Mol Gen Genet (1981) 183:181–186

MGG

Altered Transcriptional Termination in a Rifampicin-Resistant Mutant of *Escherichia coli* which Inhibits the Growth of Bacteriophage T7

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Summary. A spontaneous rifampicin-resistant mutant of *E. coli* K12, RpoB26, which inhibits the growth of bacteriophage T7 has been isolated. The mutation is an RNA polymerase mutation; it also restores the wild-type effect of polar mutations in a rho-deficient strain, probably by restoring transcriptional termination. The efficiency of plating (e.o.p.) of wild-type T7, and of some early region deletion and point mutants of T7 tested, is reduced on RpoB26 by a factor of 10^{-4}. However, some deletion mutants are inhibited more severely (up to 10^{-7}) on RpoB26. We argue that these differences may reflect variations in the frequency of transcriptional termination before gene 1, an essential gene which codes for the T7 RNA polymerase (Summers and Siegel 1970; Chamberlin et al. 1970). We also present data which suggest that the product of a late T7 gene plays a role, by some interaction with the product of gene 1, in the inhibition of T7 in RpoB26. We suggest that different levels of expression of gene 1 may lead to different degrees of inhibition of T7 strains in RpoB26.

Introduction

It is known that the host RNA polymerase interacts in several ways with functions coded by the T7 genome. There are initiation and termination sites at specific sequences in the T7 early region (Dunn and Studier 1973; Minkley and Pribnow 1973; McConnell 1979a, b) and the host RNA polymerase is affected by at least 3 phage-coded proteins. It is phosphorylated and inactivated by the T7 protein kinase, the product of gene 0.7 (Brunovskis and Summers 1972; Rothman-Denes et al. 1973; Hesselbach and Nakada 1977a, b); it is inactivated also by the product of gene 2 (Hesselbach and Nakada 1977a, b); and, in vitro, the product of gene 0.3 has been shown to bind to RNA polymerase (Ratner 1974).

In a study of the role of RNA polymerase in the transcription of the T7 virus early region, we have isolated an *E. coli* RNA polymerase rifampicin-resistant (Rif^r) mutant which is defective for the growth of T7. We show that the mutant probably terminates transcription at a higher frequency than normal. In this respect, it resembles some other *rif*^{r} mutations (Guarente et al. 1977; Guarente and Beckwith 1978; Das et al. 1978; Guarente 1979).

We present genetic evidence that the mutation causes RNA polymerase to respond in a different way to sequences on T7 DNA in the region of the C-promoter before gene 1, a region at which the wild-type enzyme terminates in vitro (McConnell 1979a, b) and possibly in vivo (Hercules et al. 1976). It appears that the *rif*^{r} mutation may increase the frequency of termination in this region, reducing the level of transcription of the distal gene 1 and thereby inhibiting phage development. We also present evidence that in T7 strains lacking the main terminator at the end of the early region, incorrect premature transcription of a late gene may lead to a decrease in the expression of gene 1 and so aggravate the growth defect caused by the *rif*^{r} mutation.

Materials and Methods

Bacterial Strains

The bacterial strains are described in Table 1. The four linked markers in the region of *rpoBC* which we have used in strain construction and genetic analysis are *metB, argBC, purD* and *bfe*^{r} (Bachmann and Low 1980).

The Rif^r mutant RpoB26 was derived from *E. coli* ED8612, which was used as the routine host strain for T7 growth and as the *sup* strain for growth of T7 amber mutants. The strain *E. coli* YS302 was used to test the effect of the *rpoB*26 mutation on termination in a rho-deficient background. It had been constructed as follows: A *metB*$^+$ derivative of AS2 (YS101) was used as a donor to transduce *metB*$^+$ into ED8612. The transductants were screened for co-transduction of *argΔBC* and *pur*D; the strain YS201 is a *metB*$^+$ *argΔBC bfe*^{s} *purD* transductant from this cross.

The *bfe*^{r} strain YS202 was selected from YS201 as resistant to colicins E1 and E2 (Nagel de Zwaig and Luria 1967) in the presence of stabs of the colE1-producing strain *E. coli* K12 420 and the col E2-producing strain *S. typhimurium* 437. Colonies growing in the halo surrounding the stabs were picked and tested for growth on plates with ϕ BF23. YS202 was resistant to col E1, col E2, col E3 and ϕBF23. The *bfe*^{r} marker – now renamed *btuB* (Bachmann and Low 1980) – selected in YS202 cotransduced with markers from the *rpoBC* region, as expected for a mutation in *bfe* (Buxton 1971). The markers in the *rpoBC* region were transduced into W3110*rho102*. First, *bfe*^{r} was transduced from YS202 into W3110, giving the transductant

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Abbreviations. Gor$^-$ signifies grow *on* rif$^-$ (Snyder and Montgomery 1974)
Gor$^-$ strains of T7 are inhibited to the same extent as wild-type T7 on the Rif^r mutant RpoB26. The inhibition is relieved by 30 mM NaCl
Gor$^=$ signifies more severe inhibition than T7 wild-type on RpoB26. This inhibition is relieved by 75 mM NaCl
e.o.p. = efficiency of plating

0026-8925/81/0183/0181/$01.20

Table 1. Bacterial strains

Strain	Genotype	Source of reference
E. coli K12 ED8612	*metB trpBE9 supE44 trpR hsdR* ($r^- m^+$)	Dr. W. Brammar
E. coli K12 AS2	*metB argΔBC sup102 purD his strA thi*	Dr. J. Scaife
E. coli K12 YS101	*argΔBC sup102 purD his strA thi*	This work
E. coli K12 YS201	*trpBE9 supE44 trpR hsdR argΔBC purD*	This work
E. coli K12 YS202	a *bfe*^{r} derivative of YS201	This work
E. coli K12 YS203	a *srl* derivative of YS202	This work
E. coli K12 LS289	*pro-48 trpA9605 trpR55 his-85 ilv-632 tsx84*	Soll and Berg 1969
E. coli K12 W3110	*trpR lacZU118 trpE trpA azi*^{r} *val*^{r}	Korn and Yanofsky 1976
E. coli W3110 *rho102*	*trpR lacZU118 trpE trpA azi*^{r} *rho102*	Korn and Yanofsky 1976
E. coli K12 YS301	*trpR lacZU118 trpE trpA azi*^{r} *val*^{r} *bfe*^{r} *argΔBC*	This work
E. coli K12 YS302	*trpR lacZU118 trpE trpA azi*^{r} *bfe*^{r} *argΔBC rho102*	This work
E. coli K12 Y20	*thr leu thi* (col El-K30)	Nagel de Zwaig and Luria 1967
E. coli B BR3	Inhibits the growth of gene 0.7 and gene 0.3 mutants of T7 and T3	Studier 1973a
E. coli B – L2	*Ligase*$^-$	Studier 1973a
E. coli K12 RpoB26	a Rif^r derivative of ED8612	This work
S. typhimurium 437	*cys-26* (col E2 – P9)	Nagel de Zwaig and Luria 1967

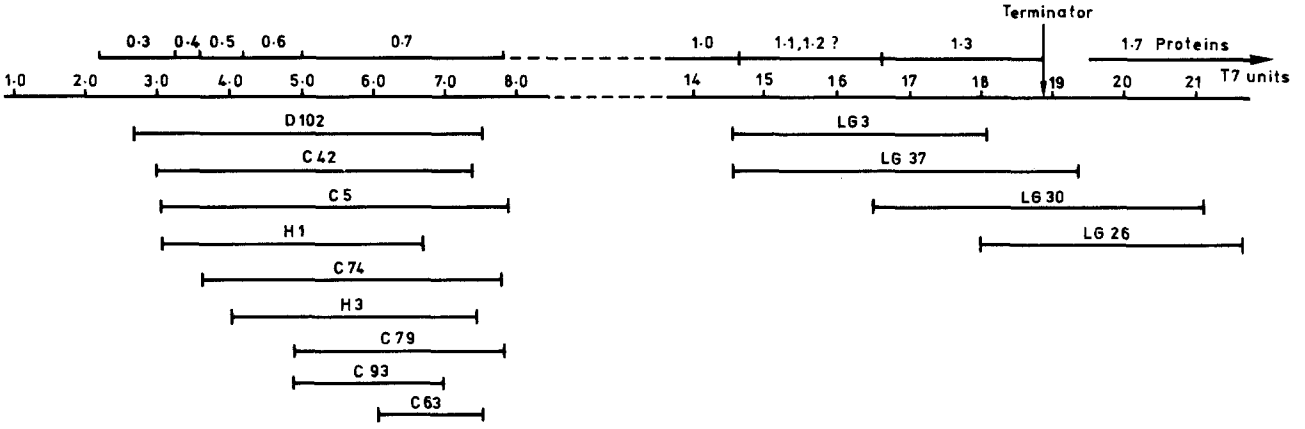

Fig. 1. Map of the positions of T7 deletions on the T7 genetic map (derived from Studier et al. 1979). The extent of different deletions is shown by horizontal bars

YS301, which was also *argΔBC*. Second, the *rho102* marker was transduced from W3110 *rho102* into YS301, selecting for growth on indole. The transductants were tested for growth on melibiose. The strain YS302 was indole$^+$, melibiose$^+$, indicating that it was *rho102*. It was used as a recipient for transduction of the *rif*^{r} marker *rpoB*26. In this transduction selection was for Arg$^+$.

Phage Strains

A map of T7 deletions used (Studier et al. 1979) is shown in Fig. 1. T7 gene 0.7 deletions and point mutants were routinely crossed with a T7 strain containing an amber in gene 15 to establish isogenic strains. The amber derivatives were plated on LS289 (the routine Sup$^+$ host) to select revertants of the gene 15 amber. The presence of the gene 0.7 mutations was confirmed by inability to grow on *E. coli* BR3 and these strains were used in subsequent work. This procedure was necessary because several strains were found to contain secondary mutations which affected their growth on RpoB26.

Media

Nutrient agar contained 2.5% Difco Nutrient Agar and stated amounts of NaCl. Nutrient broth (0.8% Oxoid Lab Lemco broth) was solidified with 0.65% Difco Agar to give soft nutrient agar. LB broth (1% Difco Bacto tryptone, 0.5% yeast extract, 0.12% NaOH and 0.5% NaCl) was solidified with 1.5% Difco Agar to give LB agar. LBCG Agar was LB Agar containing 2.5 mM $CaCl_2$ and 0.1% glucose. Indole was added at 5 μg/ml when required. T2 buffer was used to dilute bacteria and phages. It contained KH_2PO_4(0.15%), Na_2HPO_4 (0.3%), $MgSO_4$ (0.012%), $CaCl_2$ (0.0012%), and gelatin (0.001%). Minimal agar contained an equal volume of 3.3% Difco Minimal Agar and Vogel and Bonner's mineral solution (Vogel and Bonner 1956). Glucose or lactose (0.2%) or melibiose (0.1%) was added; also amino acids (0.002%) and vitamins (0.0002%) as required. Rifampicin was a gift from Lepetit, Milan. Nitrofurantoin, trimethoprim and sulphonamide were purchased from Sigma Chemical Company. For assaying RNA polymerase activity, dithiothreitol was obtained from Calbiochem; ATP, CTP, GTP and UTP were supplied by Sigma Chemical Company and guanosine-8-^{3}H-5′ triphosphate, ammonium salt, was obtained from the Radiochemical Centre, Amersham.

Methods

Rif^r mutants were isolated as spontaneous mutants resistant to 50 μg/ml rifampicin in nutrient agar at 30° C. T7 was grown, assayed and crossed (in LB or nutrient media) essentially as described by Studier (1969). P1 was grown and assayed on LBCG plates. Transduction using P1 Vir A was carried out as described by Miller (1972). When rifampicin-resistant transductants were selected, time was allowed for expression of the rifampicin-resistant phenotype before plating in the presence of rifampicin. RNA polymerase was prepared by the rapid micromethod of Gross et al. (1976) and assayed as described by McConnell and Bonner (1972).

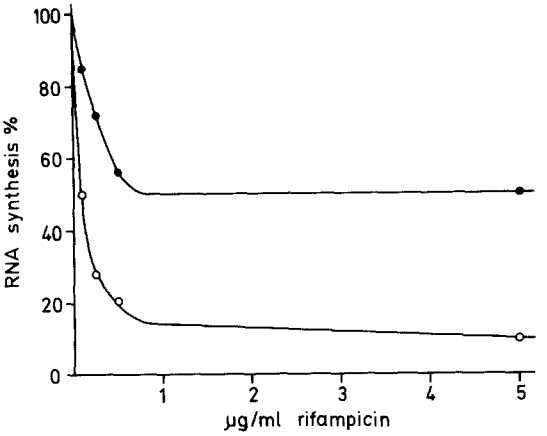

Fig. 2. Rifampicin-resistance in vitro of RNA polymerase purified from ED8612 and RpoB26. RNA synthesis was measured by incorporation of ^{3}H-guanosine (20 Ci/mmol) into RNA at 37° C for 15 minutes. 5% trichloroacetic acid was added. The samples were filtered, dried at 100° C and counted. ○ = ED8612; ● = RpoB26

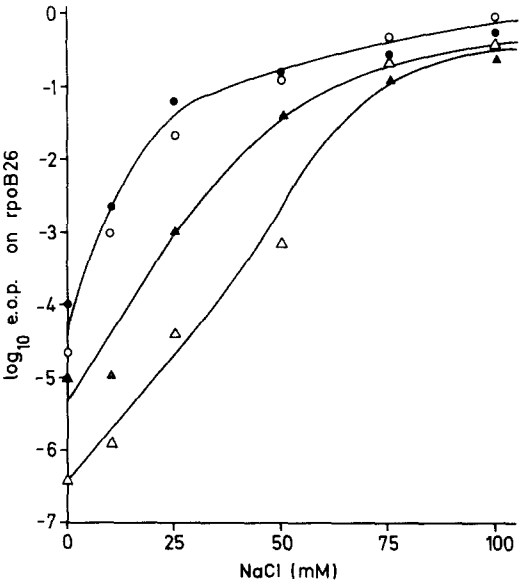

Fig. 3. Salt-dependence of the growth of T7 strains on RpoB26. The phages were assayed at different salt concentrations on RpoB26 and ED8612. The e.o.p. on ED8612 in each case was taken to be 1.0. B79, C79, H1, JS218, JS78 and LG3 were Gor$^-$, like C5 and T7; C42, C63, C74, D102 and H3 were Gor$^=$, i.e. similar to C93; LG26 and LG30 were similar to LG37. ● = T7; ○ = C5; △ = LG37; ▲ = C93

Results

Rif^r Mutants Defective for the Growth of T7

In screening about 1,500 independent spontaneous rifampicin-resistant mutants (Rif^r) of *E. coli* ED8612, we found about 80 which were defective for the growth of T7 when plated at 30° on nutrient agar. The efficiency of plating (e.o.p.) of T7 was less than 10^{-4} on the Rif^r mutant RpoB26 when compared to growth on the parent Rif^s strain ED8612; the plaques were pin-prick in size and took 24 h to appear. Two other Rif^r mutants, RpoB27 and RpoB28, were isolated and were shown to have the same properties as RpoB26. Spontaneous T7 mutants which grew well on RpoB26 also grew well on RpoB27 and RpoB28, indicating that the three *rif^r* mutations probably affect the same functions.

The rif^r Mutation Maps in rpoBC

All *rif^r* mutations which have been mapped have been located in the region containing the *rpoB* and *rpoC* structural genes for the β and β' sub-units of RNA polymerase (reviewed by Scaife 1976). In the cases examined, the rifampicin-resistance has been assigned to the β subunit of the purified RNA polymerase (Heil and Zillig 1970; Boyd et al. 1974).

The *rpoBC* genes are at 89.5 minutes on the *E. coli* genetic map (Bachmann and Low 1980), linked to *argBC, purD, metB* and *btuB*. The *rif^r* mutation *rpoB*26 was mapped by P1 transduction, using as recipient *E. coli* YS201. Rifampicin-resistant transductants were purified and tested for the donor markers *arg$^+$*, *met$^+$*, *pur$^+$* and *btuB*. The co-transduction frequencies of these markers with *rif^r* were 54%, 16%, 28% and 77% respectively. These results demonstrated that the donor markers all cotransduced with *rif^r* as expected if the *rif^r* mutation were in *rpoB*.

Purified RNA polymerase from RpoB26 was rifampicin-resistant (Fig. 2). We therefore conclude that this *rif^r* mutation lies in the *rpoB* structural gene.

Inhibition of T7 Caused by the rif^r Mutation

The *rif^r* mutation *rpoB*26 was transduced by P1-mediated transduction into the parent Rif^s strain ED8612, selecting for Rif^r on rif-NB agar plates. 100 Rif^r transductants were purified and assayed for their ability to grow T7. All gave small plaques for T7 with e.o.p.s. of about 10^{-4}. In addition, rifampicin-sensitive revertants of RpoB26 were isolated which all grew T7 normally. These two sets of data, taken together, lead us to conclude that a single mutation causes both rifampicin resistance and inhibition of T7 in RpoB26.

Salt Dependence of T7 Inhibition

The inhibition of T7 was medium-dependent and temperature dependent, occurring only at 30° and on nutrient medium, which contains no added salt. To test whether salt relieves the inhibition, T7 was assayed on RpoB26 on nutrient agar containing different amounts of NaCl, from 0–100 mM (Fig. 3). The results show that the e.o.p. of T7 on RpoB26 increased with increasing salt concentration. Plaque size increased in parallel (from very small to normal).

Addition of KCl, NH_4Cl, $NaNO_3$ or NH_4NO_3 to nutrient medium relieved the inhibition of T7 on RpoB26 in the same way as addition of NaCl (data not shown).

Effect of Early Gene Mutations on T7 Growth on RpoB26

Most of the T7 sites with which host RNA polymerase is known to interact in vivo, as well as the genes coding for the protein kinase and the 0.3 protein, which interact with it, lie within the early region. Many deletion and point mutations which affect this region have been isolated (Studier 1972, 1973a, b, 1975; Simon and Studier 1973; Studier et al. 1979). Deletions of T7 early genes fall into two groups: those which delete genes to

Table 2. E.o.p. of T7 and T7 mutants on RpoB26 (nutrient agar, 30° C)

10^{-4}	10^{-5}	$0.5\text{–}1 \times 10^{-7}$
T7 wild type	C42	LG26
B79	C63	LG30
C5	C74	LG37
C79	C93	
HI	D102	
JS78	H3	
JS218		
LG3		

the left of gene 1, and those which delete genes to its right. When a selection of these was tested for growth on RpoB26, three broad classes of e.o.p. were discovered. The results for a representative of each class are shown in Fig. 3; the complete classification is shown in Table 2.

Several gene 0.7 deletion mutants – C42, C63, C74, C93, D102 and H3 – were inhibited by a factor of about ten more than T7 wild-type. The degree of inhibition of each phage strain changed over a characteristic, relatively narrow, range of salt concentration. All of these deletion mutants required more salt than wild-type T7 in order to display an equivalent e.o.p. on RpoB26, and are described as Gor$^{=}$.

Except for C63, which deletes only gene 0.7, all of these deletions affect several early proteins. However, the only protein affected by each deletion is 0.7 (Studier et al. 1979). This suggested that T7 growth on RpoB26 at certain salt concentrations and at 30° C had a partial requirement for gene 0.7. If so, all deletion and point mutants of gene 0.7 were expected to behave in the same way when plated on RpoB26. However, this was not the case. Two gene 0.7 point mutants, B79, which maps near the left end of gene 0.7 and JS78, which maps near the right end (Studier 1973a; Studier et al. 1979), grew like wild-type on RpoB26. Hence, the relative growth deficiency of the above deletion mutants on this host was not due to the lack of gene 0.7. This conclusion was supported by the observation that three gene 0.7 deletion mutants, H1, C5 and C79 (Simon and Studier 1973; Studier et al. 1979), grew significantly better than the above deletion mutants, and nearly as well as wild-type, on RpoB26.

Four deletion mutants affecting genes to the right of gene 1 were also tested for growth on RpoB26 under the same conditions. Three of these – LG26, LG30 and LG37 – were severely Gor$^{=}$, that is, they were inhibited by a factor of 500–1,000 more than T7 wild-type. These deletions all affect gene 1.3 in addition to other non-essential genes in the early and late regions of T7 (Studier 1972; Simon and Studier 1973; Studier et al. 1979). However, a gene 1.3 deletion mutant, LG3 (Studier 1972; Studier et al. 1979) and a gene 1.3 point mutant, JS218 (Studier 1973a), grew significantly better than the above LG deletion mutants, and nearly as well as wild-type T7, on RpoB26. We conclude that the relative growth defect of LG37 and its analogues was not due to any lack of early gene functions, especially as the LG3 and LG37 deletions have identical left-hand end points.

Restoration of Polarity by the rif^r Mutation rpoB26 in a rho-Deficient Strain

The Gor^{-} deletion mutants C5 and C79, which grew almost as well as wild-type T7 on RpoB26, differ from the Gor$^{=}$ deletion mutants of gene 0.7 in that their deletions extend into a region just to the left of gene 1 which contains the C promoter (Minkley and Pribnow 1973; Stahl and Chamberlin 1977; McConnell 1979a) and two transcriptional termination sequences at which termination has been observed in vitro (Minkley and Pribnow 1973; McConnell 1979b) and possibly in vivo (Hercules et al. 1976).

The C5 and C79 deletions remove the HaeIII site at the left of the HaeIII. 48 restriction fragment (Gordon et al. 1978; McConnell 1979a, b; Studier et al. 1979) and the RNase III cleavage site between genes 0.7 and 1 (Simon and Studier 1973; Studier et al. 1979). These sites overlap with the two termination sites identified in vitro (McConnell 1979b): they are not deleted by any of the other mutations studied, including C74 which extends almost as far as C79. This difference between C74 on the one hand and C5 and C79 on the other (Table 2) could explain their different behaviour on RpoB26.

It has been reported that about 3% of all *rif^r* mutations increase the frequency of transcriptional termination (Guarente and Beckwith 1978; Das et al. 1978; Guarente 1979). This estimate is near the percentage of Rif^r mutants found in our study to inhibit T7 (6.5%). Thus, the poor growth of T7 on RpoB26 might be caused by an increased frequency of transcriptional termination in this host, particularly at the "C" terminator (McConnell 1979b). The deletion of these terminators in the mutants C5 and C79 might enhance their growth on RpoB26 relative to that of other gene 0.7 deletion mutants.

We therefore asked whether the *rif^r* mutation *rpoB26* restores polarity in a rho-deficient strain. The parent rho-deficient strain was *E. coli* K12 W3110 *rho102* (Korn and Yanofsky 1976) (Table 1), which carries polar mutations in the *trpE* and *lacZ* genes. In the *rho$^+$* isogenic strain (W3110), the distal genes are not expressed and the strain is indole$^-$ and melibiose$^-$. In the presence of *rho102*, they are expressed, and the strain grows on indole and melibiose.

An *argΔBC rho102* derivative of W3110 was constructed (see Materials and Methods). This strain, YS302, was used as a recipient in a transduction with RpoB26 as donor. Arg$^+$ transductants were selected and tested for the unselected phenotypes of rifampicin resistance and ability to grow on indole and melibiose.

All Rif^r transductants tested (43) were indole$^-$ melibiose$^-$, while all Rif^s transductants tested (123) were indole$^+$ melibiose$^+$. No indole$^-$ melibiose$^-$ transductants were found among 54 Arg$^+$ transductants tested when ED8612 was used as donor. These results indicate that the *rif^r* mutation *rpoB26* restores polarity in vivo in the *lac* and *trp* operons, suppressing the rho-deficient phenotype.

Polarity is considered to be due to rho-dependent transcription termination, which occurs after translation has ceased after a nonsense codon (reviewed by Adhya and Gottesman 1978). There is reduced polarity in rho-deficient strains. Therefore, the most likely explanation for the restoration of polarity by *rpoB26* in a rho-deficient strain is that the Rif^r RNA polymerase terminates transcription more frequently than the wild-type enzyme. There are numerous reports on *rif^r* and other RNA polymerase mutations which affect the rate of transcriptional termination negatively or positively (reviewed by Adhya and Gottesman 1978). However, an effect by *rpoB26* on initiation, rather than termination, of transcription has not yet been ruled out.

Discussion

We have shown that the *rif^r* mutation *rpoB26* restores polarity in a rho-deficient strain, probably by restoring normal levels of transcriptional termination. In addition, we have shown that

the degree of growth inhibition observed with wild-type T7 is increased in the case of several T7 deletion mutants. Deletion of the C transcriptional termination sequences before gene 1 (Minkley and Pribnow 1973; McConnell 1979b), on the other hand, appears partially to relieve the growth defect on RpoB26 of the deletion mutants C5 and C79, relative to other deletions in this region. These data suggest that the inhibition of T7 on RpoB26 may be due to excessive transcriptional termination by the mutant host RNA polymerase within the T7 early region, particularly at the C termination sites. This could lead to insufficient expression of gene 1, an essential gene which codes for the T7 RNA polymerase (Chamberlin et al. 1970; Summers and Siegel 1970). Deletion of the C termination sites in C5 and C79 (McConnell 1979b; Studier et al. 1979) could then lead to reduced inhibition on RpoB26 relative to other gene 0.7 deletion mutants. The fact that C5 and C79 nevertheless show as much inhibition as wild-type T7 on RpoB26 may be due to excessive transcriptional termination at other sites in the early region, coupled with the fact that synthesis of gene 1 protein is in any case reduced in C5, and possibly also in C79, due to interference with the initiation of protein synthesis (Studier 1973b; Simon and Studier 1973; Studier et al. 1979).

The only other gene 0.7 deletion mutant of T7 found to be Gor$^-$, rather than Gor$^=$, on RpoB26 was H1. The deletions in Gor$^=$ mutants may be behaving as polar frameshift mutations, which are expected to increase the rate of distal transcriptional termination (for review, see Adhya and Gottesman 1978), in this case before gene 1. On average, two-thirds of all large deletions are expected to cause frameshifts. There is evidence that the new peptide made in H1 is the same length as the new mRNA, indicating that the entire mRNA is translated and that the H1 deletion does not cause a frameshift (Simon and Studier 1973; Studier et al. 1979). In contrast, the new peptides made in the other deletion mutants are shorter than the new mRNA in every case measured (Simon and Studier 1973; Studier et al. 1979), suggesting that these deletions do cause a frameshift. As predicted by this explanation, H1, and the gene 0.7 missense mutants B79 and JS78, were Gor$^-$ on RpoB26; no polarity is caused by missense mutations.

We have presented data demonstrating that T7 gene 1.3 deletion mutants – LG26, LG30 and LG37 – were severely Gor$^=$ on RpoB26 (Fig. 3). In contrast, the gene 1.3 deletion mutant, LG3, and a gene 1.3 missense mutant JS218 (Studier, 1972, 1973a) were Gor$^-$. The only property of LG3 not shared by at least one of the other deletions tested, LG26, LG30 and LG37, is that LG3 alone contains the major terminator at the end of the early region, recently mapped at 18.89% (Studier et al. 1979). In strains in which this terminator is deleted, host RNA polymerase continues transcription into the late region (Studier 1972). This is expected to lead to premature expression of late genes. In particular, the "early-late" or class II genes, which are normally expressed between about 6 and 15 min after infection at 30° C, are observed several minutes earlier when wild-type cells are infected with LG37 (Studier 1972).

These data suggest that premature expression of a Class II gene could aggravate the effect of the *rpoB*26 mutation on T7 growth. An obvious candidate would be gene 2. The product of this gene shuts off early gene expression by inhibiting host RNA polymerase (Hesselbach and Nakada 1977a, b). Premature expression of gene 2 in strains lacking the major early terminator could lead to premature shutoff of early gene expression, including that of gene 1, so increasing the growth defect of these T7 strains on RpoB26.

In the accompanying paper (Yeats et al.), we present data suggesting that a Class II gene whose product affects the expression of *rpoB*26 codes for the T7 DNA unwinding protein, a single-stranded DNA binding protein (Reuben and Gefter 1973, 1973; Scherzinger et al. 1973), suggesting that this protein may affect transcription termination.

Acknowledgements. We thank Drs. F.W. Studier, B. Bachmann, W. Brammer, J. Scaife, R. Hausmann and C. Yanofsky for bacterial and phage strains, and Lepetit, Milan, for a gift of rifampicin. We also thank F.W. Studier for communicating results before publication, and Dr. R.S. Hayward for helpful discussion. We thank P. Blaney, H. Milner and D. Sullivan for technical assistance. T.F.R.S. was a recipient of a Medical Research Council of Ireland grant. S.M.Y. and P.C. were recipients of Goverment and T.C.D. postgraduate grants.

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Communicated by E. Bautz

Received February 12 / May 5, 1981