Molec. gen. Genet. 179, 55–61 (1980)

MGG

Studies on the *E. coli groNB* (*nusB*) Gene which Affects Bacteriophage λ *N* Gene Function

C.P. Georgopoulos[1], J. Swindle[1], F. Keppel[2], M. Ballivet[2], R. Bisig[2], and H. Eisen[2]

[1] Department of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City, Utah 84132, USA
[2] Department of Molecular Biology, University of Geneva, Geneva, Switzerland

Summary. *Escherichia coli* mutants, called *groNB*, which block the growth of bacteriophage λ at the level of action of the gene *N* product, have been isolated as survivors at 42° C of bacteria carrying a) the defective prophage λ*bio*11 i^{λ}*cI*857 *V*H1 or b) the pcR1 plasmid containing the *Eco*RI immunity fragment of phage λ*cI*857. In addition, *groNB* bacterial mutants have been isolated at 37° C, as large colony formers in the presence of λi^{λ} *cI* h^{434}, λi^{λ} *cI* h^{λ}, and λi^{λ} *cI* h^{80} phage. The *groNB* locus is located at 9 minute of the *E. coli* genetic map with the order of the neighboring loci being *proC tsx groNB purE*. Most *groNB* mutations isolated at 42° C were found to interfere in addition with bacterial growth at low temperatures, since (a) the GroNB phenotypes of λ growth inhibition and bacterial cold sensitivity cannot be separated by P1 transduction, and (b) some cold resistant revertants simultaneously become Gro^+ for λ growth. Lambda transducing phages carrying the $groNB^+$ bacterial gene have been isolated. *GroNB* mutant bacteria lysogenized by the transducing phage acquire the Gro^+ phenotype and simultaneously the cold resistant phenotype, suggesting that the *groNB* mutations are recessive to the wild-type gene.

Introduction

The *N* gene product of bacteriophage λ, a 13,500-dalton polypeptide (Shaw et al., 1978; Greenblatt et al., 1980) is required for early mRNA transcription, originating at promotors P_L and P_R, to proceed beyond certain termination sites on the phage λ DNA (Herskowitz, 1973). Roberts (1969) has suggested that the *N* gene product antagonizes the bacterial termination factor ρ, thus allowing mRNA transcription to proceed past the rho-sensitive sites. The mode of action of the *N* gene product is not known, although it appears that the sites of *N* recognition (*nut*) are different from the sites of *N* action (t_{R1}, t_{R2} etc.) (Friedman et al., 1973b, 1976; Rosenberg et al., 1978; Salstrom and Szybalski, 1978). Recently, it has been shown that the *N* gene product will functionally antiterminate transcription at t_{R1} when provided with a nut_R site which has been dissociated from the lambda rightward promotor P_R (de Grombrugghe et al., 1979). Evidence has been presented suggesting that the *N* gene product interacts with the host RNA polymerase. This evidence is derived chiefly from the isolation of bacterial mutants which map in the *rif* region and which interfere with proper *N* gene expression (Georgopoulos, 1971; Ghysen and Pironio, 1972; Baumann and Friedman, 1976; Sternberg, 1976; and Epp and Pearson, 1976) and in the σ subunit of *E. coli* RNA polymerase (Nakamura et al., 1979). In addition to this class of bacterial mutants, Friedman et al. (1973a) have reported the isolation of another class, called *nusA*, which maps at minute 68 near *arg*G, and which also interferes with *N* gene expression. Keppel et al. (1974), and Friedman et al. (1976), have reported the existence of yet another locus, called *groNB* and *nusB* by the respective authors, which maps near *tsx* and which also affects *N* gene expression. Here we report on our further studies of *groNB* mutants, and show that they are analogous to the *nusB* mutant of Friedman et al. (1976), and to the *E. coli* mutant M5374 isolated originally by Greer (1975b) and thought to be resistant to the action of the *kil* gene product (Greer, 1975a).

Offprint requests to: Dr. C.P. Georgopoulos

Materials and Methods

Bacterial Strains

E. coli B178 is a K12 *sup*$^+$ *gal*E derivative obtained from D. Kaiser. Strains M72 *lacam trpam*, M72 *lacam trpam* (*bio*11 i^{λ}cI857 *V*H1) and M5374 were described by Greer (1975a, b). Strains LC102 F^- *leu purE trp his metA argG proC araB lac xyl mtl gal* str^r *tsx* and

0026-8925/80/0179/0055/$01.40

LC607 F⁻ *pur*E *trp lys metE proC leu lac xyl ara tonA str*^{r} *thi* were obtained from L. Caro. Strain M72 (pcRI·*Eco*RI immunity fragment of λ*c*I857) has been described before (Hedgpeth et al., 1978). MF185 *leu thr att*$^{\lambda}$*V* was obtained from Mike Feiss. K12 *nusB5* was obtained from D. Friedman.

Bacteriophage Strains

Bacteriophage 434*c*I, carrying the h^{434} host range was obtained from E. Signer. Bacteriophage T6 was from S.E. Luria's collection. The transducing phage λ*c*I857 *nusB*$^+$ was provided by D. Friedman. Bacteriophage P1L4, used in transduction studies was obtained from L. Caro. The rest of the lambdoid phage strains originally came from the collection of D. Kaiser.

Media

TM broth contains 10 g tryptone, 5 g NaCl, and 2 g maltose per liter. L-broth contains 10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter. EMB plates without added sugar source, and minimal agar plates, used for selection in transductions, were as described by Miller (1972). To assure that the pcRI, *kan*^{R} carrying plasmid, is retained, cultures carrying pcRI were grown in the presence of 20 μg/ml Kanamycin sulfate.

Bacterial Mutant Selection

Method A. The selection of large colony formers in the presence of λi^{λ} *c*I h$^{\lambda}$, i^{λ} *c*I h^{434} and λi^{λ} *c*I h^{80} phage has been described before (Georgopoulos, 1971). *Method B.* M72 (*bio*11 i^{λ}*c*I857 *V*H1) bacteria were grown in L-broth to 5×10^8 cells/ml. About 10^7 bacteria were spread on L-agar plates and incubated at 42° C for 20 h. Survivors, obtained at a frequency of 5×10^{-5}, were replica-plated on EMB plates seeded with $10^9 \lambda i^{\lambda}$ *c*I h^{λ} and $10^8 \lambda i^{434}$ *c*I h^{434}. Colonies which were not readily killed as judged by colony morphology and absence of coloration were purified and further tested. *Method C.* M72 bacteria carrying a pcRI plasmid into which the *Eco*RI immunity fragment of λ*c*I857 was incorporated were grown in L-broth supplemented with 20 μg/ml Kanamycin at 30° C to 5×10^8 cells/ml. About 5×10^8 bacteria were spread on L-plates supplemented with 20 μg/ml Kanamycin, and incubated at 42° C for 24 h. Survivors, obtained at frequency of 10^{-7} (10^{-5} when Kanamycin was omitted) were processed as described in Method B. A large fraction of the small colony formers at 42° C turned out to be *groNB* mutant bacteria.

P1 Transductions

Phage P1 growth and phage P1 transductions were carried out as described by Miller (1972).

*Selection of λc*I857 *Lysogens in the tsx Gene*

MF185 bacteria were infected with phage λ*c*I857 at a multiplicity of infection of 10 at 30° C. The infected bacteria were grown in L-broth at 30° C for 8 h to allow expression of the Tsx phenotype (resistance to bacteriophage T6). About 10^8 bacteria were challenged at 30° C with 10^9 T6 and $10^9 \lambda c I h^{434}$ phage to isolate bacteria which became simultaneously resistant to phage T6 and lysogenic for λ*c*I857. Six such lysogens were obtained which fulfilled these criteria, three of which, upon heat induction, gave phage progeny capable of propagating on *groNB* bacteria.

Results

Isolation of groNB Bacterial Strains

Method A. We repeated our original selection technique (Georgopoulos, 1971), which involved the isolation of large colony formers of *E. coli* B178 *galE* bacteria in the presence of λi^{λ} *c*I h^{λ}, λi^{λ} *c*I h^{434}, and λi^{λ} *c*I h^{80}. The λ*c*I h^{80} phage was included in our new selection technique to eliminate further the occurrence of bacterial colonies resistant to phage adsorption. The titer of the phage added on the plate is such that the majority of the B178 *galE* bacteria will form very small colonies. However, bacteria which for one reason or another will not propagate the bacteriophages present on the plate will form large colonies under these conditions. About 700 large colony formers thus isolated after nitrosoguanidine mutagenesis (Adelberg et al., 1965) were cross-streaked against phages λi^{λ} *c*I h^{434} and λi^{λ} *c*I h^{434} *nin*5, and two colonies *groNB20* and *groNB47* were isolated, which did not allow growth of the λi^{λ} *c*I h^{434} phage but did allow growth of the λi^{λ} *c*I h^{434} *nin*5 phage. Since the *nin*5 mutation is an *N*-bypass mutation (Court and Sato, 1969), it appeared likely that these two bacterial mutants did not propagate bacteriophage λ because they interfered with the action of the lambda *N* gene product. The plating properties of the *groNB47* mutation shown in Table 1 were identical with those of *groNB20;* only those of *groNB47* are included because both isolates were obtained from the same culture and may be identical (see below).

Method B. We have briefly reported this technique in our previous publication (Keppel et al., 1974). It involves the isolation of colony formers of unmutagenized M72 (*bio*11 i^{λ}*c*I857 *V*H1) bacteria at 42° C and screening the survivors for inability to propagate λi^{λ} *c*I h^{434} and λi^{434} *c*I h^{434} phage. Such bacterial lysogens die at 42° C because the *c*I857 repressor is inactivated and the defective prophage manufactures the *kil* gene product whose production results in cell death (Greer, 1975a, b). Among all the possible combinations of bacterial survivors at 42° C (such as *kil* gene mutations, *N* gene mutations, λ*c*I857 → λ*c*I$^+$ revertants, deletions, etc.) only bacterial mutants which interfere with the expression of the λ*N* gene product will be unable to propagate $i^{434}cI$ phage upon subsequent testing. Using this criterion seven *groNB* mutants were isolated. The plating properties of two of them, M72 *groNB101* and *groNB128*, are shown in Table 1. It can be seen that *groNB128* also interferes with the growth of phage λi^{21} *c*I h^{434} whose

Table 1. Efficiency of plating[a] of various lambdoid phages on *groNB* bacterial strains

Bacterial strain	Temperature	i^{λ}cI857	i^{λ}_{c}I857 *nin*5	i^{21}cI	i^{80}cI h^{80}	i^{434}cI	i^{434}cI *nin*5	i^{λ}cI857 vA1[f]
M72 *gro*$^{+}$	37° or 42°	1.0	1.0	1.0	1.0	1.0	1.0	1.0
M72 *groNB101*[b]	42°	6×10^{-3}	0.9	0.8	0.9	$<10^{-4}$	0.8	1.0
M72 *groB128*[b]	42°	$<10^{-4}$	0.6	$<10^{-4}$	0.9	$<10^{-4}$	0.9	0.7
M72 *groNB105*[c]	42°	2×10^{-3}	0.8	0.7	1.0	$<10^{-4}$	0.9	0.9
M72 *groNB108*[c]	42°	$<10^{-4}$	0.7	$<10^{-4}$	0.9	$<10^{-4}$	0.8	1.0
B178 *groNB47*[d]	37°	1.5×10^{-3}	1.0	1.0	1.0	$<10^{-4}$	1.0	1.0
M72 *M5374*[e]	42°	$<10^{-4}$	0.6	$<10^{-4}$	1.0	$<10^{-4}$	0.9	0.8

[a] Efficiency of plating denotes the number of plaques on a given bacterial host, normalized to that on M72 at the same temperature. The host range of the phages was h^{434} unless otherwise indicated
[b] Isolated as survivors of strain M72 (*bio*11 i^{λ}cI857 ∇H1) at 42° C
[c] Isolated as survivors of strain M72 (pcrR1·*Eco*RI immunity fragment of i^{λ}cI857) at 42° C
[d] Isolated as large colony formers at 37° C in the presence of λ clear phages
[e] Isolated by Greer, 1975b
[f] A λ mutant in gene *N* near *Nsus7*, isolated as able to overcome the *groN785* block (Georgopoulos, 1971)

N gene product is functionally different from that of λ and 434 (Friedman et al., 1973b).

Method C. The best selection of *groNB* bacterial mutants discovered during the course of this work was obtained from the 42° C bacterial survivors of a M72(pcR1) bacterial strain carrying the *Eco*RI immunity fragment of λcI857. Such lysogens die at 42° C primarily because of the production of the phage λ *kil* gene product as mentioned above. If the bacteria are plated at 42° C in the absence of Kanamycin, about 5×10^{-5} to 10^{-5} survivors are found, the majority of which are *kan*^{s}. They presumably represent bacterial clones cured of their resident pcR1-i^{λ} hybrid plasmid. When Kanamycin is included in the selection at 42° C, however, only 3×10^{-8}–10^{-7} bacterial survivors are found. The large colony formers are invariably *gro*$^{+}$, whereas one quarter (42/200) of the small colony formers were found to be *groNB* bacterial mutants. The plating properties of *groNB105* and *groNB108* isolated by this procedure are included in Table 1. Interestingly, *groNB105* allows growth of the heteroimmune phage i^{21}, but *groNB108* does not. It appears that *groNB* bacterial mutants, such as *groNB47*, *groNB101*, and *groNB105* which do not restrict λ growth tightly (Table 1), also do not severely restrict i^{21} growth. Other *groNB* isolates such as *groNB108* and *groNB128* interfere strongly with i^{21} growth. All *groNB* bacterial hosts in our collection were found able to propagate phage i^{80}cIh^{80} whose *N* gene product is functionally different from those of λ and 434 (Franklin, 1974). Furthermore, they all propagate phages carrying the *N*-independent deletion-substitution *nin*5 (Court and Sato, 1969; Fiandt et al., 1971) as well as some of phage λ mutants, called *v*A, which were isolated as plaque formers on *groN785* bacteria and which map in gene *N* (Georgopoulos, 1971; Table 1).

The Characterization of Strain M5374 as a groNB Mutant

Greer (1975b), starting with bacterial strain M72 (*bio*11 i^{λ} cI857 ∇H1) carrying in addition prophage λ*gal*18 *bio*11 cIII631 i^{λ}cI857 *Pam*3, has also isolated and analyzed bacterial survivors at 42° C. These were inferred to be bacterial mutants resistant to the lethal action of the *kil* gene product. Such a survivor, strain M5374, was kindly given to us by H. Greer and we analyzed its plating properties with regard to several lambdoid phages. We found in agreement with Greer (1975b) that M5374 bacteria were resistant to adsorption by λi^{λ} cI h^{λ} but not by λi^{λ} cI h^{434} phage (see below). Although adsorption was not blocked, phage λi^{λ} cI h^{434} did not propagate on M5374 bacteria, but its *nin*5 derivative phage did (Table 1). We tentatively concluded that M5374 bacteria possessed a *groN*-like mutation and not *grokil* mutation. Mapping data (see below) verified this conclusion.

Growth Properties of groNB Bacterial Mutants

The striking features of most *groNB* bacterial mutants isolated by Methods B and C mentioned above and that of strain M5374 were; a) their inability to form colonies or very slow growth at 30° C, and b) their partial resistance to adsorption by phages carrying the host range h^{λ}. To bypass the complications associated with resistance to adsorption, we crossed the host range of phage 434 onto most of our phage derivatives. This enabled us to classify our isolates as *groNB*, inasmuch as only phage carrying the *nin*5 mutation were able to form plaques on these bacterial hosts (Table 1). The cold sensitivity of the *groNB* bacteria was manifested on minimal as well as rich broth media. Curing of the *groNB* strains of their

Table 2. P1 transduction studies with the *groNB* bacterial locus

Donor strain	Recipient strain	Selected marker	Distribution
proC$^+$ *tsx groNB101*	LC607 *proC*$^-$ *tsx*$^+$	*proC*$^{+a}$	0 *groNB*; 320 *gro*$^+$
proC$^+$ *tsx groNB101*	LC607 *proC*$^-$ *tsx*$^+$	*proC*$^{+b}$	10 *groNB*; 287 *gro*$^+$
proC$^+$ *tsx groNB101*	LC102 *proC*$^-$ *tsx*$^+$	*proC*$^+$ *tsx*^{b}	15 *groNB*; 10 *gro*$^+$
proC$^+$ *groNB101*	LC102 *purE*$^-$	*purE*$^{+b}$	0 *groNB*; 200 *gro*$^+$
proC$^+$ *groNB47*	LC607 *proC*$^-$	*proC*$^{+b}$	22 *groNB*; 344 *gro*$^+$
proC$^+$ *groNB108*	LC607 *proC*$^-$	*proC*$^{+b}$	14 *groNB*; 192*gro*$^+$
proC$^+$ *groNB5374*	LC607 *proC*$^-$	*proC*$^{+a}$	0 *groNB*; 190 *gro*$^+$
		proC^{+b}	13 *groNB*; 197 *gro*$^+$

[a] Tested after selecting *proC*$^+$ transductants for 2 days at 42° C
[b] Tested after selecting *proC*$^+$ transductants for 4–5 days at 42° C

resident prophages as described before (Keppel et al., 1974) did not alleviate the cold sensitivity. The degree of cold sensitivity, as judged by the rate of increase in the optical density of the culture, varied, depending on the particular *groNB* isolate. For example, *groNB108* bacteria growing in T-broth doubled their optical density at 30° C every 90 min, *groNB128* every 80 min, *groNB101* every 64 min, and the *groNB*$^+$ isogenic strain (derived from *groNB* by P1 transduction to *gro*$^+$, see below) every 35 min; at 42° C their corresponding doubling times were 40, 37, 34, and 26 min, respectively. The growth of other *groNB* isolates was similarly or not as severely affected at low temperature. Some of the *groNB* isolates formed long filaments as a consequence of growth at low temperature, but others, equally severely affected for growth, did not, revealing no discernible pattern.

The GroNB and Cold Sensitive Phenotypes are due to One Mutation

One of the difficulties encountered in working with the cold sensitivity of *groNB* mutant bacteria was their tendency to 'adapt' to faster growing derivatives, upon continuous doublings. When plated at 30° C or 22° C (room temperature), a culture of *groNB* bacteria would give rise to variants able to form a small colony after 24 h at a frequency of 10^{-4}–10^{-6}. Most of these variants, upon retesting, retained their GroNB phenotype. When precautions were taken to keep *groNB* bacteria at 42° C continuously before plating at room temperature on T-plages, fast colony formers arose at a frequency of 10^{-7}. About 10% of such cold resistant clones simultaneously acquired the Gro$^+$ phenotype, suggesting that the cold sensitive phenotype and the GroNB phenotype are probably due to a single mutation. The nature of the majority of the cold resistant clones which retain the GroNB phenotype is not known; they probably represent inter- or intragenic suppressors of the *groNB* mutation.

The GroNB Phenotype is due to a Single Mutation Mapping Near tsx

In our preliminary publication on *groNB* mutant bacteria, we found that when *lac groNB* bacteria were transduced to *lac*$^+$ by bacteriophage P1 grown on *lac*$^+$ *gro*$^+$ bacteria, about 3% of the *lac*$^+$ transductants simultaneously became *gro*$^+$ for λ phage growth. In a similar cross, we now show that the contransduction between *proC* and *groNB* is, on the average, seven percent (Table 2). However, when the donor and recipient genotypes are reversed, that is P1 is grown on *proC*$^+$ *groNB* bacteria and used to transduce *pro gro*$^+$ bacteria to *proC*$^+$, none of the transductants simultaneously acquired the GroNB phenotype. This was interpreted before to mean either that the *groNB* phenotype is made up of two unlinked mutations, both of which are necessary for the expression of the GroNB phenotype, or that the expression of the GroNB phenotype necessitated the presence of the parental M72 genetic background (Keppel et al., 1974). Another plausible explanation entertained was that since *groNB* bacteria grow more slowly than their *gro*$^+$ isogenic strains, *pro*$^+$ *groNB* transductants may not form visible colonies at 42° C after 2 days incubation but may do so after longer incubation. This turned out to be the correct explanation. As Table 2 shows, after 4 to 5 days incubation at 42° C, *proC*$^+$ *groNB* transductants appeared at a frequency of 5–10%, as opposed to none after 2 days. All *groNB* isolates tested, irrespective of their mode of isolation, mapped in this region of the *E. coli* chromosome (Table 2). All *proC*$^+$ *groNB* transductants (50/50) retained the parental cold-sensitive phenotype, suggesting again that a single mutation mapping near the *tsx* locus simultaneously confers the GroNB phenotype for phage growth and cold-sensitive bacterial phenotype. Since no mutagenesis was used in isolating these *groNB* mutations, the possibility that two closely linked mutations separately confer each of the two phenotypes in all cases tested is very unlikely. The

Table 3. Plating properties[a] of i^{λ} *groNB*$^+$ transducing phages at 42° C

	$i^{\lambda}cI857$	$i^{\lambda}cI857$ *groNB*$^+$			$i^{434}cI$
		#1	#3	#9	
LC607	1.0	1.0	1.0	1.0	1.0
LC607 *proC*$^+$ *groNB101*[b]	6×10^{-3}	1.0	0.9	0.9	$<10^{-4}$
LC607 *proC*$^+$ *groNB108*[b]	$<10^{-4}$	0.9	0.8	0.9	$<10^{-4}$
LC607 *proC*$^+$ *groNB108* [i^{λ}*groNB*$^+$ #1][c]	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	1.0
LC607 *proC*$^+$ *groNB5374*[b]	$<10^{-4}$	0.8	0.85	0.7	$<10^{-4}$

[a] As defined by the efficiency of plating on LC607; all phages carry the h^{434} host range

[b] Constructed by transducing strain LC607 *proC* to *proC*$^+$ *groNB* by phage P1 pregrown on the corresponding M72 *proC*$^+$ *groNB* strains

[c] Strain LC607 *proC*$^+$ *groNB108* stably lysogenized by phage $i^{\lambda}cI^+$ *groNB*$^+$ #1

Table 4. The *groNB* and *nusB* loci are probably identical

Bacterial strain	Temperature (°C)	$i^{\lambda}cI857$	$i^{\lambda}cI856$ *nusB*$^+$	$i^{\lambda}cI857$ *groNB*$^+$ #1
M72 *gro*$^+$	37, 42	1.0	1.0	1.0
M72 *groNB101*	24	6×10^{-3}	0.9	1.0
M72 *groNB108*	42	$<10^{-4}$	0.8	0.9
B178 *groNB47*	37	1.5×10^{-3}	1.0	1.0
K12 *nusB5*	37	$<10^{-4}$	0.9	1.0

mapping experiments reported in Table 2 establish the genetic order *proC tsx groNB*.

The Isolation of λgroNB$^+$ *Transducing Phages*

To isolate λ-transducing phages carrying the *groNB*$^+$ bacterial locus we used the Shimada et al., technique (1973). We proceeded to lysogenize MF185 *att*$^{\lambda}$ *V* bacteria with λi^{λ} *c*I857 h^{434} at 30° C. Bacteria which were both lysogenic for λi^{λ} *c*I h^{434} phage and resistant to bacteriophage T6 were isolated at 30° C in the presence of λi^{λ} *c*I h^{434} and T6 phages. Out of 50 such isolates (which occurred at a frequency of 2 to 5×10^{-6} per λ*c*I857 infected bacterium) three gave rise to λ*c*I857 *groNB*$^+$ transducing phages upon heat induction. These lysogens were presumably formed by the integration of the λ*c*I857 prophage in the *tsx* locus, thus simultaneously conferring the resistance to T6. In a typical experiment, about 10^5 λ*c*I857 plaque formers per ml were produced upon induction of such lysogens, 10–20 percent of which were able to form plaques on *groNB* mutant bacteria. The plating properties of a typical λi^{λ} *groNB*$^+$ transducing phage are shown in Table 3. These represent true *groNB*$^+$ transducing phage isolates because *groNB* bacteria, upon stable lysogenization by these phages, become phenotypically Gro$^+$ for λi^{434} heteroimmune phage growth (Table 3) and simultaneously lose their cold-sensitive phenotype. This result also suggests that the *groNB*$^+$ locus carried on the transducing phage is dominant over its corresponding *groNB* mutant bacterial locus.

The groNB and *nusB* loci are probably identical

In addition to our own group, Friedman et al. (1976) have also isolated *groNB*-like mutants, called *nusB*, which also interfere with the expression of the λ *N* gene and map near *tsx*. We tested the identity of the *groNB* and *nusB5* loci by testing the growth of the λ *groNB*$^+$ transducing phages on these hosts. The results presented in Table 4 show that both sets of transducing phages are able to propagate on both sets of bacterial hosts. This in turn suggests that the *groNB*$^+$ and *nusB*$^+$ bacterial loci are simultaneously being carried on both sets of transducing phages and hence are probably identical. This conclusion has been reinforced by the recent isolation of an amber mutation in the *groNB* bacterial gene carried on an λi^{λ} *groNB*$^+$ transducing phage (J.S. and C.P., unpublished results). This λi^{λ} *groNB am* phage strain plates well on *gro*$^+$ *sup*$^+$ and *groNB supF* bacteria but not on *groNB sup*$^+$ and *nusB sup*$^+$ bacteria.

Discussion

We have isolated bacterial mutants blocking the expression of the λ *N* gene product by three different selection techniques. These bacterial mutants do not propagate phage λ, but do propagate its *N*-independent derivative λ*nin5* (Court and Sato, 1969). In addition to blocking λ growth, the majority of the *groNB* mutants interfere with bacterial growth, especially at

low temperatures. Both manifestations of the GroNB phenotype, that is the inability to grow at low temperature and the inability to propagate phage λ, are due to a single bacterial locus which maps near the *tsx* locus of the *E. coli* chromosome, since (a) they were isolated without mutagenesis, (b) both phenotypes are simultaneously cotransducible, and (c) some bacterial revertants, able to grow at low temperature, simultaneously recover their ability to propagate bacteriophage λ, that is their Gro^+ phenotype. In addition to the mutants isolated during the course of this work, a bacterial mutant isolated as a *kil*-tolerant mutant (Greer, 1975b) was also shown to belong to the same class of *groNB* bacteria.

Using the technique of Shimada et al. (1973), we isolated λ prophages inserted in the *tsx* gene. Induction of these prophages gave rise to plaque-forming λ $groNB^+$ transducing phages. These phages propagated on all *groNB* bacteria isolated, including the *kil* tolerant mutant of Greer (1975b), and the *nusB5* bacterial mutant of Friedman et al. (1976). EDTA-resistant deletion derivatives of the λ $groNB^+$ transducing phages which retained their ability to propagate on *groNB108* bacteria were found to be able to propagate on all *groNB* bacterial strains mentioned above; deletion derivatives which lost their ability to propagate on *groNB108* bacteria simultaneously lost their ability to propagate on the rest of the *groNB* bacterial strains (J.S. and C.G., unpublished results). This suggests that the *groNB* bacterial mutations are all located in a very small region of the *E. coli* chromosome, most likely in a single locus.

Since our *groNB* mutations are probably in the same locus as the *nusB5* mutation of Friedman et al. (1976), the differences in our P1 transduction frequencies between *groNB* and *proC* (about 7% average) from those of Friedman et al. (1976) between *proC* and *nusB5* (about 2%) remain to be accounted for. A possible explanation may be our observation that since *groNB* bacteria grow slower than their gro^+ isogenic strains, one has to allow the $proC^+$ *groNB* transductants a longer time (up to five days) to form a colony on the minimal selection plates than their $proC^+$ gro^+ counterparts. Failure to do this would result in a biased distribution in favor of the $proC^+$ gro^+ transductants since they grow faster (Table 2).

What role does the *groNB* gene product play in allowing proper expression of the λ *N* gene product? These studies reported here do not address this question directly. However, they do suggest that the $groNB^+$ bacterial product is also necessary for *E. coli* viability in addition to its role in *N* gene function. A possible clue to the $groNB^+$ gene product mode of action is suggested by its close proximity to the *deg* (*lon*) locus (Gottesman and Zipser, 1978) of *E. coli*. Both *groNB* and *deg* (*lon*) loci cotransduce with *pro*C about 7% of the time (Markovitz, 1964). Assuming that the two loci perform analogous functions, it could be that the *groNB* product is involved in 'processing' the *N* product to an active form. *GroNB* bacterial mutants could either fail to carry out such processing or, alternatively, could 'overprocess' the *N* product to an inactive form. The work of Epp and Pearson (1976) is consistent with such speculations about processing of the *N* gene product. It has recently been shown that the *c*II gene product's half-life is considerably longer upon infection of *groNB103* bacteria than upon infection of gro^+ bacteria (Shaw, J. and Pearson, M., personal communication). The discovery of the mechanism by which the bacterial *groNB* product blocks *N* gene product action will undoubtedly shed light on the role that the *groNB* gene product itself plays in *E. coli* viability.

Acknowledgements. We would like to thank Dave Friedman and Helen Greer for sending us bacterial and phage strains, Kit Tilly for comments on the manuscript, and Mrs. Jerri Cohenour for typing the manuscript. Support came from NIH Grant GM23917 and Grant 3.519.75 Fonds National Suisse de la Recherche Scientifique.

References

Adelberg, E.A., Mandel, M., Chen, G.C.C.: Optimal conditions for mutagenesis by N-methyl-N-nitro-N-nitroguanidine in *Escherichia coli* K 12. Biochem. Biophys. Res. Commun. **18**, 788–795 (1965)

Baumann, M.F., Friedman, D.I.: Cooperative effects of bacterial mutations affecting λ *N* gene expression. II. Isolation and characterization of mutations in the *rif* region. Virology **73**, 128–138 (1976)

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

De Crombrugghe, B., Mudryj, M., DiLauro, R., Gottesmann, M.: Specificity of the bacteriophage lambda *N* gene product (p*N*): *Nut* sequences are necessary and sufficient for antitermination by p*N*. Cell **18**, 1145–1151 (1979)

Epp, C., Pearson, M.L.: Association of bacteriophage lambda *N* gene protein with *E. coli* RNA polymerase. In: RNA polymerase (R. Losick and M. Chamberlin, eds.), pp. 667–691. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory 1976

Fiandt, M., Hradecna, Z., Lozeron, H.A., Szybalski, W.: Electron micrographic mapping of deletions, insertions, inversions, and homologies in the DNAs of coliphages lambda and phi80. In: The bacteriophage lambda. (A.D. Hershey, ed.) pp. 329–354. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory

Franklin, N.C.: Altered reading of genetic signals fused to the *N* operon of bacteriophage λ: Genetic evidence for modification of polymerase by the protein product of the *N* gene. J. Mol. Biol. **89**, 33–60 (1974)

Friedman, D.I., Baumann, M., Baron, L.S.: Cooperative effects of bacterial mutations affecting λ *N* gene expression. I. Isolation and characterization of a *nus*B mutant. Virology **73**, 119–127 (1976)

Friedman, D.I., Jolly, C.T., Mural, R.J.: Interference with the

expression of the *N* gene function of phage λ in a mutant of *Escherichia coli*. Virology **51**, 216–226 (1973a)
Friedman, D.I., Wilgus, G.S., Mural, R.J.: 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 (1973b)
Georgopoulos, C.P.: Bacterial mutants in which the gene *N* function of bacteriophage lambda is blocked have an altered RNA polymerase. Proc. Nat. Acad. Sci. USA **68**, 2977–2981 (1971)
Ghysen, A., Pironio, M.: Relationship between the *N* function of bacteriophage λ and host RNA polymerase. J. Mol. Biol. **65**, 259–272 (1972)
Gottesman, S., Zipser, D.: Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**, 844–851 (1978)
Greenblatt, J., Malnoe, P., Li, J.: Purification of the gene *N* transcription anti-termination protein of bacteriophage lambda. J. Biol. Chem. **255**, 1465–1470 (1980)
Greer, H.: The *Kil* gene of bacteriophage lambda. Virology **66**, 589–604 (1975a)
Greer, H.: Host mutants resistant to phage lambda killing. Virology **66**, 605–609 (1975b)
Hedgpeth, J., Ballivet, M., Eisen, H.: Lambda phage promoter used to enhance expression of a plasmid-cloned gene. Mol. Gen. Genet. **163**, 197–203 (1978)
Herskowitz, I.: Control of gene expression in bacteriophage lambda. Ann. Rev. Genetics **7**, 289–324 (1973)
Keppel, F., Georgopoulos, C.P., Eisen, H.: Host interference with expression of the lambda *N* gene product. Biochimie **56**, 1503–1509 (1974)
Markovitz, A.: Regulatory mechanisms for synthesis of capsular polysaccharide in mucoid mutants of *E. coli*. Proc. Nat. Acad. Sci. USA **51**, 239–246 (1964)
Miller, J.H.: Experiments in molecular genetics. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory 1972
Nakamura, Y., Kurihara, T., Saito, H., Uchida, H.: Sigma subunit of *Escherichia coli* RNA polymerase affects the function of λ *N* gene. Proc. Nat. Acad. Sci. USA **79**, 4593–4597 (1979)
Roberts, J.W.: Termination factor for RNA synthesis. Nature **223**, 480–482 (1969)
Rosenberg, M., Court, D., Shimatake, H., Brady, C., Wulff, D.L.: The relationship between function and DNA sequence in an intercistronic regulatory region in phage λ. Nature **272**, 414–423 (1978)
Salstrom, J.S., Szybalski, W.: Coliphage λ*nut*L$^-$: A unique class of mutants defective in the site of *N* utilization for antitermination of leftward transcription. J. Mol. Biol. **124**, 195–221 (1978)
Shaw, J.E., Jones, B.B., Pearson, M.L.: Identification of the *N* gene protein of bacteriophage λ. Proc. Nat. Acad. Sci. USA **75**, 2225–2229 (1978)
Shimada, K., Weisberg, R.A., Gottesman, M.: Prophage lambda at unusual locations. II. Mutations induced by bacteriophage lambda in *Escherichia coli*. J. Mol. Biol. **80**, 297–314 (1973)
Sternberg, N.: A class if *rif*^{R} RNA polymerase mutations that interferes with the expression of coliphage λ late genes. Virology **73**, 139–154 (1976)

Communicated by T. Yura

Received February 16 / April 5, 1980