The EMBO Journal vol.14 no.13 pp.3252–3261, 1995

The stability of *Escherichia coli lacZ* mRNA depends upon the simultaneity of its synthesis and translation

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We have used either *Escherichia coli* or T7 RNA polymerase to transcribe in *E.coli* a series of *lacZ* genes that differ in the nature of their ribosome binding sites (RBS). Each T7 RNA polymerase transcript yields from 15- to 450-fold less β-galactosidase than its *E.coli* polymerase counterpart, the ratio being larger when weaker RBS are used. The low β-galactosidase yield from T7 transcripts reflects their low stability: the *ams-1/rne-50* mutation, which inactivates RNase E, nearly equalizes the β-galactosidase yields from T7 and *E.coli* RNA polymerase transcripts. T7 RNA polymerase transcribes the *lacZ* gene ~8-fold faster than the *E.coli* enzyme. We propose that this higher speed unmasks an RNase E cleavage site which is normally shielded by ribosomes soon after its synthesis when the slower *E.coli* enzyme is used. This leads to degradation of the T7 transcript, unless the leading ribosome comes in time to shield the cleavage site: the weaker the RBS, the lower this probability and the more severe the inability of T7 RNA polymerase transcripts for β-galactosidase synthesis.

Key words: transcription–translation synchronization/T7 RNA polymerase/mRNA stability/RNase E/ribosome binding site

Introduction

Due to the absence of a nuclear membrane, *Escherichia coli* mRNAs can bind ribosomes before their synthesis is complete (Stent, 1966). Moreover, as shown by electron microscopy observations (Miller *et al.*, 1970), this binding usually occurs before the RNA polymerase has moved far downstream from the corresponding ribosome binding sites (RBS; Steitz, 1969). Subsequently, transcription and translation proceed with the same average elongation speed (Jacquet and Kepes, 1971). This synchronization, which reflects an active adjustment of the RNA polymerase speed to that of ribosomes (Jacquet and Kepes, 1971; Gupta and Schlessinger, 1976; Vogel *et al.*, 1992; Vogel and Jensen, 1994), originates from dedicated global regulatory mechanisms (Jensen and Pedersen, 1990; Vogel *et al.*, 1992; Sørensen *et al.*, 1994), as well as from properties of individual coding sequences. Thus, *in vitro* the RNA polymerase pauses at specific sites along its DNA template (von Hippel *et al.*, 1984) and it is believed that *in vivo* these stalled polymerases resume transcription only when caught up by the leading ribosome, ensuring the periodic synchronization of transcription and translation (Landick *et al.*, 1985; Jørgensen, 1992).

As a result of this synchronization, no large ribosome-free mRNA stretch usually exists behind the transcribing *E.coli* RNA polymerase (Miller *et al.*, 1970). Such naked mRNA regions are expected to be unstable (Morse and Yanofsky, 1969; Belasco and Higgins, 1988; Petersen, 1993), especially if no translating or stalled ribosome is present upstream of them (Bechhofer and Dubnau, 1987; Nilsson *et al.*, 1987; Bechhofer and Zen, 1989; Wagner *et al.*, 1994). Therefore, synchronization of transcription and translation might be essential for the stability of nascent bacterial mRNAs. This point, however, has rarely been examined so far, perhaps because those situations that tend to desynchronize transcription from translation often result in premature transcription termination (polarity; see Adhya and Gottesman, 1978; Platt, 1986; Stanssens *et al.*, 1986; Richardson, 1991).

The bacteriophage T7 RNA polymerase travels ~8-fold faster in *E.coli* than the host enzyme or than the translating ribosomes (Iost *et al.*, 1992) and yet it does not show polarity effects (Studier, 1972; Studier and Moffatt, 1986; Chevrier-Miller *et al.*, 1990). Therefore, by the time of the first ribosome binding, the T7 RNA polymerase has synthesized a much longer stretch of naked, potentially nuclease-sensitive RNA than the *E.coli* enzyme. Subsequently, the gap between the leading ribosome and the T7 polymerase continuously expands with time. As noted above, both features might destabilize the transcripts. Recently we compared the expression of several genes that are transcribed either by *E.coli* RNA polymerase or by the T7 enzyme and we observed that the polypeptide yield per transcript was greatly reduced in the latter case (Lopez *et al.*, 1994). Here, focusing on the *lacZ* gene, we show that this difference in yield stems from a large difference in transcript stability. In the light of these findings, we discuss the role of transcription–translation synchronization in mRNA stability and, more practically, its bearing for the optimization of *E.coli* expression systems.

Results

lacZ transcripts synthesized by the T7 RNA polymerase are inefficiently utilized for β-galactosidase synthesis

Recently we compared the efficiency of *lacZ* transcripts synthesized either by T7 or by *E.coli* RNA polymerase in driving β-galactosidase expression in *E.coli* (Lopez *et al.*, 1994). To this end, the *lacZ* gene (in which, for construction purposes, the original RBS had been replaced by the *lamB* gene RBS, which is nearly as efficient; Yarchuk *et al.*, 1992) was fused upstream of a tRNA gene, the expression of which is taken as a measure of transcription. Down-

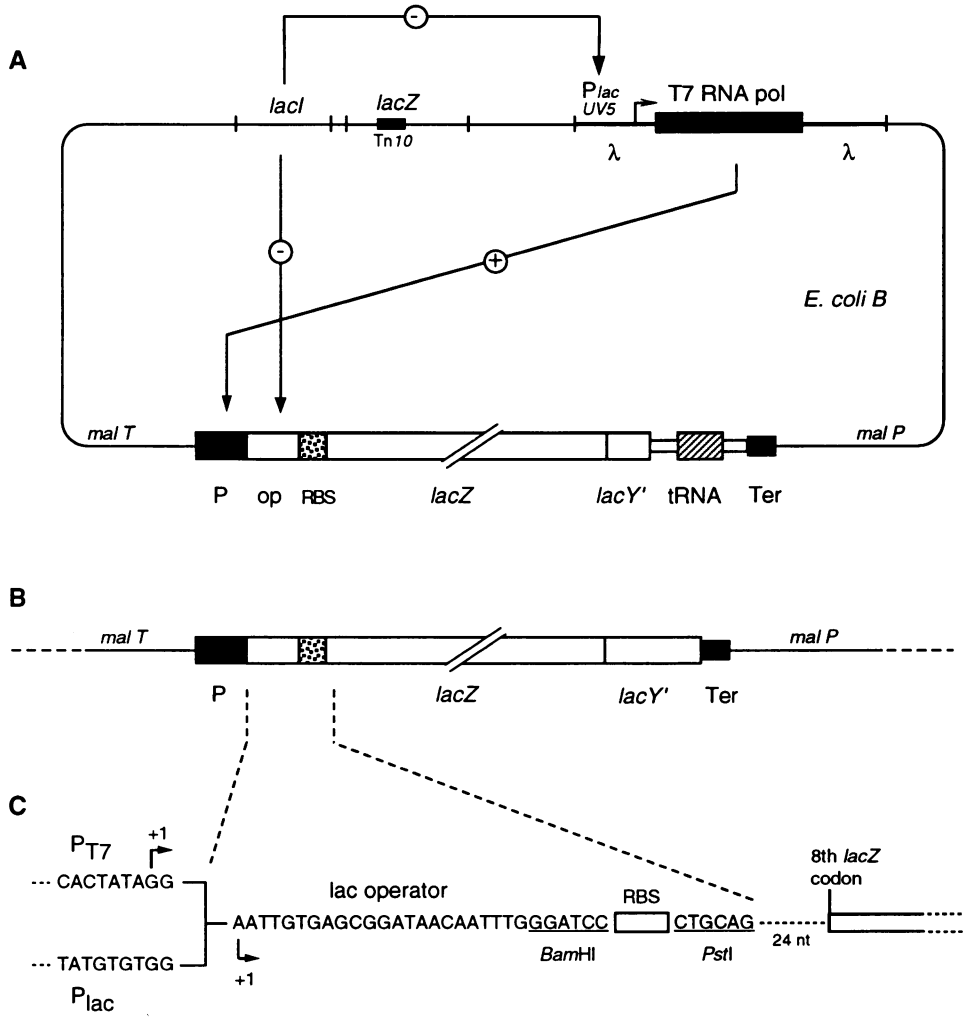

Fig. 1. Schematic representation of the chromosome from the strains used in this work. (**A**) Strains ENS133 and ENS134 carrying the tRNA reporter gene. Along with the phage λ DE3, which harbours the T7 RNA polymerase gene (black box), these strains carry a single copy *lac* cassette inserted upstream from the *malP* gene. The endogenous *lacZ* gene has been inactivated by Tn*10* insertion, as indicated. The symbols + and – stand for positive and negative control by the T7 RNA polymerase and *lac* repressor respectively. P ('promoter') stands for either the promoter of the bacteriophage T7 late gene *10* (P_{T7}, strain ENS134) or the *lac* operon promoter (P_{lac}, strain ENS133). Op ('operator') is the *lac* repressor binding site; Ter ('terminators') stands for the two tandemly arranged transcription terminators from the T7 late region and from the *E.coli trpA* gene. The dotted box denoted 'RBS' stands for a 51 bp fragment encoding the ribosome binding site from the *lamB* gene (cf. Figure 4). The thick, open box corresponds to the coding sequence of the *lacZ–lacY* gene, whereas the hatched box stands for the $tRNA^{Arg5}$ gene, which is used here as a transcriptional reporter. Other non-coding sequences are shown as thin boxes. (**B**) Structure of strains ENS33–ENS50. Odd numbered strains carry the P_{lac} promoter and even numbered ones P_{T7}. These strains harbour the different RBS depicted in Figure 4 (Table II). Aside from these RBS replacements, they differ from those depicted in (A) essentially by the absence of the reporter tRNA (see Materials and methods). (**C**) Sequence of the 5′-region of the P_{lac} and P_{T7} constructs in the strains shown in (B). The transcription start points (arrows) have been checked by primer extension. The sequence of the 24 nt polylinker is identical to that of pEMBLΔ46 (Dreyfus, 1988).

stream from the tRNA gene we inserted two tandemly arranged transcriptional terminators. This construct was then fused either to the T7 late gene *10* promoter (P_{T7}) or to the *E.coli lac* operon promoter (P_{lac}), in such a way that the sequence transcribed from either promoter is precisely the same. These constructs were then introduced as single copies into the chromosome of a Lac^- *E.coli* strain producing saturating amounts of T7 RNA polymerase (Figure 1A) and tRNA and β-galactosidase expression were recorded in exponentially growing cells at 37°C. tRNA expression from P_{T7} was 20-fold higher than from P_{lac}, reflecting a 20-fold higher transcription frequency. Yet, paradoxically, β-galactosidase expression from P_{T7} was 2-fold lower, demonstrating a 40-fold drop in polypeptide yield per transcript. Obviously, the replacement of the *E.coli* RNA polymerase by the T7 enzyme creates a large post-transcriptional defect (Lopez *et al.*, 1994; cf. Figure 2).

This low β-galactosidase yield might somehow be due to the unnatural presence of the excisable tRNA on the transcript. To settle this point, we assayed similar strains lacking the tRNA. These strains, which had been constructed previously (Iost *et al.*, 1992), are briefly described in Materials and methods and in Figure 1B. β-Galactosidase activity (Table I) and the accumulation of β-galactosidase polypeptide, as detected by Western analysis, were indistinguishable in strains carrying or lacking the tRNA. Similarly, the mRNA pattern on Northern blots was very similar whether the tRNA was present or not (not shown). Presumably, excision of the tRNA from the primary transcript is a relatively slow process that affects neither the chemical nor the functional lifetime of this transcript

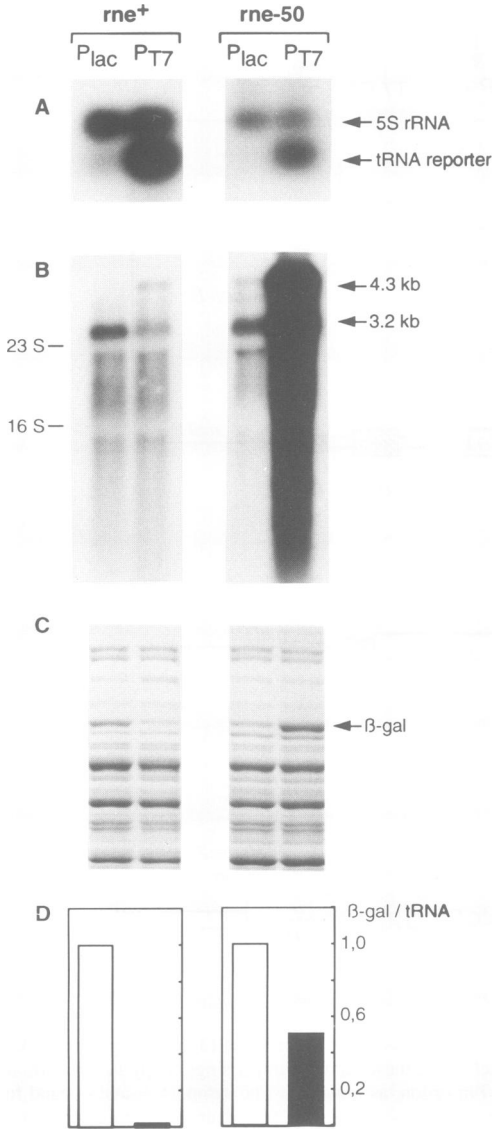

Fig. 2. (**A**) Northern blot showing expression of the reporter $tRNA^{Arg5}$ in strains ENS133 (P_{lac}) and ENS134 (P_{T7}) in the presence or absence of the *rne-50* mutation (*rne-50* and rne^+ respectively). Cells were grown at 28°C and IPTG-induced at 42°C. To correct for lane-to-lane variations in RNA loading, 5S rRNA is also visualized. Unmatured precursors of both the tRNA and 5S rRNA accumulate in *rne-50* cells, though their total abundance remained less than that of the matured species. For clarity, most of them have been cut out from the panel shown, but they are included in the quantification in (D). (**B**) Northern blot showing the *lacZ* mRNA pattern in the same RNA samples as above. The positions of the full-length operon transcript (4.3 kb) and of a processed species (3.2 kb), as well as those of 16S and 23S rRNA, are indicated. See Materials and methods for the growth conditions, preparation of the RNA samples and hybridization protocols used in (A) and (B). (**C**) Coomassie stained SDS gel showing synthesis of the β-galactosidase polypeptide (β-gal) in the same experiment as above. (**D**) Ratio of β-galactosidase activity to tRNA expression in the above experiment. These ratios reflect the β-galactosidase yield per transcript in each case. Values for P_{lac} were arbitrarily set to unity in both the rne^+ and *rne* contexts.

(see Lopez *et al.*, 1994, for a discussion). In any event, the tRNA is not responsible for the low β-galactosidase yield from T7 RNA polymerase transcripts and strains carrying or lacking it are regarded here as equivalent. For simplicity, strains lacking the tRNA are used unless determination of the transcription frequency is essential.

Table I. β-Galactosidase activities of strains carrying different RBS and either P_{lac} or P_{T7}[a]

RBS	P_{lac}	P_{T7}
lamB	5000 ± 700	2500 ± 300
lamB708	1900 ± 400	211 ± 30
lamB713	500 ± 100	88 ± 14
lamB701	340 ± 80	15 ± 2
galE	10 200 ± 2000	9700 ± 1200
malP	4400 ± 400	1550 ± 140
metF	2300 ± 600	2100 ± 200
thrA	1000 ± 180	300 ± 25
gene 10	19 000 ± 4000	25 000 ± 3500

[a]Strains lacking the tRNA (ENS33–ENS50 in Table II). The values (nmol ONPG hydrolysed/min/mg protein) are averaged from three to six independent measurements at 37°C. Uncertainties arise mainly from systematic day-to-day variations; the relative values from the different strains were more reproductible. See Materials and methods for assay conditions. The activities observed in the strains carrying the tRNA together with the *lamB* RBS [ENS133(P_{lac}) and ENS134(P_{T7}); Figure 1A] are 4000 ± 400 and 2100 ± 200 units respectively (Lopez *et al.*, 1994).

We shall use the qualifiers 'P_{lac}' or 'P_{T7}', coupled with 'tRNA' when the latter is present, to briefly designate constructs, strains, transcripts, etc. carrying P_{lac} or P_{T7} or originating from them. Unless explicitly stated, the *lacZ* gene is translated from the *lamB* RBS, which was present in our original constructs.

P_{T7} transcripts are very sensitive to RNase E-dependent cleavage

We next compared the chemical stability of the P_{T7} and P_{lac} transcripts. The stability of a mRNA, together with the frequency of its synthesis, determines its steady-state concentration. Therefore, to compare the relative stabilities of the P_{lac} and P_{T7} transcripts, we merely compared their steady-state concentration, taking into account the different transcription frequencies in both cases. A growth temperature of 42°C was used in these experiments to facilitate interpretation of the effect of the *ams-1/rne-50* mutation (see below).

Under these conditions, expression of the reporter tRNA, when analysed on Northern blots with a specific oligonucleotide probe, was 15-fold higher in the P_{T7}-tRNA than in the P_{lac}-tRNA strain (Figure 2A, left). The steady-state concentrations of the P_{lac} and P_{T7} transcripts were then assayed using a probe internal to the *lacZ* gene. Qualitatively, the pattern obtained was the same in the P_{lac} and the P_{T7} strains: aside from a smear arising from growing and decaying molecules, it consisted of the full-length transcript of the operon (4.3 kb in length) and in an ~3.2 kb species. The latter corresponds to a processed species encompassing the *lacZ* mRNA proper (Kennell, 1986; McCormick *et al.*, 1991; Murakawa *et al.*, 1991; Yarchuk *et al.*, 1992). Quantitatively, in spite of the 15-fold higher transcription frequency, the transcript pattern was fainter in the P_{T7} cells than in the P_{lac} cells. The 3.2 kb species was particularly faint in the P_{T7} cells, whereas the full-length species was slightly more abundant than in the P_{lac} cells, possibly because *E.coli* RNA polymerase transcription is sensitive to polarity as it procceds through the untranslated 0.5 kb separating the tRNA from the terminators (Figure 1A). Taken together,

these observations show that the P_{T7} transcripts are far less stable than their P_{lac} counterparts.

The chemical decay of most *E.coli* mRNAs, including *lacZ* mRNA, is controlled, presumably directly (Carpousis *et al.*, 1994), by the endonuclease RNase E (Mudd *et al.*, 1990; Babitzke and Kushner, 1991; Melefors and von Gabain, 1991; Taraseviciene *et al.*, 1991; Yarchuk *et al.*, 1992). At 42°C, RNase E can be inactivated by the conditional *ams-1* mutation (Ono and Kuwano, 1979), now renamed *rne-50* (Mudd *et al.*, 1990). When introduced into the P_{lac} and P_{T7} strains, this mutation yielded a moderate and parallel decrease in tRNA expression in both strains (Figure 2A, right). In contrast, the concentration of the 4.3 and 3.2 kb species increased in both the P_{lac} and P_{T7} strains, indicating transcript stabilization. However, the magnitude of the increase was very much larger in the P_{T7} than in the P_{lac} strain (Figure 2B, right). In fact, these concentrations now paralleled the corresponding tRNA concentrations, showing that the P_{lac} and P_{T7} transcripts had nearly the same stability. We conclude that in rne^+ cells the P_{T7} transcripts are much more sensitive to RNase E-dependent cleavage than the P_{lac} transcripts, explaining their comparatively low abundance.

The rne-50 mutation increases the low β-galactosidase yield from P_{T7} transcripts

To determine whether the difference in β-galactosidase yield between P_{T7} and P_{lac} transcripts originates from their different stabilities, we recorded this yield under conditions where these stabilities are equalized, i.e. in the presence of the *rne-50* mutation. At 42°C, in the absence of the mutation, P_{T7} cells produced only 25% as much β-galactosidase as their P_{lac} counterpart (Figure 2C), despite the 15-fold higher frequency of transcription. This implies a 60-fold lower β-galactosidase yield per transcript (Figure 2D). The *rne-50* mutation resulted in a 4-fold decrease and a 7-fold increase in β-galactosidase expression from P_{lac} and P_{T7} respectively. As a consequence, P_{T7} cells now produced seven times more β-galactosidase than P_{lac} cells. Given the ratio of the tRNA concentrations under these conditions, we calculate that the β-galactosidase yield per transcript amounts to ~50% of that from P_{lac} (Figure 2D). This residual difference might reflect the fact that P_{T7} transcripts are slightly less translatable than P_{lac} transcripts (see Discussion). Either way, most of the difference in β-galactosidase yield between the P_{T7} and P_{lac} transcripts is eliminated by the mutation. We conclude that in rne^+ cells the low β-galactosidase yield from P_{T7} transcripts arises primarily from their extreme sensitivity to RNase E-dependent cleavage(s).

Functional lifetimes of the P_{lac} and P_{T7} transcripts

The functional lifetime of a mRNA is the time during which it can support protein synthesis. To determine whether the unequal stabilities of the P_{lac} and P_{T7} transcripts are reflected in their functional lifetimes, the P_{lac} and P_{T7} promoters were switched off by rapidly removing IPTG, exploiting the fact that both promoters are flanked by the *lac* repressor binding site (Lopez *et al.*, 1994; Figure 1). The decay of β-galactosidase-synthesizing capacity was subsequently recorded using a pulse-labelling technique. This capacity faded with the same half-life for both promoters (2.5 ± 0.5 min; Figure 3). Hence, the very low chemical stability of the P_{T7} transcript, even though it severely limits β-galactosidase yield, does not result in a shortened functional lifetime. This apparent paradox is tentatively explained in the Discussion.

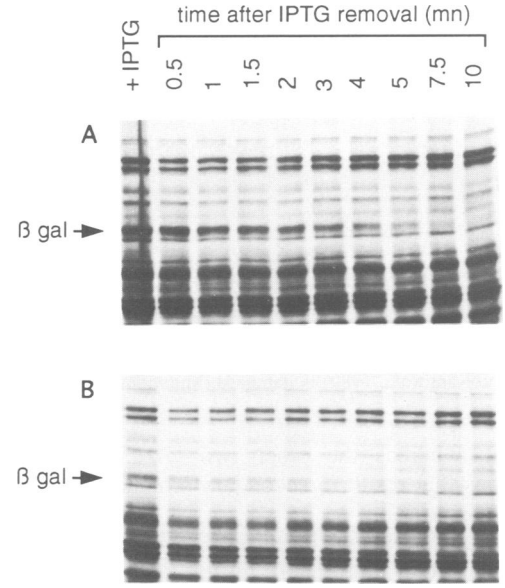

Fig. 3. Autoradiogram showing functional decay of the P_{lac} (**A**) and P_{T7} (**B**) transcripts at 37°C following IPTG removal. At the indicated time following this removal, aliquots of the cultures were labelled for 15 s with [^{35}S]methionine and then treated with excess cold methionine. Extracts of the labelled cells were separated on SDS gels and the β-galactosidase polypeptide (arrow) was quantified from the autoradiogram.

The stability and β-galactosidase yield of the P_{T7} transcript are more sensitive to changes in RBS efficiency than those of the P_{lac} transcript

The stability of the *lacZ* mRNA depends not only upon the *rne* allele, but also upon the efficiency of the RBS (Yarchuk *et al.*, 1992). Analogously, this efficiency might modulate the stability of the P_{T7} transcripts and, hence, their β-galactosidase yield. This point is examined here, taking the P_{lac} transcripts as a control.

Substitution of the 35 nt encompassing the original *lacZ* RBS by fragments carrying RBSs from other *E.coli* genes generally results in variations in β-galactosidase expression, presumably due to changes in the efficiency of translation initiation (Dreyfus, 1988; Guillerez *et al.*, 1991). We therefore replaced the region containing the *lamB* RBS in the P_{lac} and P_{T7} strains (Figure 1) by fragments containing RBS from other genes. We first chose RBS from typical *E.coli* biosynthetic and catabolic genes, i.e. *galE*, *malP*, *metF* and *thrA* (Dreyfus, 1988; Figure 4). We also attempted to construct a particularly efficient RBS by replacing the untranslated region of the *metF* RBS with the corresponding region of the T7 gene *10*, including the long Shine–Dalgarno (SD) and the so-called 'enhancer' elements (Olins and Rangwala, 1989; 'T7 gene *10* RBS', Figure 4). At the other extreme, we used three fragments encompassing mutated alleles of the *lamB* RBS which are known to be inefficient in translation initiation (Hall *et al.*, 1982; Yarchuk *et al.*, 1992; Figure 4). One of them (*713*) carries a mutation in the SD

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galE          GTCTATTTCATACCATAAGCCTAATGGAGCGAATTATGAGAGTTCTGGTTACCGGTGGTAGCGAC
malP        GTCGTTGTGTTGAAAATCTAAGAAAAGTGGAACTCCTATGTCACAACCTATTTTTAACGATAAGCAATTTCAGGAAGAC
metF     GTACAACACAACATATAACTACAAGCGATTGATGAGGTAAGGTATGAGCTTTTTTCAC
thrA       GTCCTTTTTTTTTCGACCAGAGGTAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGAC
T7 gene 10   AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAGCTTTTTTCAC
lamB                    AAAAATGACTCAGGAGATAGAATGATGATTACTCTGCGCAAACTT
                              *  *                *
                              G  A                A
                           701 713             708
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Fig. 4. Sequence of five fragments encompassing RBS from various *E.coli* genes and of one artificial fragment carrying elements of the T7 gene *10* RBS (see text). The fragments are bracketed by *Bam*HI and *Pst*I sites (not typed) and are inserted upstream of the *lacZ* gene (cf. Figure 1). Initiator ATGs are in bold type, Shine–Dalgarno elements are underlined and the region of the gene *10* RBS which has been proposed to act as an enhancer (Olins and Rangwala, 1989) is italicized. Mutations *701*, *708* and *713* are starred below the *lamB* RBS sequence (see text).

element, whereas the other two (*701* and *708*) carry mutations that result in secondary structures sequestering this sequence.

Generally speaking, the β-galactosidase activities from individual RBS correlated in the P_{lac} and P_{T7} series. Quantitatively, however, these activities spanned less than two orders of magnitude in the P_{lac} series, whereas in the P_{T7} series they spanned more than three. Thus, on average, a RBS substitution which changes the expression from P_{lac} by 10-fold changes the expression from P_{T7} by 50-fold (Table I and cf. Figure 5 below). In addition, some RBS deviated somewhat from this regular correlation. For example, the *lamB 701* and the *lamB 708* RBS, which in the P_{lac} series are nearly as efficient as the *lamB 713* and *metF* RBS respectively, are weaker than them in the P_{T7} series (Table I). In all cases we checked that these activities reflect the accumulation of β-galactosidase polypeptide (not shown).

We next introduced the *rne-50* mutation into the P_{lac} and P_{T7} strains harbouring different RBS and measured the corresponding β-galactosidase activities at 42°C (see Materials and methods). The activities from the parental *rne*$^+$ P_{lac} and P_{T7} cells were also assayed under the same conditions; they correlated at 42°C, much as they did at 37°C, with RBS changes still affecting the expression from P_{T7} more than that from P_{lac} (Figure 5). The mutation caused a decrease in the expression from P_{lac} and a marked increase in the expression from P_{T7}, as for the P_{lac}-tRNA and P_{T7}-tRNA strains (cf. Figure 2). However, the increase was not uniform for all P_{T7} strains: in general, the less efficient the RBS, the larger the increase. As a consequence, in the presence of the *rne-50* mutation the β-galactosidase activities from P_{lac} and P_{T7} became nearly proportional for all RBS. This is illustrated in Figure 5, which shows that the activities from P_{lac} and P_{T7} correlate linearly on a logarithmic scale, with a slope approaching one. In other words, in the *rne-50* context, RBS substitutions affect these activities to the same extent.

Finally, we compared the stability of the P_{lac} and P_{T7} transcripts carrying different RBS. Previously we reported that when the original RBS of the *lacZ* gene is replaced by the *galE* RBS or the wild-type and mutant *lamB* RBS, the transcript stability varies with the efficiency of the RBS, whereas the level of polarity remains almost constant (Yarchuk *et al.*, 1992). As a consequence, the steady-state concentration of the transcript parallels gene expression. To test whether the P_{lac} and P_{T7} transcripts carrying different RBS behave similarly, we compared their steady-state concentrations on Northern blots. At 42°C in *rne*$^+$ cells these concentrations did vary with the RBS used. This variation is illustrated in Figure 6, in which two very efficient RBS (*gene 10* and *galE*) and two moderately efficient ones (*malP* and *lamB 708*) were used. The summed concentrations of the 4.2 and 3.2 kb species spanned a 7-fold range in the corresponding P_{T7} strains and correlated well with the corresponding β-galactosidase expression (Figure 6, upper). In the P_{lac} series the variation was smaller (3-fold), consistent with the smaller variation in expression (cf. Figure 5). The introduction of the *rne-50* mutation eliminated these RBS-dependent variations in mRNA concentration, confirming that they reflect changes in transcript stability (Figure 6, lower). Thus, like the corresponding β-galactosidase expression, the stability of the P_{lac} or P_{T7} transcripts depends on the RBS used and this dependency is stronger for P_{T7} than for P_{lac}. In addition, the large effect of RBS changes upon P_{T7} β-galactosidase expression is a consequence of its large effect upon P_{T7} transcript stability, since it is no longer observed when the stabilities of all P_{lac} and P_{T7} transcripts are equalized by the *rne-50* mutation.

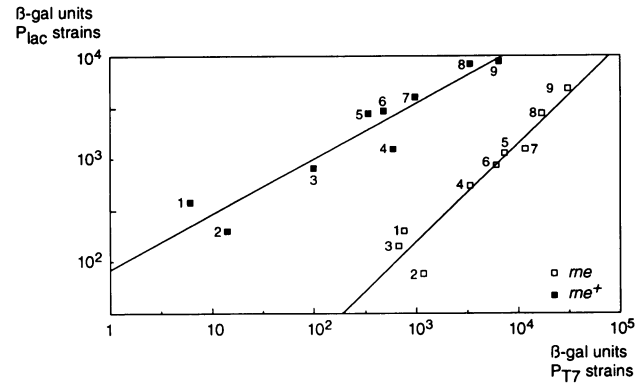

Fig. 5. β-Galactosidase activities from the P_{lac} strains harbouring different RBS are plotted on a logarithmic scale versus those from the cognate P_{T7} strains. Open and closed squares correspond to *rne-50* and *rne*$^+$ strains respectively. Cells were grown at 28°C and induced at 42°C (see Materials and methods). RBS are numbered as follows: 1, *lamB 701*; 2, *lamB 713*; 3, *thrA*; 4, *metF*; 5, *lamB 708*; 6, *malP*; 7, *lamB*; 8, *galE*; 9, gene *10*.

Discussion

The low β-galactosidase yield from P_{T7} transcripts reflects their low stability

While at 37°C P_{T7} produces 20-times more *lacZ* transcripts than P_{lac}, it does not yield a proportionally higher β-

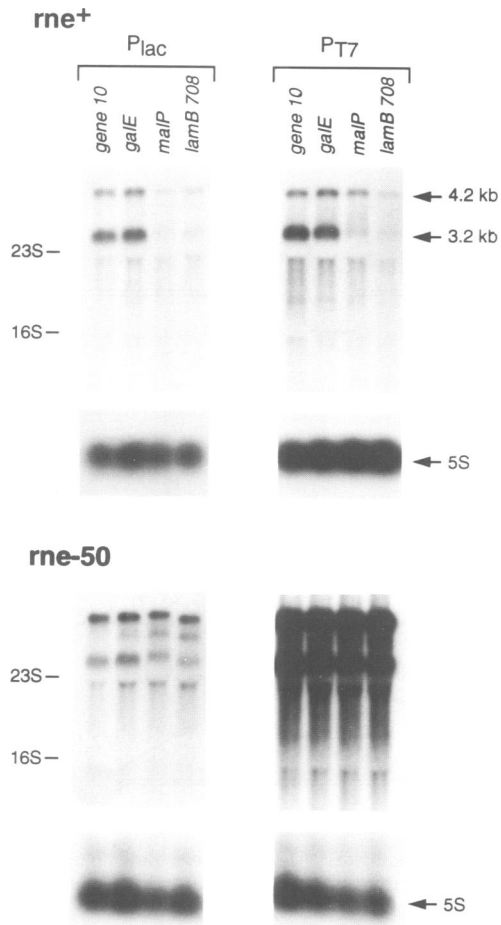

Fig. 6. (Upper) Northern blots showing accumulation of *lacZ* mRNA from P_{lac} (left) and P_{T7} (right) *rne*$^+$ strains harbouring different RBS. The RBS used are noted above each lane; they are ranked according to decreasing β-galactosidase expression. (Lower) As above, except that the strains carry the *rne-50* mutation. Below each panel is shown reprobing of the same membrane with the 5S rRNA probe (cf. Figure 2). The growth conditions and the preparation and probing of RNA samples were as in Figure 2. Aside from the RBS replacements, the strains used here differ from those used in Figure 2 by the absence of the reporter tRNA (cf. Figure 1). All symbols as in Figure 2.

galactosidase expression, implying a low polypeptide yield per transcript (Lopez *et al.*, 1994). In general, the weaker the RBS in front of *lacZ*, the more severe this reduction in yield from P_{T7} transcripts. Quantitatively, the β-galactosidase yield per transcript can be calculated from the observed β-galactosidase expression and the corresponding transcription frequency (see Materials and methods, calculation of the polypeptide yield per transcript). When harbouring the efficient gene *10* RBS, each P_{lac} transcript yields an average of 99 polypeptides, while the corresponding P_{T7} transcript yields 6.5, i.e. 15 times less. For the *lamB* RBS, which has been mostly used here, these figures are 26 and 0.65, i.e. a ratio of 40. For the very weak *lamB 701* RBS, the corresponding figures drop to ~1.8 and 0.004, yielding a ratio of 450. Therefore, unless it harbours a very efficient RBS, each P_{T7} transcript yields an average of less than one polypeptide. Most of them must remain untranslated altogether.

The low β-galactosidase yield from P_{T7} transcripts, particularly when carrying weaker RBS, stems from their extreme instability. Indeed, the *rne-50* mutation, which inactivates RNase E, markedly raises this yield, which now nearly matches that from P_{lac} transcripts. Independent evidence for the instability of P_{T7} transcripts has been reported elsewhere (Iost and Dreyfus, 1994). Yet, paradoxically, the functional lifetime of the P_{T7} transcripts in *rne*$^+$ cells is relatively long and indistinguishable from that of P_{lac} transcripts. We now propose a straightforward interpretation of all these observations.

A plausible model (Figure 7)

As noted in the Introduction, due to its high speed, transcribing T7 RNA polymerase creates an ever expanding stretch of naked mRNA behind itself. We propose that this naked region is sensitive to an RNase E-controlled endonuclease (which for simplicity we assume to be RNase E itself). Most P_{T7} transcripts will therefore be prematurely cleaved and, because the cleavage occurs ahead of the leading ribosome, they will not contribute to protein synthesis. This explains the low β-galactosidase yield from P_{T7} transcripts. The leading ribosome may sometimes come in time to protect the RNase E-sensitive site(s) and rescue the transcript: the more efficient the RBS, the higher this probability. Hence, the efficiency of the RBS not only determines the number of translation events that occur on each rescued transcript, as for P_{lac} transcripts, but also the proportion of transcripts that are rescued. Because of this dual role, transcript stability and β-galactosidase expression from P_{T7} should be more dependent upon the efficiency of the RBS than those from P_{lac}, as indeed they are.

Once rescued, P_{T7} transcripts are equivalent to their P_{lac} counterparts for β-galactosidase synthesis, explaining their identical functional lifetimes. Finally, inactivation of RNase E by the *rne-50* mutation allows all nascent P_{T7} transcripts to be rescued, raising the β-galactosidase yield to the same level as for P_{lac} transcripts, whatever the RBS used. Again, this prediction is borne out by our experiment.

Implications and limitations of the model

How, mechanistically, can ribosomes protect nascent P_{T7} transcripts against RNase E attack? Our observations are compatible with two models. First, the mere binding of a ribosome onto the RBS could protect the whole nascent transcript, whatever its length and the location of the cleavage sites. This view, which is consistent with the proposal that RNase E is a 5′→3′ scanning enzyme (Bouvet and Belasco, 1992) which can be halted by bound ribosomes, fits several other observations (Bechhofer and Dubnau, 1987; Nilsson *et al.*, 1987; Bechhofer and Zen, 1989; Wagner *et al.*, 1994). P_{T7} transcripts would then be prone to RNase E attack only before the first ribosome binds (Figure 7B, left). The stronger the RBS, the shorter the average mRNA stretch synthesized prior to this binding and the lower the probability that it harbours an RNase E site. Strong RBS would therefore allow more P_{T7} transcripts to be rescued, as is observed. Alternatively, it is possible that protection of the RNase E-sensitive sites requires their direct shielding by the leading ribosome (Figure 7B, right). It should be realized, however, that this hypothesis puts a constraint on the possible location of these sites: given the relative kinetics of translation initiation and elongation (Bremer and Dennis, 1987) they should be located close to the RBS. Indeed, the time

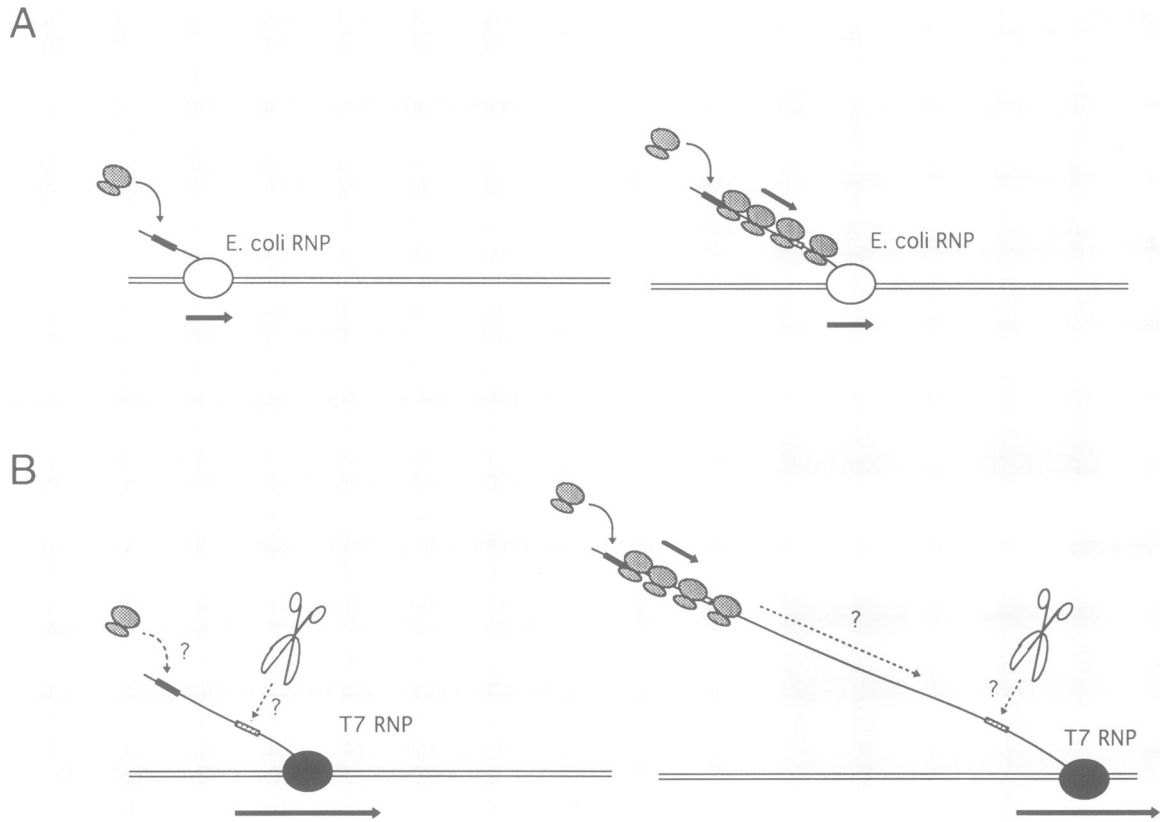

Fig. 7. Schematic drawing illustrating how stability of the *lacZ* mRNA might be adversely affected by desynchronization of transcription and translation and partially restored by the use of efficient RBS. (**A**) Synchronization between *E.coli* RNA polymerase (RNP) and the translating ribosomes during transcription. (**B**) The fast-transcribing T7 RNA polymerase outruns the ribosomes, unmasking endonuclease-sensitive sites (shaded boxes). Eventually, the leading ribosome may come in time to protect these sites. This protection might be provided by its mere binding onto the RBS (left); alternatively, it might be delayed until the ribosome directly shields the sensitive sites (right) (see text for a discussion of these two hypothesis). The endonuclease (scissors) is controlled by RNase E and may be RNase E itself. RBS are shown as closed boxes. Full arrows with different lengths symbolize the unequal elongation speed of ribosomes and RNP. The broken arrows and associated question marks denote a temporal competition between ribosomes and RNase E.

needed for translation initiation must be a sizeable part of the time needed to protect the sites if the probability of rescue is to depend upon RBS efficiency.

This model does not explain why certain RBS, particularly *lamB 701* and *708*, deviate from the general correlation between P_{lac} and P_{T7} expression (Table I). In these RBS, which yield abnormally low β-galactosidase expression in the P_{T7} series, the SD region interacts with the downstream coding sequence (Hall *et al.*, 1982). We have proposed elsewhere that transcription speed can modulate the efficiency of such structured RBS by setting the delay between synthesis of the SD and its sequestration (Jacques *et al.*, 1992). Replacing the *E.coli* RNA polymerase with the faster T7 enzyme would shorten this delay, decreasing the overall translation efficiency. According to this view, the high speed of elongation might not only depress the stability of mRNAs, as documented in this article, but also in some cases their translatability. Further work is needed to document this point.

Comparison of the decay of the P_{T7} transcript with that of the original lacZ mRNA

Previously we reported that the stability of the original *lacZ* mRNA is decreased when its RBS is replaced by a less efficient one and that it can be restored by the *rne-50* mutation (Yarchuk *et al.*, 1992). As shown in Figure 6, this also holds true in the case of the P_{lac} transcripts. The detailed mechanism of this formal competition between translation and RNase E-controlled degradation and, in particular, the nature and location of the cleavage sites are unknown. However, they are unlikely to be identical to those involved in premature degradation of nascent P_{T7} transcripts. Indeed, like most *E.coli* mRNAs (Mudd *et al.*, 1990), the original *lacZ* mRNA is stabilized chemically, but not functionally, by the *rne-50* mutation, i.e. the stabilization does not extend over the period of time during which β-galactosidase synthesis occurs (Petersen, 1993). Consistently we find no increase in β-galactosidase yield per P_{lac} transcript following stabilization by the *rne-50* mutation (compare Figure 2A and C). Apparently, in this case RNase E only scavenges transcripts that have already been inactivated non-nucleolytically and it is this non-nucleolytic inactivation, rather than the degradation itself, that competes with translation (see Petersen, 1993, for a discussion). In contrast, when RNase E cleaves nascent P_{T7} transcripts ahead of the leading ribosome, it destroys potentially functional transcripts. In the first case the chemical degradation follows inactivation, whereas in the second it causes it. That individual mRNAs can differ in this respect is not unprecedented (Petersen, 1993), but in our case the two mRNAs that behave differently are identical in sequence: only their kinetics of synthesis differ.

Table II. Bacterial strains used in this work

Nomenclature	Relevant genotype	Origin
BL21(DE3)	F⁻ *hsdS gal* (λ*imm21* Δ*nin5 int*::T7 gene *1*)	Studier and Moffatt, 1986
MO00	BL21(DE3) *lacZ*::Tn*10 aroB glpD*	Chevrier-Miller *et al.*, 1990
CH1828	MC1061 *ams* (*ts*), zce-*726*::Tn*10*	Mudd *et al.*, 1990
MO20	MO00Δ*lacZ*::Tn*10*	Lopez *et al.*, 1994
MO20-1	MO20 *ams* (*ts*), zce-*726*::Tn*10*	This work
ENS133	BL21(DE3) *lacZ*::Tn*10 malP*pΔ*534*::P_{lac}*lacZ*::RBS^{lamB}-*Arg5*	Lopez *et al.*, 1994
ENS134	BL21(DE3) *lacZ*::Tn*10 malP*pΔ*534*::P_{T7}*lacZ*::RBS^{lamB}-*Arg5*	Lopez *et al.*, 1994
ENS33	BL21(DE3) *lacZ*::Tn*10 malP*pΔ*534*::P_{lac}*lacZ*::RBS^{lamB}	Iost *et al.*, 1992[a]
ENS34	BL21(DE3) *lacZ*::Tn*10 malP*pΔ*534*::P_{T7}*lacZ*::RBS^{lamB}	Iost *et al.*, 1992[a]

[a]ENS34 was named IM4 in Iost *et al.* (1992). Derivatives of ENS33 and ENS34 carrying alternate RBS (Figure 4) were also constructed for this work. Consecutive odd and even numbering is used for naming strains carrying a given RBS and either P_{lac} or P_{T7}. The nomenclature is as follows (RBS in parentheses): ENS35 (*lamB 708*); ENS37 (*lamB 713*); ENS39 (*lamB 701*); ENS41 (*galE*); ENS43 (*malP*); ENS45 (*metF*); ENS47 (*thrA*); ENS49 (*gene 10*).

Concluding remarks

Desynchronizing transcription from translation will destabilize all mRNAs that are unstable when naked; this presumably holds true for many mRNAs aside from the *lacZ* transcript (see, for example, Morse and Yanofsky, 1969; Cole and Nomura, 1986; Nilsson *et al.*, 1987; Klug and Cohen, 1991; Petersen, 1993). Interestingly, rRNAs synthesized *in vivo* by T7 RNA polymerase also appear to be unstable, perhaps because the kinetics of ribosome assembly cannot cope with the high transcription speed (Lewicki *et al.*, 1993).

Late T7 mRNAs are synthesized from P_{T7} and yet they are stable (Summers, 1970). This stability may originate from the presence of *cis*-acting stabilizing elements on these mRNAs, as well as from their high ribosome binding efficiency. Concerning stabilizing elements, we note that the untranslated leaders from several T7 late mRNAs fold into stable secondary structures extending up to the 5′-end (Rosa, 1981), which might protect these mRNAs from degradation (Bouvet and Belasco, 1992). Consistently we have found that the gene *10* leader sequence (nt 1–22) can confer a significant stability on the *lacZ* transcript, even if completely untranslated (Chevrier-Miller *et al.*, 1990; O.Yarchuk and M.Dreyfus, unpublished). Concerning now ribosome binding efficiency, T7 late mRNAs harbour unusually long SDs (Rosa, 1981; Dunn and Studier, 1983) and a so-called translational 'enhancer' (Olins and Rangwala, 1989). It is striking that amongst all RBS tested here, the 'gene *10* RBS', which retains these features, yields the highest β-galactosidase expression from both P_{lac} and P_{T7}. The intrinsic stability of late T7 transcripts, together with their outstanding ribosome binding ability, might ensure that they are translated rather than degraded, despite the desynchronization of transcription and translation. Alternatively, the host translation and mRNA degradation machineries might be modified to increase the stability and translatability of these transcripts (Robertson and Nicholson, 1992; Robertson *et al.*, 1994).

Several efficient systems use T7 RNA polymerase transcription for expressing foreign genes in *E.coli* (Tabor and Richardson, 1985; Rosenberg *et al.*, 1987). The design of these systems may explain their success. Thus the target gene, borne on a multicopy plasmid, is transcribed to such a high level that it might exhaust the mRNA degradation machinery (Studier and Moffatt, 1986); in any case, this high level will compensate for an inefficient utilization of individual transcripts. In addition, the target gene is often fused to the T7 gene *10* leader/RBS sequence, which might maximize mRNA stability and translatability (see above). Nevertheless, our results suggest that the yield from these systems might occasionally be improved by introducing the *rne-50* mutation into the host cells.

Materials and methods

Bacterial strains

Strains ENS133 and ENS134 are described in detail elsewhere (Lopez *et al.*, 1994). Briefly, Tn*10* was introduced into the *lacZ* gene of strain BL21(DE3), which harbours the T7 RNA polymerase gene under the control of an IPTG-inducible promoter (Studier and Moffatt, 1986), yielding MO00 (Chevrier-Miller *et al.*, 1990). Into the *malA* chromosomal region of MO00 we inserted a cassette which carries sequentially (Figure 1A): (i) the *lacZ* gene (in which a 35 bp fragment encompassing the original RBS has been replaced by a 51 bp *Bam*HI–*Pst*I fragment encompassing the *lamB* RBS) followed by part of the *lacY* gene down to the *Bcl*I site (Genebank Ecolac nt 5256); (ii) the gene encoding $tRNA^{Arg5}$, a very minor arginine isoacceptor (this gene is inserted within *lacY*, the coding sequence of which has been interrupted 88 nt upstream of the insertion point, and bears a mutation at position 73 so as to be able to distinguish it from the genuine $tRNA^{Arg5}$ by hybridization); (iii) two tandemly arranged transcription terminators, the T7 late terminator (Rosenberg *et al.*, 1987) and the rho-independent *trpA* terminator (Christie *et al.*, 1981). The cassette is fused to either P_{T7}, a fragment encompassing the T7 gene *10* late promoter from nt –21 to +2 (strain ENS134), or P_{lac}, which encompasses the lactose operon promoter from nt –122 to +22 (strain ENS133) (numbering is from the main transcription start in each case). Both P_{lac} and P_{T7}, being followed by the *lac* operator site, are IPTG inducible (Figure 1A).

ENS33 and ENS34 have been described elsewhere under a different nomenclature (IM3 and IM4 in Iost *et al.*, 1992). They have been constructed along the same guidelines as for ENS133 and ENS134 and are isogenic to them, except in the following respects (Figure 1B): (i) the tRNA reporter gene is absent, i.e. the *lacY* sequence is unmodified; (ii) the fragment harbouring P_{lac} carries the natural promoter–leader junction (see below); (iii) the *trpA* terminator is present in ENS33 only. Derivatives of ENS33 and ENS34 in which the *lamB* RBS is replaced by alternate ones were also constructed for this work. *Bam*HI–*Pst*I fragments carrying the *malP*, *metF*, *galE* and *thrA* RBS were from Dreyfus (1988), those carrying the mutated *lamB* RBS from Chevrier-Miller *et al.* (1990), whereas the fragment encompassing the gene *10* RBS was synthesized for this work (cf. Figure 4). They were inserted in front of *lacZ* as for the fragment carrying the *lamB* RBS. The nomenclature used for these strains is given in Table II.

In ENS133, 2 nt had been inserted between P_{lac} and the *lacZ* untranslated leader to ensure that the 5′-end of the *lacZ* transcript is the same as in ENS134 (Lopez *et al.*, 1994). In contrast, ENS33 carries the natural P_{lac}–leader junction, resulting in a 2 nt shorter transcript (Figure 1C). We have checked that this shortening does not change the

β-galactosidase yield per transcript (O.Makarova and M.Dreyfus, unpublished).

To introduce the *rne-50* mutation into the above strains, this mutation was first P1-transduced from CH1828 (Mudd *et al.*, 1990) into MO20, a Tet^s derivative of MO00 (Lopez *et al.*, 1994), selecting for tetracycline resistance and testing for thermosensitive growth. This yielded MO20-1. The engineered *lacZ* cassettes were then introduced onto the chromosome of MO20-1 as with MO00 (Chevrier-Miller *et al.*, 1990; Lopez *et al.*, 1994).

The sequence of the promoter/RBS region from the engineered *lacZ* cassettes was checked in all final strains after PCR amplification of the relevant chromosomal region. Controls showed that the parental strains MO00 and MO20-1 do not by themselves produce β-galactosidase or *lacZ*-specific mRNA.

Growth of cells and β-galactosidase assays

Unless otherwise stated, bacteria were grown at 37°C in MOPS medium containing all amino acids, nucleic acid precursors and vitamins (Neidhart *et al.*, 1974) plus glycerol (0.2%) and IPTG (1 mM) for *lac* repressor inactivation. At 37°C the doubling time ($t_{1/2}$) in this standard medium was ~37 min, whether IPTG was present or not. For β-galactosidase assays, cells were grown for at least four generations, harvested at an OD_{600} of 0.2–0.4 and treated as in Yarchuk *et al.* (1992). β-Galactosidase activities are expressed in nmol *o*-nitrophenyl-β-D-thiogalactopyranoside (ONPG) hydrolysed/min/mg protein. For experiments with the *rne-50* mutation, *rne* cells and control *rne*$^+$ cells were grown at 28°C to an OD_{600} of 0.3–0.4 in the standard medium lacking IPTG and then transferred to 42°C. IPTG was added 6 min later and cells were harvested after an additional 39 min.

The β-galactosidase polypeptide was visualized on 7.5% SDS gels (Laemmli, 1970) and further characterized by Western analysis (Lopez *et al.*, 1994).

RNA analysis

For the quantification of $tRNA^{Arg5}$ in *rne* or *rne*$^+$ cells, aliquots of the same cultures used for β-galactosidase determination (cf. *rne* conditions) were rapidly lysed as in Uzan *et al.* (1988). After extraction, RNA samples (5 μg) were separated on 2% agarose–formaldehyde gels and vacuum blotted onto nylon membranes (Lopez *et al.*, 1994). The conditions used for probing the blot with 5′-end-labelled oligonucleotides complementary to the reporter tRNA and to 5S rRNA, as well as washing conditions, were as in Lopez *et al.* (1994), except that the two oligonucleotides were labelled to 5000 and 20 Ci/mmol respectively and used together at a 1/250 molar ratio. Hybridization signals were quantified with a PhosphorImager (Fuji or Molecular Dynamics).

For visualisation of the *lacZ* transcripts from P_{lac} and P_{T7} in *rne* and *rne*$^+$ cells, RNA samples prepared as above were analysed on a 1% agarose–formaldehyde gel. After blotting, the membrane was probed with the 1.8 kb *Hin*cII–*Hin*cII fragment internal to the *lacZ* gene (Genebank Ecolac nt 2349–4176). This fragment was uniformly ^{32}P-labelled to ~10^9 d.p.m./μg, using the BRL multiprime labelling kit. Membranes were processed as in Yarchuk *et al.* (1992) and autoradiographed without amplifying screens.

Functional mRNA lifetimes

For this experiment the growth medium lacked methionine and cysteine and contained all other amino acids at 50 μg/ml ($t_{1/2}$ 50 min at 37°C). To switch off P_{lac} and P_{T7}, exponentially growing cells ($OD_{600} \approx 0.5$) were rapidly transferred from IPTG$^+$ to IPTG$^-$ medium, as in Lopez *et al.* (1994). This transfer took <30 s. At timed intervals, aliquots of the filtered cultures were treated for 15 s with [^{35}S]methionine (50 μCi/ml, 1000 Ci/mmol; Amersham) and then with excess cold methionine (5 min). The labelled β-galactosidase was quantified on SDS gels and the functional mRNA lifetime was estimated from its decay versus time, assuming exponential kinetics.

Calculation of the polypeptide yield per transcript

Let a be the β-galactosidase activity in a given strain (in nmol ONPG hydrolysed/min/mg protein). The activity of pure β-galactosidase is 400 000 in the same units (Fowler and Zabin, 1983). The fraction of total protein mass which consists of β-galactosidase is then $(2.5\times 10^{-6})a$. Under the growth conditions used ($t_{1/2}$ = 37 min or 2200 s), the protein content per replication origin is equivalent to 3.8×10^8 amino acid residues (Bremer and Dennis, 1987). Therefore, the number of β-galactosidase monomers (1023 amino acids) per replication origin is $(3.8\times10^8)\times(2.5\times10^{-6})a/1023$, or $0.93a$. This number also approximates to the number of β-galactosidase polypeptides per P_{lac} or P_{T7} cassette, because this cassette and the replication origin, being only 9 min apart on the chromosome, have nearly the same copy number. In order to maintain $0.93a$ polypeptides per cassette, the rate of β-galactosidase synthesis must be $0.93a(\ln 2/t_{1/2})$, or $0.29\times10^{-3}a$ polypeptides/s/cassette. If t is the delay between successive *lacZ* transcriptions, the polypeptide yield per transcript is then $0.29\times10^{-3}a.t$. For P_{T7} and P_{lac} t is 0.9 and 18 s respectively when the *lamB* RBS is used (Lopez *et al.*, 1994) and we assume here that it is invariant whatever the RBS. Although transcription by *E.coli* RNA polymerase varies with the level of translation, due to polarity effects, the RBS used here are strong enough that these variations are minimal (Yarchuk *et al.*, 1992).

Acknowledgements

We are indebeted to Drs J.Guillerez, P.J.Lopez, O.Makarova and E.Makarov for stimulating discussions and to Drs P.Regnier and E.Meyer for their constructive criticisms of the manuscript. This work was funded by the CNRS and the Ministère de l'Education Nationale (DRED). I.I. was supported by the Ministère de la Recherche et de l'Espace (MRE) and the Association pour la Recherche sur le Cancer (ARC).

References

Adhya,S. and Gottesman,M. (1978) Control of transcription termination. *Annu. Rev. Biochem.*, **47**, 967–996.

Babitzke,P. and Kushner,S.R. (1991) The *ams* (altered mRNA stability) protein and ribonuclease E are encoded by the same structural gene of *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **88**, 1–5.

Bechhofer,D.H. and Dubnau,D. (1987) Induced mRNA stability in *B. subtilis*. *Proc. Natl Acad. Sci. USA*, **84**, 498–502.

Bechhofer,D.H. and Zen,K.H. (1989) Mechanism of erythromycin-induced *ermC* mRNA stability in *Bacillus subtilis*. *J. Bacteriol.*, **171**, 5803–5811.

Belasco,J.G. and Higgins,C.F. (1988) Mechanisms of mRNA decay in bacteria: a perspective. *Gene*, **72**, 15–23.

Bouvet,P. and Belasco,J.G. (1992) Control of RNase E-mediated RNA degradation by 5′-terminal base pairing in *E. coli*. *Nature*, **360**, 488–491.

Bremer,H. and Dennis,P.P. (1987) Modulation of chemical composition and other parameters of the cell by growth rate. In Neidhart,F.C., Ingraham,J.L., Low,K.B., Magasanik,B., Schaechter,M. and Umbarger,H.E. (eds), *Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 1527–1542.

Carpousis,A.J., Van Houwe,G., Ehretsmann,C. and Krisch,H.M. (1994) Copurification of *E. coli* RNAase E and PNPase: evidence for a specific association between two enzymes important in RNA processing and degradation. *Cell*, **76**, 889–900.

Chevrier-Miller,M., Jacques,N., Raibaud,O. and Dreyfus,M. (1990) Transcription of single-copy hybrid *lacZ* genes by T7 RNA polymerase in *Escherichia coli*: mRNA synthesis and degradation can be uncoupled from translation. *Nucleic Acids Res.*, **18**, 5787–5792.

Christie,G.E., Farnham,P.J. and Platt,T. (1981) Synthetic sites for transcription termination and a functional comparison with tryptophan operon termination sites *in vitro*. *Proc. Natl Acad. Sci. USA*, **78**, 4180–4184.

Cole,J.R. and Nomura,M. (1986) Changes in the half-life of ribosomal protein messenger RNA caused by translational repression. *J. Mol. Biol.*, **188**, 383–392.

Dreyfus,M. (1988) What constitutes the signal for the initiation of protein synthesis on *Escherichia coli* mRNAs? *J. Mol. Biol.*, **204**, 79–94.

Dunn,J.J. and Studier,F.W. (1983) Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J. Mol. Biol.*, **166**, 477–535.

Fowler,A.V. and Zabin,I. (1983) Purification, structure, and properties of hybrid β-galactosidase proteins. *J. Biol. Chem.*, **258**, 14354–14358.

Guillerez,J., Gazeau,M. and Dreyfus,M. (1991) In the *Escherichia coli lacZ* gene the spacing between the translating ribosomes is insensitive to the efficiency of translation initiation. *Nucleic Acids Res.*, **19**, 6743–6750.

Gupta,R.S. and Schlessinger,D. (1976) Coupling of rates of transcription, translation and mRNA degradation in streptomycin-dependent mutants of *E. coli*. *J. Bacteriol.*, **125**, 84–93.

Hall,M.N., Gabay,J., Débarbouillé,M. and Schwartz,M. (1982) A role

for mRNA secondary structure in the control of translation initiation. *Nature*, **295**, 616–618.

Iost,I. and Dreyfus,M. (1994) mRNAs can be stabilized by DEAD-box proteins. *Nature*, **372**, 193–196.

Iost,I., Guillerez,J. and Dreyfus,M. (1992) Bacteriophage T7 RNA polymerase travels far ahead of ribosomes *in vivo*. *J. Bacteriol.*, **174**, 619–622.

Jacques,N., Guillerez,J. and Dreyfus,M. (1992) Culture conditions differentially affect the translation of individual *Escherichia coli* mRNAs. *J. Mol. Biol.*, **226**, 597–608.

Jacquet,M. and Kepes,A. (1971) Initiation, elongation and inactivation of *lac* messenger RNA in *Escherichia coli* studied by measurement of its β-galactosidase synthesising capacity *in vivo*. *J. Mol. Biol.*, **60**, 453–472.

Jensen,K.F. and Pedersen,S. (1990) Metabolic growth rate control in *E. coli* may be a consequence of subsaturation of the macromolecular biosynthetic apparatus with substrates and catalytic components. *Microbiol. Rev.*, **54**, 89–100.

Jørgensen,F. (1992) Variable RNA polymerase rates couple *lacZ* transcription to translation in *Escherichia coli*. PhD Thesis, Uppsala, Sweden.

Kennell,D.E. (1986) The instability of messenger RNA in bacteria. In Reznikoff,W.S. and Gold,L. (eds), *Maximizing Gene Expression*. Butterworths, Stoneham, MA, pp. 101–142.

Klug,G. and Cohen,S.N. (1991) Effects of translation on degradation of mRNA segments transcribed from the polycistronic *puf* operon of *Rhodobacter capsulatus*. *J. Bacteriol.*, **173**, 1478–1484.

Laemmli,U.K. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

Landick,R., Carey,J. and Yanofsky,C. (1985) Translation activates the paused transcription complex and restores transcription of the *trp* operon leader region. *Proc. Natl Acad. Sci. USA*, **82**, 4663–4667.

Lewicki,B.T.U., Margus,T., Remme,J. and Nierhaus,K.H. (1993) Coupling of rRNA transcription and ribosomal assembly *in vivo*. *J. Mol. Biol.*, **231**, 581–593.

Lopez,P.J., Iost,I. and Dreyfus,M. (1994) The use of a tRNA as a transcriptional reporter: the T7 late promoter is extremely efficient in *Escherichia coli* but its transcripts are poorly expressed. *Nucleic Acids Res.*, **22**, 1186–1193 and 2434.

McCormick,J.R., Zengel,J.M. and Lindahl,L. (1991) Intermediates in the degradation of mRNA from the *lac* operon of *Escherichia coli*. *Nucleic Acids Res.*, **19**, 2767–2776.

Melefors,Ö. and von Gabain,A. (1991) Genetic studies of cleavage-initiated mRNA decay and processing of ribosomal 9S RNA show that the *Escherichia coli ams* and *rne* loci are the same. *Mol. Microbiol.*, **5**, 857–864.

Miller,O.L., Hamkalo,B.A. and Thomas,C.A. (1970) Visualization of bacterial genes in action. *Science*, **169**, 392–395.

Morse,D.E. and Yanofsky,C. (1969) Polarity and the degradation of mRNA. *Nature*, **224**, 329–331.

Mudd,E.A., Krisch,H.M. and Higgins,C.F. (1990) RNase E, an endoribonuclease, has a general role in the chemical decay of *Escherichia coli* mRNA: evidence that *rne* and *ams* are the same genetic locus. *Mol. Microbiol.*, **4**, 2127–2135.

Murakawa,G.J., Kwan,C., Yamashita,J. and Nierlich,D.P. (1991) Transcription and decay of the *lac* messenger: role of an intergenic terminator. *J. Bacteriol.*, **173**, 28–36.

Neidhart,F.C., Bloch,P.L. and Smith,D.F. (1974) Culture medium for Enterobacteria. *J. Bacteriol.*, **119**, 736–747.

Nilsson,G., Belasco,J.G., Cohen,S.N. and von Gabain,A. (1987) Effect of premature termination of translation on mRNA stability depends on the site of ribosome release. *Proc. Natl Acad. Sci. USA*, **84**, 4890–4894.

Olins,P.O. and Rangwala,S.H. (1989) A novel sequence element derived from bacteriophage T7 mRNA acts as an enhancer of translation of the *lacZ* gene in *Escherichia coli*. *J. Biol. Chem.*, **264**, 16973–16976.

Ono,M. and Kuwano,M. (1979) A conditional lethal mutation in an *E. coli* strain with a longer chemical lifetime of mRNA. *J. Mol. Biol.*, **129**, 343–357.

Petersen,C. (1993) Translation and mRNA stability in bacteria: a complex relationship. In Belasco,J.G. and Brawerman,G. (eds), *Control of mRNA Stability*. Academic press, San Diego, CA., pp. 117–145.

Platt,T. (1986) Transcription termination and the regulation of gene expression. *Annu. Rev. Biochem.*, **55**, 339–372.

Richardson,J.P. (1991) Preventing the synthesis of unused transcripts by Rho factor. *Cell*, **64**, 1047–1049.

Robertson,E.S. and Nicholson,A.W. (1992) Phosphorylation of *E. coli* translation initiation factors by the bacteriophage T7 protein kinase. *Biochemistry*, **31**, 4822–4827.

Robertson,E.S., Aggison,L.A. and Nicholson,A.W. (1994) Phosphorylation of elongation factor G and ribosomal protein S6 in bacteriophage T7-infected *Escherichia coli*. *Mol. Microbiol.*, **11**, 1045–1057.

Rosa,M.D. (1981) Structure analysis of three T7 late mRNA ribosome binding sites. *J. Mol. Biol.*, **147**, 55–71.

Rosenberg,A.H., Lade,B.N., Chui,D.-S., Lin,S.-W., Dunn,J.J. and Studier,F.W. (1987) Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene*, **56**, 125–135.

Sørensen,M.A., Jensen,K.F. and Pedersen,S. (1994) High concentrations of ppGpp decrease the RNA chain growth rate. *J. Mol. Biol.*, **236**, 441–454.

Stanssens,P., Remaut,E. and Fiers,W. (1986) Inefficient translation initiation causes premature transcription termination in the *lacZ* gene. *Cell*, **44**, 711–718.

Steitz,J.A. (1969) Polypeptide chain initiation: nucleotide sequences of the three ribosome binding sites in bacteriophage R17 RNA. *Nature*, **224**, 957–964.

Stent,G.S. (1966) Genetic transcription. *Proc. R. Soc. Ser. B*, **164**, 181–197.

Studier,F.W. (1972) Bacteriophage T7. *Science*, **176**, 367–376.

Studier,F.W. and Moffatt,B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.*, **189**, 113–130.

Summers,W.C. (1970) The process of infection with coliphage T7. IV. Stability of RNA in bacteriophage-infected cells. *J. Mol. Biol.*, **51**, 671–678.

Tabor,S. and Richardson,C.C. (1985) A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Natl Acad. Sci. USA*, **82**, 1074–1078.

Taraseviciene,L., Miczak,A. and Apirion,D. (1991) The gene specifying RNase E (*rne*) and a gene affecting mRNA stability (*ams*) are the same gene. *Mol. Microbiol.*, **5**, 851–855.

Uzan,M., Favre,R. and Brody,E. (1988) A nuclease that cuts specifically in the ribosome binding site of some T4 mRNAs. *Proc. Natl Acad. Sci. USA*, **85**, 8895–8899.

Vogel,U. and Jensen,K.F. (1994) The RNA chain elongation rate in *Escherichia coli* depends on the growth rate. *J. Bacteriol.*, **176**, 2807–2813.

Vogel,U., Sørensen,M., Pedersen,S., Jensen,K.F. and Kilstrup,M. (1992) Decreasing transcription elongation rate in *Escherichia coli* exposed to amino acid starvation. *Mol. Microbiol.*, **6**, 2191–2200.

von Hippel,P.H., Bear,D.G., Morgan,W.D. and McSwiggen,J.A. (1984) Protein–nucleic acid interactions in transcription: a molecular analysis. *Annu. Rev. Biochem.*, **53**, 389–446.

Wagner,L.A., Gesteland,R.F., Dayhuff,T.J. and Weiss,R.B. (1994) An efficient Shine–Dalgarno sequence but not translation is necessary for *lacZ* mRNA stability in *Escherichia coli*. *J. Bacteriol.*, **176**, 1683–1688.

Yarchuk,O., Jacques,N., Guillerez,J. and Dreyfus,M. (1992) Interdependence of translation, transcription and mRNA degradation in the *lacZ* gene. *J. Mol. Biol.*, **226**, 581–596.

Received on November 7, 1994; revised on March 15, 1995