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Transcribing of *Escherichia coli* genes with mutant T7 RNA polymerases: Stability of *lacZ* mRNA inversely correlates with polymerase speed

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ABSTRACT **When in *Escherichia coli* the host RNA polymerase is replaced by the 8-fold faster bacteriophage T7 enzyme for transcription of the *lacZ* gene, the β-galactosidase yield per transcript drops as a result of transcript destabilization. We have measured the β-galactosidase yield per transcript from T7 RNA polymerase mutants that exhibit a reduced elongation speed *in vitro*. Aside from very slow mutants that were not sufficiently processive to transcribe the *lacZ* gene, the lower the polymerase speed, the higher the β-galactosidase yield per transcript. In particular, a mutant which was 2.7-fold slower than the wild-type enzyme yielded 3.4- to 4.6-fold more β-galactosidase per transcript. These differences in yield vanished in the presence of the *rne-50* mutation and therefore reflect the unequal sensitivity of the transcripts to RNase E. We propose that the instability of the T7 RNA polymerase transcripts stems from the unmasking of an RNase E-sensitive site(s) between the polymerase and the leading ribosome: the faster the polymerase, the longer the lag between the synthesis of this site(s) and its shielding by ribosomes, and the lower the transcript stability.**

In *Escherichia coli* mRNAs start being translated while their synthesis is still in progress (1). Moreover, the newly synthesized ribosome binding sites (2) usually bind a ribosome before the RNA polymerase has moved far downstream (3). Following this binding, the elongation speed of transcription and that of translation are matched (4–6), so that no large ribosome-free mRNA stretch normally exists between the RNA polymerase and the leading ribosome.

In contrast, the T7 RNA polymerase is, on average, 8-fold faster than the ribosomes that translate its message, creating a continuously expanding ribosome-free mRNA stretch behind itself (7). Dreyfus and coworkers (8) compared expression in *E. coli* of several genes that are transcribed either by host or by T7 RNA polymerase and found that the polypeptide yield per transcript was reduced in the latter case. For the *lacZ* gene, the reduction was especially large and could be traced to the greater instability of the T7 RNA polymerase transcripts (9). Since naked mRNA is often nuclease-sensitive in *E. coli* (10), it was proposed that the instability of these transcripts originates from their ribosome-free region. More generally, it was hypothesized that the stability of many *E. coli* mRNAs during their synthesis rests upon the synchronization of RNA polymerase and ribosomes.

According to this view, the instability and low β-galactosidase yield of the T7 RNA polymerase transcripts arise solely from the high speed of the polymerase. This leads to the prediction that *any* RNA polymerase which is slower than the T7 enzyme should yield *lacZ* transcripts that are more stable and more efficiently expressed. Many mutants of the T7 RNA polymerase that are altered in the motifs conserved among various DNA- and RNA-dependent DNA and RNA polymerases have been characterized (11, 12). *In vitro*, most of these mutants bind to the T7 promoter with nearly wild-type affinity but have altered polymerization kinetics. We have used some of them to transcribe the *lacZ* gene in *E. coli*. We compare the β-galactosidase yields from the corresponding transcripts and discuss the results in light of the above interpretation.

MATERIALS AND METHODS

Genetic Constructions. A previous report (8) described the construction of a truncated *lac* operon consisting of the *lacZ* gene and part of the *lacY* gene, preceded by the T7 gene *10* late promoter (P_{T7}), and followed by the $tRNA^{Arg5}$ gene for use as a transcriptional reporter. By standard protocols (13), this hybrid operon has been transferred as a single copy onto the *malA* chromosomal region of the *E. coli* strain BL21 (14). The resulting strain, ENS0134, also carries Tn*10* within the genuine *lacZ* gene. The sequences of the promoter–RBS and $tRNA^{Arg5}$–terminator regions were checked after PCR amplification of the relevant genomic segments. To introduce the *rne-50* mutation into ENS0134, Tn*10* was first excised (15). This removal did not restore β-galactosidase expression. The mutation was then P1-transduced from CH1828 (16) into this strain, and transductants were selected for tetracycline resistance and tested for thermosensitive growth.

Phagemid pDPT7 and derivatives carry wild-type or mutated alleles of T7 gene *1* encoding RNA polymerase on a 4.3-kb *Bam*HI insert (11). For this work, these inserts were cloned into pBR322, yielding pAR1219 derivatives (17) harboring mutated gene *1* alleles. All mutant T7 RNA polymerases used here carry single amino acid changes and are named accordingly—e.g., Y639F has phenylalanine in place of the wild-type tyrosine-639.

Cell Growth and Protein Assays. Cells were grown at 37°C in glycerol/amino acids Mops medium (8) containing ampicillin (100 μg/ml) and, unless otherwise stated, 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). The generation time (≈40 min) was unaffected by IPTG. For β-galactosidase assays, cells were harvested at an OD_{600} of 0.5. To study the effect of the *rne-50* mutation, *rne-50* and control rne^+ cells were grown at 28°C without IPTG to an OD_{600} of 0.3–0.4 and then transferred to 42°C. IPTG was added 6 min later, and cells were harvested after an additional 39 min.

Cells were lysed by sonication, and the protein concentration and the β-galactosidase activity were measured in the cleared lysate (18). To visualize β-galactosidase and T7 RNA polymerase, lysates were fractionated in SDS/7.5% polyacryl-

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Abbreviation: IPTG, isopropyl β-D-thiogalactopyranoside.

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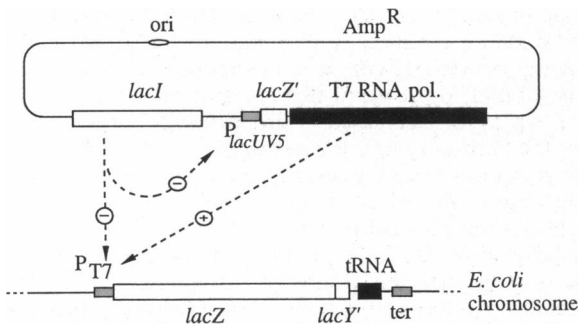

FIG. 1. Outline of the experimental system. The lower part represents the relevant chromosomal region of the strain ENS0134. The open box stands for a truncated *lac* operon encompassing *lacZ* and part of *lacY*, whereas the black box corresponds to the $tRNA^{Arg5}$ gene, used here as a transcriptional reporter. Thinner, gray boxes correspond to the bacteriophage T7 gene *10* promoter (P_{T7}) and to two tandemly arranged transcriptional terminators (ter) (8). The upper part represents the plasmids carrying the T7 RNA polymerase genes. These pAR1219 derivatives (17) sequentially carry the *lacI* gene, the *lacUV5* promoter (thin gray box) followed by the first 478 nt of the *lacZ* gene, and finally the different alleles of the T7 RNA polymerase gene (black box). The *lacI* and *lacZ* sequences are shown as thick open boxes. Symbols + and − stand for positive and negative control by the T7 RNA polymerase and *lac* repressor, respectively. ori, Origin of replication; Amp^R, ampicillin-resistance gene.

amide gels, which were stained with Coomassie blue or processed by the Western blot technique (8).

RNA Isolation and Analysis. For the $tRNA^{Arg5}$ assay, RNA was extracted from samples of the same cultures as for protein assays. RNA electrophoresis, blotting, and visualization of the 5S rRNA and $tRNA^{Arg5}$ were as described (8), except that the oligonucleotide probes complementary to these two species were mixed together at the molar ratio indicated in the figure captions. For visualization of the *lacZ* transcripts, the same membrane used for tRNA assay was reprobed with a uniformly ^{32}P-labeled *Hin*cII fragment internal to *lacZ* (GenBank Ecolac nt 2349–4176) (18). Hybridization signals were quantified with a PhosphorImager (Molecular Dynamics).

The growth of nascent T7 RNA polymerase transcripts vs. time was visualized on Northern blots as described (7), except that the experiment was done at 37°C in the standard growth medium. The probe used was a 0.6-kb *Hpa* I fragment (GenBank Ecolac nt 1725–2348; nt 480–1103 with respect to the *lac* transcription start). Though close to the *lacZ* 5′ end, this probe is located downstream of the *lacZ* region borne on pAR1219 and therefore should not hybridize with it.

RESULTS

Experimental System. To compare the β-galactosidase yields from *lacZ* transcripts that are synthesized by different mutant T7 RNA polymerases, we first fused the *lacZ* gene to the T7 gene *10* late promoter (P_{T7}). This hybrid operon was placed into the chromosome of a Lac^- strain devoid of T7 RNA polymerase expression, yielding strain ENS0134. We then introduced into this strain plasmids encoding the various mutant T7 RNA polymerases and compared in each case the β-galactosidase expression and the frequency of transcription through *lacZ*. The experimental system is outlined in Fig. 1.

Two features of the hybrid operon, which is described elsewhere (8), are noteworthy. First, P_{T7}, being flanked by the *lac* repressor binding site, can be switched on or off by adding or removing the *lac* operon inducer IPTG. Second, a gene encoding a tRNA, the *E. coli* $tRNA^{Arg5}$, is inserted downstream from the *lacZ* gene. This stable RNA "tag" is quantitatively recovered from the transcript, so that its accumulation reflects the frequency of transcription through *lacZ*.

Among the T7 RNA polymerase mutants isolated by Bonner *et al.* (11), eight mutants retaining significant *in vitro* activity were selected. Their *in vitro* elongation speed over T7 DNA and their processivity over a synthetic DNA template (12) are listed in Table 1. The genes encoding these mutant polymerases were substituted for the wild-type gene in pAR1219 (17). This pBR322-based plasmid carries (*i*) the T7 RNA polymerase gene under the control of the IPTG-inducible P_{lacUV5} promoter and (*ii*) the *lacI* gene, which ensures that enough *lac* repressor is present in the cell to switch off both P_{T7} and P_{lacUV5} when IPTG is absent (Fig. 1).

Extracts of IPTG-induced ENS0134 cells harboring the pAR1219 derivatives showed a prominent 100-kDa protein which was absent when pBR322 was used (Fig. 2*A*). The size of this protein matches that of T7 RNA polymerase. Its abundance is alike in all cases, showing that all mutant enzymes are equally stable in the strain used.

Frequency of Transcription Through *lacZ*. The frequency of *lacZ* transcription was measured in exponentially growing ENS0134 cells harboring the T7 RNA polymerase mutants. To this end, the expression of the $tRNA^{Arg5}$ reporter gene was quantified on Northern blots with an oligonucleotide probe (Fig. 2*C*). To correct for lane-to-lane differences in total RNA loading, the membrane was simultaneously probed with an oligonucleotide complementary to the 5S rRNA. The reporter tRNA accumulated to high levels in cells expressing the wild-type T7 RNA polymerase. In contrast, in cells lacking T7 RNA polymerase (pBR322 lane), its expression was at least 50-fold lower. The mutant polymerases fell into two groups. Mutants R551S, Y639F, Q649S, G645A, and F644A all produced tRNA expression significantly above that observed in cells lacking T7 RNA polymerase. The expression decreased in this order, ranging from 110% to 6% of that observed with the wild-type enzyme. In contrast, mutants R627S and I810S yielded no tRNA expression (Fig. 2*C*). Notably, these two enzymes were also the least processive *in vitro* (Table 1).

Table 1. β-Galactosidase yield from *lacZ* transcripts synthesized by mutant T7 RNA polymerases

Mutant	*In vitro* speed,* nt/sec	*In vitro* processivity*	tRNA (37°C)†	β-Galactosidase (37°C)†	β-Galactosidase yield per synthesized transcript†		
					37°C	42°C	42°C, *rne-50*
Wild type	240	0.94	100	100	100	100	100
Y639F	240	0.93–0.94	45 ± 5	13.5 ± 1.0	30 ± 4	31 ± 1	46 ± 10
R551S	240	ND	110 ± 5	114 ± 20	105 ± 15	79 ± 20	75 ± 10
F644A	170	0.90–0.93	5.5 ± 1	7.5 ± 1.0	136 ± 30	290 ± 40	92 ± 10
Q649S	160	0.88–0.91	25 ± 5	50 ± 8	200 ± 10	230 ± 10	113 ± 12
G645A	90	0.81–0.87	20 ± 4	68 ± 7	340 ± 30	460 ± 20	100 ± 12
R627S	80	0.81–0.85	<2	<0.5	ND	ND	ND
I810S	40	0.70–0.75	<2	<0.5	ND	ND	ND

*From ref. 12 [processivity on poly(dA-dT); speed value for R551S (unpublished) communicated by R.S.].

†Mean of four (37°C) or two (42°C) independent determinations, normalized to that from the wild-type enzyme (=100). With the wild-type enzyme, the β-galactosidase activity is 1975 ± 200 units (37°C), 1440 ± 30 (42°C), and 9300 ± 700 (42°C, *rne-50*) nmol ONPG hydrolyzed/min/mg protein; the β-galactosidase yield per synthesized transcript is 6.5-fold higher in *rne-50* cells than in rne^+ cells (42°C).

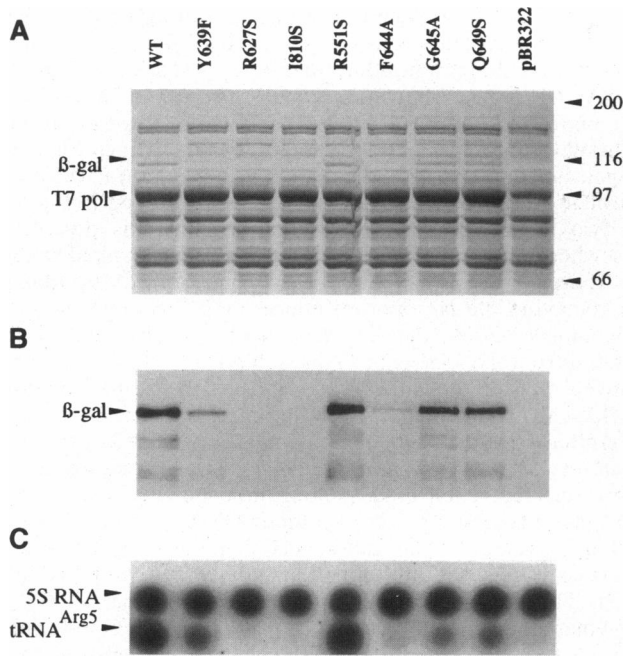

FIG. 2. (*A*) Total proteins (20 μg per lane) from exponentially growing ENS0134 cells harboring various T7 RNA polymerase mutants were separated in an SDS/polyacrylamide gel and stained with Coomassie blue. Arrowheads on the left show the position of β-galactosidase and T7 RNA polymerase, whereas those on the right point to positions of molecular size markers (kDa). The mutant T7 RNA polymerases used are indicated above each lane. WT, wild type. (*B*) Same as *A*, except that proteins (1 μg) were analyzed by the Western technique with a polyclonal anti-β-galactosidase antibody (8). (*C*) Northern blot showing $tRNA^{Arg5}$ expression in the same cultures as in *A* and *B*. The membrane was probed simultaneously with oligonucleotides complementary to $tRNA^{Arg5}$ and 5S rRNA, which were end-labeled with [γ-^{32}P]ATP to specific activities of 600 and 15 Ci/mmol (1 Ci = 37 GBq), respectively, and mixed at a 1:40 molar ratio.

As a control, the same membrane was reprobed with a *lacZ*-specific probe. Only with those mutants that produced tRNA were *lacZ* transcripts observed. The corresponding pattern was qualitatively similar in all cases, showing that the mutants did not differ grossly in their promoter or terminator recognition (data not shown; see Figs. 3 and 4).

β-Galactosidase Yield per Synthesized Transcript. We then measured the β-galactosidase activities in the same cultures (Table 1). In all cases, these activities reflected the level of the β-galactosidase polypeptide, as judged from Western blots (Fig. 2*B*). Cells harboring the wild-type T7 RNA polymerase gene yielded ≈2000 β-galactosidase units, and those lacking any T7 RNA polymerase gene (pBR322 lane) yielded ≈10 units. With the mutant T7 RNA polymerases, those that expressed the tRNA also expressed β-galactosidase above background, whereas those failing to express the tRNA did not, as expected. However, the different mutants in the former group did not rank alike for expressing either β-galactosidase or tRNA. As a consequence, the ratio between these expressions, which reflects the β-galactosidase yield per transcript, spanned a 10-fold range. Only for mutant R551S was this yield indistinguishable from that observed with the wild-type enzyme. For Y639F, it was distinctly lower, whereas for F644A and Q649S it was modestly but reproducibly higher. Mutant G645A exhibited the highest β-galactosidase yield per transcript, 3.4-fold more than the wild type (Table 1).

Even though it is present as a single copy, the *lacZ* gene is transcribed to high levels by the most active T7 RNA polymerases, which might artificially reduce the β-galactosidase yield per transcript by saturating the translational machinery. To rule this out, the IPTG concentration in the medium was incrementally decreased from 1 mM to 0.06 mM. For the wild-type and G645A enzymes, this reduction caused a large (up to 10-fold) decrease in tRNA expression, but β-galactosidase expression decreased in proportion (data not shown). Thus, for both enzymes, the β-galactosidase yield per transcript is insensitive to the level of transcription, excluding an overloading of the translational machinery. This finding fits the conclusions reached previously (8).

Stability of the G645A vs. Wild-Type Transcripts. Previous work showed that the transcripts from the *E. coli* RNA polymerase yielded ≈40-fold more β-galactosidase than those from the wild-type T7 enzyme (8), due to their higher stability (9, 19). Since the transcripts from several mutant T7 RNA polymerases also showed significantly improved β-galactosidase yields, we compared their stability to that of the transcripts from the wild-type enzyme. We focused primarily on the G645A mutant, since it showed the highest β-galactosidase yield per transcript.

The stability of an mRNA, together with the frequency of its transcription, determines its steady-state concentration. Therefore, the relative stability of the wild-type and G645A transcripts can be assessed by comparing their steady-state concentrations, taking into account the different transcription frequencies. A growth temperature of 42°C was used in these experiments to facilitate the interpretation of the effect of the *rne-50* mutation (see below). Under these conditions, exponentially growing ENS0134 cells expressing the wild-type polymerase contained 8-fold more reporter tRNA than cells expressing the G645A enzyme (Fig. 3*A*). The same blot which was used for tRNA assay was then reprobed with a *lacZ* probe. The resulting pattern was qualitatively alike for both polymerases, consisting of the full-length (4.3-kb) transcript and a processed 3.2-kb species corresponding to the *lacZ* mRNA proper (18, 20), together with a smear arising from incomplete molecules. Quantitatively, the 4.3- and 3.2-kb species were ≈2.5-fold more abundant in cells expressing the wild-type enzyme than in cells expressing G645A. This figure is lower than the ratio of the transcription frequencies, indicating that the transcripts from the wild-type enzyme are less stable than those from G645A (Fig. 3 *B* and *C*).

The low stability of *lacZ* transcripts synthesized by the wild-type T7 RNA polymerase compared with their *E. coli* polymerase counterparts stems from their hypersensitivity to RNase E (9). The following experiment shows that the stability difference between the wild-type and G645A transcripts also reflects their unequal RNase E susceptibility. RNase E can be inactivated at 42°C by the *ams-1* mutation (21), renamed *rne-50* (16). When this mutation was introduced into ENS0134 cells expressing either the wild-type or the G645A polymerase, the expression of the reporter tRNA was not grossly altered (Fig. 3*A*). In contrast, the *lacZ* transcript pattern was considerably enhanced, demonstrating a large stabilization (Fig. 3*B*). Moreover, this enhancement, and hence the stabilization, was more marked for the transcript made by the wild-type enzyme than for the transcript made by the G645A enzyme. Indeed, the concentration of the transcripts from the two enzymes was now proportional to the corresponding tRNA expression, indicating equal stability (Fig. 3*C*).

To determine whether the difference in β-galactosidase yield from the wild-type and G645A polymerase transcripts originates from their different stabilities, we measured these yields under conditions where these stabilities are alike—i.e., in the presence of the *rne-50* mutation. In *rne*$^+$ cells at 42°C, the β-galactosidase yield per synthesized transcript was 4.6-fold higher for the G645A polymerase than for the wild-type enzyme, a figure even larger than at 37°C (Table 1). The *rne-50* mutation caused this yield to increase for both enzymes, but the increase was larger for the wild-type enzyme. As a result, the difference in yield between the two transcripts vanished

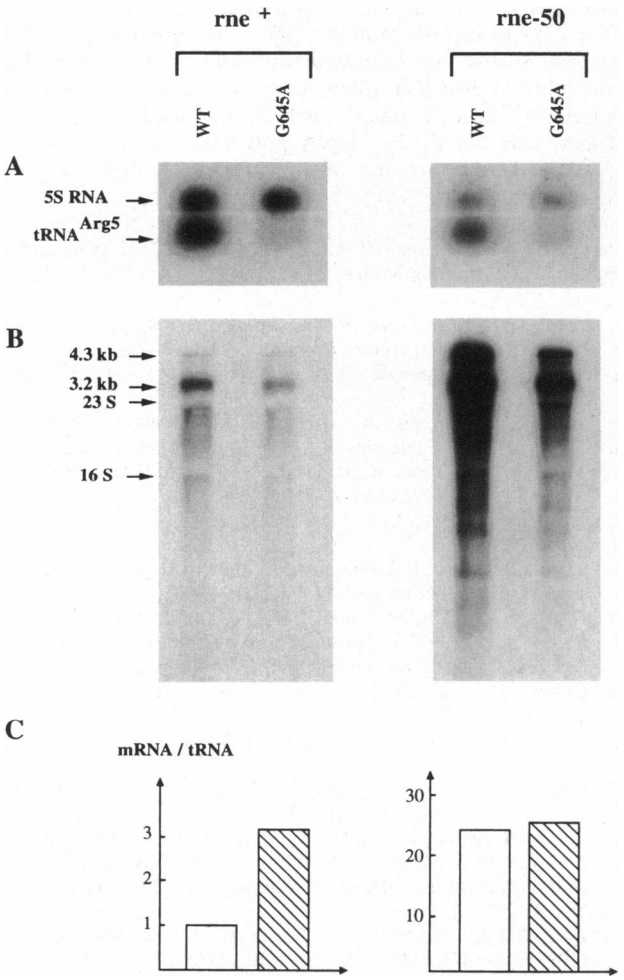

FIG. 3. (*A*) Northern blot showing the expression (at 42°C) of the reporter $tRNA^{Arg5}$ and 5S RNA, in ENS0134 cells expressing either the wild-type (WT) or the G645A T7 RNA polymerase in the absence or presence of the *rne-50* mutation (*rne*$^+$ and *rne-50*, respectively). Hybridization conditions were as in Fig. 2*C*, except that the two oligonucleotides were labeled to 2000 and 16 Ci/mmol, respectively, and mixed at a 1:125 molar ratio. In the *rne-50* but not in the *rne*$^+$ cells, the probes also detected unmatured 5S rRNA precursors and stabilized transcripts still harboring the tRNA. For clarity, these precursors (which altogether are less abundant than the mature species) have been cut out, but they are included in the quantification in *C*. (*B*) The same membrane as above was probed with a *lacZ* internal probe. The full-length transcript (4.3 kb), the processed *lacZ* mRNA (3.2 kb), and the 23S and 16S rRNAs are indicated. (*C*) Summed abundance of the 4.3- and 3.2-kb species, divided by the tRNA expression. This ratio reflects transcript stability (see text). The value for the wild-type polymerase in *rne*$^+$ cells is arbitrarily set to 1. Note the different scales of the left and right diagrams.

(Table 1). We conclude that the difference in yield in *rne*$^+$ cells arises entirely from the unequal susceptibilities of the wild-type and G645A transcripts to RNase E-dependent cleavage.

The β-galactosidase yield per transcript from all other T7 RNA polymerase mutants was similarly measured in *rne*$^+$ and *rne-50* cells at 42°C (Table 1). In *rne*$^+$ cells, these yields spanned an even larger range (15-fold) than at 37°C. These variations generally disappeared in *rne-50* cells, just as was seen for the wild-type and G645A enzymes. Only the yield from Y639F transcripts remained ≈2-fold lower than for other mutants (Table 1), pinpointing a unique property of this enzyme. Recently, Y639F has been shown to be relaxed in its ability to distinguish between dNTPs and NTPs as substrates *in vitro* (22). Possibly such misincorporation also occurs *in vivo*, lowering the translatability of the transcripts.

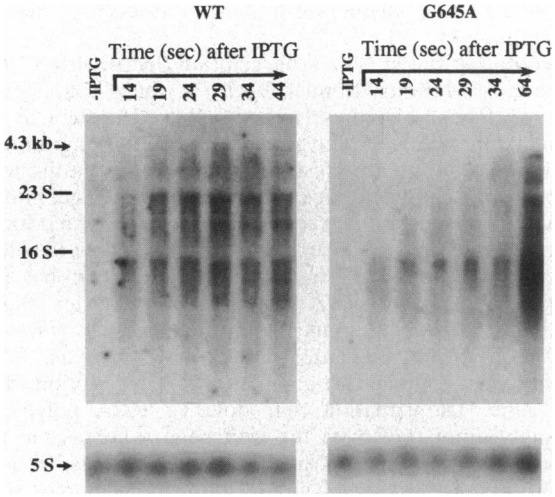

FIG. 4. Northern blots showing the growth of the transcripts from wild-type (WT) or G645A T7 RNA polymerase vs. time following IPTG induction at 37°C. The position of the 16S and 23S rRNAs and of the full-length operon transcript (4.3 kb) is indicated.

***In Vivo* Speed of the Wild-Type and G645A Polymerases.** It has been proposed that the difference in the *in vivo* speed of the *E. coli* and wild-type T7 RNA polymerases is responsible for the difference in β-galactosidase yield from their transcripts (8). We therefore compared the *in vivo* speed of the mutant T7 RNA polymerases, again focusing primarily on the wild-type and G645A enzymes. To this end, the growth of the *lacZ* transcript vs. time after IPTG induction was recorded on Northern blots (7). With the wild-type polymerase, the full-length operon transcript (4.3 kb) appeared ≈19 sec after IPTG addition (Fig. 4). This delay is shorter than in a previous report (7), due presumably to the higher temperature used here. With mutant G645A, the nascent chains grew distinctly slower and the full-length species appeared only after >30 sec, implying an ≈2-fold reduction in speed. This figure agrees reasonably with the 2.7-fold reduction observed *in vitro* (Table 1).

These experiments were repeated with two other mutants, Y639F and Q649S. The former was as fast as the wild-type enzyme, whereas Q649S, like G645A, was slower (data not shown). Again, these *in vivo* results fit the measurements obtained *in vitro* (Table 1).

DISCUSSION

Why Should Polymerase Speed Affect *lacZ* Transcript Stability? When endogenous RNA polymerase is replaced by the faster T7 enzyme for transcription of the *lacZ* gene in *E. coli*, the resulting transcripts are more RNase E-sensitive and yield little β-galactosidase. Dreyfus and coworkers (8, 9, 19) proposed that the ribosome-free mRNA region lagging behind this enzyme is sensitive to RNase E, causing premature cleavage unless the leading ribosome comes in time to protect it. Two mechanisms can be envisioned for this protection. The mere binding of a ribosome onto the ribosome binding site might suffice to protect the whole downstream sequence, as observed in several systems (23, 24). Alternatively, protection may require direct shielding of individual RNase E-sensitive sites by ribosomes. Both mechanisms agree, however, in predicting that the transcripts should be stabilized if T7 RNA polymerase were slowed down. The mRNA stretch synthesized prior to the first ribosome binding would then be shorter, reducing the probability that it harbors RNase E-sensitive sites. Similarly, the delay between the synthesis of any RNase E-sensitive site and its shielding by ribosomes would be reduced, lowering the probability of cleavage. The availability

of slow T7 RNA polymerase mutants allowed us to test this prediction.

Modulating the *in Vivo* Transcription Speed with T7 RNA Polymerase Mutants. Bonner *et al.* (11) have characterized many T7 RNA polymerase mutants altered in their *in vitro* polymerization kinetics, but retaining normal promoter affinity. The mutations are clustered within three motifs which together form the active site of the enzyme (25). Eight mutants that were still substantially active *in vitro* were selected for this work; their elongation speeds span a 6-fold range (Table 1). Their *in vivo* ability to transcribe the *lacZ* gene has been compared by using a tRNA transcriptional reporter (Fig. 1). Strikingly, the two mutants that were slowest *in vitro*—i.e., R627S and I810S, the latter hardly faster than the *E. coli* enzyme *in vivo*—failed to complete the transcription of the *lacZ* gene. The situation that slow T7 RNA polymerase mutants cannot transcribe the *lacZ* gene, whereas the even slower *E. coli* enzyme can, presumably arises from the lower stability of the T7 RNA polymerase elongation complex, which dissociates within minutes instead of hours (26, 27). The T7 polymerase then *must* be fast to synthesize long transcripts: as its speed drops, the length of the transcripts completed within the lifetime of the elongation complex drops concurrently. In addition, the processivity of this enzyme is not uniform, increasing abruptly as its transcript reaches $\approx$10 bases in length. For slow mutants, few enzyme molecules ever reach the more processive phase (11, 12). These features restrict the number of mutants that can transcribe the *lacZ* gene *in vivo*: put simply, only relatively fast mutants transcribe that far.

Within the latter category, mutants R551S and Y639F, which are as fast as the wild-type enzyme, show an equal or even a decreased β-galactosidase yield per transcript, respectively; the low yield from Y639F may reflect its unique propensity to misincorporate dNTPs in place of NTPs (22). Mutants F644A and Q649S, which are slightly slower *in vitro* ($\leq$1.5-fold), exhibit a significant increase in yield compared with the wild-type enzyme, particularly at 42°C (Table 1). However, the strongest test of the hypothesis came from G645A, the slowest mutant still able to transcribe efficiently the *lacZ* gene. This enzyme is 2.7-fold slower than the wild-type enzyme *in vitro* (and $\approx$2-fold slower *in vivo*). Significantly, its transcripts are less RNase E-sensitive than those of the wild-type enzyme and they yield 3.4- to 4.6-fold more β-galactosidase. By comparison, the *E. coli* enzyme is 8-fold slower than the wild-type T7 polymerase and its transcripts yield 40-fold more β-galactosidase. Therefore, among these three polymerases—the wild-type T7, G645A, and *E. coli* enzymes—the variations in yield parallel the variations in speed. This result supports the proposal that the high speed of the T7 RNA polymerase is responsible for the lability of its transcripts.

Concluding Remarks. We have shown here that the elongation speed of an RNA polymerase can affect mRNA stability. We further hypothesize that in other systems it might also affect the kinetics of transcript folding and even the choice between alternative folding pathways, insofar as base pairing between two RNA segments cannot occur before the downstream one has been synthesized. In this way, the polymerase speed can influence the temporal window during which a nascent RNA can interact with antisense RNAs (28), ribosomes (29), or specific proteins (30) or during which it can fold into functionally important structures such as attenuators. The availability of different polymerases with unequal speeds for transcribing *E. coli* genes—such as the wild-type T7, the G645A, and the *E. coli* RNA polymerases—will facilitate assessing the importance of this parameter upon *in vivo* properties of RNA.

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