Both RNase E and RNase III control the stability of sodB mRNA upon translational inhibition by the small regulatory RNA RyhB

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ABSTRACT

Previous work has demonstrated that iron-dependent variations in the steady-state concentration and translatability of sodB mRNA are modulated by the small regulatory RNA RyhB, the RNA chaperone Hfq and RNase E. In agreement with the proposed role of RNase E, we found that the decay of sodB mRNA is retarded upon inactivation of RNase E in vivo, and that the enzyme cleaves within the sodB 5′-untranslated region (5′-UTR) in vitro, thereby removing the 5′ stem–loop structure that facilitates Hfq and ribosome binding. Moreover, RNase E cleavage can also occur at a cryptic site that becomes available upon sodB 5′-UTR/RyhB base pairing. We show that while playing an important role in facilitating the interaction of RyhB with sodB mRNA, Hfq is not tightly retained by the RyhB–sodB mRNA complex and can be released from it through interaction with other RNAs added in trans. Unlike turnover of sodB mRNA, RyhB decay in vivo is mainly dependent on RNase III, and its cleavage by RNase III in vitro is facilitated upon base pairing with the sodB 5′-UTR. These data are discussed in terms of a model, which accounts for the observed roles of RNase E and RNase III in sodB mRNA turnover.

INTRODUCTION

RNA processing and decay play important roles in controlling the level of particular transcripts under various growth conditions (1–3). In Escherichia coli, the degradation of mRNA is generally triggered by endoribonucleolytic cleavage, and the resulting intermediate products are further degraded by endo- and exoribonucleases [reviewed in (4,5)]. E. coli RNase E seems to initiate the decay of many, if not most, mRNAs (6–10). In some cases, the initial cleavage of transcripts can also be performed by RNase III (11). Although the subsequent steps of mRNA decay may sometimes require endoribonuclease RNase P (12), further degradation is believed to be accomplished by two major exoribonucleases, PNPase and RNase II, and oligoribonuclease (13). It has also been shown that auxiliary factors, such as RNA helicases and Hfq (14–18), that modulate RNA structure, can significantly affect mRNA stability.

Recent studies of the mechanisms, which are involved in cellular responses to numerous stress conditions, revealed that mRNA stability can be modulated by the action of small regulatory RNAs (sRNAs) (19). By base pairing with target mRNAs, sRNAs affect their translation and/or stability (20). In contrast to the regulation by classical antisense RNAs (21), base pairing of sRNA with their targets does not require a high degree of complementarity and, therefore, provides a possibility for sRNAs to affect multiple transcripts.

One of the best-studied E. coli sRNAs, RyhB, has been recently shown (17,20) to regulate the level of the sodB mRNA (Figure 1) encoding the iron superoxide dismutase [FeSOD (22)]. The in vivo level of RyhB is in turn controlled (23) by the transcriptional repressor Fur [ferric uptake regulator (24)], whose ability to repress RyhB transcription is iron-dependent. Similar to the action of other sRNAs (25–27), the interaction of RyhB with sodB mRNA is mediated by the E. coli RNA chaperone Hfq, which has been shown to induce structural rearrangements within the sodB 5′-untranslated region (5′-UTR) (Figure 1B) (28).

Although the formation of inhibitory complexes with sRNAs is believed to functionally inactivate their target mRNAs, the steps leading to subsequent disassembly and recycling of these complexes are poorly understood (29). The aim of this study is to recapitulate the functional inactivation of the E. coli sodB mRNA by the regulatory RNA RyhB in vitro.

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MATERIALS AND METHODS

Bacterial strains, plasmids, media and growth conditions

The following *E. coli* strains were used in this study: N3433 [rne⁻ (30)], N3438 [rne-3071, recA (31)], SDF204 [W3110 rnc⁺ TDI-17::Tn10 (32)] and SDF205 [W3110 rnc105 TDI-17::Tn10 (32)]. The *E. coli* strains were grown in Luria–Bertani (LB) medium at 28°C.

To construct plasmid pUsod, the DNA fragment corresponding to the entire *sodB* gene was amplified from chromosomal DNA of *E. coli* strain MC4100 (33) by PCR using the primers SODBfw (5'GCTCTAGATATACGACTCACTTAGATAGCACAATAAAGGCTATTGTACGTATG) containing extra nucleotides corresponding to the T7 promoter (in bold) (34) and an XbaI site (underlined) and SODBrev (5'CGGATCCGGATGCGCGGA-GTGCGCTATCC)
containing a BamHI site (underlined). The PCR product was cleaved with XbaI and BamHI and ligated into plasmid pUC18 (35) digested with the same endonucleases. The plasmid pURyhB (17) was used for RyhB RNA synthesis.

**In vitro synthesis and labelling of RNA and single-stranded DNA**

The 204 nt long single-stranded anti-sod DNA, containing the region complementary to the first 182 nt of sodB mRNA, was amplified using the XbaI-linearized plasmid pUsod and the 5'-[32P]end-labelled primer Sod-rev (5'-CAGGTTGTCCAGGTTAGTGAC) by an asymmetric PCR, and then gel-purified. RyhB RNA was transcribed from HindIII-linearized plasmid pURyhB (17) using T7 RNA polymerase. The sodB192 mRNA (Figure 1C), containing the sodB 5' untranslated region and part of the coding region (nucleotides --55 to +137) and one G nucleotide at the 5' end, was transcribed from Asp718I-linearized plasmid pUsod. The sodB151 mRNA (Figure 1C), containing the truncated sodB 5' untranslated region and part of the coding region (nucleotides --21 to +127) and three extra G nucleotides at the 5' end, was transcribed using PCR-amplified SodB2 template. Plasmid pUsod was used for the amplification of SodB2 template with the following primers: 5'-AATTAATCGACTCATATAGGTAAATATGGATGGCAGATGGCAA TG-TCATTCG (forward primer) and 5'-GGAAGAGTAGCAA TG-TCATTCG (reverse primer). Underlined nucleotides correspond to the T7 RNA polymerase promoter (34). The RNAs were synthesized using the MEGscript T7 kit (Ambion), gel-purified and 5' end-labelled as described previously (36).

**Northern blot analysis**

Strains were grown at 30°C up to an OD_{600} of 0.4, and then the temperature was shifted to 44°C for 10 min before the addition of rifampicin (0.25 mg/ml). In some experiments (for details, see Figure legend 2), LB medium was also supplemented with 250 μM FeSO_4 that was added 16 min before rifampicin treatment. Total RNA was prepared from aliquots of the cultures withdrawn at various times using the hot phenol method (37). The RNA samples were fractionated on 4–8% polyacrylamide gels, transferred to Zeta-Probe membranes (Bio-Rad), and then separately hybridized with [32P]labelled RNA probes specific for sodB mRNA, RyhB RNA or SS rRNA as described previously (17). The signal intensities obtained with the radioactively labelled probes were quantitated on a PhosphorImager (Molecular Dynamics). The relative amount of sodB mRNA and RyhB at each time point was calculated by normalizing their signals to SS rRNA signal.

**Gel shift assays–western blot analysis**

Aliquots containing 100 fmol of 5'-[32P]labelled RNA were incubated for 10 min at 37°C in binding buffer (10 mM Tris–HCl, pH 7.5, 5 mM magnesium acetate, 100 mM NH_4Cl and 0.5 mM DTT). After cooling on ice and addition of glycerol to a final concentration of 5%, the samples were loaded on a non-denaturing 4% polyacrylamide gel. Electrophoresis was performed in 0.5X TBE buffer at 40 V for 10–16 h, and the radioactive bands were visualized using a PhosphorImager (Molecular Dynamics). Thereafter, the gel was electroblotted onto Immobilon-P membrane (Millipore). The blotting was carried out in TBE buffer at 200 V for 30 min. After blotting, the membranes were blocked in 10% non-fat dry milk suspension and probed with anti-Hfq antibodies followed by development using the enhanced chemiluminescence detection kit (Amersham) as recently described (17).

**RNase E cleavage of sodB192 mRNA**

Aliquots containing 100 fmol of 5'-[32P]labelled RNA were incubated for 10 min at 37°C in binding buffer (10 mM Tris–HCl, pH 7.5, 5 mM magnesium acetate, 100 mM NH_4Cl and 0.5 mM DTT) in the presence or absence of Hfq and unlabelled RNAs as indicated in Figure legend 3. Then, 20 ng of Rne498 (39) or 150 ng of degradosome (40) were added, and aliquots withdrawn at various time points were further extracted with phenol, mixed with an equal volume of loading dye solution (90% formamide, 0.5 mM EDTA, 0.025% xylene cyanol FF and 0.025% bromophenol blue), and then fractionated on 8% polyacrylamide gels containing 8 M urea. The cleavage sites for RNase E and RNase III were mapped using endonuclease Ti and nuclease S1 digests of the corresponding RNAs. Briefly, 200 fmol of 5'-[32P]labelled RNA were incubated with 4 U of ribonuclease S1 (MBI Fermentas) in 1× S1 buffer (MBI Fermentas) at 70°C for 1–8 min or with 0.1 U of ribonuclease T1 (MBI Fermentas) in 1× AT buffer (10 mM Tris–HCl, pH 7.5, 10 mM MgCl_2) at 37°C for 1–8 min.

**RNase III cleavage of RyhB**

Aliquots containing 40 fmol of 5'-[32P]labelled RNA were pre-incubated with 6-fold molar excess of Hfq for 5 min at 37°C in 1× RNase III buffer (Ambion) in the absence or presence of increasing quantities of unlabelled sodB192 RNA as indicated in Figure legend 7. Subsequently, 0.1 U of RNase III (Ambion) was added, and aliquots withdrawn at various time points were further extracted with phenol, mixed with an equal volume of loading dye solution (90% formamide, 0.5 mM EDTA, 0.025% xylene cyanol FF and 0.025% bromophenol blue), and then fractionated on 6% polyacrylamide gels containing 8 M urea.

**Toeprinting assay**

The toeprinting assays were carried out using 30S ribosomal subunits and tRNA^Met as described previously (41). The 5'-[32P]labelled sodB-specific oligonucleotide Sod-rev (5'-CAGGTTGTCCAGGTTAGTGAC) complementary to nucleotides +137 to +117 of the sodB mRNA was used as a primer for cDNA synthesis in the toeprinting reactions. The sodB192 RNA annealed to the primer was separated from free oligonucleotides on a MicroSpin G-50 column according to the manufacturer’s instructions (Amersham Biosciences). An aliquot of 0.04 pmol of sodB192 mRNA annealed to Sod-rev oligo was pre-incubated at 37°C for 5 min without or with 0.5 pmol of 30S subunits and 10 pmol of tRNA^Met.

To compare the affinity of the 30S subunits for sodB192 RNA and its truncated version sodB151 mRNA, increasing amounts of either sodB192 or sodB151 mRNA were added to the reaction mixtures after the annealing step before the addition of 30S ribosomal subunits and tRNA^Met.
addition of 30S subunits, as indicated in the legend to Figure 3. The rationale was that the RNA with a higher affinity for 30S ribosomal subunit would compete with the sodB192 RNA annealed to the 5'-[32P]labelled oligonucleotide Sod-rev and, therefore, would reduce the toeprint signal more efficiently than the mRNA with a lower affinity.

RESULTS AND DISCUSSION

RNase E- and RNase III-dependence of sodB mRNA decay in vivo

Previous work has shown that the steady-state level of sodB mRNA is dependent on the E.coli chaperone Hfq, the small regulatory RNA RyhB, the transcriptional regulator Fur as well as on RNase E (17,42). To differentiate between direct and indirect roles of RNase E in determining the stability of the sodB transcript, northern blot hybridization to a [32P]labelled riboprobe complementary to sodB mRNA was used to detect the full-length transcript in wild-type E.coli cells as well as in an rne<sup>+</sup> mutant strain at the non-permissive temperature after rifampicin treatment (Figure 2A). Both measurements were performed not only when E.coli cells were cultivated in medium with low or moderate iron content (LB) [i.e. under conditions leading to the coupled decay of the sodB and RyhB transcripts (42)] but also under conditions (LB + 250 μM FeSO<sub>4</sub>) that should increase the concentration of activated Fur, the repressor of RyhB transcription (23). The latter was performed with the rationale to monitor the RyhB-independent decay of sodB mRNA. In both the cases (see Figure 2A and B), the decay of sodB mRNA was retarded upon inactivation of RNase E, thus suggesting that the RyhB-dependent (Figure 2A) as well as RyhB-independent (Figure 2B) decay of this transcript is mediated by RNase E. Nevertheless, none of the degradative intermediates could be detected in addition to the full-length species (data not shown), which may not be surprising because many E.coli mRNAs are known to decay without any detectable accumulation of intermediate products.

The same probe was also used to compare the rates of sodB mRNA decay in the wild-type and its isogenic rnc mutant lacking functional RNase III (Figure 2C and D). Interestingly, the RyhB-dependent decay of sodB mRNA was more efficient in the rnc mutant when compared with the wild-type strain (Figure 2C), suggesting that RNase III does not cleave this transcript in vivo but instead may affect the decay of this mRNA indirectly, e.g. by changing the steady-state level of RyhB. Consistently, the rate of sodB mRNA decay at reduced levels of RyhB was found to be the same in the wild-type and rnc mutant strains after 8 min of rifampicin treatment (Figure 2D). Given that the addition of FeSO<sub>4</sub> does not eliminate RyhB immediately (data not shown), RyhB apparently affects sodB mRNA decay during the initial period (from 0 to 8 min), thereby resulting in different decay rates observed in these strains at early time points (Figure 2D).

RNase E cleavage of sodB mRNA in vitro

The RNase E-mediated degradation of E.coli mRNAs is usually initiated within their 5'-UTRs, which are normally not protected by ribosomes and, therefore, serve as primary targets for RNA-binding proteins and endonucleases. To test whether RNase E also cleaves within the 5'-UTR of the sodB transcript, we separately incubated 5'-end-labelled sodB192 RNA, which corresponds to nucleotide −56 to +136 of E.coli sodB mRNA (see Figure 1C), with affinity-purified RNase E (Rne498, residues 1–498) and E.coli degradosome (Figure 3A and B, respectively). As shown in Figure 3, RNase E cleavage of sodB192 occurred at position U<sub>−21</sub> and the cleavage efficiency at this site was decreased in the presence of Hfq (Figure 3A, lanes 13–15). The latter is consistent with the previously documented ability of Hfq to bind in close vicinity of RNase E cleavage sites, thereby protecting RNase E substrates from the nuclelease activity of this enzyme (15).

Moreover, we found that the structural changes, which are induced in the 5'-UTR of sodB mRNA upon binding to the regulatory RNA RyhB (28), affect the cleavage pattern. As shown in Figure 3A (compare lanes 18–20 with lanes 13–15) and Figure 3B (compare lanes 8–10 with lanes 3–5), an additional RNase E cleavage site was mapped downstream of the translational start, namely at position A<sub>−12</sub> (Figure 3D). This observation suggests that base pairing with RyhB, which stimulates RNase E cleavage downstream of the start codon (see also Figure 1A), can trigger another pathway for sodB mRNA turnover in vivo.

RNase E cleavage within the 5'-UTR of sodB mRNA decreases its affinity for Hfq and ribosomes

Given that the sodB 5'-UTR is the location of the Hfq (28) and ribosome binding sites, we next investigated whether RNase E cleavage at the 5' end of the sodB mRNA (see Figure 1B) leads to functional inactivation of the transcript, i.e. whether the truncated form of the sodB mRNA generated by cleavage at position U<sub>−21</sub> (Figure 1C, sodB152) is still able to bind Hfq and to interact with 30S ribosomes. First, the affinity of sodB192 and its truncated form sodB151 to Hfq was compared by means of gel-shift assays. As shown in Figure 4A, although both [32P]labelled transcripts (sodB192 and sodB151) can bind Hfq, the truncated form (sodB151) has lower affinity. This suggested that the 5' terminal hairpin structure facilitates Hfq binding. Likewise, the presence of this structure appears to confer higher affinity for ribosomes. This was revealed by comparing the ability of both transcripts (sodB192 and sodB151) to interfere with translation inhibition on sodB192 RNA (Figure 4B and C). As shown by toeprint analysis (Figure 4B), sodB151 RNA was required in higher concentrations than sodB192 RNA to achieve the same inhibitory effect on ribosome binding (Figure 4C).

Analysis of Hfq binding to and recycling from the RyhB-sodB 5'-UTR complex

Previous work has suggested that Hfq binding induces structural changes within the 5'-UTR of the sodB transcript, which facilitate base pairing with the small regulatory RNA RyhB (28). To study in more detail the composition of the inhibitory complex formed between RyhB and sodB mRNA, we employed gel-shift assays. Radioactively labelled sodB192 RNA (Figure 1C) was incubated with increasing amounts of RyhB in the absence (Figure 5A, lanes 2–5) or presence (Figure 5A, lanes 7–10) of Hfq, and the resulting complexes were analysed on a native polyacrylamide gel. As anticipated,
base pairing of RyhB with sodB192 RNA is strongly facilitated by Hfq. To test whether the resulting complex also contained Hfq, selected samples, which are shown in lanes 1, 6 and 10 of Figure 5A, were resolved on a separate gel, and the gel was blotted onto a PVDF membrane followed by probing with anti-Hfq antibodies (Figure 5B). As revealed by immunodetection, Hfq was part of the complex (marked by asterisks) formed by the base paired RNAs. Vice versa, labelling of
RyhB confirmed that base pairing with sodB192 is inefficient in the absence of Hfq (Figure 5C, compare lanes 2–5 with lane 7). Moreover, by the addition of increasing amounts of sodB192 to the preformed ternary complex containing both RNAs and Hfq (lane 7), Hfq was apparently released from the ternary complex (lanes 8–10), resulting in a species (indicated by a single circle) that migrates at the position of the binary RyhB–sodB192 complex. Therefore, although Hfq-mediated base pairing of sRNAs with their target does not simultaneously result in its release from the complex, these data might suggest that Hfq recycling occurs via interactions with other Hfq ligands.

**RNase E-independent and RNase III-dependent decay of RyhB in vivo**

Masse and Gottesman (20) have recently shown that inactivation of RNase E results in an increase in the steady-state level of sodB 5'-UTR. 5'-[^32P]labelled sodB192 was incubated either with RNase E polypeptide (Rne498, residues 1–498) or with RNA degradosome (40) in the presence or absence of Hfq and RyhB at 37°C (A and B, respectively). Aliquots were withdrawn at the times indicated above each lane, phenol extracted and analysed on an 8% sequencing gel. The graph on (C) shows the relative amount of sodB192 mRNA remaining at each time point of (A) as determined by phosphorimaging and plotted as a function of time. (D) Mapping of the RNase E cleavage sites within the sodB 5'-UTR in the presence (+) or absence (−) of RyhB. The molar ratio of Hfq-hexamer:RyhB:sodB192 was 8:8:1, respectively. The precise position of RNase E cleavage sites was determined from concomitantly run S1 and T1 digests of the same RNA.

**Figure 3.** RNase E cleavage within the sodB 5'-UTR. 5'-[^32P]labelled sodB192 was incubated either with RNase E polypeptide (Rne498, residues 1–498) or with RNA degradosome (40) in the presence or absence of Hfq and RyhB at 37°C (A and B, respectively). Aliquots were withdrawn at the times indicated above each lane, phenol extracted and analysed on an 8% sequencing gel. The graph on (C) shows the relative amount of sodB192 mRNA remaining at each time point of (A) as determined by phosphorimaging and plotted as a function of time. (D) Mapping of the RNase E cleavage sites within the sodB 5'-UTR in the presence (+) or absence (−) of RyhB. The molar ratio of Hfq-hexamer:RyhB:sodB192 was 8:8:1, respectively. The precise position of RNase E cleavage sites was determined from concomitantly run S1 and T1 digests of the same RNA.
We additionally found that the rates of RyhB decay in the wild-type E. coli strain and in an rne50 mutant are indistinguishable at non-permissive temperature (Figure 6A), suggesting that the above increase in the steady-state level of RyhB (42) does not stem from RyhB stabilization, but instead may be a consequence of transcriptional regulation. For example, the elevated level of RyhB could be, at least in part, brought about by a higher level of Hfq in the rne50 mutant (see Figure 6C), which is known to decrease the intracellular concentration of Fur (17), thereby facilitating RyhB transcription.
Figure 6. Effects of RNase E and RNase III inactivation on RyhB stability in vivo. (A and B) RNA samples prepared from wild-type E. coli cells (wt) and their isogenic RNase E (rne) and RNase III (rnc) mutants at various time points before and after rifampicin treatment were analysed by northern blotting using probes specific for RyhB and 5S rRNA. The latter was employed as an internal standard for normalization of RyhB-specific signals. The graph at the bottom of each panel shows the relative amount of RyhB remaining at each time point as determined by phosphorimaging and plotted as a function of time. (C) Equal amounts of total protein cell extracts prepared from wild-type E. coli cells (wt) and their isogenic RNase E (rne) or RNase III (rnc) mutants were fractionated on a 15% SDS–polyacrylamide gel followed by western blot analysis using anti-Hfq antibodies. The position of Hfq is indicated. (D) RNA samples prepared from wild-type E. coli cells before (0) and after (4) 2,2'-dipyridyl treatment (dip) at 28°C were analysed by northern blotting using a probe specific for RyhB. An asterisk indicates the position of RyhB decay intermediates. (E) RNA samples prepared from wild-type E. coli cells (wt) and their isogenic RNase E (rne) and RNase III (rnc) mutants at various time points before and after 2,2'-dipyridyl treatment (dip) were analysed by northern blotting using probes specific for RyhB and 5S rRNA. The latter was employed as an internal loading control. The molar ratio of Hfq:RyhB was 8:1, respectively. An asterisk indicates the position of RyhB decay intermediates.
Given that RyhB is apparently targeted for degradation via an RNase E-independent pathway, we also investigated whether the absence of functional RNase III, a double-strand specific E.coli endoribonuclease [reviewed in (43)], affects the stability of RyhB in vivo. Figure 6B shows that the decay rate of RyhB was slightly decreased in an RNase III-deficient strain, indicating that although the main fraction of this transcript still remained unaffected, yet some portion of it seems to be cleaved by this endonuclease in vivo. While testing this idea further, we found that RyhB degradative intermediates can be easily detected in vitro when E.coli cell cultures are treated with 2,2'-dipyridyl (Figure 6D). Moreover, their accumulation is not affected by inactivation of RNase E (Figure 6E) but is impaired in an RNase III-deficient strain (Figure 6E). Collectively, these data suggest that RyhB degradation in vivo involves its cleavage by RNase III.

RNase III cleavage of RyhB is facilitated by base pairing with its mRNA target

Our in vivo data (Figure 6) and the reported interdependence of RyhB and sodB mRNA decay (42) suggest that a 9 bp region, which is formed upon RyhB-sodB 5'UTR base pairing (see Figure 7B) (28), together with other double-stranded RNA structures of RyhB can be potentially used by RNase III to bind to the RyhB-sodB 5'UTR complex and to cleave RyhB, thereby targeting it for degradation. To test whether RNase III cleavage of RyhB can be detected in vitro, we incubated 5' end-labelled RyhB with RNase III in the presence of Hfq and increasing quantities of sodB192 RNA. As shown in Figure 7A, RyhB alone is relatively resistant to RNase III cleavage (lanes 3–5). In contrast, an increase in the concentration of sodB192 facilitates RNase III cleavage of RyhB at U26 and at some minor sites (Figure 7A). Taken together, our in vivo and in vitro data (Figures 6 and 7, respectively) strongly suggest that the second major E.coli endoribonuclease RNase III is involved in RyhB turnover and, therefore, plays an indirect role in RyhB-mediated decay of the sodB transcript.

CONCLUSIONS

It is generally believed that the majority of E.coli mRNAs are targeted for degradation following initial endonucleolytic cleavages, which often occur within their 5'-UTR (4). In agreement with this model, we demonstrated that decay of sodB mRNA is retarded upon inactivation of RNase E in vivo (Figure 2) and RNase E cleaves within the 5'-UTR of this transcript in vitro (Figure 3). As depicted in Figure 8 (left panel), by eliminating the 5'-end stem–loop structure of the transcript containing the 5'-triphasate group of the sodB transcript, the initial cleavage(s) should render 5'-monophosphorylated intermediate products. As RNA substrates bearing 5'-monophosphates are known to be better substrate than triphosphorylated ones for E.coli RNase E (44,45) and poly(A) polymerase I (46), the initial cleavage can apparently facilitate the action of these enzymes at subsequent steps of mRNA turnover. In addition to the above role, RNase E cleavage at position U21 reduces the affinity of the sodB translation initiation region for ribosomes. Given that a decrease of ribosome loading onto a transcript is known to destabilize the entire mRNA (18,47–49) resulting in its complete decay, our data suggest that functional and chemical inactivation of the sodB mRNA are interdependent and are both initiated by RNase E cleavage.

Besides general mechanisms controlling mRNA decay in bacteria, the stability of many transcripts is regulated by environmental factors, such as temperature (50–52), pH (53) and the availability of various chemicals important for bacterial growth and survival (54). Previous work has shown that, during adjustments of E.coli to decreasing concentrations of iron, the level of sodB mRNA is decreased (55). This regulation involves translational inhibition of sodB mRNA by RyhB (17,23,42). We observed that sodB/RyhB base pairing

Figure 7. RNase III cleavage of RyhB is stimulated by base pairing with its mRNA target. (A) Radioactively labelled RyhB pre-incubated with a 6-fold molar excess of Hfq was further incubated with RNase III in the absence (lanes 1–5) or presence of increasing quantities of sodB192 RNA (5-, 20- and 40-fold molar excess; lanes 6–14, respectively), and aliquots withdrawn at times indicated above each lane were analysed on a 6% (w/v) polyacrylamide sequencing-type gel. The major nucleotide (U46) at which RNase III cleaves RyhB RNA was determined from concomitantly run S1 and T1 digests of the same RNA (data not shown). (B) Model for sodB mRNA–RyhB interaction adopted from Geissmann and Touati (28). The major RNase III site of RyhB [see (A)] and RNase E cleavage sites within the 5'-leader of the sodB transcript (this study) are shown.
promotes RNase E cleavage at position $A_{+12}$, i.e. in the immediate coding region of the $sodB$ transcript (Figure 8, right panel). This cleavage most likely results from structural rearrangements in the 5′-UTR of $sodB$ mRNA upon interaction with Hfq and RyhB. Moreover, although after base pairing of these RNAs Hfq remained associated with the RyhB–$sodB$ mRNA complex, it could be released from it through interaction with other ligands. Thus, titration of Hfq by other ligands could free the RNA chaperone from ‘death end’ complexes.

Finally, although *E. coli* RNase E is often considered as the major enzyme controlling the metabolic stability of mRNA and regulatory RNAs [i.e. RNAI (8)], we showed here that the degradation of RyhB and perhaps that of other sRNAs is not always affected by this enzyme *in vivo* but is dependent on RNase III (Figure 6) known to be involved in rRNA processing and stability control of certain mRNAs (56). Moreover, RNase III cleavage of RyhB was also observed *in vitro* under conditions facilitating RyhB–$sodB$ 5′-UTR base pairing, thus suggesting a role for RNase III in the coupled degradation of these transcripts *in vivo*. These data together with another example of post-transcriptional control mediated by the small regulatory RNA IstR (57), which is cleaved by RNase III upon
its base pairing with mRNA, suggest that E. coli RNase III may play a much more significant role in bacterial gene regulation.

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