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Genetic Characterization of a Bacterial Locus Involved in the Activity of the N Function of Phage λ

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We report the genetic mapping of a locus of the *Escherichia coli* chromosome involved in the expression of the N gene function of phage λ. This phage specified function regulates the subsequent transcription of most of the λ genome. The bacterial locus involved in N expression, called *nus* for N *u*tilization *s*ubstance, maps between *aspB* at minute 62 and *argG* at minute 61 of the *E. coli* chromosome.

Two different bacterial variants in which λ N function is not active have been used in mapping the *nus* locus, a mutant of *E. coli* K12, Nus, and a hybrid bacterium formed by genetic transfer between *E. coli* and *S. typhosa*. Although these two bacterial variants exhibit slightly different phenotypes, chromosome transfer studies demonstrate that the same genetic region is involved in the observed N-ineffective phenotype.

Dominance studies show that in the case of the Nus mutant, the nus^+ allele is dominant. This suggests that the nus^+ allele is responsible for the expression of a function necessary for *N* product activity. In the case of transfer of the *nus* region from a Nus mutant to an *E. coli-S. typhosa* hybrid, the resulting hybrid assumes the phenotype of the Nus mutant. Genetic studies using P1 transduction demonstrate that the same genetic region is involved in the N-ineffective phenotype of the two bacterial variants.

INTRODUCTION

The N gene of coliphage λ specifies a protein which regulates the expression of most other λ functions (Echols, 1971). This regulation occurs at the level of transcription, since in the absence of N function, transcription of the λ genome is significantly reduced (Skalka *et al.*, 1967; Kumar *et al.*, 1969; Kourilsky *et al.*, 1968). Two classes of bacterial strains have been isolated and found to limit λ growth by inhibiting the action of the N protein. This has led to the conclusion that a host factor is necessary for this protein to be functionally active. One class is made up of mutant bacteria derived from strains of *Escherichia coli* which normally permit λ growth (Pironio and Ghysen, 1970; Georgopoulos, 1971a; Friedman, 1971; Friedman *et al.*, 1973a). A second class is made up of hybrid bacteria constructed by mating *E. coli* K12 donors with *Salmonella* recipients (Baron *et al.*, 1970, 1972). In this study, we report the genetic characterization of a representative of each class; K95, an *E. coli nus*-mutant and WR4255, an *E. coli–S. typhosa* hybrid.

Although the Nus mutant, K95, and *S. typhosa* hybrid, WR4255, were obtained in uniquely different ways, both bacterial strains share the same major phenotypic characteristics; each bacterial type does not permit the growth of N-dependent λ, but does permit the growth of N-independent or partially N-independent λ. The complete inhibitory effect of the Nus mutant, however, is conditional, being expressed only at high temperature, 42°, while the inhibitory effect of WR4255 is observable at all temperatures. A further difference is the fact that N function is partially active in the Nus mutant,

whereas there is no evidence that N function is active, to any extent, in WR4255. Consistent with these inferences is the observation that one class of mutant phage selected for ability to grow in the Nus mutant at 42° carries mutations mapping in the N gene and does not grow in WR4255 (Mural and Friedman, in preparation). The isolation of this class of phage mutants also shows that N protein can be synthesized in the Nus mutant at the restrictive temperature. In addition, studies in other laboratories (N. Franklin; S. Hu and W. Szybalski; personal communications) demonstrate that the N gene is transcribed normally in the Nus mutant under conditions where N function is not expressed. We therefore conclude that in the case of Nus, the mutation involved inhibits N expression at the level of N action and not at the level of N synthesis.

Previous studies have shown that the introduction of an additional *E. coli* chromosomal segment into hybrid WR4255 enabled this strain to produce λ suggesting the presence of a λ replication (λ*rep*) locus in this segment (Baron *et al.*, 1972). We now report that the nus mutation maps in precisely the same genetic region which on transfer renders WR4255 able to plate λ. Further, these studies show that the Nus$^+$ phenotype is dominant when both the *nus*$^+$ and *nus*$^-$ alleles are present.

MATERIALS AND METHODS

Bacteria. The characteristics of the bacterial strains employed in this study are listed in Table 1.

Bacteriophages. Preparation of phage lysates and other phage procedures were as previously described (Baron et al., 1970; Friedman *et al.*, 1973). Lysates of P1*vir* were obtained by the confluent lysis procedure (Adams, 1959). λ*c*I90*c*17 (the *c*17 mutation permits constitutive N-independent transcription of genes *c*II-O-P), λ*c*I857, and λ^{++} were supplied by M. E. Gottesman. P1*vir* was supplied by J. L. Rosner. λ*c*I857 *byp* constructed in these laboratories is a partially N-independent phage. The *byp* mutation (Hopkins, 1970; Butler and Echols, 1970) frees Q expression from N control; the Q gene product, in turn, stimulates transcription of late λ functions. λsx, an N-independent phage selected for its ability to plate on the *S. typhosa* hybrid bacterium WR4255, carries a number of mutations, one mapping in the region of the λ genome where the *byp* mutation is located (Baron *et al.*, 1970).

Media. The media utilized have all pre-

TABLE 1

CHARACTERISTICS OF STRAINS[a]

Strain	Description	Pertinent markers	Source or derivation
WR2010	*E. coli* K12 Hfr	O-*tna*$^+$-*xyl*$^+$-*malA*$^+$···	E. A. Adelberg, AB313
WR2020	*E. coli* K12 Hfr	O-*argG*$^+$-*malA*$^+$-*xyl*$^+$···	E. A. Adelberg, AB312
WR2029	*E. coli* K12 F′141	F-*malA*$^+$-*aspB*$^+$-*argG*$^+$	K. B. Low, KLF-41
WR2030	*E. coli* K12 F′ internal deletion of F′140	F-*pyrE*$^+$-*xyl*$^+$-*aspB*$^+$-*argG*$^+$	Baron *et al.* (1972)
WR2033	*E. coli* K12 Hfr	O-*argG*$^-$-*malA*$^+$-*xyl*$^+$···	W. Maas, Ma 1065 (AB312 type Hfr)
WR2034	*E. coli* K12 Hfr	O-*argG*$^+$-*nus*$^-$-*xyl*$^+$···	Cotransduction of *argG*$^+$-*nus*$^-$ from K206 to WR2034 with P1*vir*
WR3050	*E. coli* K12 F$^-$	*nus*$^+$	E. A. Adelberg, AB1133
K95	*E. coli* K12	*nus*$^-$	Friedman (1971)
K206	*E. coli* K12 F$^-$	*nus*$^-$	WR3050
WR2045	*E. coli* K12 F$^-$	*argG*$^-$ *nus*$^+$ *aspB*$^-$	N. Glansdorff
WR4255	*S. typhosa* hybrid	*xyl*$^-$ λ*rep*$^-$	Baron *et al.* (1972)
WR4021	*S. typhimurium* Hfr	O-*ilv*$^+$-*xyl*$^+$-*argG*$^+$···	K. Sanderson, Hfr K-10

[a] Abbreviations: *arg*, arginine; *ilv*, isoleucine and valine; *asp*, aspartic acid; *mal*, maltose; *st*^{r}, streptomycin; *xyl*, xylose; *nus*, *N* utilization substance; λ*rep*, replication of λ; *pyr*, pyrimidine; +, utilized or present; −, not utilized or absent.

viously been described in detail (Baron *et al.*, 1970; Friedman *et al.*, 1973a). Bacterial hybrids were initially selected and purified on appropriate minimal media, as were transductants. The selective techniques for obtaining particular hybrids depend on differences in nutritional requirements and/or resistance to streptomycin (500 μg/ml) between donor and recipient strains. Instability of hybrid strains for fermentation markers as an indication of diploidy was tested on MacConkey agar base (Difco or BBL) containing 1% of the appropriate carbohydrate.

Identification of Nus Phenotype. The Nus phenotype can be identified by its effect on λ growth in either of two ways (Friedman, 1971). Bacteria carrying the *nus* mutation inhibit λ growth at 42°, but not at 32° and inhibit the growth of λ carrying the *c*17 mutation at all temperatures. Thus, λ will form plaques on a "lawn" of the Nus mutant at 32°, but not at 42°. On the other hand, λ*c*17 (or λ*cI*c17) will not form plaques on a "lawn" of the Nus mutant at any temperature.

Transduction. Standard method for P1 transduction was used (Lennox, 1955).

RESULTS

Genetic Mapping Using Hfr Donors

In a previous study using Hfr donors, it was demonstrated that in the *S. typhosa* hybrid bacterium, WR4255, a region of the *E. coli* genome located between minutes 60 and 73 must be present in order for the N gene product of λ to be functional (Baron *et al.*, 1972). In these experiments, two *E. coli* Hfr strains were used which transfer the region of the *E. coli* chromosome between minutes 60 and 73 from opposite directions (see Fig. 1); WR2010 which transfers genetic markers in a counterclockwise direction originating at minute 73 and WR2020 which transfers genetic markers in the opposite direction originating at minute 60. Each transfers the information necessary for λ viability (activity of the N gene product) to *S. typhosa* hybrid WR4255 as an early marker. The *E. coli* locus involved was referred to as λ*rep*.

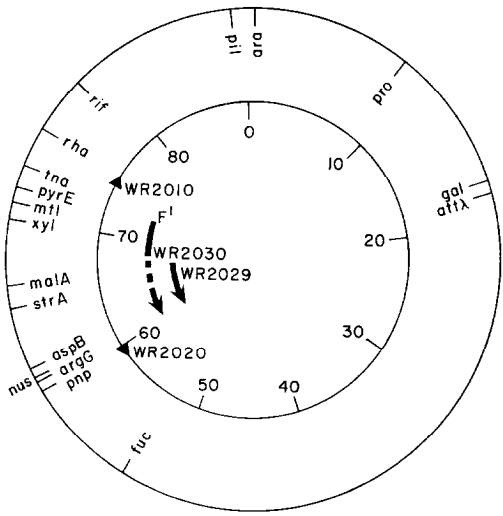

FIG. 1. Chromosome map of *Escherichia coli* K12. Included are the locations of pertinent genes and origins and directions of transfer of donor strains employed (Taylor and Trotter 1972).

Using these two Hfr donors in mating experiments with a Nus recipient, we have found that the Nus$^+$ phenotype (ability to support λ growth at 42°) is transferred early by both Hfr strains. Therefore, it is concluded that the *nus* gene is located between minutes 60 and 73 of the *E. coli* chromosal map.

Genetic Mapping Using Episomes

The genetic information necessary for WR4255 to support λ growth can be supplied by an F′ strain derived from F′140 (Baron *et al.*, 1972). The derivative F′ in WR2030 was thought to contain a region extending from minutes 66 to 72 since it was shown to carry the *pyrE-mtl-xyl* segment of F′140, and was found to be deleted for the *malA-strA* region also carried by F′140. In light of these observations, it was suggested by Baron and his co-workers (1972) that the region of the *E. coli* genome needed to permit WR4255 to plate λ locates between minutes 66 and 72.

In order to perform analogous experiments with the Nus mutant, we constructed a *nus* derivative, K206, which carries a *xyl*$^-$ marker. The F′ carried by WR2030, was transferred to this Xyl$^-$ recipient and Xyl$^+$ exconjugants were obtained using streptomycin to select against the *str*^{s} donor. The

Xyl$^+$ exconjugants became Nus$^+$ as indicated by the observation that they would now plate λ at 42 C. Therefore, we conclude that as in the case of WR4255, the Nus mutant is able to plate λ under normally inhibitory conditions if the genetic region covered by the F′ of WR2030 is introduced into the bacterium.

However, experiments with another F′ containing-strain, WR2029, obtained as KLF41 (Low, 1968), suggested that the *nus* locus was not located in the minutes 66–72 region of the *E. coli* chromosome. Transfer experiments using the F′ contained in WR2029, which includes the genetic region extending from *malA* (minute 66) through *argG* (minute 61) (see Fig. 1), showed that this F′ also carries the *nus*$^+$ allele. In these experiments, a *malA*$^-$ derivative of the Nus mutant was used as a recipient in a mating with the WR2029 donor. Again, the donor was *str*^{s} and the recipient was *str*^{r}, and Mal$^+$ recipients were obtained by using streptomycin to select against the donor. Although only thirteen of eighteen Mal$^+$ recipients became Nus$^+$, the fact that so many Mal$^+$ recipients did carry the *nus*$^+$ allele indicated that the F′ of WR2029 carries the *nus*$^+$ region. This suggests that either WR2029 contains genetic information extending into the minutes 66–72 region or that WR2030 carries additional genetic material beyond that reported by Baron *et al.* (1972).

Our present studies demonstrate that WR2030 does in fact carry additional genetic material. We have found that although the episome contained in WR2030 is deleted for the *malA . . . strA* region, approximately minutes 64–66 (Baron *et al.*, 1972), it carries genetic markers located on either side of this region. Thus, in addition to the *pyrE . . . xyl* region (minutes (70–72), WR2030 transfers the *argG* gene which is located at minute 61 of the *E. coli* chromosome. We therefore conclude that WR2030 resulted from an internal deletion of F′140 and that the region between minutes 60–65 contains the *nus*$^+$ allele.

Dominance Patterns of nus$^+$/nus$^-$ Diploids

Most of the Xyl$^+$ exconjugants isolated from the cross of the Xyl$^-$ Nus mutant with the WR2030 donor yielded Xyl$^-$ segregants at a high frequency and therefore were diploid for the *xyl* region. This region of diploidy also included the gene(s) controlling the Nus phenotype, since all Xyl$^-$ segregants simultaneously became Nus$^-$.

The isolation of these *nus$^+$/nus$^-$* merodiploids permitted us to determine the dominance patterns of the *nus* mutation. The Nus$^+$ and Nus$^-$ phenotypes are easily distinguishable since at 42° λ N-dependent phages grow in a Nus$^+$ host, but not in a Nus$^-$ host (Friedman, 1971). Figure 2 depicts the results of single-step growth experiments at 42° following induction of three hosts that carry the N-dependent prophage, λ*c*I857. These hosts were all derived from WR3050 and had the following genotypes: *nus*$^+$, *nus$^+$/nus$^-$* diploid, and *nus*$^-$ (derived

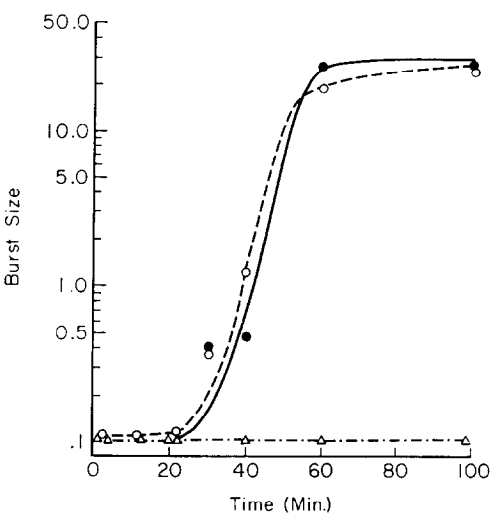

FIG. 2. Dominance of *nus*$^+$ allele. The following isogenic lysogens carrying the thermoinducible prophage, λ*c*I857, were used to determine the dominance patterns of the *nus* mutation: K291, no episome/chromosome-*nus*$^+$; K292, no episome/chromosome-*nus*$^-$; K290, episome-*nus*$^+$/chromosome-*nus*$^-$. Lysogens were grown until they reached "log-phase" growth conditions in M-9 medium containing 0.25% casamino acids with xylose as the carbon source. Lysogens were then induced by diluting cultures into LB broth which had been prewarmed to 42°. Cultures were maintained at 42° and aliquots were removed at the indicated times, treated with chloroform, and plated on a sensitive lawn using tryptone top agar and tryptone plates. Plates were incubated overnight at 40°. Burst size was computed as the number of phage released per bacterium. O- - -O, K291; ●——●, K290; △·-·-△, K292.

as a Xyl⁻ segregant from the *nus*⁺/*nus*⁻ diploid). As shown in Fig. 2, the growth of λcI857 in the *nus*⁺ and *nus*⁺/*nus*⁻ diploid strain was precisely the same. In comparison, there was no observable phage production following induction of the *nus*⁻ segregant which carried a λcI857 prophage. We conclude that the *nus*⁺ allele is dominant.

Genetic Mapping by Transduction

In order to map the location of the *nus* gene(s) more precisely within the 60 to 65 minute region of the K12 chromosome, we used phage P1*vir* to transduce suitable markers in this region. P1 lysates prepared on K206 (*argG*⁺ *nus*⁻ *aspB*⁺) were used to infect WR2045 (*argG*⁻ *nus*⁺ *aspB*⁻) with subsequent selection being made for *argG*⁺ and *aspB*⁺ as well as for double transductants (*argG*⁺ *aspB*⁺). After purification, the transductants were screened for the presence of the Nus⁻ trait as reflected by an inability to plate λcIc17. The results listed in Table 2 indicate that the *nus* locus is very closely linked (approximately 90% cotransduction) to the *argG* gene at minute 61 of the K12 chromosome. The results also show that the *nus* locus is 36% cotransducible with the *aspB* marker near minute 62. The data in Table 2 as depicted in Fig. 3 also indicate that the *nus* locus maps between the *argG* marker at minute 61 and the *aspB* marker at minute 62. These results are supported by the fact that all of 108 doubly (*argG*⁺ *aspB*⁺) selected transductants obtained also had acquired the *nus*⁻ trait.

TABLE 2

MAPPING OF *asp*, *nus*, AND *argG* BY P1 TRANSDUCTION

Selected marker	Scored markers	% Scored markers	
asp⁺	*argG*⁻ *nus*⁺	45	
asp⁺	*argG*⁻ *nus*⁻	19	100
asp⁺	*argG*⁺ *nus*⁺	2	
asp⁺	*argG*⁺ *nus*⁻	34	
argG⁺	*nus*⁺	10	100
argG⁺	*nus*⁻	90	

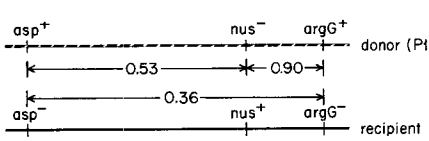

FIG. 3. Cotransduction frequencies of *aspB*, *nus*, and *argG*.

Dominance of nus in S. typhosa Hybrids

Previously, it was shown that the ability to plate λ was dominant in *S. typhosa* hybrids made diploid for the 60–72 minute region of the K12 chromosome (Baron *et al.*, 1972). In order to directly compare the effects of the *nus* and *rep* loci we determined if the *nus* trait, recessive to *nus*⁺ in *E. coli* K12 merodiploids, is dominant in *S. typhosa* hybrids. Establishment of the precise location of the *nus* marker enabled us to construct a suitable Hfr strain for transfer of the *nus* allele to *S. typhosa* hybrid WR4255. This was accomplished by cotransduction by P1*vir* of the closely linked *argG*⁺ *nus*⁻ markers to an *argG*⁻ Hfr strain having the transfer orientation of WR2020. The resulting Hfr strain WR2034, which transfers its chromosome in the order O-*argG*⁺-*nus*⁻-*malA*⁺-*xyl*⁺ . . . *metC*⁺-F, was then used for conjugal transfer of the chromosomal segment containing the *nus*⁻ locus to WR4255. In this cross, hybrids of WR4255 were selected on minimal xylose media using nutritional counterselection of the donor. Although occasional stable Xyl⁺ hybrids were obtained, as in similar reported experiments (Baron *et al.*, 1972), the Xyl⁺ WR4255 hybrids isolated were predominantly unstable diploids yielding frequent Xyl⁻ segregants. These merodiploids exhibit the Nus⁻ phenotype in that they plate λ and will not plate λcIc17 at 32 C. In addition, Xyl⁻ segregants lose the ability to plate λ at all temperatures. From these results we infer that the *xyl*⁺/*xyl*⁻ WR4255 merodiploids also carry the *nus*⁻ marker on the K12 diploid segment, and further that the *nus* and *rep* loci are either identical or closely linked.

Transfer of λrep from Salmonella to E. coli

The phenotypic similarity of the λ*rep*⁻ and *nus*⁻ alleles as well as their close linkage suggested that these markers represent the same locus. We therefore used P1 transduction to map the λ*rep*⁻ loci of *Salmonella* strains. Although it is possible to transduce some markers from *S. typhosa* hybrid

WR4255 to *E. coli* K12 recipients with P1*vir*, we were unable to recover any *argG*$^+$, transductants using an *argG*$^-$ *E. coli* recipient. A less direct approach was therefore undertaken to map the location of the λ*rep*$^-$ marker based on the assumption that λ*rep* may in fact be an equivalent locus to *nus*. This procedure involved transferring the *arg*$^+$ region to an *E. coli* recipient by conjugation with an appropriate *S. typhimurium* Hfr strain. A cross was therefore performed between *S. typhimurium* Hfr WR4021 (transfer orientation O-*ilv*$^+$-*xyl*$^+$-*argG*$^+$ . . . *metE* . . F) and an *argG*$^-$ *E. coli* recipient with selection for *argG*$^+$ recombinants. The small number of *argG*$^+$ hybrids which appeared were tested after purification for sensitivity to λ, λcIc17, λ*sx*, and λ*byp*. All these *argG*$^+$ hybrids proved to be insensitive to λ and λcIc17, but were able to plate λ*sx* and λ*byp* at 37°. This pattern is identical to that exhibited by *Salmonella* hybrid WR4255 and indicates that these exconjugants had acquired the *Salmonella* λ*rep*$^-$ locus.

Transduction studies, using one such *argG*$^+$ λ*rep*$^-$ *E. coli* exconjugant, demonstrated that like the *nus* allele, the λ*rep* allele cotransduces at a high frequency with the *argG* locus. In these experiments, lysates of P1*vir* propagated in the *argG*$^+$ λ*rep*$^-$ strain were used to transduce an *aspB*$^-$ *nus*$^+$ *argG*$^-$ recipient WR2045. Analysis of the resulting *argG*$^+$ transductants revealed that 92% also carried the λ*rep*$^-$ marker derived from *S. typhimurium*. However, none of over 100 *aspB*$^+$ transductants carried the λ*rep* locus nor were any double (*aspB*$^+$ *argG*$^+$) transductants recovered. Thus it was impossible for us to order the location of λ*rep* with respect to the *aspB* and *argG* loci. We assume that these latter results reflect the genetic inhomology apparent in transduction of genes between *E. coli* and *Salmonella*. Nevertheless, it appears likely on the basis of all of the accumulated data that λ*rep* and *nus* represent equivalent loci.

DISCUSSION

Previously reported experiments using bacterial variants which inhibit λ growth have demonstrated that in order for the phage-specified N function to be active, a bacterial-specified function must be present. In the case of the usual host for λ, *E. coli* K12, two mutations, *gro*N and *ron*, which affect N activity, map near the *rif*^{r} genetic locus, minute 79 of the *E. coli* chromosome (Georgopoulos, 1971a; Pironio and Ghysen, 1970). Mutations to *rif*^{r} are associated with changes in RNA polymerase (Wehrli *et al.*, 1968). Since the N gene product is involved in the transcriptional process, it is not surprising that functional and biochemical studies indicate that both the *ron* and *groN* mutations are located within structural genes for RNA polymerase (Georgopoulos, 1971b; Ghysen and Pironio, 1972).

In contrast to the studies on the *groN* and *ron* mutation, our studies with another mutant of *E. coli* K12, Nus, and an *E. coli*–*S. typhosa* hybrid, WR4255, demonstrate that an allele mapping in a region of the *E. coli* chromosome distant from the *rif* locus also is involved in permitting N function to be active. This was shown by our observation that the ability to plate N-dependent phages could be transferred to either the Nus mutant or to the WR4255 hybrid when the chromosomal region corresponding to minute 61 of the *E. coli* chromosome was introduced either by P1 transduction or by F′ episome transfer.

The isolation of various strains diploid for the *nus* region permitted us to answer two crucial questions regarding the *nus* mutation: First, does the *nus* mutation actually define a function needed for N activity? Second, is the same allele involved in rendering N function active in Nus and WR4255?

The first question was answered by our studies with *nus*$^+$/*nus*$^-$ diploid strains. In such diploids, N-dependent phage grow as well as they do in *nus*$^+$ strains. Thus, the *nus*$^-$ mutation does not result in the formation of an inhibitor of N activity, but rather must cause an undersupply of a substance needed for N utilization, a component which can be supplied by the *nus*$^+$ allele. We will therefore continue to refer to this allele as *nus*, *N* utilization substance.

As to the second question, the transduction studies made possible by the isolation of appropriate diploids demonstrate that the

nus and λ*rep* alleles cotransduce at the same frequency with the *argG* locus, which locates at minute 61 of the *E. coli* K12 chromosome. In addition, functional studies with the *E. coli–S. typhosa* diploid formed by transfer of the *argG* region from a *nus* donor demonstrated that the new diploid assumed the characteristics of the *nus* mutant. That is, the resulting merodiploid plates λ, but not λcIc17. In contrast, the original hybrid bacterium, WR4255, which does not carry the minute 61 region from *E. coli*, plates neither phage. Therefore, we conclude that the allele mapping near *argG* which is involved in N utilization is identical in both the Nus mutant and the *E. coli–S. typhosa* hybrid WR4255.

Although the nature of the interaction between the Nus function and the N protein is not known, we do know something about the nature of the specificity of this interaction. Previous studies have shown that in order to be active N products with different recognition specificities require the presence of the Nus function. Thus, in addition to λ, a hybrid phage, 21*hy*5 (Liedke-Kulke and Kaiser, 1967) does not grow in the *nus* mutant at 42° (Friedman, 1971), nor does it grow in WR4255 (unpublished observation). This hybrid phage carries the immunity region of phage 21 and expresses the 21 N function, an N function whose specificity of action differs from that of λ (Friedman *et al.*, 1973b). This difference in specificity is shown by the observations that the N of λ is not active in stimulating expression of the 21 genome (Friedman *et al.*, 1973b), and the N of 21 is not active in stimulating the expression of the λ genome (Couturier and Dambly, 1970; Herskowitz and Signer, 1970). Since the expression of both 21 and λ N functions are interfered with equally in Nus and WR4255, we conclude that the Nus substance itself has no specificity for either N product or for DNA with either the immunity region of λ or the immunity region of 21. The observed specificity for N must be determined by the N function itself or by an N-nus complex.

Although the Nus function is essential for λ growth, we have not been able to determine whether the *nus* mutation affects any host physiological processes. In unreported experiments by Friedman and Jolly it was found that the growth rate of the *nus* mutant under a wide variety of conditions did not vary from that of the *nus*$^+$ parent strain. In addition, in the case of an induced operon, the kinetics of induction of β-galactosidase in the two strains was precisely the same. It did not escape our attention that the *pnp* gene is located near *argG* (Reiner, 1969). Since polynucleotide phosphorylase can, under some conditions, polymerize mononucleotides into polynucleotides (Grunberg-Manago, 1963), it seemed possible that *pnp* might be *nus*. However, two separate studies suggest that this is not the case. First, polynucleotide phosphorylase actively appears to be normal in extracts of the Nus mutant (unreported observations) and second, the *pnp* and *nus* alleles map on opposite sides of the *argG* gene.

In conclusion, we report the mapping of a new gene *nus*, the product of which is involved in the utilization of the phage specified regulatory function, the N product.

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