JOURNAL OF BACTERIOLOGY, Jan. 1992, p. 619–622 Vol. 174, No. 2
0021-9193/92/020619-04$02.00/0
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Bacteriophage T7 RNA Polymerase Travels Far Ahead of Ribosomes In Vivo

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Received 2 July 1991/Accepted 24 October 1991

We show that in *Escherichia coli* at 32°C, the T7 RNA polymerase travels over the *lacZ* gene about eightfold faster than ribosomes travel over the corresponding mRNA. We discuss how the T7 phage might exploit this high rate in its growth optimization strategy and how it obviates the possible drawbacks of uncoupling transcription from translation.

In *Escherichia coli*, transcription occurs simultaneously with translation, and both processes are often tightly coupled. Thus, the average rate of transcription matches that of translation, even though it can theoretically be higher (3, 11a). Moreover, in the absence of translation, transcription usually terminates prematurely (1). Based on in vitro studies, it is believed that this coupling reflects the existence in most genes of specific pause sites where RNA polymerase waits for the first translating ribosome (12). If the ribosome delays and if the naked mRNA sequence behind the polymerase is suitable for rho factor binding, transcription termination occurs (1, 17, 20). Aside from its role in synchronizing the RNA polymerase with the leading ribosome in attenuators (24), the coupling of transcription and translation helps in avoiding the wasteful synthesis of mRNA during amino acid shortage (17). This coupling has also been proposed to prevent the sequestration of ribosome binding sites (RBSs) within the structure of mRNAs (11, 25); insofar as naked RNA is prone to degradation (2), it may protect the nascent transcripts against nucleolytic attacks.

The late genes of the coliphage T7 are transcribed by a phage-encoded RNA polymerase that consists of a single polypeptide chain of 98,092 Da (7). The in vitro turnover of this enzyme and those of similar polymerases encoded by related phages range from 200 to 300 nucleotides (nt) per s (4, 9). Should this also hold in vivo, transcription would travel far ahead of translation, as ribosome motion is known to be considerably slower (3). Herein, we have tested this intriguing possibility by comparing these two rates in vivo. The *lacZ* gene was chosen as the test gene for this experiment because, with its length, its transcription and translation last longer than those of most bacterial genes. Moreover, its product is readily identified on one-dimensional polyacrylamide-sodium dodecyl sulfate (SDS) gels.

We recently reported the construction of *E. coli* strains that harbor on their chromosome (i) the T7 gene *1*, encoding the RNA polymerase, under the control of the *lacUV5* promoter, which is inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) (22), and (ii) an engineered *lacZ* gene bracketed on one side by the T7 gene *10* promoter and leader sequences and on the other by a terminator for T7 RNA polymerase (5). Because it is present as a single copy, the *lacZ* gene can be transcribed constitutively from the very active T7 promoter without affecting cell growth. For the purpose of this study, we replaced the gene *10* leader sequence by a synthetic DNA fragment encompassing the *lac* operator (Fig. 1). Thereby, not only the transcription of the T7 gene *1* but also that of the target *lacZ* gene can be blocked by the *lac* repressor (8). Furthermore, various RBSs can be inserted between the operator and the coding sequence (5, 6). For purposes of comparison, we also prepared similar strains in which the genuine *lac* promoter replaced the T7 gene *10* promoter. In this case, a 28-bp synthetic sequence encompassing the strong *trp* operon transcriptional terminator was placed immediately downstream of the T7 terminator. These constructs will be described in detail elsewhere (10a).

To measure the in vivo elongation rate of T7 RNA polymerase, we exploited the fact that transcription of the *lacZ* gene can now be repressed even in the presence of T7 RNA polymerase. Cells were first grown in the presence of 1 mM IPTG to allow buildup of the T7 polymerase pool. When the culture reached an optical density at 600 nm of 1.0, the inducer was filtered out and the cells were resuspended for 90 min in IPTG-free medium to allow the preexisting *lacZ* message to decay and the cells to recover. The culture was then reinduced with IPTG. At timed intervals, samples were rapidly lysed by mixing for 10 s with an equal volume of boiling lysis buffer (5% SDS, 12 mM EDTA, 1.5 M sodium acetate [pH 7]). The phenol-extracted RNAs were then analyzed on Northern blots by using a DNA probe corresponding to the 5′ end of the *lacZ* gene (Genebank Ecolac 1305 to 2124) to visualize the growing RNA chains. Whatever the strength of the RBS in front of *lacZ*, the full-length message (4.2 kb) was clearly visible 25 s after induction (Fig. 2A and A′). In contrast, when the host RNA polymerase was used, this same species did not appear until 3 min (Fig. 2B). No transcripts longer than 4.2 kb were detected, indicating that the T7 and *trp* terminators are highly efficient within our constructs.

The time that it takes for RNA polymerase to transcribe the hybrid gene is not strictly equal to the time elapsed at the onset of full-length transcription. Before transcription starts,

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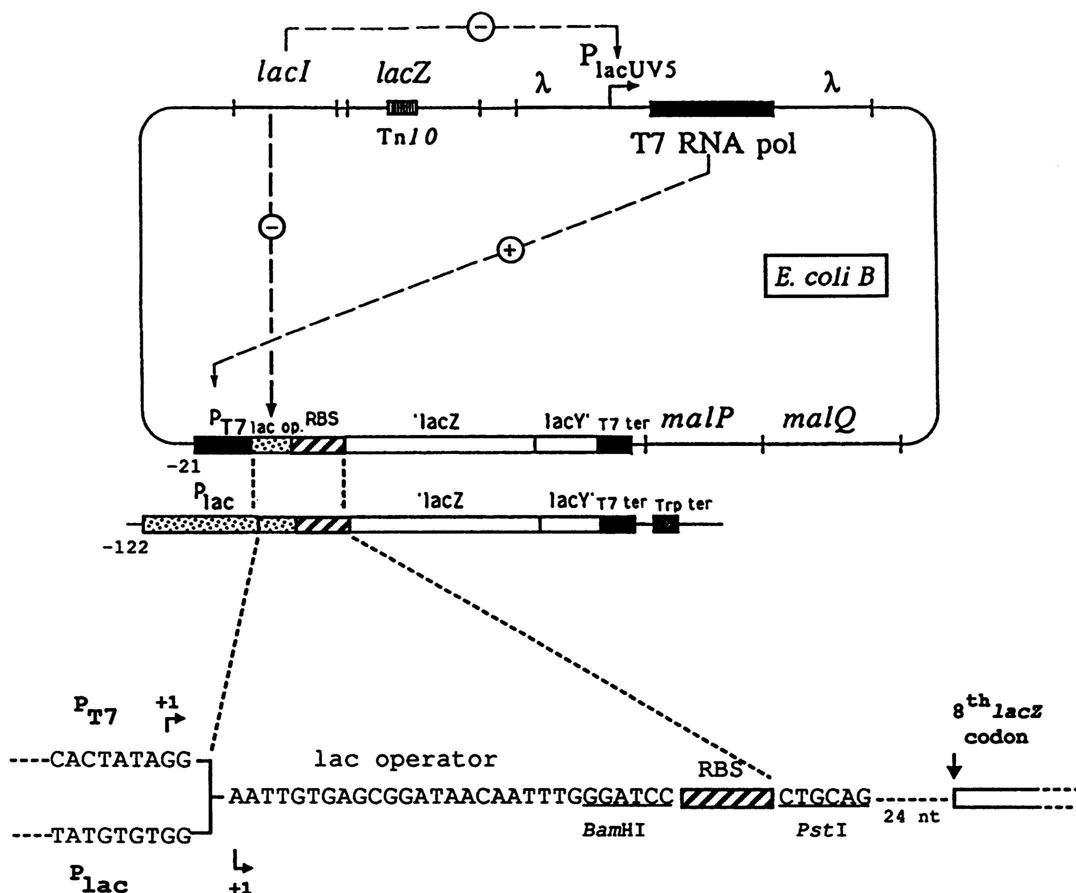

FIG. 1. Structure of the chromosome of *E. coli* B strains carrying a single-copy *lac* cassette transcribed by the T7 RNA polymerase (5). Positive (+) and negative (−) control by the T7 RNA polymerase and *lac* repressor are indicated. The endogenous *lacZ* gene is inactivated by an inserted transposon (vertical hatches). The open boxes represent *lac* operon sequences cloned in the T7-transcribed cassette. P_{T7} encompasses the sequence of the T7 gene *10* promoter from positions −21 to +2. Downstream from the second transcribed nucleotide we inserted a synthetic sequence (lac op.) that carries the *lac* repressor binding site. The 50- to 80-nt DNA fragments carrying RBSs from various genes are indicated (5,6; the exact RBSs used in this work are described in the legend of Fig. 2). Below this cassette is depicted an alternate cassette in which the genuine *lac* promoter replaces the T7 gene *10* promoter. T7 ter is a 133-nt fragment encompassing the major T7 RNA polymerase terminator that is responsible for the termination of transcription at position 24209 of the phage genome (19), and Trp ter is a 28-nt synthetic fragment encompassing the rho-independent terminator of the *trp* operon. The enlarged part of the figure illustrates the sequence of the upstream region of both cassettes. The bent arrows indicate the start points of the T7 and *E. coli* RNA polymerase transcripts. Nucleotides are numbered from these start points; positive numbers correspond to transcribed nucleotides. The sequence of the 24-nt polylinker was published previously (5). These strains (IM strains) are derivatives of BL21(DE3) (22) and have the genotype F^- *hsdS gal* (*λimm-21 Δnin-5 int*::T7 gene *1*) *lacZ*::Tn*10* $malP_p$ *Δ534*::*lacZ*. IM strains carrying the T7 gene *10* promoter and the *lac* promoter in front of the *lacZ* gene are given even and odd numbering, respectively. The strains used in the experiment shown in Fig. 2 are named IM4 (*lamB* RBS) and IM25 and IM26 (gene *10* RBS).

the inducer has to enter the cells, the repressor has to drop off, and the RNA polymerase has to bind proficiently to the promoter. Conversely, transcription does not stop at the exact time of harvest, because the lysis is not immediate. These unknown lags are particularly important in the case of the very fast T7 transcription. However, they cancel out if one compares the sizes of the growing mRNA at different times. For T7 transcription, no transcripts longer than 1.5 kb were seen at 10 s after induction, whereas the 4.2-kb transcript appeared between 20 and 25 s. For the endogenous enzyme the corresponding times were 1 and 3 min. On this basis, the elongation rates are ca. 200 and 25 nt/s for the T7 and host RNA polymerases, respectively. This eightfold difference is unmistakable. The value observed for the *E. coli* enzyme fits that previously reported for a similar temperature and generation time (13).

As shown in Fig. 2, several diffuse bands are seen upon the background of nascent transcripts, specially in the 0- to 1,500-nt range. Moreover, this banding pattern is similar regardless of the polymerase used. Since, in the *lacZ* gene, growing mRNA chains largely outweigh decaying fragments retaining the 5′ end of the message (15), it seems unlikely that these bands arise from the accumulation of degraded or processed species. Rather, they might correspond to regions that are transcribed comparatively slowly by both polymerases. This raises the intriguing possibility that certain sequences are intrinsically prone to pausing, regardless of which polymerase is used. Should this hold true, then the rates calculated above would correspond to average values, with local rates being eventually different.

It was of interest to estimate the rate of ribosome movement in the same experiment. We labeled aliquots of the

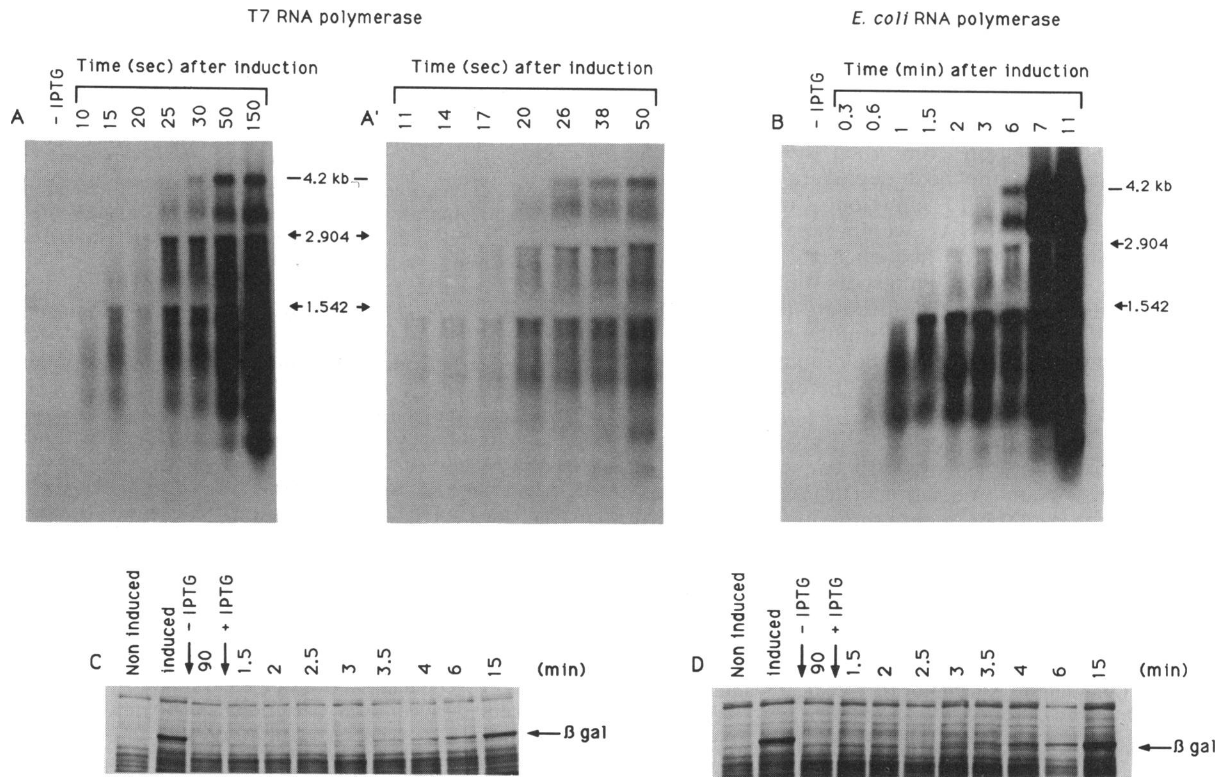

FIG. 2. (A and A') Northern blots showing the T7 RNA polymerase transcripts as a function of time after the *lac* repressor block over the T7 promoter was removed by the addition of IPTG. With this procedure, transcription from the cassette was repressed while a high pool of T7 RNA polymerase was maintained in the cell (see the text). The cells were grown in morpholinopropanesulfonic acid-based medium containing glycerol as the carbon source and all amino acids except Met and Cys; the generation time at 32°C was 80 min, i.e. intermediate between those observed in minimal and fully supplemented glycerol media. The strain used in panel A harbors in front of the *lacZ* gene an artificial RBS (denoted gene *10* RBS) encompassing the T7 gene *10* sequence from nt +28 to +63 (+1 being the transcriptional start point), followed by the first five codons of the *E. coli metF* gene. Its nucleotide sequence is AATAATTTTGTTTAACTTTAAGAAGGAGATATA CAT ATG AGC TTT TTT CAC (the Shine-Dalgarno element and enhancer sequence [16] are underlined). In panel A', we used the previously described *lamB* RBS (5), which, in the present context, directs fourfold less β-galactosidase synthesis than does the gene *10* RBS. (B) As in panel A, except that the cassette was now transcribed from the *lac* promoter by the *E. coli* RNA polymerase (Fig. 1). The positions of the 16S and 23S rRNAs and of the transcripts that terminate at the T7 or *trp* terminators (4.2 kb) are indicated. The major band (ca. 3.2 kb in length) that appears just above the 23S rRNA in the last samples from each experiment presumably corresponds to transcripts processed between *lacZ* and *lacY* (13, 15). (C and D) Autoradiograms of SDS gels showing the onset of β-galactosidase production during the time course of the experiments illustrated in panels A and B, respectively. Just before harvest and lysis, the cells were pulse-labeled with [^{35}S]methionine as indicated in the text. The times elapsed (1.5 to 15 min) between the readdition of the inducer and the end of the labeling period are indicated above the corresponding lanes. The lane marked 90 corresponds to a sample labeled 90 min after the removal of the inducer and just before the reinduction. The position of β-galactosidase is marked with an arrow.

cultures with [^{35}S]methionine at various times after IPTG reinduction. Translation was quenched 30 s later with chloramphenicol, and the labeled proteins were visualized on SDS gels. With both polymerases, the β-galactosidase polypeptide (ca. 1,040 amino acids) was just visible 2.5 min after induction and clearly seen at 3 min (Fig. 2C and D). Neglecting the induction lag, the average translation rate is then 7 codons (21 nt) per s. This matches the rate of elongation of the *E. coli* RNA polymerase but falls considerably short of that of the T7 enzyme. Therefore, transcription by T7 RNA polymerase generates extensive ribosome-free mRNA regions behind the polymerase. Although these regions may bind the rho factor, it is not known whether this can induce termination of transcription. However, should this occur, the rho effect upon T7 RNA polymerase would still differ radically from that upon the *E. coli* enzyme, insofar as ribosomes, lagging far behind transcription, are now unlikely to interfere with rho action. Indeed, the susceptibility of T7 RNA polymerase to termination is the same whether or not its transcript is translated (5, 21).

Why should the T7 phage elongate its late transcripts so fast? As the elongation rate rises, the polymerase molecules spend less time during each transcription cycle, and this tends to expand the pool of free RNA polymerase. This may be important for achieving efficient transcription, since T7 late promoters have comparatively weak affinities for their cognate polymerase (10). Maximizing the frequency of late transcription is rather crucial for T7, because most of the late transcripts required for the phage burst are synthesized from a limited amount of (not yet replicated) DNA template and within the very few minutes that separate the onset of late transcription from that of replication (14).

As pointed out above, uncoupling transcription from translation in *E. coli* may have some disadvantages that bacteriophage T7 must obviate. Presumably, it is not greatly concerned with subtle mechanisms such as attenuation or

with the necessity of sparing its host's resources in the case of amino acid shortage. However, it must somehow avoid the negative effects that uncoupling may have upon the stability and translatability of its mRNAs. Late T7 mRNAs are known to be stable (23), and therefore they may be tailored to resist degradation even when they are free of ribosomes; alternatively, the host degradation machinery may be inactivated during T7 infection. Concerning the translatability of late T7 mRNAs, we note that they harbor unusually long Shine-Dalgarno sequences (7, 18), which, furthermore, are preceded by enhancers that raise their frequency of translation (16). We speculate that T7 RBSs have evolved to keep translation efficient despite its uncoupling from transcription. Possibly, their unusual features allow them to rapidly catch ribosomes before the mRNA becomes highly folded.

Irrespective of its consequences for T7 growth, the high rate of T7 RNA polymerase provides a convenient way to uncouple transcription and translation. The consequences of this uncoupling for bacterial gene expression will be analyzed elsewhere (10a).

This work has been funded by the Centre National de la Recherche Scientifique, the Ministère de l'Education Nationale, and the Fondation pour la Recherche Medicale. I.I. received a long-term fellowship from the Ministère de la Recherche et de la Technologie.

We thank M. Uzan and L. Martin for their critical reading of the manuscript.

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