Ribosomal protein L4 and transcription factor NusA have separable roles in mediating termination of transcription within the leader of the S10 operon of *Escherichia coli*

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Ribosomal protein L4 of *Escherichia coli* autogenously regulates both transcription and translation of the 11-gene S10 operon. Transcription regulation occurs by L4-stimulated premature termination at an attenuator hairpin in the S10 leader. This effect can be reproduced in vitro but depends on the addition of transcription factor NusA. We show that NusA is required to promote RNA polymerase pausing at the termination site; such paused transcription complexes are then stabilized further by r-protein L4. The L4 effect is observed even if the protein is added after the NusA-modified RNA polymerase has already reached the pause site. Genetically separable regions of the S10 leader are required for NusA and L4 action: The attenuator hairpin is sufficient for NusA-dependent pausing, but upstream elements are necessary for L4 to prolong the pause.

[Key Words: Transcription; termination; r-protein L4; NusA; attenuation]

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The S10 operon of *Escherichia coli* contains the genes for 11 ribosomal proteins. Like other r-protein operons in *E. coli*, the S10 operon is autogenously regulated by one of its products, the 50S subunit protein L4 (Lindahl and Zengel 1979; Yates and Nomura 1980; Zengel et al. 1980). This protein, which is encoded by the third gene of the operon, regulates the operon by two genetically distinct mechanisms: It inhibits transcription by causing RNA polymerase to terminate within the S10 leader (Lindahl et al. 1983; Freedman et al. 1987; Zengel and Lindahl 1990a), and it inhibits translation by preventing initiation of translation of the most proximal gene of the S10 operon (Yates and Nomura 1980; Freedman et al. 1987; Lindahl et al. 1989).

Both regulatory processes are induced when the synthesis of r-protein L4 exceeds the synthesis of 23S rRNA. Because L4 is a "primary binding protein" that binds directly to 23S rRNA in the early steps of 50S assembly (Spillmann et al. 1977), newly synthesized L4 is normally rapidly consumed by the ribosome assembly process. If the protein's target on 23S rRNA becomes limiting, free L4 accumulates and inhibits expression of its own operon. Similar autogenous control mechanisms regulate expression of other r-protein operons and thereby help to maintain balanced expression of r-protein and rRNA genes (for review, see Lindahl and Zengel 1986; Jinks-Robertson and Nomura 1987). However, these other r-protein operons are regulated only at the

level of translation: Apparently only the S10 operon exhibits control of transcription termination.

We have been focusing on L4-mediated transcription control to learn more about the general process of termination, as well as the specific mechanism by which an r-protein regulates expression of its own operon. Our earlier experiments showed that, both in vivo and in vitro, excess L4 stimulates termination of transcription ~140 bases from the start of transcription, within a string of U's on the descending side of a stable hairpin structure (Lindahl et al. 1983; Zengel and Lindahl 1990a,b). This process is independent of translation control and requires only sequences contained within the first 150 bases of the S10 leader (Freedman et al. 1987; Zengel and Lindahl 1990a). Furthermore, in vitro studies (Zengel and Lindahl 1990b, 1991) have revealed that L4-mediated attenuation control is dependent on protein NusA, a transcription factor required for efficient N- and Q-mediated antitermination in bacteriophage λ (Friedman and Gottesman 1983; Grayhack et al. 1985).

Because NusA is known to generally slow the elongation rate and to enhance RNA polymerase pausing at specific sites in a variety of transcription units (Yager and von Hippel 1987), we wanted to know whether pausing is involved in the L4-mediated attenuation control. Here, we report kinetic studies showing that NusA does induce RNA polymerase to pause within the S10 leader. Unlike other examples of regulation of transcription ter-

mination and antitermination where NusA-enhanced pausing occurs well upstream of the termination site (Landick and Yanofsky 1984; Chan and Landick 1989; Roesser and Yanofsky 1990; Andersen et al. 1991), the pause in the S10 leader is at the site of in vivo L4-stimulated termination. L4 greatly prolongs the pause, even if the r-protein is added after RNA polymerase has already reached the site. Furthermore, we show that genetically separable regions of the S10 leader are required for establishing the NusA-dependent pause and for L4 stabilization of the paused complex.

Results

Termination of transcription on plasmids pLL226 and 39B

The plasmids used to characterize the NusA-dependent, L4-stimulated termination of transcription are pLL226 and its derivative pLL226-39B, hereafter called 39B (Zengel and Lindahl 1990a,b). Plasmid pLL226 contains the S10 operon promoter and the proximal 165 bases of the S10 leader upstream of the terminator from the *rrnC* transcription unit (Fig. 1A). Plasmid 39B is a deletion derivative of pLL226 containing the promoter and proximal 149 bases of the leader followed by the *rrnC* terminator (Fig. 1A). The secondary structure of the S10 leader RNA encoded by these plasmids is shown in Figure 1B.

Previous in vivo studies indicated that the portion of the S10 leader contained on both pLL226 and 39B is sufficient for L4-stimulated termination of transcription (Zengel and Lindahl 1990a). Moreover, in vitro transcription studies showed that for both plasmids the addition of purified L4 protein stimulated transcription termination in the region of in vivo termination (Zengel and Lindahl 1990b, 1991). Although transcription from both pLL226 and 39B was inhibited to about the same extent by L4 in vivo and in vitro, the experiment shown in Figure 2 illustrates a subtle but reproducible difference

Figure 2. Effect of L4 on in vitro transcription from plasmids pLL226 and 39B. Single-round transcription reactions (40 μ l) contained NusA, 5 μ Ci of [32 P]UTP, the indicated DNA template, and 160 nM r-protein L4 (+L4) or, as a control, r-protein S7 (-L4; S7 has no effect on the transcription reaction [Zengel and Lindahl 1990b]). After 5 min of incubation at 37°C, the reactions were terminated and the transcription products were analyzed on a denaturing urea/polyacrylamide gel (for details, see Materials and methods). Bands corresponding to read-through (RT) and attenuated (ATT and ATT') RNAs are indicated.

between the two plasmids in the relative amounts of attenuated transcripts ending at the ATT and ATT' sites (Fig. 1B). The ATT site [which corresponds to the in vivo end of L4-stimulated attenuated transcripts (Zengel and Lindahl 1990a)] was preferentially used by RNA polymerases transcribing plasmid 39B DNA, whereas both sites were used by RNA polymerases transcribing pLL226. Because these two templates have identical S10 operon sequences throughout the transcribed region, these differences probably reflect an effect of the terminator-distal DNA sequence on elongation. Downstream

Figure 1. The S10 ribosomal protein operon. (A) Genetic map of the 11-gene S10 operon (top). Below are expanded maps of plasmids pLL226 and pLL226-39B carrying the indicated regions of the S10 leader cloned upstream of the *rrnC* terminator (Zengel and Lindahl 1990a,b). The site of L4-stimulated termination is shown by the arrow labeled att. (B) The secondary structure of the S10 leader (Shen et al. 1988). Sites of in vivo (ATT; Zengel and Lindahl 1990a) and in vitro (ATT and ATT'; Zengel and Lindahl 1990b) termination are indicated. The 3' ends of the leader carried in deletion derivatives 39B and 2B are also indicated. The shaded region shows the portion of the leader deleted in mutant $\Delta 72$. The boxed sequence GGAG indicates the Shine-Dalgarno sequence of the S10 gene.

sequence effects on termination and pausing have also been reported in other systems (Telesnitsky and Chamberlin 1989; Lee et al. 1990; Reynolds and Chamberlin 1992). For quantitation of the amount of "attenuated" transcripts, we pooled the two RNA classes.

L4 stabilizes a NusA-dependent pause at the attenuation site

Our previous experiments showed that transcription factor NusA is required *in vitro* for the S10 attenuator hairpin to function as a terminator (Zengel and Lindahl 1990b, 1991). NusA is known to modify the behavior of RNA polymerase, causing reduced transcription elongation rates and pausing at specific sites (Yager and von Hippel 1987). To determine whether NusA stimulated pausing of RNA polymerase at any positions within the S10 leader, we analyzed the kinetics of RNA polymerase transcription elongation through the S10 leader. The reactions were synchronized by allowing RNA polymerase to initiate transcription in the presence of all components except ATP and UTP. Because the S10 leader transcript begins with the sequence GGCU, these conditions allow RNA polymerase to initiate transcription but to incorporate only the first three nucleotides. A single round of elongation was then started by adding ATP and UTP together with rifampicin. At various times after the start of transcription, aliquots were removed and analyzed by gel electrophoresis.

The effects of NusA and r-protein L4 on the kinetics of transcription from plasmid 39B are shown in Figure 3. We observed that RNA polymerase does pause in the attenuator (ATT) region, where termination occurs *in vivo* and where we assumed termination was occurring *in vitro*. The pause was barely detectable in the absence of NusA, even in the presence of L4 (Fig. 3A, a and b).

With NusA, pausing was very efficient: Within 30 sec of the start of transcription, virtually all of the RNA polymerases were paused at the ATT/ATT' site, whether or not L4 was present in the reaction (Fig. 3A, c and d; Fig. 3B). In the absence of L4, most RNA polymerases were then released slowly and proceeded quickly to the *rrnC* terminator (Fig. 3A, d). When L4 was included in the assay, the paused complex was stabilized further, so that even after 8 min only 30% of the RNA polymerases had elongated past the attenuator (Fig. 3A, c; Fig. 3B). Using plasmid pLL226 as template we observed kinetics of transcription that were very similar to the 39B data both with and without NusA (not shown).

The attenuator hairpin is sufficient for NusA-dependent pausing but not for L4 stabilization of the paused complex

Previous *in vivo* experiments showed that a 72-base deletion removing the 5' half of the S10 leader ($\Delta 72$; see Fig. 1B) eliminates transcription inhibition by L4 (Freedman et al. 1987). The experiments described above show that our *in vitro* transcription system can distinguish two steps in the L4-mediated attenuation control: NusA-dependent pausing and L4 stabilization of the paused complex. To learn which of these steps is defective in the $\Delta 72$ mutant, we used a derivative of pLL226 carrying the 72-base deletion as template in the *in vitro* system. Our results suggest that the proximal half of the S10 leader is dispensable for NusA-dependent pausing: In the presence of NusA, RNA polymerase paused at the ATT/ATT' region of the $\Delta 72$ leader with about the same efficiency and stability as the wild-type leader plasmid (Fig. 4B), although the intensity of the ATT/ATT' bands is reduced because the molecules are only half as long as the corresponding pLL226 bands (Fig. 4A). However, the

Figure 3. Kinetics of transcription of the S10 leader. (A) Single-round transcription reactions were performed using 25 nM plasmid 39B DNA, 160 nM L4 (+ L4) or S7 (– L4), 20 μ Ci of [35 S]UTP and, where indicated, 40 nM NusA. The RNA products after the indicated times of incubation were analyzed by gel electrophoresis. (B) Radioactivity in the readthrough (RT) and attenuated (ATT and ATT') transcripts from the NusA-containing transcription reactions shown in A was measured as described previously (Zengel and Lindahl 1990b, 1991). The fraction of RNA polymerases at ATT/ATT' was then calculated as the ratio of the radioactivity in ATT and ATT' bands divided by the radioactivity in ATT, ATT', and RT bands, corrected for background radioactivity and for the number of U's in each transcript.

Figure 4. Effect of deleting the 5' half of the S10 leader on L4-stabilized, NusA-dependent pausing. (A) Single-round transcription reactions containing 10 μ Ci of [32 P]UTP were performed using the standard reaction conditions, except the templates (pLL226 or pLL226- Δ 72) were at 50 nM. The RNA products were analyzed by gel electrophoresis after the indicated times of incubation. The transcript labeled X is synthesized from a promoter in the vector portion of the pLL226 and pLL226- Δ 72 plasmids (data not shown) and, hence, is irrelevant for the current study. (B) The fraction of RNA polymerases at ATT/ATT' was calculated from the NusA-containing reactions shown in A.

paused complex was not stabilized further by the addition of L4 (Fig. 4B). These results suggest that the attenuator hairpin contains information sufficient for NusA-dependent pausing, but a structure or sequence upstream of this region is necessary for L4 to mediate its effect on the stability of the paused complex.

Protein L4 stabilizes the paused complex after RNA polymerase has already reached the pause site

The kinetic analysis suggests that NusA is required to program RNA polymerase to pause at the S10 attenuator; only such paused complexes are then vulnerable to L4 stimulation of termination. To further elucidate the role of L4, we asked whether the r-protein needs to be present at the initiation step or during RNA polymerase elongation through the S10 leader, or, alternatively, if L4 can stabilize an already formed paused complex. A syn-

chronized transcription reaction containing NusA and plasmid pLL226 as template was started as described above; protein L4 was then added to aliquots at various times after the start of elongation. The results, shown in Figure 5A, suggest that L4 need not be present during the initiation step or early transcription of the S10 leader. When L4 was added at 0.6 min after the start of transcription, essentially all RNA polymerases still paused at the attenuator were prevented from continuing to the *rnc* terminator. Protein L4 added as late as 2 min after the start of transcription "froze" those RNA polymerases still paused at the attenuator. The quantitation of the effect of late L4 addition is shown in Figure 5B.

Paused complexes can be chased by increasing UTP concentration

Our standard reactions contain 100 μ M UTP and 500 μ M

Figure 5. Effect of late L4 addition on the stability of the paused transcription complex. (A) A single-round transcription reaction was performed using standard conditions, including NusA at 40 nM. The template was pLL226. At 0.6 and 2 min after transcription elongation was started by the addition of rifampicin/ATP/UTP, aliquots of the -L4 reaction mixture were removed to prewarmed tubes containing L4 (to a final concentration of 160 nM). Samples were removed from the various reaction tubes at the times indicated and analyzed by gel electrophoresis. Each 40- μ l reaction equivalent contained 20 μ Ci of [32 P]UTP. (B) The fraction of RNA polymerases at the attenuator was calculated using the results from the experiment shown in A, as well as the results of an independent reaction to which L4 was added at 0.3 min after the start of transcription elongation.

each ATP, GTP, and CTP. We have observed that the stability of the paused complex is sensitive to the UTP concentration: For example, at 500 μ M UTP, the pause is briefer but is still NusA-dependent and sensitive to L4 (data not shown). To determine whether the ATT/ATT' transcripts found late in the transcription reaction in the presence of L4 are actually released or still associated with a stalled RNA polymerase, we took advantage of the sensitivity of readthrough to the UTP concentration. Transcripts were synthesized at 100 μ M UTP. At 6 min after the start of elongation, the UTP concentration was increased to 1 mM. We found that even in the presence of L4, many of the attenuated transcripts were actually in paused complexes, as much of the radioactivity corresponding to these molecules could be chased into readthrough transcripts when the UTP concentration was increased (Fig. 6). These results indicate that our transcription system does not efficiently carry out the final step in termination—release of the nascent RNA molecule. Interestingly, in the presence of L4, transcripts with 3' ends in the ATT' region were much less sensitive to the UTP chase than were the ATT RNAs, suggesting that ATT' transcripts might represent truly terminated transcription products.

Discussion

L4-mediated transcription control of the S10 operon is the result of premature termination of transcription at an attenuator within the S10 leader (Lindahl et al. 1983). When excess L4 accumulates in the cell, the termination efficiency of this attenuator increases by about fourfold (Lindahl et al. 1983; Freedman et al. 1987). Even during exponential growth, when L4 synthesis is presumably balanced with the synthesis of its 23S rRNA target, at least 50% of the RNA polymerases appear to terminate at the S10 attenuator (Lindahl et al. 1983; Zengel et al. 1984). This attenuation control can be reproduced in a cell-free system but only if protein NusA is included in the assay (Zengel and Lindahl 1990b, 1991).

The kinetic analysis of the *in vitro* transcription of the S10 leader presented here, together with our earlier *in vivo* experiments (Lindahl et al. 1983; Zengel et al.

1984), suggests that L4-mediated attenuation control consists of at least three separable steps, illustrated by the cartoon in Figure 7. The first step is a NusA-dependent transcription pause at the attenuator site. This process is highly efficient, as our quantitation indicates that virtually every RNA polymerase pauses. In the second step, L4 stabilizes the paused complex. Only NusA-modified RNA polymerase is susceptible to L4 action; RNA polymerase ignores the attenuation signal in the absence of NusA, even if L4 is added. However, L4 need not be present during the first step, as the effect of L4 is observed even if this protein is added after the NusA-modified RNA polymerase has already reached the pause site in the attenuator region. Thus, NusA and L4 appear to have sequential effects on RNA polymerase. The third step, which we can only infer from *in vivo* measurements of transcription rates (see below), is the release of the nascent RNA and RNA polymerase.

The sequential action of NusA and L4 proposed in our model is consistent with our finding that different regions of the S10 leader are required for the NusA and L4 effects on transcription. The $\Delta 72$ mutation removing almost all of the 5' half of the S10 leader has no effect on NusA-dependent pausing. Thus, the attenuator hairpin region contains all of the information necessary for efficient NusA-dependent pausing. However, this deletion removes information necessary for L4 stabilization of the paused complex. The lack of L4 effect in the $\Delta 72$ mutant probably reflects a specific sequence or structure requirement in the RNA upstream of the attenuator structure, rather than a need for a minimum length of RNA, as recent genetic studies indicate that the proximal three hairpins of the S10 leader are dispensable for L4-stimulated pausing, but a deletion of the small hairpin immediately upstream of the attenuator hairpin (Fig. 1B) has the same effect as the 72 base deletion (experiments in progress). We are currently analyzing what features of this hairpin region are required for L4 action. In any case, it is interesting that L4-dependent stabilization of the paused transcription complex requires a sequence upstream of the attenuator hairpin. We do not know the function of this sequence. It could constitute, at least in part, the target for L4 binding (see below); or transcrip-

Figure 6. Effect of the addition of increased UTP on the stability of paused transcription complexes. (A) A single-round transcription reaction was performed using pLL226 DNA (25 nM) as template. Transcription elongation was initiated as usual by the addition of rifampicin/ATP/UTP (5 μ Ci of [32 P]UTP/40- μ l reaction equivalent). The starting concentration of UTP was 100 μ M. After 0.6 min, L4 (to 160 nM) was added to a portion of the reaction mixture. At 6 min after the start of elongation, aliquots of both reaction mixtures (+L4 and -L4) were transferred to tubes containing additional UTP (to 1 mM final

concentration). Samples were removed from the various tubes at the indicated times after the start of elongation and analyzed by gel electrophoresis. The RNA products at the indicated times after the start of transcription elongation are shown. (B) The fraction of RNA polymerases at ATT/ATT' was calculated as described. (■) +L4, 100 μ M UTP; (●) +L4 + 1 mM UTP chase; (□) -L4, 100 μ M UTP; (○) -L4 + 1 mM UTP chase.

Figure 7. Model for L4-mediated attenuation of the S10 operon. (A) Elongating complexes plus NusA: RNA polymerase pauses at the att site. (B) Elongating complexes minus NusA: The pause signal is ignored.

tion of this sequence could “sensitize” the RNA polymerase to L4, possibly because that part of the nascent transcript remains bound to the RNA polymerase during the pause.

The specific features of the attenuator region required for the NusA-dependent pause are not yet known. It is likely that the upper GC-rich hairpin structure is involved, because such structures have been implicated in other pausing/termination regions (Platt 1986; Yager and von Hippel 1987). Mutations disrupting this helix eliminate L4-stimulated termination of transcription in vivo (Freedman et al. 1987) and reduce the efficiency and/or stability of RNA polymerase pausing in the in vitro transcription system (experiments in progress). The sequence or structure at the pause site itself also appears to have some influence, because a deletion extending from 3' of the pause site to just 7 bases upstream of ATT (Fig. 1B, mutant 2B) eliminates L4 control of transcription in vivo (Zengel and Lindahl 1990a) and inactivates the attenuator in vitro (Zengel and Lindahl 1990b).

Two important questions are not addressed in our simple model for L4-mediated attenuation control (Fig. 7): What does L4 interact with and how does it modulate RNA polymerase activity? Given the known function of the r-protein as a 23S rRNA-binding protein during ribosome assembly (Spillmann et al. 1977), we assume that L4 recognizes a specific target on the nascent leader RNA, perhaps a structure similar to its 23S recognition site. Once bound to the leader RNA, L4 might effect a structural alteration in the RNA that stimulates termination. However, experiments such as gel retardation or filter-binding studies have not detected a direct interaction between L4 and the leader (J.M. Zengel and L. Lindahl, unpubl.). Moreover, its precise target on 23S rRNA is still not known. Nevertheless, we have demonstrated that a fragment of 23S rRNA containing the proximal 840 bases can eliminate in vitro L4-mediated attenuation (Zengel and Lindahl 1991). These results suggest that this region of 23S rRNA contains information sufficient for L4 binding and, furthermore, as predicted by the generic model for autogenous control of r-protein synthesis, that L4 bound to its rRNA target is not active in regulating transcription of the S10 operon. It is also possible that L4 stabilization of the paused transcription complex involves direct interaction with the RNA poly-

merase, perhaps owing to similarities between peptides in the RNA polymerase and r-proteins with which L4 may interact in the ribosome. However, it is unlikely that the L4 target is made up entirely by the RNA polymerase, because only interactions with the S10 leader transcript could explain the specificity of L4 for regulating the S10 operon.

Even though our purified transcription system is useful for dissecting attenuation control of the S10 operon, the in vitro and in vivo results differ in two respects. First, all of the attenuated transcripts isolated in vivo have 3' ends in the ATT region (Zengel and Lindahl 1990a,b), whereas the in vitro-synthesized transcripts end at ATT and ATT' (Zengel and Lindahl 1990b). Second, the attenuator appears to function as an efficient terminator in the cell (Zengel et al. 1984); but in the purified transcription system, most of the RNA polymerases pause at this site but do not actually terminate. Our conclusion that transcription is terminated in vivo (i.e., that the pausing step is followed rapidly by dissociation of nascent leader RNA and RNA polymerase) is based on the observation that L4 specifically reduces transcription of the structural genes of the S10 operon but has no effect on transcription of the leader (Lindahl et al. 1983). These results could not be accounted for by pausing alone: A pause lasting less than the time between two consecutive transcription initiations should have no effect on the synthesis of either leader or structural gene RNA, and a pause lasting longer should create a queue of RNA polymerases in the leader that would quickly reach the promoter region and reduce the transcription initiation rate, inhibiting transcription of leader and structural genes equally. An interesting speculation accounting for this discrepancy between in vivo and in vitro results is that the release constitutes a third independent step in the attenuation pathway (Fig. 7), which is stimulated by an additional factor not present in our in vitro system. In this connection, we analyzed the effect of the addition of termination factors ρ (Platt and Richardson 1992) and NusG (Sullivan and Gottesman 1992) to our transcription assays: We observed no effect on the kinetics of pausing in the ATT/ATT' region (data not shown). According to the current model for ρ action (Platt and Richardson 1992), the S10 leader RNA is probably too structured for ρ access.

The significance of the two classes of attenuated molecules, ending at ATT and ATT', is not clear. The relative abundance of the two types of molecules is not only affected by downstream DNA sequences (this paper) but also by the source of RNA polymerase (Zengel and Lindahl 1990b). Interestingly, in vitro-synthesized transcripts ending in the ATT' region were less sensitive to the UTP chase and, hence, might represent truly terminated (released) transcripts. Perhaps termination in vivo also occurs in the ATT' region, but the released transcripts are quickly nibbled back to the base of the hairpin, that is, the ATT region.

Materials and methods

Plasmids

Plasmids pLL226 and pLL226-39B carrying most of the S10 leader cloned upstream of the *rrnC* terminator have been described (Zengel and Lindahl 1990a,b). Plasmid pLL226-Δ72 is a derivative of pLL226 containing a deletion of 72 bases from the 5' half of the S10 leader (Freedman et al. 1987). Mutant 2B has been described (Zengel and Lindahl 1990b).

Materials

RNA polymerase and NusA were gifts from E. Morgan (Roswell Park Memorial Institute, Buffalo, NY). Purified r-proteins L4 and S7 were gifts from K. Nierhaus and P. Nowotny (Max Planck Institute, Berlin, Germany). ρ-Factor was from T. Platt (University of Rochester, NY) and NusG was from J. Greenblatt (University of Toronto, Canada). Ultrapure nucleoside triphosphates were purchased from Pharmacia.

In vitro transcription reactions with E. coli RNA polymerase

Standard 40-μl transcription reactions contained 20 mM Tris-acetate (pH 7.9), 4 mM Mg-acetate, 0.1 mM EDTA, 100 mM K-glutamate, 20 mM RNA polymerase, and 20–25 mM supercoiled plasmid DNA. Where indicated, NusA was added to 40 nM, and L4 or S7 was added to 120–160 nM. These reaction components were mixed together with 500 μM each CTP and GTP and incubated at 37°C for 10 min to allow formation of the initiation complex and incorporation of the proximal three nucleotides (pppGGC). A single round of transcription elongation was then started by the addition of ATP (to 500 μM), UTP (to 100 μM), 5–10 μCi of [³²P]UTP or 20 μCi of [³⁵S]UTP and rifampicin (to 10 μg/ml). Reactions were terminated at the indicated times by the addition of 40 μl of 50 mM EDTA containing 10 μg of yeast carrier RNA, 40 μl of phenol, and 40 μl of chloroform/isoamylalcohol (24 : 1). The samples were then processed and analyzed as described previously (Zengel and Lindahl 1991). Reaction products were quantitated by scintillation counting of the radioactivity in gel bands corresponding to the indicated RNA transcripts (Zengel and Lindahl 1990b, 1991).

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