RNase III Controls the Degradation of corA mRNA in Escherichia coli

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In Escherichia coli, the corA gene encodes a transporter that mediates the influx of Co2+, Mg2+, and Ni2+ into the cell. During the course of experiments aimed at identifying RNase III–dependent genes in E. coli, we observed that steady-state levels of corA mRNA as well as the degree of cobalt influx into the cell were dependent on cellular concentrations of RNase III. In addition, changes in corA expression levels by different cellular concentrations of RNase III were closely correlated with degrees of resistance of E. coli cells to Co2+ and Ni2+. In vitro and in vivo cleavage analyses of corA mRNA identified RNase III cleavage sites in the 5′-untranslated region of the corA mRNA. The introduction of nucleotide substitutions at the identified RNase III cleavage sites abolished RNase III cleavage activity on corA mRNA and resulted in prolonged half-lives of the mRNA, which demonstrates that RNase III cleavage constitutes a rate-determining step for corA mRNA degradation. These findings reveal an RNase III–mediated regulatory pathway that functions to modulate corA expression and, in turn, the influx of metal ions transported by CorA in E. coli.

The degradation and processing of mRNA involve numerous cis- and trans-acting factors. Among them, the RNase III family of enzymes plays a pivotal role in the control of mRNA stability in both prokaryotes and eukaryotes (14, 26, 32, 38, 43, 45). All RNase III family members contain a characteristic RNase domain commonly called the RNase III domain. RNase III is encoded by the rnc gene in Escherichia coli and was the first double-stranded RNA endoribonuclease to be described (36). This enzyme requires Mg2+ to cleave phosphodiester bonds, creating 5′-phosphate and 3′-hydroxyl termini with an overhang of 2 nucleotides (nt) (9). Several in vivo mRNA substrates for E. coli RNase III have been identified, including rnc mRNA (5, 28), pnp mRNA (35), bdm mRNA (19, 38), and proU mRNA (16). However, an analysis of mRNA species whose abundance was downregulated by increased cellular RNase III activity indicated that nearly 100 mRNA species could be potential targets of RNase III (38).

The functional importance of the regulation of E. coli RNase III activity has been best characterized for its role in RNA processing (6). Recent studies have further emphasized its role in the degradation of bdm and proU mRNAs in response to osmotic stress (16, 38). Those studies suggested that RNase III activity may contribute to the adaptation of E. coli cells to environmental changes by rapidly controlling the abundance of related mRNA species.

In a previous study, we found that the alteration of RNase III concentrations in the cell changed the steady-state levels of corA mRNA more dramatically than most other potential targets of RNase III (38). The corA gene was initially discovered by its cobalt resistance phenotype in E. coli (30). Its protein product, the CorA protein, and its homologous proteins are well conserved across kingdoms (17). The CorA protein has been characterized as a transporter for Mg2+ and Co2+ with high affinities of 15 to 20 μM and 20 to 40 μM, respectively (12, 13, 18, 39). The CorA protein can also transport Ni2+ with an affinity of 200 to 400 μM. The functional role of CorA in Salmonella species has been well studied, and those studies showed that the CorA protein is constitutively expressed in a manner independent of Mg2+ concentrations in the medium (39). In Salmonella, Mg2+ is also transported by MgtA and MgtB, which are required for growth in low concentrations of Mg2+ (40), and E. coli has only an MgtA homolog (46). However, a recent study showed that levels of the CorA protein increase in stationary-phase Salmonella enterica cells when grown in LB or N-minimal medium with low concentrations of Mg2+ (10 μM), although the CorA protein content does not correlate with the transport of Mg2+ and Ni2+, indicating that an unknown mechanism regulates CorA function, which also affects virulence in mice (31).

In E. coli and S. enterica, the affinity of CorA for Ni2+ is in the toxic concentration range, which is much higher than physiologically optimal concentrations for either species. However, the transport of Ni2+ by CorA affects the sensitivity of E. coli to oxidative stress induced by the lactoperoxidase system (37), indicating that the transport of Ni2+ by CorA may be physiologically relevant.

While the physiological roles of CorA have been relatively well studied, the factors directly affecting the expression of the corA gene have not been identified. We investigated the functional role of RNase III activity in corA expression in E. coli and reported experimental evidence showing that RNase III controls the degradation of corA mRNA by cleaving its 5′-untranslated region (5′-UTR), which in turn influences the influx of metal ions transported by CorA into the cell.

MATERIALS AND METHODS

Strains and plasmids. E. coli strain MG1655rnc-14::ΔTn10 was constructed by the P1 transduction of the rnc-14::ΔTn10 allele from E. coli strain H1115, which was obtained from Donald L. Court. MG1655mgtA and MG1655rnc-14::ΔTn10mga were constructed by deleting the open reading frame of mgtA in the genomic DNA of MG1655 and MG1655rnc-14::ΔTn10 by using a procedure described previously (10). PCR primers were mgtA H1P1 (5′-TTCTGTACTGTTTCAGACAGTGCGGAGGGAC TCTTCATAATGAAATCCTCCTTA) and mgtA H2P2 (5′-CAATCGTAAAGGGGCTGCTTGAGTGGAGAGGCCTGGCAC GACCGCTGATTTCGAGGGGGGAC TCTTCATAATGAAATCCTCCTTA) and mgtA H2P2 (5′-CAATCGTAAAGGGGCTGCTTGAGTGGAGAGGCCTGGCAC GACCGCTGATTTCGAGGGGGGAC TCTTCATAATGAAATCCTCCTTA) and mgtA H2P2 (5′-CAATCGTAAAGGGGCTGCTTGAGTGGAGAGGCCTGGCAC GACCGCTGATTTCGAGGGGGGAC TCTTCATAATGAAATCCTCCTTA). BW25113corA was obtained from the E. coli stock center at Yale.

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Regulation of corA Expression by Rnase III

In vitro cleavage analyses and primer extension analysis. His-tagged Rnase III purification and cleavage assays were performed as previously described (1). The 3′-5′-end-labeled transcripts were either 5′-end labeled with [γ-32P]ATP (6,000 mCi mmol−1) and T4 polynucleotide kinase (New England Biolabs) or 3′-end labeled with [5′-32P]cytidine 3′,5′-biphosphate ([5′-32P]PcP) (3,000 mCi mmol−1) and T4 RNA ligase (New England Biolabs) and then separated on 6% polyacrylamide gels containing 8 M urea. The transcripts were eluted from the gel after mixing for 16 h in a buffer containing 30 mM Tris-HCl (pH 7.9), 10 mM NaCl, 0.1% sodium dodecyl sulfate, and 0.1 mM EDTA (pH 8.0). The transcripts were purified by using phenol-chloroform extraction and ethanol precipitation. One pico mole labeled transcript was incubated with 5 ng of purified Rnase III in the presence of 0.25 μg/ml of yeast RNA (Ambion) and 20 U of Rnase inhibitor (Takara, Otsu, Japan) in cleavage buffer (30 mM Tris-HCl [pH 7.9], 160 mM NaCl, 0.1 mM dithiothreitol [DTT], 0.1 mM EDTA [pH 8.0]). Cleavage reactions were initiated by the addition of 10 mM MgCl₂ to the mixture after 5 min of incubation at 37°C. Samples were removed at the time intervals indicated in the figure legends and mixed with an equal volume of gel loading buffer II (Ambion) containing 95% formamide, 18 mM EDTA, 0.025% SDS, 0.025% xylene cyanol, and 0.025% bromophenol blue. The samples were denatured at 65°C for 10 min and separated on a 12% polyacrylamide gel containing 8 M urea and 1× Tris-borate-EDTA (TBE). Primer extension analysis was performed by using the total RNA purified by phenol extraction and ethanol precipitation and hybridized with 5′-32P-labeled primers. The following primers were used: corA-N-3′ (5′-TTTGGCGGGCCAGACACGGCGAAC TTTCC), corA-M-3′ (5′-TTTGGCGGCGAGACACGGCGAACTT CT), corA-utr-157R (5′-AACACGGCTACGTACCGGGA), and corA-3′. RNA and labeled primers were annealed at 65°C for 15 min and then slowly cooled down to 37°C for 1 h. They were then extended at 42°C for 1 h by using avian myeloblastosis virus (AMV) reverse transcriptase (New England Biolabs). The extended fragments were separated on 12% polyacrylamide gels as described above.

RESULTS

Rnase III negatively regulates corA gene expression. To confirm the results of microarray analyses from our previous research (38), we measured steady-state levels of corA mRNA in wild-type and rnc-deleted cells of E. coli strain MG1655 using quantitative real-time PCR. Consistent with the microarray data, wild-type cells expressing Rnase III (1× Rnase III) demonstrated an approximately 4.5-fold decrease in the amount of corA mRNA compared with the amount in rnc-deleted cells (0× Rnase III) (Fig. 1A). In wild-type cells that adventitiously overexpressed Rnase III at levels 10 times the endogenous level (10× Rnase III) from plasmid pRNC1 (35), the amount of corA mRNA was further decreased by 2.6-fold compared with the amount in wild-type cells harboring an empty vector (pKAN6B). Western blot analysis also showed that levels of CorA protein were well correlated with cellular concentrations of Rnase III. Levels of Rnase III expression in the rnc-deleted cells were approximately 3 and 5 times higher than those in wild-type cells harboring pKAN6B and pRNC1, respectively (Fig. 1B). The difference in expression levels of the corA mRNA and the CorA protein was greater between E. coli cells expressing 0× and 1× Rnase III than that between E. coli cells expressing 1× and 10× Rnase III. We think that this discrepancy stems from the saturation of Rnase III activity on corA expression in E. coli cells expressing 1× Rnase III. Our previous study showed that expression levels of Rnase III at levels 0.1 to 10.0 times the endogenous levels do not greatly affect the abundance of Rnase III-targeted mRNA species and normal cellular growth in rich medium (36).

Next, we tested whether cellular levels of Rnase E, a single-stranded RNA-specific endoribonuclease E, affect the corA mRNA abundance, since it is known to play a major role in mRNA decay
FIG 1 Downregulation of corA expression by RNase III. (A) Effects of the cellular RNase III concentration on corA mRNA levels were measured by using quantitative PCR analysis. Total RNA was prepared from E. coli strain MG1655, harboring pKAN6B or pRN1, and MG1655rc-14::ΔTn10, harboring pKAN6B. We measured the relative abundance of corA mRNA by setting the amount of corA mRNA in MG1655 cells harboring pKAN6B to 1. Levels of mgtA mRNA, which were independent of the cellular concentrations of RNase III (38), were used to normalize the amount of corA mRNA. (B) Western blot analysis of the CorA protein. E. coli strains MG1655, harboring pKAN6B or pRN1, and MG1655rc-14::ΔTn10, harboring pKAN6B, were grown in LB at 37°C to an OD600 of 0.8 to obtain total proteins. The amounts of CorA, RNase III, and ribosomal protein S1 were analyzed by using Western blot analyses. The S1 protein was used to provide an internal standard to evaluate the amount of cell extract in each lane. The same membrane probed for CorA was also probed with polyclonal antibodies to RNase III and S1.

in E. coli (2, 7, 25). We utilized E. coli strain KSL2000 (22), in which the chromosomal rne gene has been deleted and complemented with a construct that expresses RNase E from an rne gene under the arabinose-inducible P Bad promoter in plasmid pBAD-RNE. In strain KSL2000, RNase E expression is controlled solely by the concentration of arabinose, and cellular RNase E levels can be conditionally knocked down to ~10% of endogenous RNase E levels without significantly affecting normal cellular growth. Steady-state levels of corA mRNA and the CorA protein were measured in KSL2000 cells in either the presence or the absence of arabinose. The results revealed no significant changes in expression levels of corA mRNA or the CorA protein in cells depleted of RNase E compared with cells that expressed endogenous levels of RNase E (see Fig. S1 in the supplemental material). These results demonstrate that RNase E is not actively involved in the decay pathway of corA mRNA.

RNase III affects the transport activity of CorA. We wished to test whether alterations in the levels of CorA expression are physiologically relevant to its normal function in E. coli by measuring the degree of resistance of E. coli cells expressing 0X, 1X, or 10X RNase III to Co2+ and Ni2+. An excessive influx of Co2+ and Ni2+ by CorA inhibits normal cellular growth in E. coli. Cobalt toxicity is due mainly to its competition with iron in iron-containing proteins such as Fe-S clusters (33, 41) or via the cobalt-mediated oxidative stress of free-thiol pools (41), whereas nickel toxicity is likely to be related to nickel-mediated oxidative stress (35). For this reason, we expected that decreased expression levels of the CorA protein would result in the transport of smaller amounts of cobalt and nickel into the cell, which would consequently render E. coli cells more resistant to cobalt and nickel. First, we measured optimal Mg2+ concentrations for the growth of mgtA-deleted E. coli cells in order to circumvent indirect effects of MgtA, another putative Mg2+ transporter in E. coli, on the degree of resistance of E. coli cells to cobalt by different expression levels of CorA. Under Mg2+ concentrations equal to or below 10 μM, the growth of mgtA-deleted E. coli cells was inhibited (Fig. 2A), indicating an important functional role of MgtA in Mg2+ transport under Mg2+-limited conditions. We obtained analogous results when an mgtA deletion was introduced into an rnc-deleted E. coli strain (Fig. 2B). As was shown previously for CorA function in magnesium transport in Salmonella species (29, 37, 38), the corA deletion did not affect the cellular growth of E. coli under Mg2+-limited conditions (Fig. 2C). BW25113 and BW25113corA were used.
TABLE 1 Cobalt contents in wild-type and rnc strains

| Strain and condition | Mean intracellular cobalt content (µM) ± SD
<table>
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<tr>
<td>MG1655</td>
<td></td>
</tr>
<tr>
<td>−Co²⁺</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>+Co²⁺ (200 µM)</td>
<td>11.3 ± 0.1</td>
</tr>
<tr>
<td>MG1655rnc-14::ΔTn10</td>
<td></td>
</tr>
<tr>
<td>−Co²⁺</td>
<td>17.2 ± 0.1</td>
</tr>
<tr>
<td>+Co²⁺ (200 µM)</td>
<td>21.4 ± 0.3</td>
</tr>
</tbody>
</table>

The cobalt content was determined by using a colorimetric assay (25). The cobalt contents for corA-deleted E. coli cells (BW25113corA) were 3.6 ± 0.1 µM (without Co²⁺) and 5.4 ± 0.1 µM (with Co²⁺).

for measurements of optimal Mg²⁺ concentrations because BW25113 is an E. coli strain that is very closely related to MG1655 (3, 4), and we were unable to obtain an MG1655-derived strain lacking corA despite several attempts to construct the strain.

Based on the results described above, we measured the MICs of cobalt and nickel for E. coli cells expressing 0×, 1×, or 10× RNase III in N-minimal medium supplemented with 1 mM MgSO₄. The MIC of cobalt for E. coli cells expressing 0× RNase III was 10.0 µM, whereas the MICs were 33.3 and 40.0 µM for E. coli cells expressing 1× and 10× RNase III, respectively (Fig. 2D). The MIC of nickel for E. coli cells expressing 0× RNase III was 33.3 µM, whereas the MICs were 160.0 and 240.0 µM for E. coli cells expressing 1× and 10× RNase III, respectively (Fig. 2E). The MICs were not significantly changed when E. coli cells deleted for mgtA were tested (Fig. 2D and E), indicating that MgtA expression in E. coli is suppressed in the presence of 1 mM Mg²⁺, as was shown previously for that in Salmonella (39). The difference in MICs was greater between E. coli cells expressing 0× and 1× RNase III than that between E. coli cells expressing 1× and 10× RNase III. This discrepancy is probably due to a slightly slow growth phenotype of E. coli cells expressing 0× RNase III (Fig. 2B) and a saturation of RNase III activity upon corA expression in E. coli cells expressing 1× RNase III. Nonetheless, these results demonstrate the correlation between levels of CorA expression and the degree of resistance to cobalt and nickel. Expression levels of CorA were also closely correlated with cellular concentrations of RNase III under the conditions of MIC measurement (Fig. 2F).

To confirm that expression levels of the CorA protein directly affect cobalt accumulation, we used a colorimetric assay method to measure cobalt concentrations in wild-type and rnc-deleted E. coli cells that were grown to the mid-log phase in LB for 30 min in the presence or absence of an additional 200 µM CoCl₂. Among cells that were grown in LB without the addition of CoCl₂, the cobalt content in rnc-deleted E. coli cells was approximately three times higher than that in the wild-type cells (17.2 µM versus 5.0 µM) (Table 1). Among cells exposed to 200 µM CoCl₂, cobalt concentrations were 11.3 µM in wild-type cells and 21.4 µM in rnc-deleted cells (Table 1). These results indicate that different levels of CorA expression in isogenic E. coli strains differing in the rnc gene affect cobalt accumulation.

Identification of RNase III cleavage sites in corA mRNA. The correlation between steady-state levels of corA mRNA and cellular concentrations of RNase III suggests the presence of cis-acting elements in corA mRNA that are responsive to RNase III. To identify RNase III cleavage sites in corA mRNA, we performed primer extension experiments using several 5′-32P-end-labeled primers and total RNA purified from wild-type and rnc-deleted cells. We observed two distinct cDNA bands that were present only in the lanes loaded with cDNA products from the reaction mixture containing total RNA from wild-type cells (Fig. 3A, bands A and B). In addition, these cDNA bands were more distinct when the reaction was carried out with total RNA prepared from E. coli cells that adventitiously overexpressed corA mRNA and RNase III (Fig. 3A, last lane). These cDNA bands corresponded to sites that were positioned in the double-stranded region in the 5′-UTR of the corA mRNA (Fig. 3B). RNase III cleavage at these sites was predicted to produce cleavage products with an overhang of 2 nucleotides at the 3′ end, which is characteristic of RNase III cleavage products. These sites were designated cleavage sites A and B.

The cleavage of corA mRNA by RNase III at cleavage sites A and B was further demonstrated biochemically by using an in vitro-synthesized model hairpin RNA and purified RNase III. The model hairpin RNA has a nucleotide sequence between nt −232 and −41 from the start codon of corA, which encompasses RNase III cleavage sites A and B in the corA mRNA (Fig. 3C). The RNase III cleavage of a 5′-32P-end-labeled model hairpin RNA in vitro generated one major and one minor cleavage product, the lengths of which corresponded to cleavage sites A and B, respectively. The predicted secondary structure of the hairpin was confirmed by analyzing the cleavage patterns of the model hairpin RNA after RNase T1 and V1 digestion. Other minor cleavage products might have resulted from the intrinsic property of RNase III to randomly cleave RNA transcripts in vitro when the RNase III concentration is relatively high (44).

The radioactivity in the cleavage product at site A was −12 times higher than that at site B. There are two possible explanations for this result. Either RNase III cleaves more efficiently at site A than at site B, or the cleavage product at site A appeared to be more abundant because the model hairpin was 5′-32P-end labeled and the cleavage product at site A accumulated during the cleavage reaction. To address this uncertainty, we synthesized a 3′-32P-end-labeled model hairpin and performed an in vitro cleavage assay. The results demonstrated that cleavage products at both sites were similarly accumulated, indicating that RNase III cleaves more efficiently at site A than at site B (Fig. 3D).

RNase III cleavage at sites A and B is a rate-limiting step for corA degradation. To test whether RNase III cleavage at sites A and B is a rate-limiting step for corA degradation, we introduced nucleotide substitutions (C-122G, U-153A, and A-152G) at the RNase III cleavage sites (Fig. 4A) in a corA overexpression plasmid (pCRS1). Wild-type and mutant corA mRNAs were expressed in E. coli cells lacking corA, and the half-lives of this mRNA and the cleavage specificity of RNase III were investigated. These nucleotides were created because they do not alter the overall secondary structure of the 5′-UTR of the corA mRNA. In addition, our previous research on RNase III cleavage site selection on bdm mRNA showed that base substitutions at scissile-bond sites are sufficient to alter RNase III cleavage activity (15). The half-life of the mutant mRNA more than doubled (~7 versus ~20 s), and RNase III was not able to efficiently cleave mutant corA mRNA at cleavage sites A and B in vivo, resulting in an increased expression level of the CorA protein from the mutant corA mRNA (Fig. 4B to D). These results demonstrate that RNase III cleavage at sites A and B is a rate-limiting step for corA degradation in vivo. The blocking of the RNase III cleavage of corA mRNA by the nucleotide substitution mutations in the corA mRNA was further demonstrated biochemically.
The results showed that RNase III was unable to cleave the model containing the corresponding mutations and purified RNase III. The expected products (indicated with short arrows in panel D), might have resulted from the RNase III digestion of RNA transcripts containing a 1-nt deletion at 5' positions. Cleavage products (A and B) were identified by using size markers generated by alkaline hydrolysis. The relative amounts of cleaved products A and B are indicated in parentheses as well as by the sizes of the arrows. Other cleavage products, whose sizes are shorter by 1 nt than those of the expected products (indicated with short arrows in panel D), might have resulted from the RNase III digestion of RNA transcripts containing a 1-nt deletion at the 5' end. Other minor cleavage products are indicated with asterisks.

**DISCUSSION**

We investigated the functional role of RNase III in the regulation of corA expression in *E. coli* and identified an RNase III-mediated regulatory pathway that controls corA expression. In *vitro* and *in vivo* analyses of corA mRNA revealed that RNase III controls the degradation of corA mRNA by cleaving the 5' UTR, which consequently affects levels of CorA protein expression (Fig. 1 and 3). The blocking of the RNase III cleavage of corA mRNA by nucleotide substitution mutations at the cleavage site in the corA mRNA further demonstrated that RNase III cleavage is a rate-limiting step for corA degradation (Fig. 4). In addition, we showed that the downregulation of corA expression by RNase III results in the reduced accumulation of cobalt, which renders *E. coli* cells more resistant to cobalt stress (Fig. 2D and Table 1). The downregulation of corA expression by RNase III also rendered *E. coli* cells more resistant to nickel (Fig. 2E). As was shown previously for CorA function in Mg2+...
transport in Salmonella species (29, 38), the corA deletion did not affect the cellular growth of E. coli under Mg\(^{2+}\)-limited conditions (Fig. 2C). These results highlight the physiological significance of the regulation of corA expression.

The rapid degradation of corA mRNA by RNase III cleavage is not likely to stem from differences in translation efficiencies between the intact and the RNase III-cleaved corA mRNAs because the RNase III cleavage site is distant from the putative ribosome binding site. In addition, RNase III cleavage does not appear to change the RNA structure of the region encompassing the putative ribosome binding site of the corA mRNA (Fig. 1B). Rather, we hypothesize that the removal of the 5′ hairpin by RNase III cleavage generates corA mRNA that is vulnerable to attack by RNases, the action of which is inhibited by the presence of 5′ hairpins and/or a triphosphate group at the 5′ end of the mRNA (11, 15, 27, 42).

Unlike the regulation of RNase III activity on bdm and proU mRNAs in response to osmotic stress (38), RNase III activity on the corA mRNA was not significantly altered in E. coli cells subjected to cobalt or nickel stress (see Fig. S2 in the supplemental material). These results indicate that RNase III does not directly control corA expression in response to cobalt/nickel stress, at least under the conditions tested. However, it is possible that under different environmental conditions, another regulatory pathway exists to modulate RNase III activity on corA and other mRNA species that encode factors related to CorA activity and/or RNase III activity upon cobalt/nickel stress. This view is supported by the observation that RNase III activity on pnp mRNA, which is a well-characterized in vivo RNase III substrate (32), is upregulated in E. coli cells exposed to cobalt stress (our unpublished data). It was also shown that the PhoPQ gene status has an effect on the activity of the CorA protein (8), the mechanism of which has not yet been characterized. The identification of these factors and conditions will advance our understanding of the role of RNase III in modulating rapid physiological adjustments to environmental changes, such as metal stress.

FIG 4 Inhibition of RNase III cleavage of corA mRNA by introduction of mutations at the cleavage site. (A) Secondary structures of the hairpin encompassing RNase III cleavage sites. Nucleotide substitutions (C-122G, U-153A, and A-152G) at the RNase III cleavage sites are shown. (B) Effects of mutations at the RNase III cleavage sites on corA mRNA decay. Plasmid pCRS1-MT expresses corA mRNA containing nucleotide substitutions (C-122G, U-153A, and A-152G) at the RNase III cleavage sites. Strain BW25113 deleted for corA (BW25113 corA) and harboring either pCRS1-WT or pCRS1-MT was grown in LB at 37°C to an OD\(_{600}\) of 0.6. Total RNA samples were prepared from the cultures 0, 5, 10, and 20 s after the addition of rifampin (1 mg ml\(^{-1}\)) and separated on 1.2 M agarose gels containing 0.6 M formaldehyde. The abundances of corA mRNA and M1 RNA, the RNA component of RNase P (21, 34), were measured by Northern blotting with 5′-end-labeled primers. The abundance of M1 RNA was measured to provide an internal standard for evaluating the total amount of RNA in each lane. (C) Effects of mutations at the cleavage sites on RNase III cleavage activity on the corA mRNA. Total RNA was prepared from BW25113 corA cells harboring no plasmid, pCRS1, or pCRS1-MT and analyzed as described in the legend of Fig. 3A. (D) Effects of mutations at the cleavage sites on levels of CorA protein expression. Total proteins were prepared from BW25113 corA harboring no plasmid, pCRS1, or pCRS1-MT and analyzed as described in the legend of Fig. 1B. (E) Effects of mutations at the cleavage sites on RNase III cleavage activity in vitro. A mutant model hairpin (MT) containing nucleotide substitutions (C-122G, U-153A, and A-152G) was synthesized and tested for RNase III activity on the RNA, as described in the legend of Fig. 3C. Cleavage products at RNase III sites A and B are shown with arrows, and other minor cleavage products are indicated with asterisks.
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