RNA Polymerase Interaction with *dnaB* Protein and Lambda *P* Protein During Lambda Replication

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The *Escherichia coli* GroP⁻ phenotype, associated with some *dnaB* mutants and measured as a decreased ability to plate λ bacteriophage, was altered by some *rpoB* mutations. The *rpoB* effect showed an allele specificity. The participation both of *dnaB* and of λ *P* alleles in the GroP⁻ phenotype was also allele specific. It was concluded that RNA polymerase, *dnaB* protein, and λ *P* protein form a functional complex required for λ replication.

The largest genetic class of *Escherichia coli* temperature-sensitive *dna* mutants is *dnaB* mutants. Excluding a single, and now well-understood exception, none of the several dozen *dnaB* mutants is capable of propagating lambda bacteriophage at the restrictive temperature (6, 11).

Some *dnaB* mutants exhibit a diminished ability to plate lambda even at temperatures permissive for the host. This characteristic, termed the GroP⁻ phenotype, was characterized by Georgopoulos and Herskowitz (3), and could be overcome by some mutations in the *P* gene of lambda. This GroP⁻ phenotype of *dnaB* mutants was originally subdivided into two classes: (i) GroPA⁻, which could be counteracted by lambda mutations classed as πA or as πB , both of which mapped within the *P* gene, and (ii) GroPB⁻, which could be counteracted by πB mutations but not by those of the πA group (3). One possible conclusion from these observations, that the lambda *P* gene protein and the *dnaB* protein interact (3), has been confirmed by recent biochemical and genetic experiments showing that these two proteins do interact in vitro and, apparently, in vivo (4, 13) also.

While studying the *dnaB* locus, we serendipitously discovered that a rifampin-resistant (Rif^r) mutation suppressed the GroP⁻ phenotype of one *dnaB* allele. The work reported here was undertaken to determine the generality of this observation and to characterize this suppression.

Several *dnaB* mutants exhibiting various GroP⁻ phenotypes were selected from our collection. The three mutants E279, E368, and E391 contain the *dnaB*279, *dnaB*368, and *dnaB*391 alleles, respectively; all are derivatives of the *dnaB*⁺ strain CR34 (12). Independent, spontaneous Rif^r mutations were isolated in each of these mutants, and the resultant *dnaB*

Rif^r derivatives were characterized with respect to GroP phenotype and to strain temperature sensitivity. All remained temperature sensitive.

To determine whether the alteration of the GroP phenotype by Rif^r mutations exhibited an allele specificity, each Rif^r mutation was transduced into all of the *dnaB* strains. Since, however, Rif^r mutations occur spontaneously at a frequency similar to the P1 transduction frequency of *E. coli* markers, it was necessary to ensure that Rif^r transductants contained the Rif^r-conferring mutation of the donor. The *zih-510::Tn10* insertion, which cotransduces at a high frequency (~86%) with *rpoB*, was transduced into the original *dnaB* Rif^r isolates, and Rif^r tetracycline-resistant (Tet^r) transductants were purified. These Rif^r *zih-510::Tn10* strains were used as donors in P1vir^s transductions with the *dnaB* recipients; selection was for Tet^r with subsequent screening for Rif^r. Since mutations in the *rpoB* locus are the only ones known to confer the Rif^r phenotype in *E. coli*, and since all of the Rif^r mutations cotransduced with *zih-510::Tn10* at frequencies consistent with their being in the *rpoB* gene, the Rif^r mutations have been assigned *rpoB* allele numbers. To rule out a variety of possible artifacts, including P1 lysogeny (despite the use of P1vir^s), the *rpoB dnaB* strains were selected for spontaneous loss of Tn10 and then transduced to *rpoB*⁺. These *dnaB rpoB*⁺ derivatives exhibited phenotypes identical to those of their original *dnaB* parents (data not shown).

The influence of various *rpoB* alleles on the GroP⁻ phenotype of *dnaB*368 is shown in Table 1. The *dnaB*368 *rpoB*⁺ strain E368 exhibits a weak but definite GroPA⁻ phenotype. The decreased efficiency of plating (EOP) of lambda is generally less pronounced for mutants isolated for their DNA replication defect rather than for

TABLE 1. Lambda plating efficiency on *dnaB368* derivatives^a

<i>rpoB</i> allele ^b	λ^+	$\lambda\pi A$	$\lambda\pi B$
+	0.3	0.82	0.97
2790	0.3	0.96	0.55
2794	0.4	1.6 ^c	0.32
3910	0.11	0.17	0.02
3911	0.82	0.27	2×10^{-3}
3913	0.23	0.1	3×10^{-3}
3914	0.23	0.1	0.44

^a Strain growth and plating of phage at 30°C were as described previously (7). All lambda strains contain the cI60 mutation and produce clear plaques. The π mutations are $\pi A1$ and $\pi B1$ used by Georgopoulos and Herskowitz (3) and obtained from those investigators. A minimum of ca. 100 plaques per plate (duplicate platings) were counted to determine the EOP. Experiments were repeated at least five times; the variation between repeats was always within acceptable experimental error. The values in Tables 1 and 2 are from separate experiments.

^b The first three digits of the *rpoB* allele numbers are identical to the number of the E series strain in which the Rif^r mutation was isolated (e.g., *rpoB2790* was isolated in E279).

^c The EOP of λ on CR34 is defined as 1. On the standard λ plating strain 594, the EOP, normalized to CR34, varies from 1.2 to 1.6. The somewhat variable lower EOP on CR34 is a plating effect; the transmission efficiencies of CR34 and 594 are identical. Apparently, minor changes in growth occur in some of the mutants and result in occasional values greater than 1.

their EOP defect. Nevertheless, the data are highly reproducible and do signal a bona fide GroP⁻ phenotype (3). For many of the host-bacteriophage combinations used in these experiments, the plaques were small. As the plating efficiencies appear to be an accurate measure of the GroP character, plaque size comparisons are not presented.

The results in Table 1 clearly show that *rpoB* alleles can significantly alter the GroP⁻ phenotype of *dnaB368* and that the change in the phenotype can be either a decrease or an increase in the efficiency of λ plating. At one extreme, the *dnaB368 rpoB3913* mutant plates all three phages very poorly, whereas at the other extreme, *rpoB3911* reverses the plating efficiency observed with the *dnaB368 rpoB⁺* strain such that λ^+ exhibits a near normal EOP and $\lambda\pi B$ plates with an efficiency of 2×10^{-3} .

The apparent *rpoB* allele specificity could reflect (i) a distinct, separate effect unrelated to GroP, (ii) a transcriptional effect on the genes involved in the GroP character, or (iii) the involvement of the RNA polymerase β subunit in a complex with the *dnaB* and λP gene products.

If the *rpoB* and *dnaB* GroP effects are completely separate, the combination of *dnaB* and

rpoB in a single strain should result in one character taking precedence or in the characters combining either as the sum or the product of the two separate effects. One example from Table 2 that is clearly inconsistent with both of these predictions is the plating of $\lambda\pi B$ on *dnaB368 rpoB⁺* (Table 2, line 1c) and on *dnaB⁺ rpoB3911*, each of which give an EOP approximately equal to one, whereas combination of the mutations (line 5c) results in an EOP of 3×10^{-3} . Another example is the EOP of $\lambda\pi B$ on *dnaB391 rpoB⁺* at 0.05 (line 1d) and on *dnaB⁺ rpoB2790* at 0.1 (line 2a) but on *dnaB391 rpoB2790* at 1.05 (line 2d). These examples plus many others in Table 2 strongly argue against the possibility that the *dnaB* and *rpoB* effects are unrelated.

As an alternative to the hypothesis that the *dnaB* and λP gene products interact, it was suggested that the original GroP data were also largely explicable by assuming that the levels of *dnaB* protein and λP product must bear some

TABLE 2. Lambda plating efficiency on *rpoB dnaB* strains^a

Line	<i>rpoB</i> allele	<i>dnaB</i> allele	λ^+	$\lambda\pi A$	$\lambda\pi B$
1a		+	1	1	1
1b		279	0.21	0.14	0.61
1c	+	368	0.28	0.93	1.03
1d		391	0.87	0.7	0.05
2a		+	0.97	0.72	0.1
2b	2790	279	0.32	0.45	0.41
2c		368	0.28	1.03	0.49
2d		391	0.65	0.51	1.05
3a		+	0.93	0.97	0.84
3b	2794	279	0.47	0.35	0.11
3c		368	0.37	1.52	0.33
3d		391	0.35	0.33	0.04
4a		+	0.99	0.71	1.04
4b	3910	279	0.16	0.16	0.04
4c		368	0.11	0.17	0.02
4d		391	0.47	0.38	1.06
5a		+	1.31	1.17	1.52
5b	3911	279	0.02	0.07	0.03
5c		368	0.82	0.23	3×10^{-3}
5d		391	0.79	0.84	1.44
6a		+	0.51	0.23	0.06
6b	3913	279	0.02	10^{-3}	0.09
6c		368	0.23	0.1	2×10^{-3}
6d		391	0.27	10^{-3}	2.5×10^{-3}
7a		+	0.73	0.11	0.32
7b	3914	279	0.03	0.36	0.28
7c		368	0.23	0.1	0.44
7d		391	0.92	0.92	1.06

^a See footnotes a, b, and c in Table 1.

fixed proportion to each other (3). This "levels hypothesis," presented as a possible explanation of the original data, assumed that the level of functional *P* product in the three strains was in the order $P^+ > \pi A > \pi B$ (3). The results obtained with either *rpoB2790* or *rpoB2794* in combination with *dnaB368* argue against this levels hypothesis (Table 1). For both of these strains, $\lambda\pi A$ plates significantly better than either λ^+ or $\lambda\pi B$.

If the GroP effects of the *rpoB* alleles were a consequence of an alteration in the level of transcription from the promoters of *dnaB* or of λP , the results would be explicable by a levels hypothesis. Since the π mutations map within the *P* gene (3; C. Georgopoulos, personal communication), the *P* gene promoters are the same in all three phages. Also, as the *dnaB* mutations used in this study are temperature-sensitive, missense mutations mapping within the *dnaB* gene and are in identical backgrounds (5, 8), the *dnaB* promoters are identical in each of the host mutants. Therefore, explanations of the EOP values on *dnaB368 rpoB3911* (Table 2, line 5c compared with 1c) and on *dnaB391 rpoB3911* (Table 2, line 5d compared with 1d), based on transcriptional modulation of product levels, must be parallel and consistent. We have been unable to devise such an explanation without invoking several additional and rather discrete assumptions. Furthermore, with or without the additional assumptions, the data for the remaining two *rpoB3911* strains could not be explained (Table 2, lines 5a and b compared with lines 1a and b).

Another case in which the transcriptional modulation of product levels fails to explain the data can be seen by comparing the effects of *rpoB*⁺, *rpoB2790*, *rpoB3910*, and *rpoB3914* on *dnaB391* (Table 2, lines 1d, 2d, 4d, and 7d). The three mutant *rpoB* alleles have nearly identical effects on the plating of $\lambda\pi B$ but significantly differing effects on the plating of both λ^+ and $\lambda\pi A$.

The GroP phenotype of *dnaB391* is, itself, unusual in that it is the inverse of the GroPB phenotype.

The data in Table 2 also show clear *dnaB* allele-specific effects with all of the *rpoB* mutations except *rpoB3913* which restricts the ability of all of the *dnaB* mutants to plate lambda. More importantly, there are significant pattern shifts in the plating efficiencies of λ^+ , $\lambda\pi A$, and $\lambda\pi B$ (compare, for example, lines 2c with 2d, 4d with 4b or 4c, 5c with 5d, etc.). As might be expected, in some cases, the *rpoB* mutations alter the ability of a *dnaB*⁺ strain to plate one or another of the λ phage.

All of the data in Table 2 are consistent with the notion that RNA polymerase (or, minimally,

TABLE 3. Transmission of λ by *dnaB rpoB* mutants^a

Relevant genotype		EOP ^b	Transmission at MOI	
Host	λ		0.2	10
<i>dnaB279 rpoB3911</i>	πB	0.03	0.03	0.3
<i>dnaB368 rpoB3911</i>	πB	3×10^{-3}	10^{-3}	1.1×10^{-2}
<i>dnaB368 rpoB3914</i>	πB	0.44	0.37	0.41
<i>dnaB368 rpoB3911</i>	+	0.82	0.80	0.75

^a Transmission efficiencies were determined by adsorbing phage to the mutant cells and plating for infectious centers on a lawn of CR34. The transmission frequency on CR34 was used for normalization.

^b From Table 2.

the β subunit) physically participates in the *dnaB*- λP protein interaction during lambda replication.

That the EOP data accurately reflect the ability of the strains to propagate lambda is shown by the fact that at low multiplicity of infection (MOI), the transmission frequencies are nearly identical to the observed EOP results (Table 3). There was a substantial improvement in transmission frequencies for the *dnaB368 rpoB3911* strain, and also for *dnaB279 rpoB3911*, when the MOI was increased to 10. These data provide an explanation for the reliability of the EOP measurements despite the low transmission efficiencies at an MOI of 0.2. After the burst emitted from the originally infected cell, surrounding cells are likely to be multiply infected, and the increased transmission frequencies under these conditions would be sufficient to produce an observable plaque. The increased transmission efficiency at high MOI could result either from increased concentration of a required λ gene product or from progeny produced solely by recombination (4, 7, 9). An additional inference to be drawn from the inability to increase the transmission frequency of *dnaB368 rpoB3911* with λ^+ and *dnaB368 rpoB3914* with $\lambda\pi B$ at high MOI is that, even when transmission is relatively efficient, a proportion of the cells in the culture are unable to produce even one infective λ . It has been shown that the GroP⁻ phenotype is a consequence of a reduced ability to replicate λ DNA (3).

Mutations in *rpoB*, then, can increase or decrease the ability of a strain to plate and to propagate λ , and the particular response of the strain to the *rpoB* allele is dependent on the resident *dnaB* allele. In addition, the λP allele also specifically affects the observed EOP. Because mutations in the *rpoB*, *dnaB*, and λP genes all exhibit clear allele-specific effects on the GroP character, we contend that the simplest explanation is that the products of each of these

genes participate in a physical complex involved in λ DNA replication.

The data were not consistent with a levels hypothesis as a distinct alternative to protein-protein interactions. It should be noted, however, that protein levels are significant within the context of complex formation. If the defect in a mutant protein is a decreased ability to complex with another protein, the defect may be overcome by increasing the concentration of the second protein and driving complex formation by mass action. An example of this is the suppression of the unique, initiation-defective *dnaB* allele by increased levels of *dnaC* (6). The converse of this result has been demonstrated by the inhibition of cellular DNA replication when the λP gene is cloned on a multicopy plasmid (4). Presumably, *P* protein competes with *dnaC* product for *dnaB* protein.

Although the narrowest conclusion from the data is that the *rpoB* product, the β subunit of RNA polymerase, is a component of this complex, we assume that the *rpoB* effect is indicative of the participation of RNA polymerase in a tripartite complex with *dnaB* protein and λP product. Genetic data suggest that the *P* and *O* gene products interact (2, 10), and thus, *O* product may also be involved in this complex. It is likely, although specific data are not available, that additional *E. coli* replication proteins are also included. Transcriptional activation of the lambda origin is known to be required to allow use of the origin for viral DNA replication (1). Inclusion of host RNA polymerase in a complex with DNA replication proteins provides a physical structure through which this transcriptional step and the lambda DNA replication process could be coordinated.

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