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RNase E Enzymes from *Rhodobacter capsulatus* and *Escherichia coli* Differ in Context- and Sequence-Dependent In Vivo Cleavage within the Polycistronic *puf* mRNA

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The 5′ *pufQ* mRNA segment and the *pufLMX* mRNA segment of *Rhodobacter capsulatus* exhibit different stabilities. Degradation of both mRNA segments is initiated by RNase E-mediated endonucleolytic cleavage. While *Rhodobacter* RNase E does not discriminate between the different sequences present around the cleavage sites within *pufQ* and *pufL*, *Escherichia coli* RNase E shows preference for the sequence harboring more A and U residues.

The polycistronic *puf* operon of *Rhodobacter capsulatus* (Fig. 1) encodes the pigment binding proteins of the light harvesting I antenna complex (LHI) (PufB and PufA) and of the reaction center complex (PufL and PufM) and the proteins PufQ and PufX, which do not bind pigments but are required for the formation of photosynthetic complexes. The stoichiometry of LHI and reaction center complexes in the membrane is in part determined by the different stabilities of individual *puf* mRNA segments (13, 15). The processing of this polycistronic mRNA species has been extensively studied over the last decade (reviewed in reference 14). It was shown that decay of the 2.7-kb *pufBALMX* mRNA species (half-life of around 8 min under low oxygen tension) is initiated by endonucleolytic cleavage by RNase E at a specific recognition sequence within the *pufL* coding region (9). After initial cleavage, extremely rapid decay occurs in the 3′-to-5′ direction as well as in the 5′-to-3′ direction. A highly stable intercistronic secondary structure localized between *pufA* and *pufL* protects the *pufBA* mRNA segments against 3′-to-5′ exonucleases and is responsible for the higher stability of the *pufBA* mRNA (around 30 min) and consequently for the 15:1 molar excess of LHI versus reaction center complexes (13). PufQ is a protein that most likely serves a regulatory function, and it is present in the cells in very small amounts (7). It is encoded by the 5′ *puf* mRNA segment that undergoes rapid turnover (half-life of less than 1 min) (11). Decay of the primary *puf* transcript is initiated by RNase E at a specific sequence at the 3′ end of the *pufQ* coding region (11a). The RNase E recognition sequences which are involved in rate-limiting endonucleolytic cleavage within the *pufQ* and *pufL* coding sequences both resemble the consensus recognition sequence suggested for *Escherichia coli* RNase E, A/GA UUA/U (5), but are not identical. Initial RNase E-mediated cleavage at the 3′ end of *pufQ* occurs at the sequence GAUU UU; within the *pufL* coding region, RNase E cleaves the sequence GGCUUU. In order to find out whether the different decay rates of the 5′ *puf* mRNA segment encompassing the *pufQ* gene and the *pufLMX* mRNA segment are due to the differences in RNase E recognition sequences, we expressed a *puf* mRNA that carries the GAUUUU sequence at the 3′ end of the *pufQ* coding region as well as around position 1205 within the *pufL* coding region.

Effect of modification of the RNase E recognition sequence at position 1205 within *pufL* on the rate of *puf* mRNA decay in *R. capsulatus*. To study the effect of different sequences on RNase E-mediated mRNA cleavage, we constructed a number of plasmids with modifications of the *puf* DNA sequence (Fig. 1). All positions mentioned relate to the *puf* transcriptional start (+1). For analysis of the RNase E cleavage site within *pufL*, plasmid pTΔMBP6 (Fig. 1) was used in previous studies (9). This plasmid allows the expression of a *puf* mRNA which has a 1.2-kb segment with putative additional RNase E cleavage sites removed but the RNase E cleavage site around position 1205 present. The RNA sequence around the RNase E cleavage site is changed from GGCUUUU**U**GC**UU**AUCCUU to GGCUUUU**GGC**C**A**AUCCUU. This sequence modification showed no effect on the stability of the *puf* mRNA species in vivo (9) but created a new *Bal*I recognition site, which was used for the introduction of further sequence modifications. Further modification of this sequence to **A**UC**GA**U**U**GGC**CA** AUCCA resulted in the prolongation of the half-life of the 2.7-kb *pufBALMX* mRNA in *R. capsulatus* from 8 to 20 min (9). We then constructed plasmid pTΔMBP6/11, which contains the sequence G**A**UUUUU**GGC**C**A**AUCCUU around position 1205. The sequence GAUUUU is identical to the sequence found directly at the RNase E recognition site at position 522 within *pufQ*. Both the RNase E recognition sequence around position 522 and the recognition sequence around position 1205 are followed by a hairpin loop structure with similar predicted stabilities ($\Delta G^{\circ\prime}$, −18.1 and −14.3 kcal/mol, respectively). Our cloning strategy retained the original locations of the hairpin loop structures. After a triparental conjugational transfer (12) with pRK2013 as a helper plasmid (8) into the *Rhodobacter* recipient strain ΔRC6 (3), which has the *puf* operon deleted from the chromosome, we analyzed the half-lives of the *pufBAL/X* mRNA in strains ΔRC6(pTΔMBP6) and ΔRC6(pTΔMBP6/11) by Northern blotting (Fig. 2). In both strains, the 1.3-kb *pufBAL/X* mRNA decayed with a half-life of 7 ± 1 min, indicating that the nucleotide sequence itself is not responsible for the different decay rates of the 5′ *puf* mRNA segment and the *pufLMX* mRNA segment. We also performed primer extension analysis in order to map the 5′ ends within and around the RNase E recognition sequences

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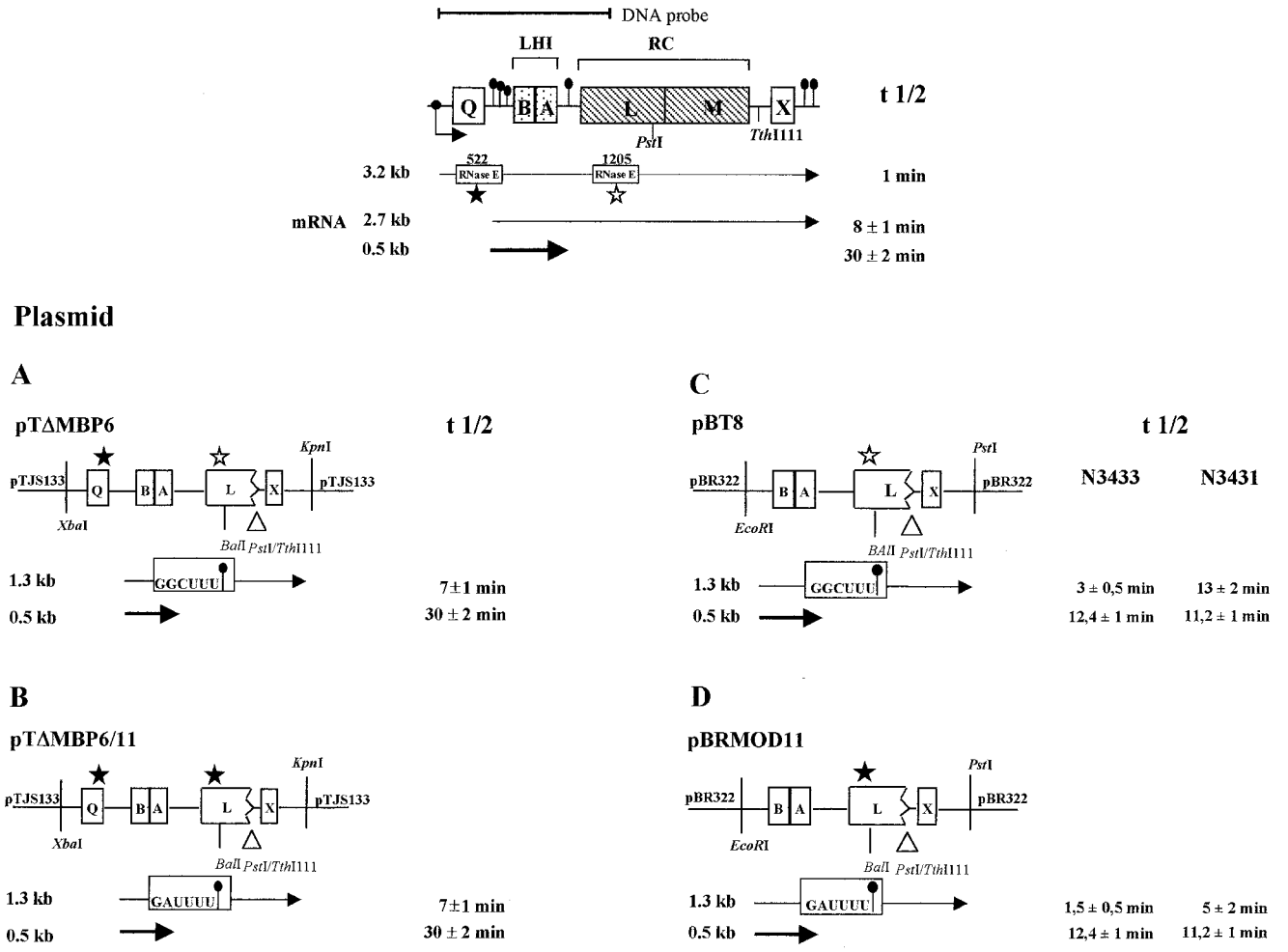

FIG. 1. Schematic map of the *R. capsulatus* wild-type *puf* operon and the *puf* DNA segments present in the plasmids used in this study. The primary transcript, the stable 0.5-kb *pufBA*, and the 2.7-kb *pufBALMX* mRNA segments are indicated by arrows, and their half-lives (t 1/2) are on the side. The location of the oxygen-dependent *puf* promoter upstream of *pufQ* is shown by the bent arrow. The hairpin-loop regions forming a secondary structure at the mRNA level are indicated by pins. The RNase E cleavage sequence at position 522 is marked by a filled star, and the sequence of the RNase E cleavage site at position 1205 is signified by an open star. All plasmids investigated in this study carried a *Pst*I-*Tth*I111 deletion, and the positions of these restriction sites are given in the schematic map. Plasmids named pT are derivatives from pTJS133 (19, 23). Plasmids named pB are derived from pBR322 (2). mRNA segments detectable in Northern blots with a 1.7-kb DNA probe (shown on top) are located below the plasmid maps, and their respective half-lives are on the side. All half-lives were measured at least three times, and the means ± standard deviations are indicated.

(Fig. 3). In strain ΔRC6(pTΔMBP6), the RNase E recognition sequence is cleaved at two sites, GG/C/UUUU. Cleavage within the recognition sequence G/AUUUUU also occurs in strain ΔRC6(pTΔMBP6/11). A similar cleavage pattern was observed for the cleavage motif GAUUUU at its original position, 522, in vitro. Cleavage sites for three 5′ ends within the putative RNase E recognition sequence were determined: between G/A, A/U, and U/U (data not shown). Additional 5′ ends at some distance from position 1205 are most likely the result of successive endonucleolytic cleavages which are involved in further mRNA degradation after rate-limiting cleavages have taken place (reviewed in reference 22). Surprisingly, an additional 5′ end occurs 13 nucleotides (nt) upstream of the RNase E cleavage site in strain ΔRC6(pTΔMBP6/11) (Fig. 3). This suggests that the sequence alteration around position 1205 can cause minor changes in the mRNA decay steps following rate-limiting cleavage.

Effect of modification of the RNase E recognition sequence at position 1205 within *pufL* on the rate of *puf* mRNA decay in *E. coli*. We also expressed the *puf* operon in *E. coli* in order to study the influence of the sequence alteration within the RNase E cleavage site around position 1205. To this end, we used plasmids pBPT8 and pBRMOD11 (Fig. 1), which allow transcription of the *puf* operon from the upstream *bla* promoter of plasmid pBR322 in *E. coli*. We transformed both plasmids into *E. coli* strain N3431, which expresses a temperature-sensitive RNase E, and into the isogenic wild-type strain N3433 (10). The decay rate of the 1.3-kb *pufBAL/X* mRNA was dependent on the sequence at the RNase E cleavage sites around position 1205 in both strains. While the presence of the sequence that naturally occurs at this position of *pufL* resulted in a half-life of the *pufL/X* mRNA segment of 3 ± 0.5 (mean ± standard deviation) min in strain N3433, the presence of the sequence that is identical to the RNase E cleavage site at position 522 within the *pufQ* coding region decreased the half-life to 1.5 ± 0.5 min (Fig. 4). The difference in cleavage rates was also observed in strain N3431 [*rne*(Ts)] at the nonpermissive temperature, at which only low RNase E activity is present. The 1.3-kb *pufBAL/X* mRNA exhibited a half-life of 13 ± 2 min in strain N3431(pBPT8) but one of only 5 ± 2 min

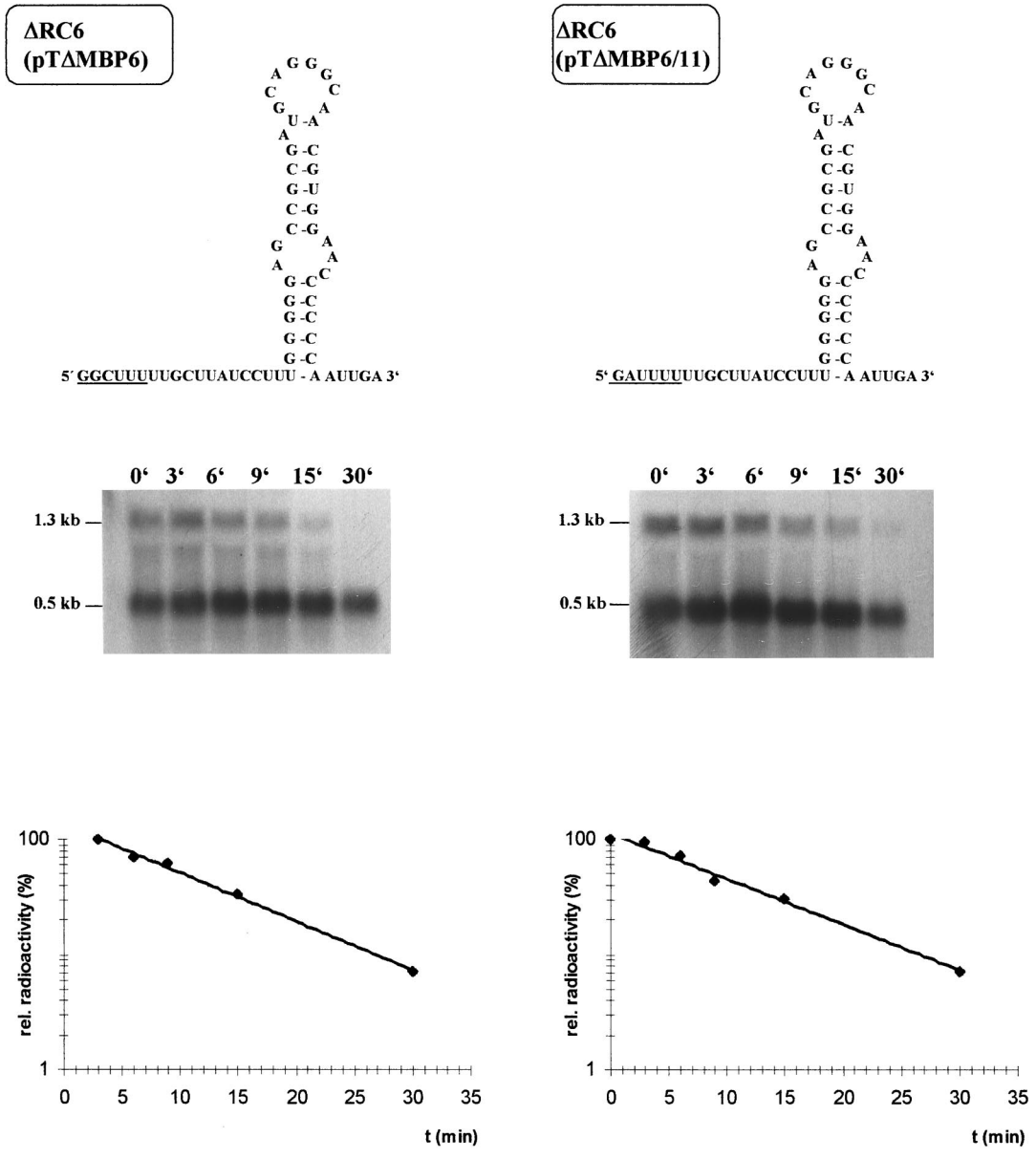

FIG. 2. Decay of *pufBAL/X* mRNA segments in control strain ΔRC6(pTΔMBP6) and mutant strain ΔRC6(pTΔMBP6/11). The wild-type RNase E site of strain ΔRC6(pTΔMBP6) and the modified RNase E site of strain ΔRC6(pTΔMBP6/11) are shown on top. Total RNA was isolated at various time points after addition of rifampin to a culture in the logarithmic growth phase (11). *puf*-specific mRNA segments were monitored by Northern blot analyses with a *pufQBA* DNA fragment as the probe as previously described (11). The half-life of the 1.3-kb *pufBAL/X* mRNA was determined by quantification of the radiolabelled bands by laser densitometry and plotting the values on a semilogarithmic scale as a function of time. The half-life for the *pufBAL/X* mRNA in both strains was measured to be 7 ± 1 (mean ± standard deviation) min.

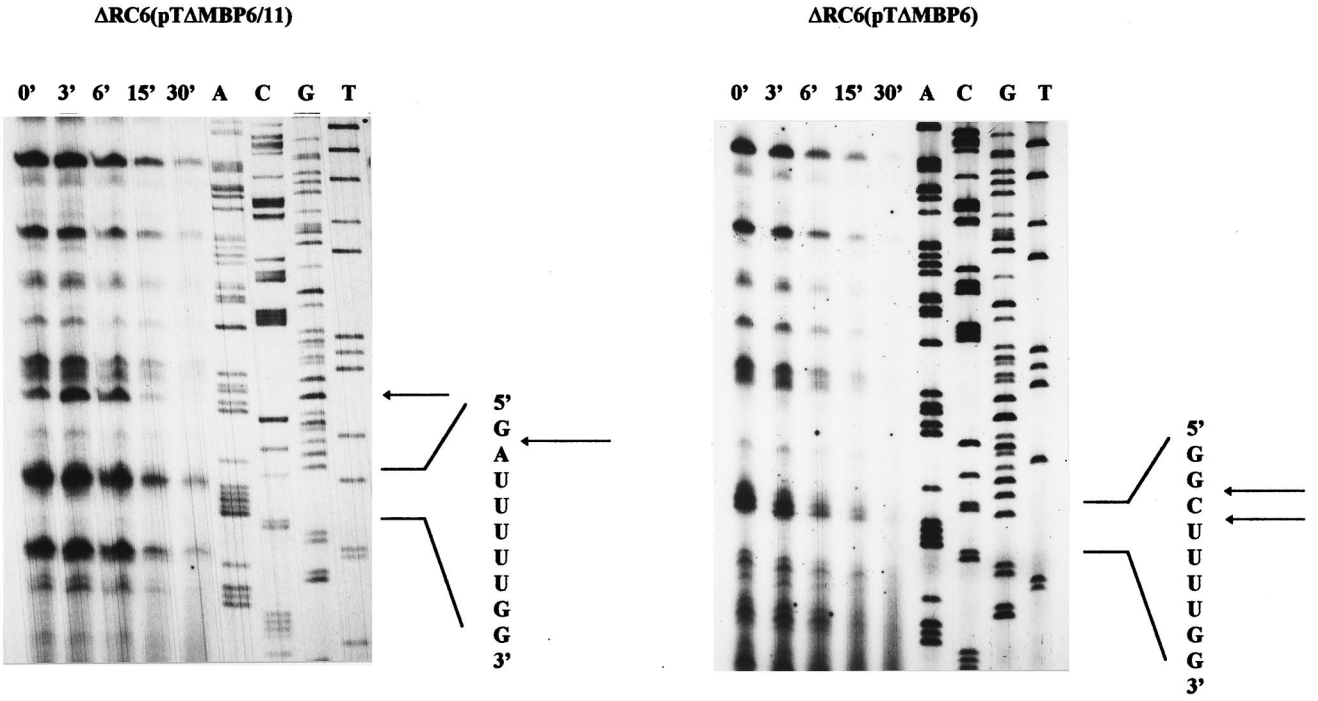

FIG. 3. Primer extension analyses to map the 5′ ends within and adjacent to the wild-type RNase E site at position 1205 in strain ΔRC6(pTΔMBP6) or the modified RNase E site in strain ΔRC6(pTΔMBP6/11) at the same position. After addition of rifampin, total RNA was isolated at the time points indicated. Each reaction contained 10 μg of total RNA. For primer extensions and the sequencing reaction as described previously (11, 16), we used the primer 5′-GGCGGCGGAAAGAT GGAGATC-3′. The arrows mark the 5′ ends within the RNase E cleavage sites [between G/C and C/U in strain ΔRC6(pTΔMBP6) and G/A in strain ΔRC6(pTΔMBP6/ 11)]. An additional 5′ end 13 nt upstream of the modified RNase E site in strain ΔRC6(pTΔMBP6/11) was mapped, which is also marked with an arrow.

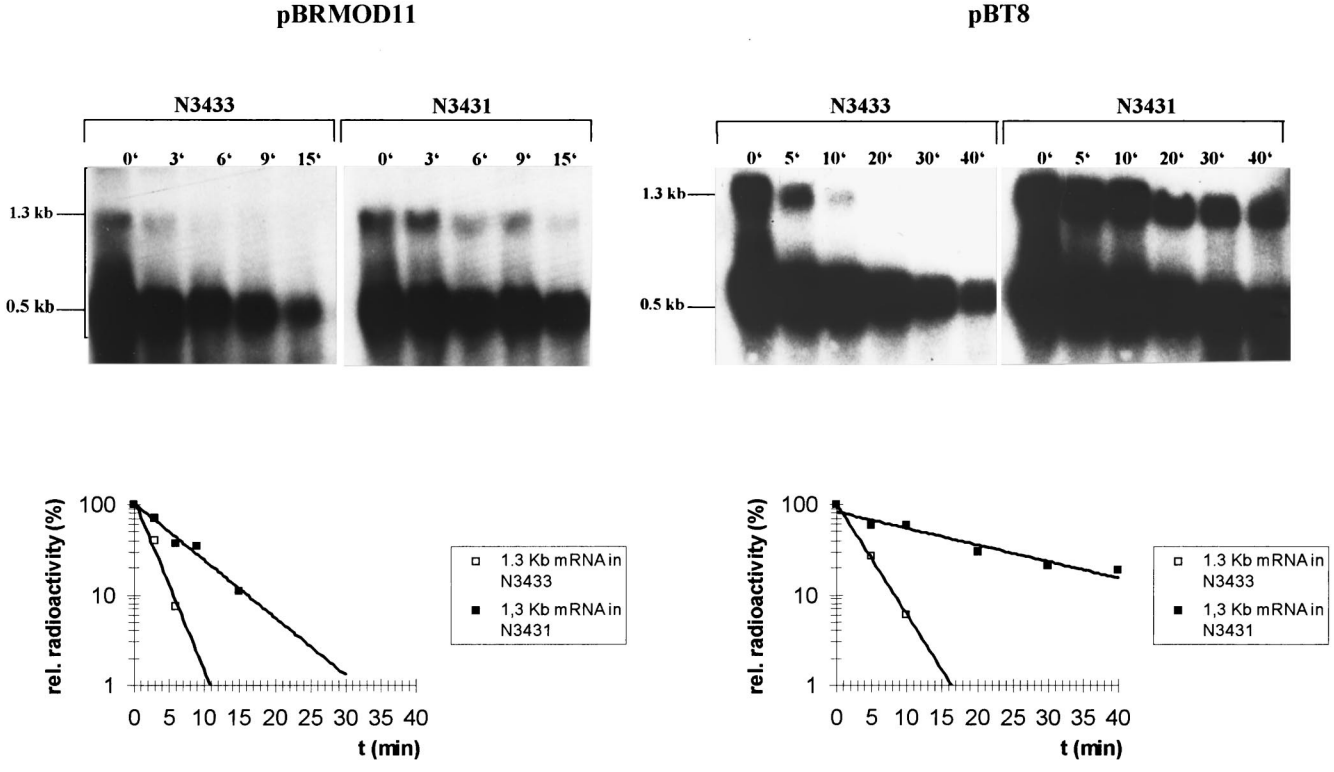

FIG. 4. *puf* mRNA decay in *E. coli*. After transferring plasmids pBT8 and pBRMOD11 into the *E. coli* strains N3433 (*rne*$^+$) and N3431 [*rne*(Ts)], total RNA was isolated at different time points after addition of rifampin. An amount of 10 μg of total RNA in each lane was separated on a 1% agarose formaldehyde gel and transferred to a nylon membrane. After hybridization with the *puf* probe, we measured a half-life of 3 ± 0.5 (mean ± standard deviation) min for the 1.3-kb *pufBAL/X* mRNA in strain N3433(pBT8), and a half-life of 13 ± 2 min was determined for the same mRNA segment in strain N3431(pBT8). The 1.3-kb mRNA species in strain N3433(pBRMOD11) decayed with a half-life of 1.5 ± 0.5 min, whereas this mRNA segment of strain N3431(pBRMOD11) degraded with a half-life of 5 ± 2 min.

in strain N3431(pBRMOD11) (Fig. 4). These results indicate that RNase E from *E. coli* discriminates between the two sequences, GGCUUU and G**AU**UUU, while RNase E from *R. capsulatus* does not. It is conceivable that RNase E from *E. coli*, an organism whose genome is 50% AT, has a stronger preference for sequences containing more A and U residues than RNase E from *R. capsulatus*, an organism whose genome is only 32% AT. The preference of *E. coli* RNase E for AU-rich sequences is in agreement with results from previous studies (5, 6, 18). When we analyzed the 5′ ends around the RNase E cleavage site at position 1205 of the *puf* mRNA by primer extension for both sequences expressed in *E. coli*, we found bands identical to those shown for the *R. capsulatus* strains ΔRC6(pTΔMBP6) and ΔRC6(pTΔMBP6/11) (data not shown).

A number of polycistronic mRNAs have been shown to undergo processing that results in differences of the stabilities of individual mRNA segments (1, 4, 20, 21). However, it was not shown for any of these polycistronic transcripts which mechanisms initiate the decay of the individual mRNA segments. We showed previously that the decay of two *puf* mRNA segments exhibiting very different stabilities is initiated by the same mechanism: internal cleavage within a *puf* coding region at a specific recognition sequence by RNase E (9, 11a). Here we show that the differences in the rate of rate-limiting cleavage occurring within the 5′ *puf* mRNA segment and the *pufLMX* mRNA segment are not determined by the sequence directly surrounding the RNase E cleavage site. Structural analysis of the RNA (11) revealed that the cleavage site around *pufL* is in a single-stranded region, as is predicted for the cleavage site in *pufQ* by computer analysis (24) (the high instability of the 5′ *puf* mRNA segment does not allow an unambigous structural analysis [data not shown]). This suggests that additional factors, like ribosome density or tertiary RNA structure, are involved in determining the cleavage rates by RNase E. Context-dependent cleavage rates for RNase E for some monocistronic transcripts were described previously (17, 18). The sequence alteration of the RNase E recognition site within *pufL* which did not affect the half-life of the *pufL/X* mRNA segment in *R. capsulatus* showed, however, a clear influence on the stability of this mRNA segment in *E. coli*. Our data indicate that the RNase E enzymes from *R. capsulatus* and *E. coli* differ in regard to their preferences for the RNA recognition sequence. This observation will be of interest for future studies addressing the RNA-protein interaction that is the molecular basis for RNA recognition and cleavage by RNase E.

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