation to the *c*I and P genes.

(a) If the $N_{21-1}$ mutation is located to the left of the *c*I gene, then a single crossover (shown with dotted lines) will result in the recombinant *c*Its100 phage carrying the *P*am3 allele.

(b) If the $N_{21-1}$ mutation is located to the right of the *c*I gene, then a single crossover (shown with dotted lines) will result in the recombinant *c*I phage carrying the $P^+$ allele.

clear phages formed by a single recombination event will carry the *P*am3 marker. If $N_{21}$ lies to the right of immunity, then a single recombination event will result in the majority of phage carrying the $P^+$ marker. Since 37 out of 40 clear phage could not plate on the $sup^0$ host, presumably because they carried the *P*am3 marker, we conclude that the $N_{21-1}$ mutation maps to the left of the *c*I gene of 21. However, the fact that $N_{21-1}$ is not an amber-type of mutation (it is not suppressible) makes it impossible to determine if the mutation is in the $N_{21}$ gene itself or in a promoter regulating the expression of that gene.

### (d) *Stimulation of gene expression in* trans *by* $N_{21}$

The N gene product of phage λ acts in *trans* to stimulate expression of phage genes (Couturier & Dambly, 1970; Herskowitz & Signer, 1970*b*; Luzzati, 1970). Couturier & Dambly (1970) showed that the N gene function can act in *trans* to turn on the synthesis of endolysin. Expression of this enzyme is only indirectly under the control of N product, since its synthesis is regulated by Q function whose expression, in turn, is controlled by N product (Dove, 1966; Thomas, 1966). In the experiments of Couturier and Dambly a *rec*$A^-$ host was co-infected with two phages, a $\lambda N^-$ and a $\lambda imm434Q^-R^-$ and the production of endolysin was determined. In the case of the co-infection, high levels of endolysin were synthesized, while in the case of single infections with either mutant no endolysin was synthesized. This stimulation was specific to the N gene of λ, since a co-infecting $\lambda imm21Q^-R^-$ did not act to stimulate endolysin synthesis.

TABLE 3

*Induction of endolysin synthesis following infection of an induced lysogen with defective homo- or heteroimmune phage*

| Bacteria | Prophage | Infecting phage | Units of endolysin† |
|---|---|---|---|
| K37 | — | $\lambda imm434$ $Q^-R^-$ | 1·0 |
| K37 | — | $\lambda imm21$ $Q^-R^-$ | 0·6 |
| K268 | $\lambda imm21$ $c$Its100 $N_{21-1}$ | — | 1·2 |
| K268 | $\lambda imm21$ $c$Its100 $N_{21-1}$ | $\lambda imm434$ $Q^-R^-$ | 1·8 |
| K268 | $\lambda imm21$ $c$Its100 $N_{21-1}$ | $\lambda imm21$ $Q^-R^-$ | 35·0 |

Cultures were grown as described in Table 2. However, where indicated they were infected with the appropriate phage at a multiplicity of 10 and incubated for 20 min to effect adsorption before the temperature shift. Samples were removed 30 min after induction, prepared and assayed for endolysin as in the legend to Table 2.

† See footnote ‡ of Table 2.

We have done analogous experiments using K268. This lysogen, which carries the $\lambda imm21$ with the $N_{21-1}$ mutation, was superinfected with either $\lambda imm21Q^-R^-$ or $\lambda imm434Q^-R^-$ and the level of endolysin synthesized was measured. The results of these experiments are given in Table 3 and show that $N_{21}$ can act in *trans* to stimulate the production of endolysin. When K268 was infected with $\lambda imm21Q^-R^-$ and immunity was lifted, significant levels of endolysin were synthesized. As in the case of $\lambda$, there is a specific requirement for a unique N product. In this case only $N_{21}$ is active. This is shown by the fact that under the same conditions superinfection with $\lambda imm434Q^-R^-$, which expresses the N gene of $\lambda$, does not result in the synthesis of endolysin.

### (e) Site of action of $N_{21}$

Since phages carrying the immunity region of 21 express an N function analogous to that of the $\lambda$N function, it might be expected that the sites of action of that product would be the same as those for $N_\lambda$. Experiments with *nus*, a bacterium which inhibits $\lambda$ growth by antagonizing the action of N function, suggest that the $\lambda imm21$ phage used in those experiments, 21*hy*5, has a site of action located between genes P and Q similar to the site in $\lambda$ defined by the *byp* mutation. This region in $\lambda$ appears to be a site of action for N function. But are these putative sites of action similar? That is, can $N_\lambda$ and $N_{21}$ relieve the same transcriptional block? This question was answered in a set of experiments outlined in Table 4. In these experiments we determined whether $byp^+$, a region carrying a site of action for $N_\lambda$, can be rescued from phage 21.

TABLE 4

*Rescue of* byp+ *from phage 21 and* λimm*21*

| Bacterium | Prophage | Infecting phage | Frequency *byp*+ recombinants (%) |
|---|---|---|---|
| K305 | 21 gp | λcI857*byp* | 1 |
| SA462 | λcI857 ∇P-Ch1A | λ*imm*21 | 8 |
| SA462 | λcI857 ∇P-Ch1A | 21gp | 9 |

Lysogens were grown in Tryptone broth to a cell density of about $10^8$/ml at 32°C, infected at a multiplicity of one with the appropriate phage, and then incubated for 20 min at 32°C to permit adsorption. Recombination was stimulated by u.v. irradiation (for method see Materials and Methods). Infected cultures were grown 90 min at 40°C and λ recombinants were isolated by plating the lysates on a lysogen carrying a 21gp prophage. The *byp*+ phenotype was determined by testing whether the isolated recombinants could plate on K95 at 42°C, a temperature at which *byp*+ phage do not plate, while *byp* phage do plate on this host.

The high recombination frequency in the case of infection of strain SA462 in comparison to that found in the infection of K305 is most likely due to the fact that the infected cultures were grown at 40°C, a temperature at which the λcI857 prophage is induced.

In the first set of experiments, a lysogen carrying a 21 prophage was infected with λ*byp*, and λ*byp*+ recombinants were obtained at a frequency of $10^{-2}$. One conclusion that might be drawn from this experiment is that a site sensitive to $N_\lambda$ can be rescued from phage 21. This conclusion assumes that the *byp* mutation removes or alters a site where gene N acts. Alternatively, the *byp* mutation might result in the formation of a new promoter that permits partial N-independence. In this case, recombination with phage 21 would yield λ*byp*+ phage by removing or substituting genetic material for the *byp* mutation. Thus, with the *byp* mutation removed, an already present λ N site would again be active. Genetic studies of Herskowitz & Signer (1970*b*) were interpreted as showing that a recombinant cannot be formed which carries the P to Q region of 21 and the immunity region of λ. These authors concluded that the "P–Q region of phage 21 does not have a suitable site for λ N regulator".

A second set of experiments demonstrates conclusively that a site sensitive to $N_\lambda$ can be rescued from phage 21 as well as λ*imm*21. A lysogen carrying a defective λ prophage deleted for all λ genes distal to the midpoint of the P gene (see Fig. 1) and thus missing completely the P–O region, was infected with phages 21 or λ*imm*21. The viable λ recombinants which were obtained were shown to be *byp*+ (Table 4). Since the λ prophage was deleted for the P–Q region, the *byp*+ information had to originate with either the 21 or λ*imm*21 phage. Thus, a site of action sensitive to $N_\lambda$ can be rescued from phage 21.

## 4. Discussion

These experiments demonstrate that a mutation mapping to the left of the *c*I gene and located within the 21 immunity region results in the same pleiotropic effects on phage physiology as do N mutants of λ. Additionally, the complementation studies demonstrate that $N_{21}$, like $N_\lambda$, can act in *trans*. But the respective N functions are

specific; $N_{21}$ only acts on phage carrying the immunity region of 21 while $N_{\lambda}$ acts only on phage carrying the immunity region of $\lambda$ or 434.

In the light of this specificity, how do we explain the observation that a site recognized by $N_{\lambda}$ can be rescued from phage 21? One possible explanation is contained in a model first proposed to explain certain features of $\lambda$ growth in the *nus* bacterial mutant (Friedman, Jolly, Mural & Wilgus, unpublished results) and subsequently modified at the suggestion of Adhya, Gottesman & De Crombrugghe (personal communication). A similar model has been independently proposed by Waclaw Szybalski (personal communication). We suggest that the sites of action of N product may be different from the sites of recognition†. N products, then, would attach somewhere within the area covered by the immunity region of 21 and outside of the area covered by the immunity region of 434 (see Fig. 1). Since N stimulates transcription, perhaps N product at some point binds with polymerase in a transcription complex. Accordingly, RNA polymerase then travels out from the promoter with the N product subsequently attaching near the promoter, but being able to act at sites distal to the initial recognition site. It should be noted that in this regard, studies by Georgopoulos (1971) and Ghysen & Pironio (1972) suggest that N product does interact with RNA polymerase. The intriguing corollary of this model is that the specificity of N action is determined by the immunity region involved and not by the sites where N may act. As an example, if N acts as an anti-terminator, the N protein would attach near the site of transcription initiation, but block the effect of termination sequences located at sites more downstream. However, there appears to be no relation between repressor and N product specificities. Hilliker & Botstein (personal communication), using complementation and heteroduplex mapping, have shown that although P22 (a temperate phage specific for *Salmonella typhimurium*) and phage 21 have the same repressor, the two phages express different N functions.

Further evidence in support of this model has been obtained by Adhya, Gottesman & De Crombrugghe (personal communication). They have shown that rho-dependent termination in the galactose operon can be overcome by $\lambda$ N function during galactose escape synthesis only if the transcription initiates at the $\lambda$ promoter. This observation demonstrates in another way that in order for $N_{\lambda}$ to relieve a transcription block, transcription must initiate at a $\lambda$ promoter.

It should be noted that $N_{21-1}$ is not an amber-like mutation, and therefore it is not possible to determine whether this mutation is actually in the structural gene specifying the $N_{21}$ function or in a promoter regulating transcription of the $N_{21}$ gene. In either case, the phenotypic manifestations would be expected to be the same and thus the conclusions concerning $N_{21}$ arrived at in this paper are valid. Further, Inokuchi & Dove (personal communication) have isolated amber mutations in $\lambda$*imm*21 that are similar to the $N_{21-1}$ mutation.

The observation that the induced lysogen carrying $\lambda$*imm*21$N^{-}{}_{21}P^{-}$ prophage goes into an anti-immune state suggests that $\lambda$*imm*21 synthesizes an anti-immunity or Cro substance (Eisen & Ptashne, 1971).

In conclusion, the isolation of the mutant discussed in this report confirms studies with the *nus* bacterial mutant (Friedman, 1971), which were interpreted as evidence that $\lambda$*imm*21 expresses an N-like function.

† This model is based on a suggestion of Philippe Kourilsky that certain observations on Cro activity might best be explained by assuming that the site of Cro action is unique from its site of recognition.

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