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Bacterial Mutants in Which the Gene *N* Function of Bacteriophage Lambda Is Blocked Have an Altered RNA Polymerase

(*GroN*/rifamycin/*N*-gene action/*E. coli*/DNA transcription)

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ABSTRACT **Bacterial mutants have been isolated, called *groN*, that block phage development by interference with the action of the product of the phage *N* gene. λ*trp* phages, which depend on the *N* product for the synthesis of tryptophan enzymes, do not make these enzymes in *groN* bacteria. Two types of phage mutants have been isolated that can overcome the *groN* block. One type makes an altered *N* product, the other contains an *N*-bypass mutation.**

The *groN* mutation is closely linked to the rifamycin-resistance locus in *Escherichia coli*. Purified RNA polymerase from the *groN* mutant is less activated by salt and more sensitive to rifamycin than is the polymerase from *gro*$^+$. This suggests that the *groN* mutation produces a structural change in the bacterial RNA polymerase such that it can no longer interact properly with the phage *N* product.

The product of gene *N* of bacteriophage λ is a positive regulator of phage development (1). It regulates by stimulating transcription of the *cII-O-P* and *cIII-exo* operons. Surprisingly, it does not act at the right (*pr*) or left (*pl*) promoters that initiate these operons, but at sites distal to them (1–4). Genetic and biochemical experiments (1, 5, 6) suggest three major sites of *N*-product action on λ DNA: one between genes *N* and *cIII*, one between genes *cro* and *O*, and one between genes *P* and *Q* (Fig. 1). Roberts (7) has proposed that the termination factor for RNA synthesis, ρ, terminates transcription at these sites. In some way the *N* product permits transcription beyond the three termination sites. Three kinds of explanation for this action can be proposed: (*a*) *N* product is a new RNA polymerase; (*b*) *N* product modifies the specificity of the host RNA polymerase; (*c*) *N*-gene product is, or modifies, a component of the transcription system other than RNA polymerase, for example, the ρ factor.

In a class of bacterial mutants called *groN*, λ growth is arrested because the *N*-gene product fails to function (8). The properties of *groN* strains, and of the RNA polymerase isolated from them, show that the *groN* mutation causes a structural change in the bacterial RNA polymerase. Since this mutation prevents the action of the *N* product, it suggests that the *N* product works through the host polymerase.

MATERIALS AND METHODS

Bacterial mutants were isolated from SKB178, a *galE*$^-$, *su*$^-$ λ-sensitive prototroph of *Escherichia coli* K12. Strain WGS6 (9) contains a defective λ prophage, in which phage genes both to the right of the immunity region and to the left of the immunity region, starting with *cIII*, are deleted. SA462 carries a different defective λ prophage, in which phage genes beginning in *P* and extending to the right are deleted. SA268 contains a defective λ prophage, in which *Q* and genes to the right are deleted. SA268 and SA462 were isolated by Sankar Adhya and were obtained from D. Court. Phage λ*trp*46*N*$^+$*fed* and λ*trp*48*N*$^+$*fed* [the *fed* mutation blocks the early turn-off of the *cIII-exo* operon, and appears to be identical to *cro* (10) and *tof*(11)] are plaque-forming phage variants that carry the genes for tryptophan biosynthesis from *E. coli*. The *trp* genes have been placed on the phage chromosome to the left of gene *N* and, as Franklin (6) has shown, their transcription originates at *pl*. The λ*trp* phage strains were a generous gift from N. Franklin. λ*imm*^{434}*susN7nin*$_5$ is an *N*-bypass mutant strain that can grow in the absence of an active *N* gene, and was obtained from D. Court.

TYM-broth is 1% Bacto-tryptone, 0.5% Yeast Extract, 0.5% NaCl, and 0.2% Maltose. λ dil is 10 mM KPO_4 (pH 7 0)–10 mM $MgSO_4$.

SKB178 cells were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (12) to 25–40% survival and allowed to grow for 5 generations in TYM-broth. 10^8–10^9 mutagenized cells were spread on TYM-agar plates, along with a mixture of 10^8 λ*b2cI* and 10^7 434*cI* phage, and incubated at 37° C for 18 hr. Under these conditions, all phage-sensitive cells give rise to microcolonies. The large colonies, which appeared at a frequency of 10^{-3}, were purified twice by single-colony isolation and further tested. They were found to be either (*a*) mucoid, (*b*) unable to adsorb λ or 434, or (*c*) to be blocked in phage development at some step after absorption. The last class of mutant, called *gro*$^-$, has been further analyzed.

The experimental conditions for assay of the activity of Component II of anthranilate synthetase (PR transferase) were

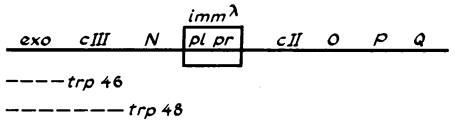

FIG. 1. Genetic map of the early region of λ. *pl* is the promoter for the *cIII-exo* operon and *pr* is the promoter for the *cII-O-P* operon. – – –indicates the phage DNA region substituted by the bacterial *trp* region.

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TABLE 1. *Phage growth on bacterial strains*

Bacteriophage	SKB178 Burst size*	*groN*785 e.o.p.†	*groN*785 Burst size*	*groN*786 e.o.p.†
λ*b2cI*	205	$<10^{-11}$	2.0	$<10^{-9}$
		2.5×10^{-8}‡	N.D.	10^{-8}‡
434*cI*	240	9×10^{-6}	1.5	3×10^{-6}
		2×10^{-6}‡	N.D.	
λ*imm*^{434}*cI*	210	5×10^{-8}	N.D.	1×10^{-8}
φ80*vir*	N.D.	1×10^{-4}	N.D.	4×10^{-4}
381*cI*, 21*cI*	N.D.	$<10^{-5}$	N.D.	$<10^{-5}$
82*c*$^+$, 424*c*$^+$, 186*c*, T1, T3, T5, T7, T2, T4, T6, P1	N.D.	1.0	N.D.	1.0
λ*imm*^{434}*cIsusN7nin*$_1$	84	0.8	33	0.45
λ*imm*$^{\lambda}$*cIνA2*	140	$<10^{-6}$	N.D.	$<10^{-6}$
		0.8‡	N.D.	0.9‡
434*cIνA1*	195	1.0	85	0.9
434*cIνB1*	183	0.5	30	N.D.

SKB178 is the parent of both *groN*785 and 786.

* Burst size, the average phage yield per infective center, was determined as described (8).

† e.o.p. denotes the number of plaques produced by a phage strain on a given bacterial host at 37°C relative to the number on SKB178.

‡ e.o.p. determined at 30°C.

§ N.D.. not determined.

those of Ito and Yanofsky (13), as modified by Franklin (6). 1 unit of activity is defined as that amount of enzyme required to consume 0.1 μmol of anthranilate in 20 min at 37°C. Specific activity is expressed as units of enzyme per mg of protein.

RNA polymerase was purified through "*step 5*" and assayed as described by Berg *et al.* (14). 1 unit of RNA polymerase activity is defined as the amount that catalyzes the incorporation of 1 nmol of UMP in an hour under standard assay conditions. [^{3}H]UTP was purchased from New England Nuclear Corp. T4 DNA was generously provided by Wai Mun Huang.

Rifamycin-resistant bacterial mutants were isolated as follows: 10^{10} SKB178 cells, grown in TYM-broth, were spread on TYM-agar plates containing 50 μg/ml of rifamycin (Rifampin, Calbiochem). Rifamycin-resistant colonies appeared after 18 hr of incubation at 37°C.

P1*vir* phage stocks were prepared and used to transduce the rifamycin-resistance marker as described by Scott (15).

RESULTS

Phage growth in *groN* strains

Bacterial mutants have been isolated that block λ development at stages after adsorption and injection. These mutants, called *gro*$^-$, can be divided into classes according to the phage function that fails as a result of the bacterial mutation. One class, called *groN*$^-$, is defective in the function normally performed by λ gene *N*. Among 500 *gro*$^-$ colonies isolated as survivors of SKB178 simultaneously infected with λ and 434, one mutant of this class, *groN*785, was isolated. Table 1 shows that λ and 434 plate with very low efficiency on *groN*785. *GroN*785 can be transduced by λ*dg*, showing that λ can adsorb to and inject its DNA into these bacteria. Nevertheless, λ- or 434-infected *groN*785 produce very few phages: both the transfer efficiency and burst size are low (Table 1). Among the λ-related phages, φ80, 21, and 381 fail to multiply, but 82 and 424 grow and lysogenize well in *groN*785. All phage unrelated to λ that were tested grew normally. A second and independent mutant, *groN*786, isolated by its ability to survive infection and convert λ to a plasmid, responds to phage infection like *groN*785 (Table 1). Friedman (16) and Pironio and Ghysen (17) have isolated bacterial mutants whose response to different phages resembles that of *groN*.

Failure of *N* to function in *groN*785

The *trp* genes of *E. coli* can be incorporated into λ DNA in such a way that expression of these genes is entirely dependent upon transcription from *pl* (6). In λ*trp*46, synthesis of the tryptophan biosynthetic enzymes requires an active *N*-gene product. Thus, fusion occurred to the left of the termination signal between *N* and *cIII*, and the amount of *trp* enzymes produced after infection by such a phage measures *N*-gene function (6). Other strains, such as λ*trp*48, express the *trp* enzymes in the absence of the *N* function, because the *trp* operon is fused to λ with loss of the mRNA-termination signal between *N* and *cIII* (6). The amount of *trp* enzymes produced after infection by λ*trp*48 is, therefore, a measure of the total transcription initiated at *pl*.

In *groN*785, the *N*-dependent λ*trp*46 fails to synthesize Component II of the anthranilate synthetase complex, the product of the *trpD* gene (Fig. 2). The rate of enzyme formation was more than 1000-fold lower in *groN*785 than in its *gro*$^+$ parent. In *groN*785, the *N*-independent λ*trp*48 synthesized Component II of the anthranilate synthetase complex at a rate comparable to that found in *gro*$^+$ (Fig. 2). These results imply that in *groN*785, transcription is initiated normally at *pl* and, therefore, that the *N* gene itself is transcribed. Nevertheless, transcription beyond the *N-cIII* termination signal fails to occur in *groN*785.

Phage mutants that can multiply on *groN* bacteria

At least two classes of phage mutant might be expected to grow on *groN* bacteria: (*a*) phage that have lost the requirement for the *N* product, and (*b*) phage that synthesize an *N*

product suitably modified to compensate for the alteration in *groN* bacteria. Both classes have been found.

One kind of *N*-independent mutant of λ, called *nin* (18), has been isolated from λ N^- mutants. λ*nin* have lost a segment of DNA between genes *P* and *Q* (19) and, as expected, they plate on *groN* bacteria (Table 1). Phage mutants called *ν* have also been isolated from λimm^{434} and 434 by their ability to grow on *groN* strains (Table 1). One class of *ν* mutants, called *νB*, is located (9/9 tested) between genes *P* and *Q*, defined by the end points of the SA268 and SA462 deletions. Thus, *νB* resembles *nin*.

The rest of the *ν* mutants, called *νA* (Table 1), map in or near the *N* gene, as shown by the following experiments. *νA* lies outside the immunity region, since it can be crossed from λimm^{434} into λ and vice versa. A νA^+ allele can be extracted from the deletion prophage in bacterial strain WGS6 or from a cross with λ*bio10*. Lambda *bio10* is deleted from the right side of the prophage attachment site through *cIII* (20). Therefore, *νA* must be located to the right of *cIII* and to the left of the immunity region. 12 crosses, each with a different *νA* mutation, of the type $imm^{434}susN7 \times imm^{\lambda}\nu A$, yielded a minority (5–30%) of the $imm^{434}N^+$ recombinants as *νA*, indicating that these *νA* mutations map to the right of *N*7. With five other *νA* mutations in similar crosses, 65–95% of the $imm^{434}N^+$ recombinants were *νA*, suggesting a closer linkage between *N*7 and these *νA* mutations.

One of the *νA* mutants, *νA*16, though it can multiply on *groN* bacteria, is unable to multiply on gro^+ bacteria. The defect of *νA*16 in gro^+ bacteria can be complemented by N^+ $susO_{29}$ or by N^+ $susP_{80}$, but not by *susN*7*N*53 O^+ P^+ phage. One step mutants of *νA*16 selected for ability to grow on gro^+ bacteria simultaneously lose their ability to plate on *groN*, suggesting that the *νA* phenotype and the production of an inactive *N* product in gro^+ cells are the consequences of a single mutation. In summary, all *νA* mutations appear to lie in or near the *N* gene and one, *νA*16, behaves as though it produces an altered *N* product.

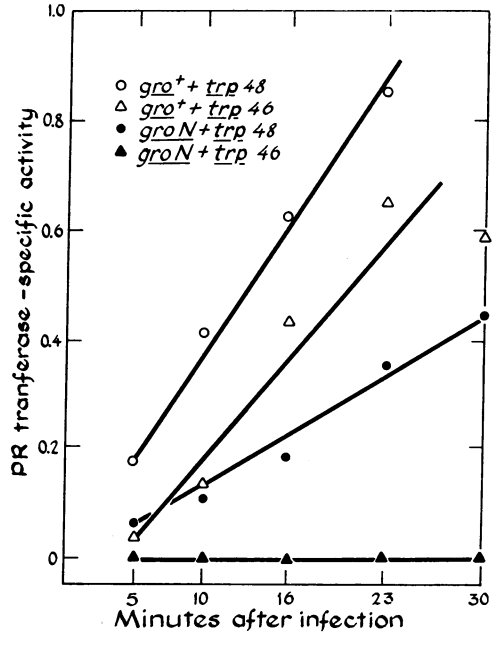

FIG. 2. Activity of Component II of the anthranilate synthetase complex induced after infection of gro^+ and *groN*785 bacteria with various λ*trp* phages. PR transferase: Component II of the anthranilate synthetase complex.

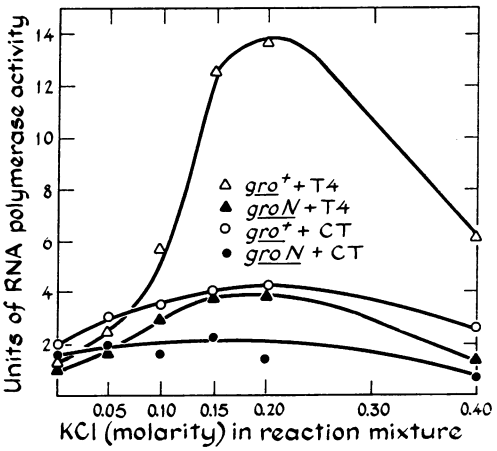

FIG. 3. Effect of KCl concentration on the activity of gro^+ and *groN*785 RNA polymerases. Assay mixtures (0.10 ml) contained 4 μmol Tris·HCl (pH 8.0), 1 μmol $MgCl_2$, 1 μmol 2-mercaptoethanol, 30 μg of T4 or calf-thymus (*CT*) DNA, 40 nmol (each) of [^{3}H]UTP, CTP, GTP, and ATP, and 2–3 μg of enzyme. Samples were incubated for 10 min at 37°C, and processed as described by Berg *et al.*[11]

Mapping of *groN*785 in *E. coli*

To find the chromosomal location of the *groN*785 mutation, two independent rifamycin-resistant (rif^r) mutant strains of SKB178 were isolated. P1*vir*, grown on these strains, was used to transduce strain *groN*785 to rifamycin resistance. Of the *groN*785 rif^r transductants, 95% (61/64) simultaneously acquired the ability to grow λ and 434, i.e., were gro^+. The actual cotransduction frequency between the *groN*785 and rif^r loci may be greater than 95%, because of the transduction frequency to rif^r was low (10^{-7}), and some of the *groN*785rif^r colonies may be spontaneous mutants of *groN*785 to *groN*-785rif^r, which occur at a frequency of 10^{-8}. In a control experiment, spontaneous rif^r mutants (50/50 tested) were found to conserve their *groN* phenotype. Thus, the locus of *groN*785 is closely linked to the locus for rifamycin resistance, which is known to be in a structural gene for one of the subunits of the RNA polymerase enzyme (21).

GroN bacteria possess an altered RNA polymerase

The strong linkage between *groN*785 and rif^r suggested that *groN* may also reside in a gene for one of the subunits of the bacterial RNA polymerase. Accordingly, RNA polymerase was purified from SKB178 (=gro^+) and *groN*785 (=*groN*) by the procedure of Berg *et al.* (14), through the DEAE-cellulose step (*step 5*). The two enzymes obtained were about 50% pure, and possessed equivalent amounts of σ factor, as judged by sodium dodecyl sulfate–gel electrophoresis and by their ability to transcribe T4 DNA (a process that requires σ). The two enzyme preparations had similar specific activities on calf-thymus (1.5–2.0 units/μg protein) or T4 (1.0–1.5 units/μg protein) DNA as template.

At high ionic strength, transcription of T-even phage DNA by *E. coli* polymerase is stimulated severalfold. High salt concentration causes more frequent initiations and release of RNA

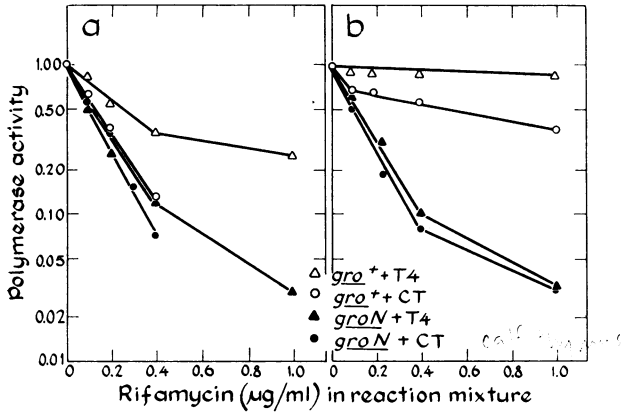

FIG. 4. Effects of various amounts of rifamycin on the activity of *gro*$^+$ and *groN*785 RNA polymerases. (*a*) The assay mixtures were as described in the legend of Fig. 3. No additional salt was added. (*b*) Conditions were similar to those in (*a*), except that 0.03 M $(NH_4)_2SO_4$ was added to the reaction mixtures.

chains, more specific initiations, and an increase in the rate of chain growth (22). The response of *gro*$^+$ and *groN* RNA polymerases to a range of KCl concentrations are compared in Fig. 3. The *gro*$^+$ RNA polymerase activity on T4 DNA was stimulated more than 10-fold by the addition of 0.2 M KCl. *GroN* polymerase was stimulated less than the *gro*$^+$ enzyme at all the KCl concentrations tested, and was stimulated only 4-fold at 0.2 M KCl.

E. coli RNA polymerase is sensitive, both *in vivo* and *in vitro*, to the antibiotic rifamycin (23). Rifamycin inhibits the enzyme by interaction with the β subunit of core polymerase, as shown by (*a*) the binding of radioactive rifamycin to purified β subunit, but not to the α or β' subunits (24) and (*b*) the fact that a rifamycin-resistant mutant possesses an electrophoretically altered β subunit (21). The exact stage of RNA synthesis at which rifamycin exerts its inhibitory effect is not known. It does not seem to inhibit RNA polymerase during chain propagation, to prevent the formation of a stable enzyme–DNA complex, or to cause its release from DNA. Goldthwait *et al.* (25) have suggested that rifamycin blocks the formation of an initiation complex of the enzyme with a purine ribonucleoside triphosphate.

The purified polymerases were tested for their relative sensitivity *in vitro* to various amounts of rifamycin. Very little, if any, difference in sensitivity to rifamycin was found between the *gro*$^+$ and *groN* RNA polymerases, with calf-thymus DNA as a template, in the absence of added salt (Fig. 4*a*). With T4 DNA as a template, in the absence of added salt, the *groN* RNA polymerase activity was more sensitive to rifamycin than was its *gro*$^+$ counterpart (Fig. 4*a*). A differential response to rifamycin was most clearly noted in the presence of 0.03 M or higher concentrations of $(NH_4)_2SO_4$. The *gro*$^+$ polymerase was relatively insensitive to low concentrations of rifamycin with calf-thymus and T4 DNA. However, the ammonium sulfate concentration had no effect on the sensitivity of the *groN* polymerase to rifamycin (Fig. 4*b*).

A preliminary experiment with phosphocellulose-treated enzyme (17), with calf-thymus DNA as template, suggested that this enhanced sensitivity of the *groN* RNA polymerase to rifamycin is a property of the core enzyme (enzyme without σ factor).

CONCLUSIONS

The properties of *groN* help to discriminate among the three ways *N* product might act: (*a*) The *N* gene might code for a viral RNA polymerase analogous to the gene 1 product of bacteriophages T7 and T3 (26, 27). This new enzyme would be able to initiate transcription past the termination signals. (*b*) The *N* product might modify bacterial RNA polymerase by combining with it, or by replacing or modifying one of its subunits, as observed in the case of bacteriophage T4 (28) and during bacterial sporulation (29). The interaction of RNA polymerase and *N* product might result either in a failure to read or obey the mRNA termination signals, or in an ability to initiate mRNA transcription at promoter sites beyond the termination signals. (*c*) Finally, *N* product might act by modifying a component of the transcription system other than polymerase, for example, the ρ factor.

The evidence described here shows that the *groN* phenotype results from change in the bacterial RNA polymerase. Thus, models (*a*) and (*c*) are probably not correct. Rather, it appears that the *N*-gene product of λ interacts with bacterial RNA polymerase to change its specificity so that it can transcribe fully the *cII-O-P* and *cIII-exo* operons.

The rarity of *groN* mutants may be explained by the constraints placed on the structure of the mutant enzyme, which must still be able to read bacterial transcription signals properly.

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