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Growth of λ Variants with Added or Altered Promoters in N-Limiting Bacterial Mutants: Evidence that an N Recognition Site Lies in the P_R Promoter

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Transcription of the λ genome, initiating at the early rightward promoter (P_R), traverses the *c*II-O-P operon and extends through the Q gene. In the absence of the λ N function, this transcription is prematurely terminated at either of two termination sites, t_{R1} and t_{R2}. The *c*II-O-P operon lies distal to t_{R1}, but proximal to t_{R2}. A number of mutations resulting in new promoter activities (e.g., *c*17 and ri^c5b) mapping distal to t_{R1}, but proximal to t_{R2}, have been isolated.

Although phages carrying the *c*17 mutation grow in a normal *Escherichia coli* host, we find that λ derivatives carrying this mutation will not grow in mutant *E. coli* K12 hosts, Nus^-, which limit λ growth by inhibiting the expression of N function. However, under the same conditions, a λ phage containing only the normal λ promoters grows significantly better in the Nus^- hosts. Our studies demonstrate that under conditions of limited N expression, phage carrying the *c*17 mutation can express functions coded for by genes in the *c*II-O-P operon, but not endolysin, a function coded for by a gene distal to t_{R2}. Thus, under conditions of low N activity, functions whose genes lie downstream from the *c*17 promoter without any intervening termination signals are expressed. On the other hand, functions whose genes lie downstream from this promoter with an intervening termination signal are not expressed.

These results are consistent with a model of N action, which has N acting only with transcription initiating at a specific class of promoters (e.g., P_R), *c*17 not being a member of this class. Although previous studies (Friedman and Ponce-Campos, 1975) have shown that the ri^c5b promoter is also not a member of the N-utilizing class of promoters, we find that λri^c5b grows on Nus^- hosts. This suggests that whereas *c*17 interferes with transcription from P_R, ri^c5b does not show such an interference.

We also find that λ variants carrying two mutations v_3 and vs_{326}, which map in the O_R − P_R region, exhibit the same growth characteristics in Nus^- hosts as phages carrying the *c*17 mutation. These observations imply that the combination of v_3 and vs_{326} interfere with N-modification of transcription initiating at P_R, and lead us to conclude that one site for N recognition is located within the P_R promoter.

INTRODUCTION

During growth of phage λ, transcription of the early regions of the phage genome initiates at two promoters, P_R and P_L (see Fig. 1). The transcription initiating from these two promoters terminates early unless a phage-specified function encoded by the N gene is present. For example, in the case of transcription of the *c*II-O-P operon, transcription initiating at P_R (Fig. 1) terminates close to that promoter, at t_{R1}, in the absence of N product, but proceeds outward toward Q in the presence of *N* product (reviewed by: Echols, 1971; Herskowitz, 1973). Another N-sensitive termination signal, t_{R2}, appears to be located to the right of the immunity region and downstream from t_{R1}. The existence of this site is inferred from the observation that a class of mutants, *byp* and *nin*, can be iso-

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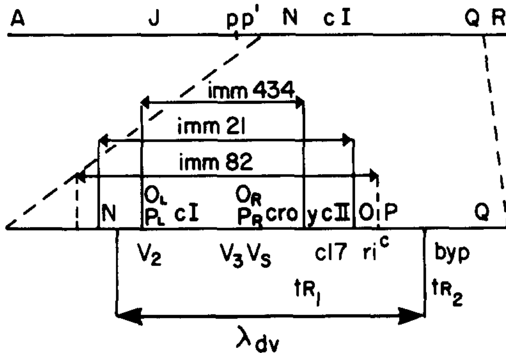


FIG. 1. Genetic map of λ showing the immunity regions of 434, 21, and 82 as well as the genetic extent of the λ*dv* plasmid. The symbol t locates the "so-called" transcription termination sites.

lated which free Q synthesis from N control and map in the P-Q region (Hopkins, 1970; Butler and Echols, 1970; Court and Sato, 1969).

Roberts has proposed that the N product acts as an "antiterminator" which inhibits the action of a host-specified termination function, "Rho" (Roberts, 1969). According to this model, Rho terminates transcription of the λ genome at specific sites (i.e., t_{L1}, t_{R1}, and t_{R2}; see Fig. 1) and N product permits continuation of transcription by inhibiting this Rho-specified termination.

The dependence on N for transcription of the *c*II-O-P operon can be overcome if the phage carries the *c*17 mutation. This mutation has been mapped in the y region, beyond t_{R1} (see Fig. 1), and has been shown to be a new promoter which permits N-independent transcription of the *c*II-O-P region (Packman and Sly, 1968). It might be expected, then, that under conditions of low N expression, phage carrying the additional *c*17 mutation would grow better than phage which carry the N-dependent promoter P_R alone. Experiments using an *E. coli* host carrying a mutation in the *nus* A gene, indicate that the opposite is the case, namely, under conditions of low N expression, the *c*17 promoter interferes with λ growth.

The *nus* A gene maps near minute 61 of the *E. coli* chromosome (Friedman and Baron, 1974). Only one mutation in the *nus* A gene (*nus* A-1) has been studied and that initially appeared to be temperature sensitive, since λ could form plaques on hosts carrying *nus* A-1 at 32°, but could not form plaques on the same host at 42° (Friedman, 1971). Later studies showed that even at 32°, the *nus* A-1 mutation reduced the activity of the N product (Friedman, Jolly, and Mural, 1973). Surprisingly, phage carrying the *c*17 mutation were found to be unable to grow on the *nus* A-1 mutant even at the low, "permissive," temperature (Friedman, 1971). Thus, under conditions of reduced N function, the *c*17 mutation interferes with λ growth.

We now report studies which indicate that in bacteria carrying *nus* mutations, the *c*17 promoter functions well, in fact too well to support λ growth. These paradoxical findings can be explained in terms of a model of N action, which has previously been outlined in another context (Friedman *et al.*, 1973). According to this model, the N protein attaches to a transcription complex at or near either of the two λ promoters P_R or P_L. Once attached the N product modifies polymerase so that transcription can transcend distal termination signals, sites of N action being distinguishable from sites of N recognition. We have previously shown that transcription initiating at the *c*17 promoter cannot utilize N product, since even in the presence of N, transcription initiating at *c*17 cannot pass through the presumed terminator sequence in the P-Q region, t_{R2} (Friedman and Ponce-Campos, 1975). We propose that in the case of λ*c*17 infecting *nus* mutants (where N function is reduced), little transcription passes t_{R2}, because most transcription of the *c*II-O-P operon can be viewed as initiating from the *c*17 promoter.

This effect of limited N expression is not unique to phage carrying the *c*17 mutation. We show a similar effect on the growth of λ variants carrying the v_3 and vs_{326} mutations. The *vs* mutations occur in a new class of λ mutants, "supervirulent," or "supervir," which have been selected using a host bacterium carrying an abbreviated λ plasmid, λ*dv* (Ordal, 1971). The *dv* plasmid contains the *c*I gene as well as replication genes O and P including the site of initiation of DNA synthesis, *ori*

(Fig. 1; Matsubara and Kaiser, 1968). Strains carrying the λ*dv* plasmid, contain around 50 copies of the plasmid/bacterium. Although λ*vir*, a phage carrying classical "virulence" mutations ($v_1v_2v_3$), mutations which permit λ growth in the presence of λ repressor, does not grow in *dv* containing strains (Matsubara and Kaiser, 1968), "virulent" mutants derived from λv_2v_3[1] can be isolated which do grow in *dv* strains. Mutations conferring supervirulence, called *vs*, map in or near O_R and between two P_R mutations, x_3 and x_{13} (Ordal and Kaiser, 1973; Ordal, 1971). Direct sequence analysis has placed the vs_{326} mutation in the O_R1 region (Ptashne, personal communication). Since the combination of the v_3vs_{326} mutations causes a reduction in N-utilization for rightward transcription, we argue that there is a site within P_R involved in N-recognition.

MATERIALS AND METHODS

Media. Tryptone media used has been previously described (Friedman, Jolly and Mural, 1973). M-9-Mal-CAA medium contains (per liter) 6 g of Na_2HPO_4, 0.5 g of NaCl, 3 g of KH_2PO_4, 1 g of NH_4Cl, 0.25 g of $MgSO_4$, 2 g of Maltose, and 0.25 g of Casamino acids.

Bacterial strains and phage stocks. The following *E. coli* K12 derivatives were used: K-37, is the nus^+ progenitor of both *nus* mutants (Friedman, 1971); K-95, which carries the *nus*-1 mutation that maps in the *nus* A locus at min 61 (Friedman *et al.*, 1973; Friedman and Baron, 1974). For clarity of discussion the *nus*-1 mutation will be referred to as the *nus* A-1 mutation. K-450, which carries the *nus* B-5 mutation and maps at min 11, will be discussed elsewhere (Friedman, Baumann, and Baron, in preparation). Lysogens of K-37 carrying λ and λ*imm*82 were constructed in this laboratory. The λ*dv* containing strain KM 424 (Matsubara, 1972) was obtained from D. Jackson. Phage stocks and sources are listed in Table 1.

[1] Although not definitively proven, the reader should be aware of the fact that the v_3 mutation associated with the vs_{326} mutation appears to be located in O_R2, while the classical v_3 mutation is located in O_R1 (Ptashne, personal communication).

TABLE 1[a]

Phage	Source
λ*c*I*c*17	M. Yarmolinsky
λ*c*I*c*17*Oam*29	W. Sly
λ*c*I*c*17*Pam*3	W. Sly
λ*imm*82	W. Dove
λ*rt*[c]5b	W. Dove
λ*c*I60	M. Gottesman
λ*imm*434	M. Yarmolinsky
λ*c*I60*byp*	This Laboratory
λ*c*17*c*II	A. Oppenheim
λ*c*I*c*17*byp*	This Laboratory
$\lambda v_2v_1v_3$	M. Yarmolinsky
$\lambda v_2v_3vs_{326}$	D. Jackson
λv_2vs_{326}	G. Gussin
λv_3vs_{326}	This Laboratory
λ*imm*434*c*17	M. Gottesman
λ*c*I*c*17	M. Gottesman
λ*imm*21 (hy5)	M. Yarmolinsky
λv_3vs_{326}*byp*	This Laboratory
λv_2v_3	S. Flashner
λv_3	S. Flashner
λvs_{326}	G. Gussin
λv_2h	M. Yarmolinsky

[a] We find that lysates of λ "supervir," which are propagated on non-λ*dv*-carrying bacterial strains, contain a high level (5%) of non-"Super vir" revertants. Lysates of λ "Super vir" with low levels of such revertants can be obtained by propagating phage in a mixed culture of KM424 (a λ*dv* carrying strain) and K-37.

DNA-DNA hybridization. The techniques of labelling and isolation of DNA, as well as the preparation of DNA-bound filter membranes and hybridization, were those described by Packman and Sly (1968). The only modifications were: (1) the temperature at which the infected cells were cultivated, 34°; (2) the medium, M-9-maltose-CAA; (3) the period of labelling, 20th to 23rd min following infection.

Endolysin. Levels of λ endolysin were measured according to the method of Butler and Echols (1970).

RESULTS

Effect of the nus A-1 mutation on the growth of λc17. E. coli K12 strains carrying the *nus* A-1 mutation (such as strain K-95) permit growth of λ at 34°, but in general, severely inhibit the growth of λ derivatives which carry the *c*17 promoter mutation at this same temperature. It

should be noted that single-step growth experiments indicate that even at 34° the *nus* A-1 mutation adversely affects λ growth (Friedman, Jolly, and Mural, 1973). As shown in Fig. 2, at the low temperature the burst of λ is delayed approximately 20 min in a Nus⁻ bacterium when compared to the burst in a Nus⁺ host.

Similar single-step growth experiments (Fig. 2) demonstrate that phages carrying the *c*17 mutation (in this case λcIc17) do not grow to any extent in a Nus⁻ host at 34°, a condition where, as previously discussed, λ can grow. In Nus⁺ strains (such as K-37), phages carrying the *c*17 mutation are able to grow. However, it should be noted that even in Nus⁺ strains the growth of *c*17 phages is less than optimum (see Fig. 2).

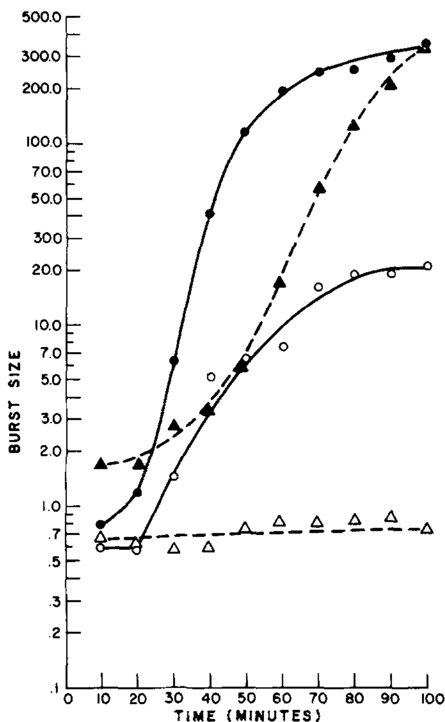


FIG. 2. Burst of λ*c*17 in the *nus* A-1 host. Bacterial strains, K-95 (*nus* A-1) and K-37 (*nus*⁺), were grown in adsorption broth and diluted to a final concentration of 10^8/ml. The bacteria were infected with either λcI60 or λcIc17 at a multiplicity of infection (m.o.i.) of 0.1. Adsorption was effected by incubating the cells for 15 min at 34°. Infected cells were then diluted into adsorption broth, incubated at 34°, and aliquots were removed at the indicated time and a few drops of chloroform were added. The lysed samples were plated using a K-37 lawn in top agar on tryptone plates. Burst size was computed as phage released per infected bacterium. ●-●-●, K-37 infected with λcI60; ○-○-○, K-37 infected with λcIc17; ▲-▲-▲, K-95 infected with λcI60; △-△-△, K-95 infected with λcIc17.

That this restrictive effect of the *c*17 mutation is not limited to the specific case of λcIc17 is evidenced by the observations that the growth of phages λcI⁺c17, λc17cII⁻, as well as a *c*17 derivative of the hybrid phage λ*imm*434 are all similarly inhibited by the *nus* A-1 mutation. The parent hybrid phage λ*imm*434, genetically λ except for the immunity region which derives from phage 434 (Fig. 1; Kaiser and Jacob, 1957), grows in the *nus* A-1 host.

Since we found no evidence of phage production following infection of K-95 with phages carrying the *c*17 mutation, we next determined if a phage carrying this mutation could express any functions in a Nus⁻ host.

Expression of λ replication functions by λc17 in the nus A-1 host. Studies by Packman and Sly (1968) demonstrated that λ phage carrying *c*17 can express replication functions O and P even in the absence of N product. They showed, in a number of ways, that λcIc17 replicated even in the presence of λ repressor, a condition where N function cannot be expressed. It therefore seemed likely that if phages carrying the *c*17 mutation expressed any functions in a Nus⁻ host, such phages would express replication functions. The extent of λcIc17 replication in the Nus⁻ host at 34°, a temperature where λ growth is permitted, but λc17 growth is not permitted, was determined in two ways, one biological and a second biochemical.

(a) *Biological evidence that λcIc17 replicates in K-95.* The design of this experiment is based on two assumptions. First, if λcIc17 replicates in the Nus⁻ bacterium, then it might be possible to supply missing functions in trans and thus package these DNA copies with a coinfecting ("helper") phage. Of course, this assumes that the helper phage is able to grow normally in the Nus⁻ host. Second, it is necessary to insure that the burst of λcIc17 actually reflects the level of its own self-directed

replication. This can be accomplished by using a helping phage which cannot supply replication functions to the *c*17 phage.

Another hybrid phage, λ*imm*82, a phage which carries the immunity region of phage 82 (Fig. 1; Dove *et al.*, 1971; Simon, Davis, and Davidson, 1971), and some genetic material of λ fulfills the above listed criteria for a proper "helper" phage in this experiment. Thus, λ*imm*82 which grows well in a Nus^- bacterium even at 42° cannot supply replication function O to a λO^- mutant (Dove *et al.,* 1971). However, λ*imm*82 can supply replication function P to a λP^- mutant. The growth of λ*imm*82 and λ*c*I*c*17 in K-95 is depicted in Fig. 3. Although λ*c*I*c*17 by itself produces no measurable progeny in the Nus^- host, in a coinfection with λ*imm*82, λ*c*I*c*17 produces a significant burst of progeny.

The burst of *c*I*c*17 in the presence of λ*imm*82 might be explained in two ways: (1) The *c*17 genome is passively replicated by λ*imm*82, such as by the formation of a tandemly joined λ-λ*imm*82 genome. (2) The λ*c*I*c*17 replicates independently, the role of the coinfecting λ*imm*82 merely being a supplier of functions necessary to process and (or) package copies of replicated *c*17 DNA. That the latter is the likely explanation is evidenced by the following experiments, also depicted in Fig. 3. When K-95 is coinfected with λ*imm*82 and λ*c*I*c*17O*am*29, there is only a rise in titer of the λ*imm*82. On the other hand, if K-95 is coinfected with λ*c*I*c*17P*am*3 and λ*imm*82 there is a rise in titer of both phages. The *c*17 phage appears in the burst only under conditions where it has available the functions it needs for self replication. Thus, the λ*imm*82 helping phage is not replicating the λ*c*I*c*17, but on the contrary, these experiments imply that λ*c*I*c*17 is capable of directing its own replication in the Nus^- host, an implication confirmed by direct measurement of λ DNA synthesis.

(b) *Biochemical evidence that λcIc17 replicates in K-95.* The synthesis of λ DNA can be directly measured by the technique of DNA-DNA hybridization (Joyner *et al.*, 1966; Packman and Sly, 1968). We have utilized this technique to determine if

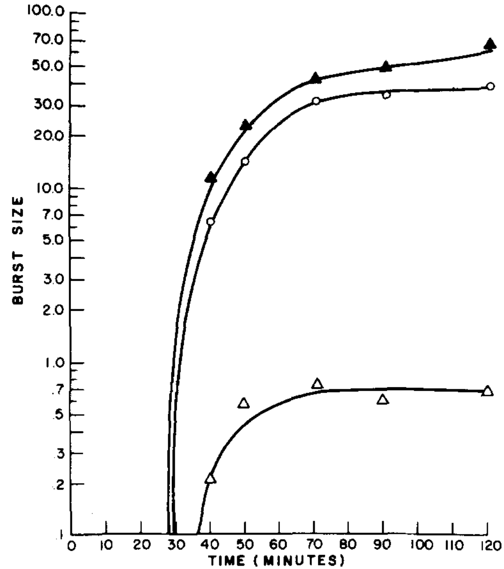


FIG. 3. Burst of λ*c*17 in the presence of a λ*imm*82 helping phage. The methods of phage growth and determination of burst were similar to those outlined in the legend for Fig. 1. In this case, bacteria (K-95) were coinfected with an m.o.i. of 5 of each phage type, and grown at 34°. The burst of λ was assayed on bacteria lysogenic for phage 82. Each determination is from a coinfection of a λ*c*17 variant with λ*imm*82 in the *nus* A-1 host, K-95. ▲-▲-▲, λ*c*I*c*17; O-O-O, λ*c*I*c*17 *Pam*3; △-△-△, λ*c*I*c*17 O*am*29.

λ*c*I*c*17 replicates in the Nus^- bacterium. In Table 2 are listed the results of an experiment in which the amount of λ DNA synthesized is measured following infection of a Nus^- host at 34° with either λ*c*I*c*17 or λ*c*I60. These results confirm that λ DNA is synthesized in the Nus^- bacterium following infection with λ*c*I*c*17. It is also evident that the level of *c*17 replication is relatively high since the amount of *c*17 DNA synthesized is comparable to the amount of λ*c*I DNA synthesized in the Nus^- host. It should be remembered that under these conditions, λ*c*I infection results in a substantial burst of phage.

Thus, in two ways we have demonstrated the replication of a phage carrying the *c*17 mutation in a Nus^- host. But, we are still faced with the question of why under these same conditions λ*c*I*c*17 is unable to direct the synthesis of viable phage progeny.

Effect of the c17 mutation on λ endolysin production in the nus A-1 host. As shown in Fig. 1, the O and P genes lie adjacent to the *c*17 promoter, with no intervening termination signal. Since the experiments in the previous section showed that the O and P products were expressed by a *c*17 phage in a Nus^- host, we next asked whether a gene distal to an intervening termination signal could be expressed by *c*17 phage. The putative N-sensitive termination signal t_{R2} lies between the *c*17 promoter and the R gene (Fig. 1). In these experiments, the level of expression of the R gene product, endolysin, was measured following infection of the Nus^- host. The data listed in Table 3 show that in the Nus^- host at 34°, λ*c*I*c*17 does not synthesize any measurable endolysin. Control experiments show that λ*c*I60 synthesizes endolysin in the Nus^- host and that λ*c*I*c*17 synthesizes endolysin in the Nus^+ host.

TABLE 2

DNA-DNA HYBRIDIZATION OF [^{3}H]DNA ISOLATED FROM *nus* A-1 BACTERIA INFECTED WITH λ[a]

Infecting phage	Percentage of [^{3}H]DNA retained by filters carrying:		
	λ DNA (25 μg)	*E. coli* DNA (100 μg)	No DNA
λ*c*I*c*17	20.3	1.7	0.7
λ*c*I60	21.0	3.1	1.3

[a] Labelled DNA and DNA-bound filters were prepared according to the method outlined by Packman and Sly (see Materials and Methods). In each case, 25 μg of labelled DNA (of approximately equal specific activities) were added to filters which had been preincubated in buffer, and incubation was continued for 12 hr at 65°. Filters were dried and counted to determine the percentage of radioactive label retained by the filter. Infection was carried out at a m.o.i. of 5. Nus^- bacterium was the *nus* A-1 mutant, K-95.

Growth of λc17 byp in the nus A-1 host. The preceding observations suggest that under conditions of reduced N-expression (in the Nus^- host at 32°), phage carrying the *c*17 promoter are unable to overcome the effect of the t_{R2} terminator. If this is true, then a mutation which reduces or eliminates the effect of t_{R2} should permit a *c*17 phage to grow in a Nus^- host. The *byp* mutation mapping between genes P and Q, in the region of t_{R2}, appears to inhibit the effect of the t_{R2} terminator. This is inferred from the observation that phages which carry both *c*17 and *byp* can grow independently of N function (Hopkins, 1970; Butler and Echols, 1970); it is argued that *c*17 renders cII-O-P expression N-independent and *byp* renders Q product

TABLE 3

EFFECT OF *nus* A-1 MUTATION ON EXPRESSION OF PHAGE FUNCTIONS BY VARIOUS DERIVATIVES OF λ[a]

Phage tested	Functions assayed 90-min postinfection				
	Phage replication[b]	Endolysin[c]		Total phage yield[d]	
		K-95 (Nus^-)	K-37 (Nus^+)	K-95 (Nus^-)	K-37 (Nus^+)
λ*c*I60	ND	170	255	200	200
λ*c*I*c*17	30	<2	74	0	20
λ*c*I*c*17*byp*	ND	ND	ND	35	30
$\lambda v_2 v_3 vs_{326}$	23	<2	60	0	70
$\lambda v_2 v_1 v_3$	ND	111	436	100	100
λrt^c5b	ND	383	549	17	200

[a] In the cases of endolysin synthesis and total phage yield, the amount produced in the *nus* A-1 host (K-95) was compared to that produced in a nus^+ host (K-37). All experiments were carried out at 34°. ND means not done.

[b] Replication was determined by calculating the burst of the tested phage at 100 min in the presence of λ*imm*82 (see legend to Fig. 3). In each case, single-step growth curves were very similar to those shown in Fig. 3.

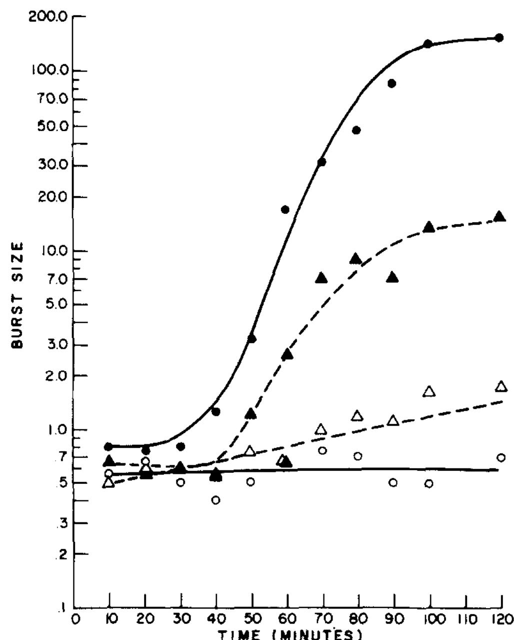
[c] Total endolysin (in cells and supernatant) was determined 90 min following infection. An m.o.i. of 5 was used throughout. One unit of activity = Δ 0.001 OD unit-min/10^8 cells.

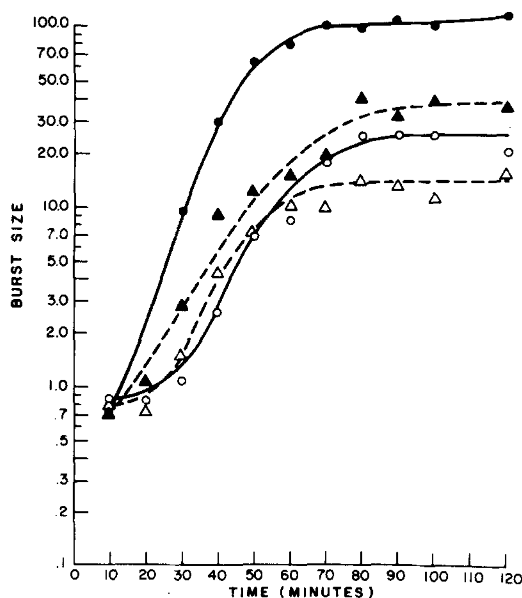
[d] The burst of phage was calculated per infected bacterium. An m.o.i. of ~0.1 was used throughout.

expression N-independent. Since the Q product, in turn, stimulates transcription of late genes (Herskowitz and Signer, 1970; Couturier, *et al.*, 1973), all functions needed for vegetative phage growth can be expressed in the absence of N. Therefore, we can ask whether the elimination of the effect of t_{R2} will permit a *c*17 phage to grow in a Nus^- host, by determining if the addition of the *byp* mutation will permit a *c*17 phage to grow in a Nus^- host. We find that λ*c*I*c*17*byp* can grow in the Nus^- host, K-95, at 34°. The phage gives a burst of 35 at 90 min following infection (Table 3). In addition, as expected for an N-independent phage, λ*c*I*c*17*byp* grows in the Nus^- host even at the high, "nonpermissive" temperature.

Dominance of the c17 promoter in a Nus^- host. We have seen that λ*imm*82 can serve as a "helper" in permitting production of viable *c*17 phage in a Nus^- host. However, similar complementation studies with phage more closely related to lambda (λ*imm*434 and λ*imm*21 as well as λ itself) demonstrate that these phages, unlike λ*imm*82, cannot serve a "helper" function in permitting the production of *c*17 phage in a Nus^- host. These latter phages differ from λ*imm*82 in that they do not grow in the *nus* A-1 host at 42°. Further, coinfection experiments demonstrate that the *c*17 phage actually inhibits their growth in the *nus* A-1 host at 34°. As shown in Fig. 4, in single-step growth experiments the *c*17 phage reduces the burst of the second phage, in this case λ, by a tenfold factor. In control experiments in a nus^+ host, the *c*17 phage exerts a substantially smaller negative influence on the coinfecting phage, about a twofold effect (Fig. 5). Very similar results are found if either λ*imm*21 or λ*imm*434 is used as the coinfecting phage.

Effect of the nus A-1 mutation on growth of phage carrying the v_3vs_{326} mutations. The location of *vs* mutations in the P_R region suggests that one way this class of mutations might exert its effect on phage growth is by influencing transcription initiating at P_R. We have already seen that growth of phage carrying a new promoter for rightward transcription, *c*17, is





FIGS. 4 and 5. Effect of λ*c*17 on a coinfecting phage. Bacteria, K-37 in Fig. 4 and K-95 in Fig. 5 were either singly infected with λ*c*I60 and λ*c*I*c*17 or coinfected with both of these phages. In each instance an m.o.i. of 5 was used. Burst sizes were determined according to the procedures outlined for Fig. 2. The temperature of growth was 34°. ●-●-●, λ*c*I60 alone; ▲-▲-▲, λ*c*I60 in presence of λ*c*I*c*17; ○-○-○, λ*c*I*c*17 alone; △-△-△, λ*c*I*c*17 in presence of λ*c*I60. The two phages were selectively distinguished by plating for λ*c*I60 on the *nus* A-1 host at 34° and for λ*c*I*c*17 on a K-37 λ lysogen at 40°.

severely inhibited at low temperatures in the *nus* A-1 host. Therefore, it seemed reasonable to assume that if *vs* mutations altered the normal pattern of transcription initiating at P_R, a similar restriction on phage growth in the Nus^- host might be observed. Indeed, we find that the one "supervir" mutant tested, $\lambda v_2 v_3 vs_{326}$, has growth characteristics in the Nus^- host (K-95) similar to *c*17-containing phage. Thus, as shown in Table 3 this "supervir" phage grows poorly in a Nus^- host at 34°, while the "classical" virulent mutant, $\lambda v_2 v_1 v_3$, grows well in the mutant host at the low temperatures. Functional studies show that λ "supervir" replicates in the Nus^- host at 34°, but produces little endolysin (Table 3). In the presence of λ*imm*82 substantial λ "supervir" progeny phage are produced in a Nus^- bacterium. Control experiments demonstrate that even in the presence of λ*imm*82, λ "supervir" O^-, does not produce progeny phage. Following the argument outlined above regarding replication of λ*c*17, we interpret these latter experiments as demonstrating that λ "supervir" replicates in the Nus^- host under conditions where it cannot express late gene products, such as endolysin.

Since λ "supervir" carries three mutations, we next determined which of these mutations were responsible for the inhibition of growth in the Nus^- host. A variety of phages carrying different combinations of the v_1, v_2, v_3, and vs_{326} mutations were tested. When the efficiency of plaque formation in a Nus^- host (K-95) is compared to that in a Nus^+ host (K-37) at 34°, we find (Table 4) that growth on the *nus* A-1 mutant at 34° is inhibited only when a phage carries both the v_3 and vs_{326} mutations. Therefore, we conclude that the inability of "supervir" to grow in the Nus^- host at low temperature results from an effect caused by a combination of the v_3 and vs_{326} mutations.

If this inhibition of growth results from a reduction in N expression (similar to that seen in the case of *c*17), we would expect that combining the *byp* mutation with $v_3 vs_{326}$ should permit phage growth in the *nus* A-1 host. This prediction was confirmed by experiments comparing the plating of $\lambda v_3 vs_{326}$ *byp* on the Nus^- host with that on the Nus^+ host. As shown in Table 4, this phage plates almost as efficiently on Nus^- as it does on Nus^+ (e.o.p. = 0.8).

TABLE 4

EFFECT OF *nus* A-1 MUTATION ON GROWTH OF λ DERIVATIVES CARRYING VARIOUS VIRULENCE MUTATIONS[a]

Phage	Titer on K-95/titer on K-37
$\lambda v_2 v_3 vs_{326}$	1.8×10^{-4}
$\lambda v_3 vs_{326}$	1.3×10^{-4}
$\lambda v_2 v_1 v_3$	1
$\lambda v_2 v_3$	0.5
λv_3	0.9
λvs_{326}	1
λv_2h	1
$\lambda v_2 vs_{326}$	1
$\lambda v_3 vs_{326}$*byp*	0.8

[a] Phage were titered using either K-95 (*nus* A-1) or K-37 (nus^+) as the indicator lawn. Plates were incubated at 33°, and the titer of each phage on K-95 and K-37 was compared.

Effect of the nus A-1 mutation on growth of phages carrying the ri^c*5b mutation.* Another class of promoter mutants, ri^c (replication inhibition constitutive), have been selected which permit constitutive transcription of some of the functions coded for by the *c*II-O-P operon (Dove *et al.*, 1969; Dove, Inokuchi and Stevens, 1971). One of these mutations, ri^c5b, maps in the O gene and permits constitutive transcription of the P gene. Unlike the case of phage carrying the *c*17 mutation, phage carrying the ri^c5b mutation produced progeny in the Nus^- host (K-95) at low temperature (Table 3). The burst of λri^c5b in the Nus^- host at 34° is significant, but it should be noted that it is still lower than that observed in the case of the infection of the Nus^+ host. A more compelling demonstration that the growth of λri^c5b in Nus^- is different from that of λ*c*17 is the level of endolysin synthesized in the Nus^- host at 34° by each of these phages. λ*c*17 synthesizes no endolysin, while λri^c5b synthesizes almost as much as it does in the Nus^+ host.

Effect of nus B-5 mutation on the growth of various λ derivatives. To test our assumption that the observed effects of the

nus A-1 mutation on the growth of the various λ derivatives is due to a reduction in N expression and not some anomalous effect specific to the *nus* A-1 mutation, we tested the growth of the phages in question on another *nus* mutant, K-450. Although K-450 displays a phenotype almost identical to that of the *nus* A-1 mutant, the mutation in K-450 maps at another locus, *nus* B, on the *E. coli* chromosome, at min 11 (Friedman, Baumann, and Baron, in preparation). As shown in Table 5, the effect of this *nus* B mutation (*nus* B-5) on the growth of phage carrying *c*17 or v_3vs_{326} is similar to that of the *nus* A-1 host. Moreover, it can be seen that other phages, λcI⁻, $\lambda v_2v_1v_3$, and λri^c5b, which plate on the *nus* A-1 (K-95) host, also plate on the *nus* B-5 (K-450) mutant.

DISCUSSION

Previous studies have shown that the *nus* A-1 mutation, which maps in the *nus* A locus, inhibits λ growth by interfering with the action of the N gene product at t_{R1}, t_{R2} and t_L (Friedman, 1971; Friedman, Jolly, and Mural, 1973; Friedman and Baron, 1974). These studies demonstrated a temperature effect of the *nus* A-1 mutation; at 34° λ is able to grow, but at 42° λ is unable to grow in the Nus⁻ host. However, phage producing low levels of N function were unable to grow in this mutant host, even at low, permissive temperatures. This suggests that even at 34°, the N protein is less active in a Nus⁻ host. One peculiar finding in these studies was the observation that if λ carries *c*17, a mutation which confers partial N-independence, the phage is unable to grow in a Nus⁻ host even at 34°.

The studies presented in this paper characterize the effect of the *nus* A-1 mutation on the growth of phages carrying mutations, *c*17 and v_3vs_{326}, both of which affect transcription of the *c*II-O-P operon. A mutation at a different locus, *nus* B, exhibits a phenotype nearly identical to that of the *nus* A-1 mutant, including similar effects on the growth of λ variants carrying these two promoter mutations. The results with the *nus* B-5 host indicate that the observed effects of the *nus* A-1 mutation are not due to some effect specific to the *nus* A-1 mutation, but are, most likely, due to a reduction of N expression. The replication functions coded by genes transcribed from the *c*17 promoter are expressed in the Nus⁻ host. On the other hand the endolysin function, coded by a gene located in an adjacent operon is not expressed by a *c*17 phage in the Nus⁻ host.

An explanation for these observations follows from a consideration of (a) the nature of N-regulation of these two operons and (b) the ability of transcription complexes initiating at various promoters to utilize N function. We focus on transcription of genes located to the right of the "immunity" region. The N product augments this transcription by relieving transcription termination at two sites, t_{R1} and t_{R2} (Fig. 1; Echols, 1971; Herskowitz, 1973). In the presence of N, transcription initiating at P_R proceeds past t_{R1}, through the *c*II-O-P operon, past t_{R2}, and through the Q gene. The Q-gene product, in turn, effects the transcription of late genes, including

TABLE 5

EFFECT OF *nus* B-5 MUTATION ON GROWTH OF λ VARIANTS CARRYING ADDITIONAL OR MUTANT PROMOTERS[a]

Bacteria *nus*	Phage tested						
	λcI_{60}	$\lambda v_2v_3vs_{326}$	λv_3vs_{326}	λv_2vs_{326}	$\lambda v_2v_1v_3$	λcIc17	λrt^c5b
+ (K-37)	+	+	+	+	+	+	+
A (K-95)	+	−	−	+	+	−	+
B (K-450)	+	−	−	+	+	−	+

[a] Bacterial strains were grown overnight in TB broth. TB plates were seeded with bacteria and then spotted with dilutions of the indicated phage. Plates were incubated at 32°. +, Means phage plated well; −, means phage plated poorly (a spot of $\sim 10^3$ phage/ml did not show any lysis). Although λrt^c5b and λcI_{60} plate at the same e.o.p. on the two *nus* mutants, it should be noted that the plaque size is reduced (particularly on the *nus* B-5 host).

the R gene (endolysin). The *c*17 mutation lies distal to t_{R1} and thus transcription initiating at that promoter is not affected by the t_{R1} terminator. However, transcription initiating at *c*17 does reach t_{R2}, where in the absence of N it is presumed that transcription termination occurs. Moreover, previous work from this laboratory (Friedman and Ponce-Campos, 1975), has shown that transcription initiating at *c*17 cannot utilize N product to overcome the t_{R2} barrier. In light of that finding, the observations presented in this paper are interpretable in terms of a previously proposed model (Friedman, Wilgus, and Mural, 1973), in which λ N protein is presumed to attach near or at P_R and travel in a complex, Nus-N-polymerase, out toward the various terminators. N function subsequently exerts its antitermination effect at t_{R1} and t_{R2}. This implies that the sites of N recognition are different from the sites of N action. In a Nus^+ host, we assume that there is sufficient N activity so that transcription complexes initiating at P_R and modified by N can proceed through Q in spite of the presence of the *c*17 promoter. However, it should be noted that λ*c*17 growth even in a Nus^+ host is suboptimal, indicating that the *c*17 mutation interferes in some way with normal λ growth. We suggest that in the case of the Nus^- host, N activity is reduced and *c*17 transcription is predominant. Since transcription complexes initiating at *c*17, which predominate in an infection of a Nus^- host, cannot utilize N, they cannot pass through t_{R2}, and, thus, functions such as endolysin, coded for by genes distal to t_{R2}, cannot be expressed. The observation that a mutation *byp*, which obviates the need for N at t_{R2}, overcomes the effect of the *c*17 mutation in an infection of a Nus^- host, is consistent with this model. In this case, there is no need for N function at t_{R2} and, thus, transcription initiating at *c*17 can pass through to Q.

Experiments from other laboratories (Franklin, 1974; Adhya *et al.*, 1974) also demonstrate that sites of N recognition differ from sites of N action. These studies show that in the case of P_L, N attaches at or near the promoter and then is capable of influencing transcription at distal termination sites. It was also shown that N exerts effects not only at termination sequences in the λ genome, but at termination sequences in bacterial genes, and reduces the effect of polar mutations in bacterial genes.

The observation that the combination of the v_3 and vs_{326} mutations mimics the effects of *c*17 in the Nus^- host, suggests that in addition to altering the operator (O_R), these two mutations result in an altered promoter activity at P_R, one with characteristics like the *c*17 promoter. This dual effect, i.e., altering both the promoter and the operator, is consistent with the mapping data of Ordal and Kaiser (1973) which places the *vs* mutations between two promoter mutations, x_3 and x_{13}. These authors infer from their mapping data that there is interpenetration of the promoter and operator, an inference confirmed by sequence analysis (Maurer, Maniatis and Ptashne, 1974; Allet and Solem, 1974; Walz and Pirrotta, 1975). There is one obvious difference between the *c*17 mutation and both the v_3 and vs_{326} mutations; the former maps distal to t_{R1}, while the latter two map proximal to t_{R1}. We have argued that the reason why transcription initiating at *c*17 traverses the *c*II-O-P region, but not the Q-R region, is that such transcription cannot utilize N protein, and thus terminates at t_{R2}. Since both v_3 and vs_{326} map proximal to t_{R1}, it seems surprising that there could be good expression of genes lying distal to t_{R1} and proximal to t_{R2}, while there is extremely poor expression of genes lying distal to both terminators. However, it has been shown that normally in the absence of N product there is some transcription of the *c*II-O-P operon (Nijkamp, Bovre, and Szybalski, 1970; Kourilsky *et al.*, 1968), as well expression of the O and P functions (Ogawa and Tomizawa, 1968; Calef and Neubauer, 1968; Eisen, Pereira da Silva, and Jacob, 1968), transcription which does not transcend the t_{R2} barrier. In light of these observations, we propose that the v_3vs_{326} mutations functionally alter P_R in two ways: first, initiation of transcription at P_R is increased, i.e., the v_3vs_{326} mutations result in a so-called "up promoter"

(Fraenkel and Banerjee, 1970; Muller-Hill, Crapo and Gilbert, 1968). Studies of λ "supervir" growth in λ *dv*-carrying strains have also suggested such an "up promoter" role for v_3vs_{326} (Reichardt, personal communication). Second, the v_3vs_{326} mutations result in decreased N attachment in the P_R region. Accordingly, under conditions of reduced N expression v_3vs_{326}-carrying phage can transcribe past t_{R1}, because of the increased levels of initiation at P_R, but not past t_{R2} because of the reduced level of production of N-modified transcripts. Such an N-inhibitory role for v_3vs_{326} suggests that the actual site of N recognition lies in the region of the P_R promoter. Supporting this contention is the sequence data placing vs_{326} in the region of RNA polymerase binding (Walz and Pirrotta, 1975; Ptashne, personal communication). Such a site for N recognition is consistent with our findings that transcription initiating at *c*17, which lies only slightly distal to the P_R–O_R region, cannot utilize N.

We now turn to the question of why the ri^c5b mutation does not cause an effect similar to that of *c*17. Might this mean that ri^c5b-initiated transcription can utilize N to overcome transcription termination? Previous studies from this laboratory demonstrate that this is not the case (Friedman and Ponce-Campos, 1975); these experiments showed that transcription initiating at ri^c5b is unable to utilize N function to overcome the t_{R2} transcription barrier. Therefore, unlike *c*17, the ri^c5b mutation does not significantly interfere with transcription initiating at P_R, the latter being able to utilize N. The interference by *c*17 under conditions of limiting N could either be due to blocking of transcription initiation at P_R or to interference in the propagation of the transcription complex past *c*17. It is not possible at this time to distinguish between these two alternative explanations.

We cannot offer an explanation for the transdominance of *c*17 in the coinfection experiments. There may be competition of λ genomes for a limiting host factor, perhaps a membrane site (Sakakibara and Tomizawa, 1971; Kolber and Sly, 1971); a competition that in the N-limiting case of the Nus⁻ host finds the *c*17 phage dominant. This argument is consistent with the findings of Murialdo (1974) who reported that only a limited number of input λ DNA molecules can be used as template for progeny phage DNA synthesis. Current studies in this laboratory are directed toward understanding the nature of this competition.

In conclusion, these studies confirm our assertion that transcription initiating at *c*17 cannot utilize N function, and further strongly suggest that a site of N recognition is located in the O_R–P_R region.

Note added in proof. Experiments from other laboratories (Franklin, 1974 and Salstrom and Szybalski, personal communication) suggest that another site of N recognition is located near or in the P_L promoter.

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REFERENCES

Adhya, S., Gottesman, M., and de Crombrugghe, B. (1974). Release of polarity in *E. coli* by gene N of phage λ: Termination and antitermination of transcription. *Proc. Nat. Acad. Sci. USA* 71, 2534–2538.

Allet, B., and Solem, R. (1974). Separation and analysis of promoter sites in bacteriophage lambda DNA by specific endonucleases. *J. Mol. Biol.* 65, 475–484.

Butler, B., and Echols, H. (1970). Regulation of bacteriophage λ development by gene N: Properties of a mutation that bypasses N control of late protein synthesis. *Virology* 40, 212–222.

Calef, E., and Neubauer, Z. (1968). Active and inactive states of the *c*I gene in some λ defective phages. *Cold Spring Harb. Symp. Quant. Biol.* 33, 765–767.

Court, D., and Sato, K. (1969). Studies of novel transducing variants of lambda: Dispensability of genes N and Q. *Virology* 39, 348–352.

COUTURIER, M., DAMBLY, C. S., and THOMAS, R. (1973). Control of development in temperate bacteriophages V. Sequential activation of the viral functions. *Molec. Gen. Genetics* **120**, 231-252.

DOVE, W. F., HARGROVE, E., OHASHI, M., HAUGLI, F., and GUHA, A. (1969). Replicator activation in lambda. *Japan J. Gen.* **44** (suppl. 1), 11-22.

DOVE, W. F., INOKUCHI, M., and STEVENS, W. R. (1971). Replication control in phage lambda. *In* "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 747-771. Cold Spring Harbor Laboratories, New York.

ECHOLS, H. (1971). Regulation of lytic development. *In* "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 247-270. Cold Spring Harbor Laboratories, New York.

EISEN, H., PEREIRA DA SILVA, L., and JACOB, F. (1968). The regulation and mechanism of DNA synthesis in bacteriophage λ. *Cold Spring Harb. Symp. Quant. Biol.* **33**, 755-764.

FRAENKEL, D. G., and BANERJEE, S. (1970). Mutation increasing the amount of a constitutive enzyme in *Escherichia coli*, glucose 6-phosphate dehydrogenase. *J. Mol. Biol.* **56**, 183-194.

FRANKLIN, N. C. (1974). Altered reading of genetic signals fused to the N operon of bacteriophage λ: Genetic evidence for the modification of polymerase by the protein product of the N gene. *J. Mol. Biol.* **89**, 33-48.

FRIEDMAN, D. (1971). A bacterial mutant affecting λ development. *In* "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 733-738. Cold Spring Harbor Laboratories, New York.

FRIEDMAN, D. I., and BARON, L. S. (1974). Genetic characterization of the bacterial locus involved in the activity of the N function of phage λ. *Virology* **58**, 141-148.

FRIEDMAN, D., and PONCE-CAMPOS, R. (1975). Differential effect of phage regulator functions on transcription from various promoters: Evidence that the P22 gene 24 and the λ gene N products distinguish three types of promoters. *J. Mol. Biol.* **98**, 537-549.

FRIEDMAN, D. I., JOLLY, C. T., and MURAL, R. J. (1973). Interference with the expression of the N gene product of phage λ in a mutant of *Escherichia coli*. *Virology* **51**, 216-226.

FRIEDMAN, D. I., WILGUS, G. S., and MURAL, R. J. (1973). Gene N regulator function of phage λ*imm*21: Evidence that a site of N action differs from a site of N recognition. *J. Mol. Biol.* **81**, 505-516.

HERSKOWITZ, I. (1973). Control of gene expression in bacteriophage lambda. *Ann. Rev. Genetics* **7**, 289-324.

HERSKOWITZ, I., and SIGNER, E. R. (1970). A site essential for expression of all late genes in bacteriophage λ. *J. Mol. Biol.* **47**, 545-556.

HOPKINS, N. (1970). Bypassing a positive regulator: Isolation of a λ mutant that does not require N product to grow. *Virology* **40**, 223-229.

JOYNER, A., ISSACS, L. N., ECHOLS, H., and SLY, W. S. (1966). DNA replication and messenger RNA production after induction of wild-type λ bacteriophage and λ mutants. *J. Mol. Biol.* **19**, 174-186.

KAISER, A. D., and JACOB, F. (1957). Recombination between related temperate bacteriophages and the genetic control of immunity and prophage localization. *Virology* **4**, 509-521.

KOLBER, A. R., and SLY, W. S. (1971). Association of lambda bacteriophage DNA with a rapidly sedimenting *Escherichia coli* component. *Virology* **46**, 638-654.

KOURILSKY, P., MARCAUD, L., SHELDRICK, P., LUZZATI, D., and GROS, F. (1968). Studies on the messenger RNA of Bacteriophage λ, I. Various species synthesized early after induction of the prophage. *Proc. Nat. Acad. Sci. USA* **61**, 1013-1020.

MATSUBARA, K. (1972). Plasmid formation from bacteriophage λ as a result of interference by resident plasmid λdv. *Virology* **47**, 619-627.

MATSUBARA, K., and KAISER, A. D. (1968). λdv: An autonomously replicating DNA fragment. *Cold Spring Harb. Symp. Quant. Biol.* **33**, 755-764.

MAURER, R., MANIATIS, T., and PTASHNE, M. (1974). Promoters are in the operators in phage lambda. *Nature (London)* **249**, 221-223.

MULLER-HILL, B., CRAPO, L., and GILBERT, W. (1968). Mutants that make more lac repressor. *Proc. Nat. Acad. Sci. USA* **59**, 1259-1264.

MURIALDO, H. (1974). Restriction in the number of infecting lambda phage genomes that can participate in intracellular growth. *Virology* **60**, 128-138.

NIJKAMP, M. J. J., SZYBALSKI, W., OHASHI, M., and DOVE, W. F. (1971). Gene expression by constitutive mutants of coliphage lambda. *Molec. Gen. Genetics* **114**, 80-88.

OGAWA, T., and TOMIZAWA, J. (1968). Replication of bacteriophage DNA. I. Replication of DNA of lambda phage defective in early functions. *J. Mol. Biol.* **38**, 217-225.

ORDAL, G. (1971). Supervirulent mutants and the structure of operator and promoter. *In* "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 565-570. Cold Spring Harbor Laboratories, New York.

ORDAL, G. (1973). Mutations in the right operator of bacteriophage lambda: Physiological effects. *J. Mol. Biol.* **79**, 723-729.

ORDAL, G., and KAISER, A. D. (1973). Mutations in the right operator of bacteriophage lambda: Evidence for operator-promoter interpenetration. *J. Mol. Biol.* **79**, 709-722.

PACKMAN, S., and SLY, W. S. (1968). Constitutive λ DNA replication by λ*c*17, a regulatory mutant related to virulence. *Virology* **34**, 778-789.

ROBERTS, J. (1969). Termination factor for RNA synthesis. *Nature (London)* **224**, 1168-1174.

SAKAKIBARA, Y., and TOMIZAWA, J. (1971). Gene N

and membrane association of lambda DNA. *In* "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 691–699. Cold Spring Harbor Laboratories, New York.

SIMON, M. N., DAVIS, R. W., and DAVIDSON, N. (1971). Heteroduplexes of DNA molecules of lambdoid phages: Physical mapping of their base sequence relationships by electron microscopy. *In* "The Bacteriophage Lambda" (A. D. Hershey, ed.), pp. 313–328. Cold Spring Harbor Laboratories, New York.

WALZ, A., and PIRROTTA, V. (1975). Sequence of the P_R promoter of phage λ. *Nature (London)* 254, 118–122.