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Genetics

σ subunit of *Escherichia coli* RNA polymerase affects the function of λ *N* gene

(transcription/antitermination/host and phage mutants/rifampicin resistance)

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ABSTRACT **A new class of *Escherichia coli* mutants, referred to as *grn*, has been isolated by localized mutagenesis. These mutations affect the σ subunit of DNA-dependent RNA polymerase (ribonucleoside 5′-triphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) by abolishing the expression of the λ *N* gene, and they are closely linked to *dnaG* in the order *dnaG-grn-uxaA*. Detailed study of one such mutant, *grn1*, yielded the following results: (*i*) *grn1* is a single mutation and the mutant cell shows cold-sensitivity in growth; (*ii*) the Grn phenotype of the mutant can easily be suppressed by secondary mutations in the β subunit gene of RNA polymerase; (*iii*) purified holoenzyme of RNA polymerase isolated from the mutant showed an altered salt-dependency *in vitro*, and the mixed reconstitution of the mutant with the wild-type subunits showed that the σ subunit of the *grn1* mutant is altered; (*iv*) λ phage mutants (λ*grg*), which overcome the *grn* mutation, can be classified into two groups, the "*nin*-deletion" and the "*N*-mutant" groups (both of these are also able to grow on the previously described *groN* mutant of Georgopoulos and *nusAB* of Friedman); (*iv*) the mutant polymerase transcribed 12S as well as 7S RNA from λ DNA in the presence of the ρ factor *in vitro*. These results indicate that the *grn* mutation alters the σ subunit of RNA polymerase and that the σ subunit participates in activating the *N*-mediated antitermination mode of λ phage transcription.**

The *N* protein of λ phage acts as a positive regulator of transcription by permitting "read-through" beyond several termination sites (i.e., t_L, t_{R1}, t_{R2}) into adjacent "delayed-early" genes of the phage (1). Several host mutations have been reported that specifically block the function of *N* (2–4). Some of these mutations mapped in the bacterial gene coding for the β subunit of RNA polymerase (*rpoB*), suggesting that *N* protein interacts with the polymerase (2). Moreover, initiation of transcription apparently affects the proper functioning of *N* protein; *N*-mediated antitermination failed to take place at known termination sites when transcription was initiated at certain bacterial promoters (5, 6) or at new promoters created by mutation (7). Furthermore, *nutL* mutants of λ phage have been described recently that are defective in *N* utilization only at the leftward termination site, t_L (8). The *nutL* site maps immediately downstream from promoter p_L at a site corresponding to the actual site of *N* protein recognition, which differs from the site where *N*-mediated antitermination takes place. These findings emphasize the importance of the initiation step(s) of transcription for the event of *N* protein-mediated antitermination. Because the function of the σ subunit of RNA polymerase is essential for proper initiation of transcription (9–11), appropriate mutational alterations of the σ subunit may affect the function of the *N* protein of λ phage. The structural gene for the σ subunit (*rpoD*) is located at 66 min on the *Escherichia coli* chromosome (12, 13), and therefore isolation of such a mutation should be feasible by the application of localized mutagenesis to the region. We report in this paper a type of GroN mutation, *grn*, which appears to be in the structural gene for the σ subunit of RNA polymerase of *E. coli*.

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MATERIALS AND METHODS

Bacterial and Phage Strains. The *E. coli* K-12 strains used are listed in Table 1. Phages used were: λ*papa*; λ*vir*; λ*cI71*; λ*gt-λC*; λ*gt-rpoD*; λ*Nsus7nin5*; λ*imm21cI*; λ*imm434*; λ*Nsus7imm434cI*; P1*vir*; P2. Those strains without indication of source are from our own collection.

Media, Buffers, and Chemicals. The broth and agar media used were as described (16). Minimal medium was medium E (17) with appropriate supplements. Buffers and chemicals used were generally as described (18–20).

Isolation of *grn* Mutants. Phage P1 grown on a wild-type strain (C600) was mutagenized with hydroxylamine (21, 22), and was used to transduce a *tolC* strain carrying λ*cI857ΔH1* (YN495). Tol$^+$ transductants (deoxycholate-resistant colonies) were selected at 33°C on tryptone/yeast agar plates containing 0.05% deoxycholate. They were replica-plated onto EMBO plates seeded with λ*cI71* (10^8–10^9 phages per plate) and incubated at 42°C for 24 hr. Temperature-resistant pink colonies not lysed by phage infection were isolated. One of these mutants, called YN524, was used for the present study.

Purification of RNA Polymerase. RNA polymerase was purified essentially according to the procedure described by Burgess and co-workers (23, 24), which consists of prior treatment with polyethylene glycol 6000, chromatography on a calf thymus DNA-cellulose column, and chromatography on a DEAE-cellulose column (18). RNA polymerase preparations thus obtained (containing 70–90% holoenzyme) were used in the present study.

Reconstitution of Holoenzyme from Core and σ. The core enzyme and σ subunit were prepared from purified RNA polymerase as described by Burgess and Jendrisak (23), with chromatography on a Bio-Rex 70 (100–200 mesh) column. Reconstitution of active holoenzyme was carried out by mixing the core and σ fractions in a stoichiometric ratio and dialyzing the mixture against the reconstitution buffer as described (18).

***In Vitro* Transcription of λDNA.** The reaction system was that of Roberts (25), with minor modifications. The template used was λ*b515b519cI857S7xis6* DNA. The reaction mixture contained 20 mM Tris·HCl (pH 7.8 at 37°C), 10 mM Mg acetate, 0.1 mM dithiothreitol, 40 μg of bovine serum albumin per ml, 80 mM KCl, 4% glycerol, 0.16 mM XTPs, and [^{3}H]ATP at 4 μCi/ml (1 Ci = 3.7×10^{10} becquerels); RNA polymerase, ρ factor, and DNA were added to give 6, 1, and 4 μg, respectively, per reaction tube containing a total volume of 0.5 ml. Transcription was carried out at 37°C for 20 min and was stopped

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Table 1. Bacterial strains

Strain	Character	Source or reference
YN203	F⁻ *trpE9829(am) tyr(am) thr pro his argA metE sup-126 uxaA dnaG3 tolC rif* (*ø80h$^{\lambda}$imm434pSuIII^{ts6}*)	This paper
YN495	F⁻ *tolC lac(am) supF str* del(*bio-ch1A*)(*λcI857ΔH1*)	This paper
YN508	F⁻ *tolC⁺ grn1* transductant constructed by YN495 × P1(YN524)	This paper
YN524	Same as in YN495 except *grn1 tolC⁺*	This paper
groN785	*groN785 galE sup⁺*	Ref. 2
nusAB27-1	*nusA nusB*	Ref. 14
C600	F⁻ *thr leu tonA lac supE44 thi*	
594	F⁻ *gal su⁻ str*	
Km605[*λdv1*]	*str his recA1 su⁻ gal thy trp* [*λdv1*]	Ref. 15

by addition of 0.25% Na dodecyl sulfate. After standing for 3 min at 37°C, the mixture was rapidly cooled in an ice bath, and the precipitates that formed were removed by centrifugation. After addition of ribosomal [^{14}C]RNA as a size marker, the supernatant was fractionated by sucrose density gradient centrifugation in a Spinco SW 41 rotor at 37,000 rpm for 14 hr. Acid-insoluble radioactivity was measured.

Electron Microscopic Analysis of Heteroduplexes. The procedure has been described (26).

RESULTS

Isolation of the *grn* Mutants. The *grn* mutants of *E. coli* were obtained by localized mutagenesis of the *tolC–rpoD* region of the chromosome and selection for cells that specifically abolish the function of the *N* gene product of λ phage. The parental strain (YN495), used for isolation of the mutants, had a *tolC* mutation and carried prophage *λcI857ΔH1*. [*ΔH1* is a deletion that eliminates from the lysogen a region extending from the *cro* gene of λ to the *chlA* gene of the bacterium, including the right-hand attachment site of λ (27).] Thus, the prophage could not be excised upon induction. Although the prophage harbored *Nsus7Nsus53* mutations, they were suppressed in the YN495 strain by introducing a *supF* mutation. Therefore, the cell could grow at high temperature (>40°C) only when the function of the *N* gene was abolished and the expression of the *kil* gene of the prophage was prevented (28). A similar rationale was previously used for selecting GroN mutants of *E. coli* (29). Localized mutagenesis was accomplished by transduction with hydroxylamine-treated P1 phage (21, 22) and selecting for Tol⁺ transductants. Among 2×10^4 transductants selected at 33°C, 10 could grow at 42°C without losing immunity to λ. These *E. coli* mutants were named *grn* mutants (for *groN*-type), and one of them, YN524[*grn1*], was studied in detail.

Table 2 lists the efficiency of plating of various derivatives of λ phage on *grn⁺* and *grn1* bacteria. On *grn1* mutant bacteria, growth of those phages that require the function of the λ *N* gene for growth was severely impaired, especially at high temperature. On the other hand, those phages that do not depend on the function of λ *N* gene (i.e., those harboring the *nin5* deletion or *imm21* substitution) grew well on the mutant bacteria. Other coliphages (i.e., T4, T5, T6, T7, P1, P2, and BF23) multiplied normally on *grn1* bacteria.

It is interesting to note that the *grn1* mutant did not form colonies at 20°C. Thus, it is cold-sensitive (Cs character).

Table 2. Efficiency of plating of various phages on *grn⁺* and *grn1* bacteria

Phage	Temperature, °C	C600 (*grn⁺*)	YN495 (*grn⁺*)	YN524 (*grn1*)
λvir	33	1	1.15	7×10^{-5}
	40	1	—	$<5 \times 10^{-10}$
λpapa	40	1	—	$<2 \times 10^{-8}$
λcI71	40	1	—	$<2 \times 10^{-8}$
λNsus7nin5	40	1	—	0.09
λgt-λC	40	1	—	0.35
λimm434	33	1	0.82	3×10^{-3}
	40	1	—	$<3 \times 10^{-7}$
λimm21cI	33	1	1.14	0.54
	40	1	—	0.39

Spontaneous Cs⁺ revertants able to grow at 20°C were selected and examined for the capacity to support development of *λpapa* or *λcI71*. Of 10 Cs⁺ revertants tested, 7 allowed phage to grow at high temperature. In another experiment, the *grn1* mutation was transduced into temperature-sensitive YN495, and Tol⁺ transductants were selected. Of 789 Tol⁺ transductants, 425 (54%) could grow at 42°C. These temperature-resistant transductants, without exception, could not grow at 20°C. On these transductants, λ *N*-dependent phages such as *λcI71* or *λvir* could not grow, but *λgt-λC* (a λ *N*-independent phage) could grow at high temperature. We conclude that a single mutation, *grn1*, is responsible for both the Cs and Grn phenotypes.

The location of the *grn1* mutation was determined more precisely by P1-transduction using the mutant as donor and strain YN203 [*tolC dnaG uxaA*] as recipient. The results presented in Table 3 indicate that the *grn1* mutation is closely linked to *dnaG* on the side of *uxaA*. The location is the same as that of the *rpoD* gene as determined in previous experiments (18, 30–32).

Alteration of the β Subunit of RNA Polymerase Suppresses the *grn1* Mutation. Mutations conferring resistance to rifampicin are known to alter the β subunit of RNA polymerase (33). If the *grn1* mutation indeed affected the structure of the σ subunit, appropriate secondary mutations in the *rpoB* gene conceivably could suppress the original mutation. Therefore, spontaneous rifampicin-resistant (*rif*) derivatives of the *grn1* mutant were isolated and examined for their Grn phenotypes. Nine of 19 *rif* derivatives were scored as Grn⁺ because they regained the capacity to allow growth of λ phages. In addition, several *rif* mutations suppressed the cold-sensitivity associated with the *grn1* mutation. The suppressor function of secondary *rif* mutations strongly suggests that there is a physical interaction between the β subunit and the *grn* gene product, which may be the σ subunit of RNA polymerase.

Mutants of λ Phage Able to Grow on *grn1* Mutant Bacteria. Mutants of λ phage able to form plaques on the *grn1* mutant (hereafter referred to as *λgrg*) were isolated by plating

Table 3. Genetic mapping of *grn1* mutation by P1 transduction*

Unselected markers			Colonies	
dnaG	*grn1*	*uxaA*	No.	% of total
1	1	1	14	14
1	0	1	1	1
1	1	0	47	47
1	0	0	4	4
0	0	0	34	34

Donor: YN524 = [*tolC⁺dnaG⁺grn1 uxaA⁺*] = [1 1 1 1]
Recipient: YN203 = [*tolC dnaG grn⁺ uxaA*] = [0 0 0 0]
* Selected marker, Tol⁺.

phages on *grn1*. Table 4 summarizes the plating efficiencies of seven independently isolated λ*grg* phages on *grn1*, *groN785* (2), and *nusAB27-1* (14) mutant bacteria. The data indicate that the mutant phages isolated on *grn1*, hereafter called "overcomer phages," are also able to develop plaques on previously described mutant bacteria in which *N* expression is prevented. Upon close examination of the λ mutants, it appears that the λ*grg* phages can be classified into two groups according to their efficiency of plating on *grn1* bacteria; three phage mutants—λ*grg2*, λ*grg3*, and λ*grg5*—plated with high efficiency, whereas the rest of the phages plated with much reduced efficiencies. Electron microscopic analysis of appropriate heteroduplexes disclosed that the former group of λ*grg* phages harbored *nin5*-like deletions eliminating the t_{R2} termination site. Therefore, they are listed as "*nin* deletion" types in Table 4. Apparently, these λ*grg* mutants acquired plating ability by losing the t_{R2} site and thus became "*N*-independent." This implies that the *grn1* mutant is in fact a GroN-type mutant in which the expression of the λ *N* gene is abolished. It should also be noted that at least the rightward promoter, p_R, is recognized by RNA polymerase of *grn1* mutant cells.

Heteroduplexes of the remaining class of λ*grg* DNA with λ*imm434* DNA were also examined by electron microscopy. No insertion or deletion could be found anywhere in the *nin5* region. Accordingly, the mutant sites in these phages were mapped by crosses with λ*Nsus7imm434cI*, selecting for *sus*$^+$*imm434* recombinants, and examining their growth on *grn1* bacteria. The results are presented in Table 5 (λ*grg6* was not examined). These findings indicate that the overcomer mutations of λ*grg1*, λ*grg4*, and λ*grg7* are closely linked to *imm434*. Because the behavior of these *grg* mutations can be most easily explained by assuming that they are mutations altering the *N* protein, they are tentatively designated "*N*-mutants" in Table 4. (On the other hand, the result of the cross involving λ*grg3* is consistent with the electron microscopic observation that the mutant phage harbors a *nin5*-like deletion which eliminates the t_{R2} termination site.)

Another set of crosses was carried out between λ*grgimm434* phages and λ*dv1* plasmids to map the *grg* mutations more precisely. The results obtained are also presented in Table 5. Although very rare, *grg*$^+$ recombinant phages were found among λ*vir* recombinants in crosses involving *grg4* and *grg7*. Therefore, these mutations are covered by the *dv1* region and are close to its left-hand terminus. The segment defined by the two left-hand ends of the λ*dv1* and *imm434* regions is about 124 base pairs long (8). *Nsus7* is known to be a promoter-distal mutation, and *N* polypeptides produced by infection of the wild-type and *Nsus7* phages have been identified as having molecular weights of 13,500 and 10,500, respectively (34). However, the exact site of the λ genome which codes for the amino terminus of the wild-type *N* protein is not known. If "*N*-mutant"-type *grg* mutations were in fact mutations within the *N* cistron, the cistron should start within the λ*dv1-imm434* segment. The segment contains one GUG initiation codon at 90–92 bases from the 5′ terminus of the p_L transcript (35). It should be noted that these "*N*-mutant" *grg* phages plate on *groN785* and *nusAB27-1* mutant bacteria with efficiencies higher than those on *grn1*, on which they were originally selected (Table 4).

Table 4. Efficiency of plating of overcomer phages on various GroN bacteria

Overcomer λ mutants	Efficiency of plating on: *grn1*	*groN785*	*nusAB27-1*	Type
λ*grg1*	0.04	0.65	0.97	*N* mutant
λ*grg2*	1.01	0.80	1.26	*nin* deletion
λ*grg3*	0.4	1.05	1.26	*nin* deletion
λ*grg4*	0.02	0.62	1.36	*N* mutant
λ*grg5*	0.78	0.84	1.37	*nin* deletion
λ*grg6*	0.003	0.01	0.79	Unknown
λ*grg7*	0.007	0.61	0.85	*N* mutant

Table 5. Genetic mapping of *grg* mutations

Cross	Selection	Recombinants, no. Total	grg$^+$(%)
λ*grg1* × λ*Nsus7imm434*	*sus*$^+$*imm434*	233	198 (85.0)
λ*grg3* × λ*Nsus7imm434*	*sus*$^+$*imm434*	200	200 (100.0)
λ*grg4* × λ*Nsus7imm434*	*sus*$^+$*imm434*	190	156 (82.1)
λ*grg7* × λ*Nsus7imm434*	*sus*$^+$*imm434*	177	138 (78.0)
λ*grg1imm434* × λ*dv1*	*vir*	320	0 (0.0)
λ*grg4imm434* × λ*dv1*	*vir*	215	2 (0.9)
λ*grg7imm434* × λ*dv1*	*vir*	235	1 (0.4)

For crosses between two phages, C600 cells were infected with phages at a multiplicity of 5 each. *sus*$^+$*imm434* recombinant phages were selected on 594(λ) and were tested for the *grg* character on the *grn1* mutant, YN524. For crosses between λ*dv1* plasmid and λ*grgimm434* phages obtained in the above crosses, Km605 carrying the plasmid was infected with phage at a multiplicity of 3. λ*vir* recombinants were selected on C600(λ*imm434*) and tested for the *grg* character.

Altered Salt-Dependency of RNA Polymerase from the *grn1* Strain. The results presented above suggest that the σ subunit of RNA polymerase may be altered in the *grn1* mutant. To test whether the *grn1* mutation directly affects the σ subunit, we measured the salt-dependence of transcription by purified enzyme from a *grn1* transductant, YN508, and its isogenic parent YN495. When equal weights of the enzyme from *grn1* and *grn*$^+$ strains were assayed with T7 DNA as a template, transcription by the polymerase from YN508 was considerably more salt resistant than that from YN495 (Fig. 1*A*). To identify the altered subunit, purified holoenzymes from mutant and wild-type cells were separated into σ and core fractions and were mixed in various combinations to reconstitute holoenzymes. Their activities were tested at a fixed salt concentration (0.16 M KCl) and at various temperatures. The results indicated that the σ subunit of the *grn1* RNA polymerase was altered (Fig. 1*B*).

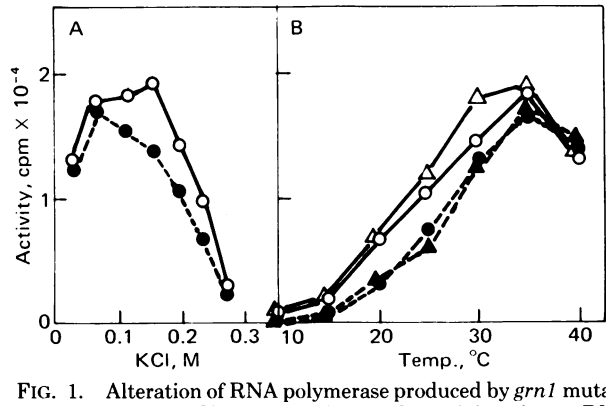

FIG. 1. Alteration of RNA polymerase produced by *grn1* mutant cells. (*A*) Effect of KCl concentration on the activity of *grn1* RNA polymerase. RNA polymerase holoenzyme was purified from *grn1* (YN508) and its parent strain (YN495). Enzyme activities were assayed with T7 DNA template at the indicated KCl concentrations for 10 min at 30°C. The reaction mixture was as described (18). ○, Mutant enzyme; ●, wild-type enzyme. (*B*) Mixed reconstitution of the mutant with the wild-type subunits. Enzyme activities were assayed with T7 DNA template at the indicated temperatures for 20 min at 0.16 M KCl. ○, Mutant core + mutant σ; △, wild-type core + mutant σ; ●, wild-type core + wild-type σ; ▲, mutant core + wild-type σ.

Leftward Transcription of λDNA by the *grn1* RNA Polymerase. The GroN character of the *grn1* mutant can be explained by postulating either that *N* protein is produced in the cell but is unable to function or that *N* protein is not produced because the p_L promoter is not recognized by the *grn1* σ subunit. To distinguish between these alternatives, *in vitro* transcripts of λDNA (produced by the mutant polymerase in the presence of ρ factor) were analyzed. Wild-type RNA polymerase produces a 7S rightward transcript and a 12S leftward transcript, encoding the polypeptide products of *cro* and *N*, respectively (25). The results presented in Fig. 2 indicate that the mutant RNA polymerase produces 12S RNA as well as 7S RNA. This shows that, at least *in vitro*, the p_L as well as the p_R promoter is recognized by the mutant polymerase and that transcription is terminated in the presence of the ρ factor at the normal termination sites. Because there is no reason to assume that translational activity is impaired in the mutant bacteria, we conclude that the *N* protein is produced but does not function in *grn1* mutant cells.

Because *grn* mutants were originally isolated by selecting bacterial mutants in which expression of the λ *kil* gene was prevented, it may be argued that we have selected bacteria tolerant to the *kil* gene function without preventing λ *N* function. To examine this possibility, we looked for expression of λ genes other than *kil*. Expression of the *spi* gene in the left arm of λ is under *N* control and is required for inhibition of λ growth by prophage P2. In fact, λ*gt*-λ*C* harboring the *nin5* deletion does not grow on a P2 lysogen of C600. However, the phage was able to grow at 40°C on P2 lysogens of *grn1* as well as *nusAB* mutants, indicating the *grn1* as well as *nusAB* mutations block leftward transcription beyond t_L, abolishing transcription and expression of the *spi* gene (data not shown). On the other hand, growth of a transducing phage, λ*gt-rpoD*, carrying *rpoD* was strongly impaired on a P2 lysogen of *grn1* cells, whereas the transducing phage grew well on a P2 lysogen of *nusAB*. These results indicate that the *spi* gene is expressed in the *grn1*(P2) cells using the wild-type σ subunit produced by λ*gt-rpoD*.

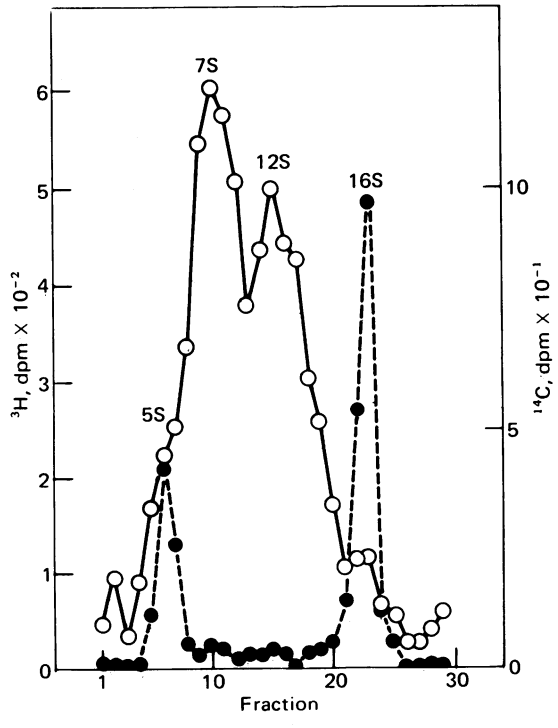

FIG. 2. Transcription of λDNA *in vitro* by the *grn1* RNA polymerase. ^{3}H-Labeled transcripts (○) of λDNA produced in the presence of ρ factor were fractionated by sucrose density gradient centrifugation. ^{14}C-Labeled *E. coli* ribosomal RNA (●) was added as reference markers.

DISCUSSION

Our studies demonstrate that a mutation called *grn1* alters the σ subunit of *E. coli* RNA polymerase and that this alteration specifically affects the antitermination function of the *N* protein of phage λ. The first inference is based on the following findings. (*i*) The genetic locus of the *grn1* mutation is at 66 min on the *E. coli* chromosome and the order is *tolC-dnaG-grn1-uxaA;* the location is exactly the same as that of *rpoD*. (*ii*) Spontaneous mutations introduced into the β subunit gene (*rpoB*) of RNA polymerase often suppressed the phenotype of the *grn1* mutation. Furthermore, *grn1* confers on *E. coli* the property of cold-sensitive growth, suggesting that the product of the *grn1* gene is unable to assemble into an active structure at 20°C. (*iii*) RNA polymerase holoenzyme purified from *grn1* cells is more salt-resistant *in vitro* than the enzyme obtained from isogeneic *grn*$^+$ cells. Enzyme reconstitution experiments indicated that the altered component is the σ subunit.

The second inference, that the *grn1* mutation specifically blocks *N* protein function, is based on the following evidence. (*i*) Although λ *N*-dependent phages do not form plaques on the mutant bacteria, "λ *N*-independent" phages harboring the *nin5* deletion or *imm21* substitution do plate on *grn1*. (*ii*) Two "delayed-early" genes of λ, *kil* and *spi*, cannot be expressed in the mutant cell. (*iii*) Phage mutants, referred to as λ*grg*, could be selected on *grn1*, and they also plate on previously described *groN785* and *nusAB27-1* cells (2, 14), in which wild-type λ phage does not multiply because λ *N* expression is abolished. Some of these λ*grg* phages were deletion mutants that have lost the rightward termination site, t_{R2}. We postulate that λ *N*-dependent phages cannot multiply in *grn1* cells because the antitermination function at t_{R2} does not operate despite the fact that rightward transcription has been initiated at p_R. (*iv*) 12S RNA as well as 7S RNA is produced normally from λDNA by the mutant RNA polymerase purified from *grn1* cells (Fig. 2). This result argues against the possibility that the mutant polymerase is unable to recognize the p_L promoter, even though it transcribes from p_R.

N protein is believed to interact with the β subunit of RNA polymerase, because mutational alteration of the β subunit blocks expression of *N* function (2). Analogously, the present results indicate that at some stage *N* protein also interacts with the σ subunit of RNA polymerase. *N* protein does not act directly on termination factors to effect antitermination, but it exerts its influence by modifying RNA polymerase at a *cis*-acting site located upstream from the terminator site on DNA (5, 6). In fact, Salstrom and Szybalski (8) described mutations that define a new kind of *cis*-acting recognition site controlling an early step in the *N*-mediated antitermination mode of leftward transcription; they designated the site *nutL*, for *N* utilization. Thus, we may visualize that σ subunit remains bound to core polymerase transcribing template DNA until the polymerase moves down to the *nutL* site where *N*-mediated modification takes place. Because *nutL* is mapped within an interval defined by the left ends of the λ*dv1* plasmid and the *imm434* substitution, the site is clearly outside the p_L promoter (8). An alternative model assumes that σ protein is replaced by *N* protein at the promoter site, but *N* does not modify RNA polymerase until the *N*-core complex moves down to the *nutL* site. The *grn1* mutation of the σ subunit prevents formation of the *N*-core complex. This model retains the presently accepted notion of the "σ-cycle" (36).

λ*imm21* grows on *grn1* cells (Table 2) but fails to grow on *groN785*, *nusA*, and *nusB* (2, 14). This appears to indicate that the block exerted by the *grn1* mutation is "weaker" than that exerted by other mutations. However, poor "overcomer" phages selected on *grn1* cells grew more easily on *groN785* and *nusAB27-1* (Table 4), indicating that *grn1* is even more restrictive than other mutations. Therefore, the block of *N* function caused by alteration of the σ subunit is specific to λ *N* protein or to a combination of the λ *N* protein and the *nutL* site.

The frequent occurrence of secondary-site suppressors of the *grn1* mutation among spontaneous rifampicin-resistant mutants suggests that alteration of the β subunit can easily compensate for alteration of the σ subunit. Thus, it may be possible to visualize that λ*grg* overcomer mutants of the "*N*-mutant" type may not necessarily confer a new modification on the σ subunit, but they may be those that confer on the β subunit modifications different from those affected by wild-type λ *N*. The altered modification of the β subunit by λ*grg* phages of the "*N*-mutant" type might be those that simultaneously suppress other bacterial mutations blocking the λ *N* function. Because the mechanism of the suppression, as now visualized, bypasses restoration of the proper interaction between the σ protein of the *grn1* mutant RNA polymerase and λ *N*, this would explain why poor overcomer mutants on *grn1* could be good overcomer mutants on *groN785* or *nusAB27-1* cells.

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