Research Article

Rapid Degradation of Hfq-Free RyhB in Yersinia pestis by PNPase Independent of Putative Ribonucleolytic Complexes

Zhongliang Deng, Zizhong Liu, Yujing Bi, Xiaoyi Wang, Dongsheng Zhou, Ruifu Yang, and Yanping Han

1 Department of Sanitary Inspection, School of Public Health, University of South China, Hengyang, Hunan 421001, China
2 State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China

Correspondence should be addressed to Ruifu Yang; ruifuyang@gmail.com and Yanping Han; yanpinghan@gmail.com

Received 26 December 2013; Revised 10 March 2014; Accepted 15 March 2014; Published 10 April 2014

Academic Editor: Ammad Ahmad Farooqi

Copyright © 2014 Zhongliang Deng et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The RNA chaperone Hfq in bacteria stabilizes sRNAs by protecting them from the attack of ribonucleases. Upon release from Hfq, sRNAs are preferably degraded by PNPase. PNPase usually forms multienzyme ribonucleolytic complexes with endoribonuclease E and/or RNA helicase RhlB to facilitate the degradation of the structured RNA. However, whether PNPase activity on Hfq-free sRNAs is associated with the assembly of RNase E or RhlB has yet to be determined. Here we examined the roles of the main endoribonucleases, exoribonucleases, and ancillary RNA-modifying enzymes in the degradation of Y. pestis RyhB in the absence of Hfq. Expectedly, the transcript levels of both RyhB1 and RyhB2 increase only after inactivating PNPase, which confirms the importance of PNPase in RNA degradation. By contrast, the signal of RyhB becomes barely perceptible after inactivating of RNase III, which may be explained by the increase in PNPase levels resulting from the exemption of pnp mRNA from RNase III processing. No significant changes are observed in RyhB stability after deletion of either the PNPase-binding domain of RNase E or rhlB.

Therefore, PNPase acts as a major enzyme of RyhB degradation independent of PNPase-containing RNase E and RhlB assembly in the absence of Hfq.

1. Introduction

Small regulatory RNAs (sRNAs) function as posttranscriptional regulators by altering translation or stability of the target mRNA, which increases their applicability in different physiological processes in bacteria [1]. The RNA chaperone Hfq is hypothesized to facilitate the access of sRNAs to their mRNA targets and stabilize sRNAs by protecting them from the attack of RNase E [2]. Given that the increasing amount of available information on sRNA-induced mRNA decay is accumulating [3–6], the sRNA degradation processes and RNases that catalyze such activities must be investigated. The multienzyme assembly of RNA degradosome is important for mRNA decay and processing in Escherichia coli. RNase E and polynucleotide phosphorylase (PNPase) are two major components of the RNA degradation process [7, 8]. RNase E is also responsible for the rapid degradation of sRNAs and competes with Hfq in accessing the same RNA sequences [9–11]. Hfq recruits RNase E by directly interacting with the RhlB-recognition region, which is hypothesized to cause the coupled cleavage of mRNA and sRNA [6, 12]. PNPase plays the protective role in the RNase E-dependent degradation in the presence of Hfq [13, 14]. Recent studies show that Hfq has a limited access to RNAs under wild-type conditions considering the dynamic interactions of Hfq with sRNAs [15–17]. A transient Hfq-free state of sRNAs may also be observed. A recent study shows that sRNAs are preferably degraded by the major exoribonuclease PNPase upon release from Hfq [14]. PNPase usually cooperates with RNase E in RNA degradation complexes [18]. RNA helicase RhlB usually facilitates RNA degradation by manipulating RNA structure and remodeling ribonucleoprotein complexes in the presence or absence of RNase E [19]. However, the relationship between the PNPase activity in Hfq-free sRNAs and RNA degradation complexes remains unknown.
The well-characterized sRNA RyhB was used as a model sRNA for this study. RyhB is an Hfq-binding sRNA that maintains iron homeostasis in bacteria [20, 21]. Besides Hfq, RyhB also becomes very stable when the overall mRNA possesses the conserved core and RyhB also becomes very stable when the overall mRNA.

### Table 1: Bacterial strains used in this study.

| Strains | Relevant characteristics | Sources or reference |
|---------|--------------------------|----------------------|
| WT      | Wild-type strain 201     | [24]                 |
| Δhfq    | hfq                      | [25]                 |
| Δhfq::hfq | hfq::pACYC184-hfq     | [25]                 |
| Δrnc    | rnc                      | This study           |
| Δhfq-rne910 | hfq-rne910-1061aa | This study           |
| Δhfq-rng | hfq-rng                 | This study           |
| Δhfq-rnc | hfq-rnc                 | This study           |
| Δhfq-ppn | hfq-ppn                 | This study           |
| Δhfq-rnb | hfq-rnb                 | This study           |
| Δhfq-rnr | hfq-rnr                 | This study           |
| Δhfq-pcnB | hfq-pcnB                | This study           |
| Δhfq-rhlB | hfq-rhlB              | This study           |
| Δhfq-rnc-rne910 | hfq-rnc-rne910-1061aa | This study           |
| Δhfq-rnc-rng | hfq-rnc-rng            | This study           |
| Δhfq-rnc-ppn | hfq-rnc-ppn           | This study           |
| Δhfq-rnc-rnb | hfq-rnc-rnb          | This study           |
| Δhfq-rnc-rnr | hfq-rnc-rnr          | This study           |
| Δhfq-rnc-rhlB | hfq-rnc-rhlB       | This study           |

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions. All strains are derivatives of *Y. pestis* strain 201, a newly established biovar, the *Microtus* [24]. Table 1 shows the bacterial strains that are used in this study. Except for the RNase E mutants, all mutant strains were constructed by replacing the entire gene with an antibiotic cassette via λ-Red homologous recombination. RNase E is essential for viability in bacteria, but deleting the C-terminal half (CTH) of this enzyme is not lethal [26]. The CTH after the 910th containing putative PNPase-binding site (1190-1221aa corresponding to 1021-1061aa in *E. coli* RNase E) [26] was deleted and designated as rne910. Bacteria were grown to midexponential phase (A_600~1.0) in BHI medium at 26°C. Iron depletion was induced by adding 100 μM 2′,2′-dipyridyl (DIP) for 20 min. Antibiotics were added when needed at the following concentrations: 34 μg/mL chloramphenicol, 50 μg/mL kanamycin, 100 μg/mL ampicillin, 20 μg/mL gentamicin, and 20 μg/mL streptomycin.

2.2. RNA Extraction and Northern Blotting Analysis. Pure bacterial cultures were mixed with RNAProtect Bacteria Reagent (Qiagen) to minimize RNA degradation. The total RNA was then extracted from *Y. pestis* using TRizol Reagent (Invitrogen). Northern blotting analysis was performed by using a DIG Northern Starter Kit (Roche) according to the manufacturer’s protocol described by Beckmann et al. [27]. RNA samples (3 μg) were denatured at 70°C for 5 min, separated on 6% polyacrylamide-7M urea gel, and transferred onto Hybond N+ membranes (GE) via electrophoretic transfer.

The membranes were then hybridized overnight at 68°C in a DIG Easy Hyb. RNA was immunologically detected and scanned according to the instructions. Multiple exposures to X-ray film were taken to achieve the desired signal strength.

2.3. RNA Half-Life Determination. Bacteria grown to exponential phase were treated with 250 μg/mL rifampicin for RNA half-life determination. Culture samples were collected at 0, 5, 10, 20, 30, and 60 min and were subject to RNA extraction and Northern blotting. Films were scanned and RNA band intensity was measured using Quantity One software. The intensities were plotted and RNA half-lives were calculated using the slope from each plot.

2.4. Quantitative RT-PCR. Total RNA was isolated from different *Y. pestis* strains grown to exponential growth phase (OD_600 = 1.2) in BHI by using Trizol Reagent (Invitrogen). DNA contaminants were removed by using DNA-free Kit.
Figure 1: Effects of RNases and an ancillary RNA-modifying enzyme on the transcriptional level of *Y. pestis* RyhB1 and RyhB2 in the Δhfq background. RyhB1 and RyhB2 were detected by Northern blotting using 5 μg of total RNA extracted from *Y. pestis* grown to exponential phase in BHI medium upon treatment with 100 μM DIP treatment for 20 min. 5S rRNA was used as a negative control. Lanes 1–8 represent WT (lane 1), Δhfq mutant (lane 2), double mutants lacking Δhfq, and another gene encoding either endoribonucleases (RNase E, RNase G) (lanes 4 and 5), exoribonucleases (RNase III, PNPase, and RNase II) (lanes 3, 6, and 8), or ancillary RNA-modifying enzyme (polyA polymerase) (lane 7).

Figure 2: Effects of various ribonucleases on the transcriptional level of RyhB1 and RyhB2 upon inactivation of Hfq and RNase III. RyhB1 and RyhB2 were detected by Northern blotting using 5 μg of total RNA extracted from *Y. pestis* grown to exponential phase in BHI medium upon treatment with 100 μM DIP treatment for 20 min. Lanes 1–7 represent WT (lane 1), Δhfq mutant (lane 2), Δhfq-rnc double mutants (lane 3) and triple mutants lacking Δhfq, rnc, and another gene encoding RNase E (lane 4), RNase G (lane 5), PNPase (lane 6), or RNase III (lane 7).

(Ambion), and the cDNA was converted by using random hexamer primers with the Superscript II system (Invitrogen). Real-time PCR was performed in duplicate for each RNA preparation by using the LightCycler system (Roche) with an appropriate dilution of cDNA as a template. Negative controls without reverse transcriptase enzyme were included in all experiments. Relative quantitative analysis across different cDNA templates was performed by using LightCycler 480 software (Bio-Rad) with the 16S rDNA as the normalized gene.

3. Results and Discussion

3.1. Influence of RNases and Ancillary RNA-Modifying Enzymes on the Regulation of Hfq-Free RyhB. BHI was selected as the growth medium for bacterial culture because some mutants that were constructed in this study experienced a slow growth upon inoculation into TMH medium, which pose a challenge to our experiments.

The expressions of RyhB1 and RyhB2 were monitored in multiple Δhfq mutants that lacked major RNases or ancillary RNA-modifying enzymes to validate the influence of endoribonucleases, exoribonucleases, and ancillary RNA-modifying enzymes on RyhB regulation in *Y. pestis* without Hfq [14]. The expression levels of RyhB1 and RyhB2 slightly increased (~1.8-fold) upon the deletion of PNPase, but no obvious changes were observed in the RNase E truncate and deletion strains of RNase G (rng), RNase II (rnb), or polyA polymerase (pcnB). In contrast, RyhB was rarely detected in the double mutants that lacked Hfq and RNase III (rnc).

The *rne* (910-1221aa), *rng*, *pnp*, and *rnb* genes were deleted from the double deletion mutant that lacked Hfq and RNase III to determine which RNases account for the degradation of RyhB1 and RyhB2, respectively (Figure 2). RyhB in the *hfq-rnc-pnp* mutant reached a similar amount of that in the *hfq* mutant, which indicates that PNPase was the main contributor in the degradation of Hfq-free RyhB [14].

The degradation of Hfq-free RyhB by PNPase tends to occur in stationary phase rather than exponential phase in *E. coli* [14]. However, the inactivation of PNPase in this study increased the RyhB levels in *Y. pestis* grown to exponential phase. Therefore, PNPase may degrade the Hfq-free RyhB in different growth-phase-dependent manners in *E. coli* and in *Y. pestis*. However, such discrepancy may also be due
3.2. The RNase-III-Inactivation-Induced mRNA Level Increase of PNPase May Be Partially Responsible for the Degradation of Hfq-Free RyhB. Few amounts of micA could be also detected in the hfq-rnc double mutant of E. coli [14]. Andrade et al. explained this phenomenon as an impairment of RNase III activity that was caused by the decreased duplex in the absence of Hfq. However, this impairment cannot explain the obvious difference in RyhB expression between hfq and hfq-rnc double mutant. RNase III can alter gene expression by cleaving dsRNA or by binding without cleaving RNA [28]. RNase III has been proved to involve in the autoregulation of PNPase in E. coli by cleaving the 5' end of pnp mRNA [29]. However, the unprocessed pnp mRNA is accumulated and can be translated into polynucleotide phosphorylase in E. coli rnc mutant [29]. To determine if the inactivation of RNase III affected the expression of PNPase, quantitative PCR was performed to estimate the relative amounts of pnp mRNA in different mutants (Figure 3). The pnp gene was upregulated from 1.9- to 3.3-fold in hfq-rnc double and triple mutants than in the hfq mutant, which further confirmed that PNPase was the main exoribonuclease responsible for the degradation of Y. pestis RyhB in the absence of Hfq. The RNase-III-inactivation-induced upregulation of PNPase could be partially responsible for the decreased expression of RyhB (Figure 2). However, the effects of RNase III on RyhB stability could not be determined through other means.

3.3. PNPase Activity on RyhB in the Absence of RNase III Is Dependent on the State of Hfq Binding. RNase III affects the stability of the Hfq-dependent sRNA, MicA, in Salmonella [30]. The expression patterns of single and double mutants of rnc and hfq were compared via Northern blotting to examine the effects of RNase III and Hfq inactivation on the rapid degradation of RyhB. RyhB was rarely detected after inactivating both RNase III and Hfq. However, the amount of RyhB could reach modest levels in the rnc and hfq single mutants as well as in the complementary strains that carried the corresponding plasmids. Therefore, the PNPase activity on RyhB in the absence of RNase III depends on the state of Hfq binding (Figure 4). RyhB was rapidly degraded by the increased levels of PNPase in the absence of Hfq because of the RNase III inactivation.

3.4. Rapid Degradation of Hfq-Free RyhB by PNPase Is Independent of the PNPase-Containing RNase E or RhlB Assembly. RyhB1 was rapidly degraded, but RyhB2 retained its stability in the absence of Y. pestis hfq grown in TMH medium [23]. In Y. pestis hfq mutant grown in BHI medium, RyhB1 obtained a 22.8 min half-life whereas RyhB2 obtained a 54.3 min half-life (Figure 5). Although the Hfq-dependent stabilities of Y. pestis RyhB1 and RyhB2 remained different in this study, RyhB1 showed a significantly higher stability in bacterial cells that were grown in rich media (with $a > 20$ min half-life) than
in bacterial cells that were grown in minimal media (with ~8 min half-life). The half-lives of both RyhB1 and RyhB2 exceeded 60 min in a WT strain that was grown exponentially in BHI medium (data not shown), which indicated that the nutrition conditions would influence the stability of \textit{Y. pestis} RyhB in the absence of Hfq.

The half-lives of RyhB in the \textit{hfq-pnp} double mutant were investigated to verify the effects of PNPase on the degradation of Hfq-free RyhB (Figure 5). The stability of RyhB slightly increased in the \textit{hfq-pnp} double mutant rather than in the \textit{hfq} single mutant, which confirmed the role of PNPase in the degradation of Hfq-free RyhB. The \textit{rnc} deletion mutation produced insignificant effects on the stability of RyhB with half-lives of 20.2 min and 49.3 min (Figure 5). However, the 14 min decrease in the half-life of RyhB2 in the \textit{hfq-rne910} double mutant remains unclear. The half-lives of RyhB dramatically reduced to 3.8 min and 6.5 min in the \textit{hfq-rnc} double mutant, whereas the deletion of the \textit{pnp} gene increased the half-life of RyhB to >30 min (Figure 5). Therefore, the RNase-III-induced PNPase increase might be responsible for the RyhB degradation in the absence of Hfq, and the PNPase served as the main enzyme in the degradation of Hfq-free RyhB.

PNPase usually forms multienzyme ribonucleolytic complexes with RNase E and/or RNA helicase RhlB during the degradation of the structured RNA [31, 32]. RNase E serves as a “scaffolding” protein of RNA degradosome that contains the binding sites of three major degradosome components, namely, PNPase, DEAD-box helicase RhlB, and enolase [8, 33]. RhlB facilitates the formation of single stranded RNA, which helps PNPase to engage in the 3’ to 5’ exoribonucleolytic degradation of RNA [15]. PNPase directly interacts with RhlB by forming the transient complex, which is not dependent on the formation of the degradosome [34]. Therefore, this study tries to determine if RNase E degradosome is involved in PNPase activity on Hfq-free RyhB. Given that the deletion of the \textit{rne} gene in the encoding of RNase E is lethal, an \textit{rne} mutant without PNP-binding domain was constructed in this study to produce an RNase E protein that was unassociated with PNPase.

The Northern blotting analysis revealed that the mutation of \textit{rne} and \textit{rhlB} had \(a > 7\) min half-life in the \textit{hfq-rnc} mutant, but its stability was substantially lower than that upon PNPase inactivation (Figure 5). Therefore, the PNPase-containing degradosome or exosome plays minor roles in Hfq-free RyhB decay, and PNPase might be involved in these
processes by itself or through other unknown mechanisms. Therefore, the degradation of Hfq-free sRNAs is far more complex than what was previously expected. An extended analysis should be performed to check if these results could be applied to other sRNAs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Zhongliang Deng and Zizhong Liu contributed equally to this work.

Acknowledgments

This study was funded by the National Natural Science Foundation of China (31171248) and the National Basic Research Program of China (2014CB744405).

References

[1] G. Storz, J. Vogel, and K. M. Wassarman, “Regulation by small RNAs in bacteria: expanding frontiers,” Molecular Cell, vol. 43, no. 6, pp. 880–891, 2011.
[2] T. Morita and H. Aiba, “RNase E action at a distance: degradation of target mRNAs mediated by an Hfq-binding small RNA in bacteria,” Genes and Development, vol. 25, no. 4, pp. 294–298, 2011.
[3] K. J. Bandyra, N. Said, V. Pfeiffer, M. W. Gorna, J. Vogel, and B. F. Luisi, “The seed region of a small RNA drives the controlled destruction of the target mRNA by the endoribonuclease RNase E,” Molecular Cell, vol. 47, pp. 943–953, 2012.
[4] K. Prévost, G. Desnoyers, J.-F. Jacques, F. Lavoie, and E. Massé, “Small RNA-induced mRNA degradation achieved through both translation block and activated cleavage,” Genes and Development, vol. 25, no. 4, pp. 385–396, 2011.
[5] A. J. Carpousis, “Degradation of targeted mRNAs in Escherichia coli: regulation by a small antisense RNA,” Genes and Development, vol. 17, no. 19, pp. 2351–2355, 2003.
[6] E. Massé, F. E. Escorcia, and S. Gottesman, “Coupled degradation of a small regulatory RNA and its mRNA targets in Escherichia coli,” Genes and Development, vol. 17, no. 19, pp. 2374–2383, 2003.
[7] A. J. Carpousis, “The RNA degradosome of Escherichia coli: an mRNA-degrading machine assembled on RNase E,” Annual Review of Microbiology, vol. 61, pp. 71–87, 2007.
[8] J. A. R. Worrall, M. Gorna, N. T. Crump et al., “Reconstitution and analysis of the multienzyme Escherichia coli RNA degradosome,” Journal of Molecular Biology, vol. 382, no. 4, pp. 870–883, 2008.
[9] I. Moll, T. Afnonyushkin, O. Vytvytska, V. R. Kaberdin, and U. Bläsi, “Coincident Hfq binding and RNase E cleavage sites on mRNA and small regulatory RNAs,” RNA, vol. 9, no. 11, pp. 1308–1314, 2003.
[10] K. S. Frohlich, K. Papenfort, A. Fekete, and J. Vogel, “A small RNA activates CFA synthase by isoform-specific mRNA stabilization,” The EMBO Journal, vol. 32, pp. 2963–2979, 2013.
[11] M. Folichon, V. Arluison, O. Pellegrini, E. Huntzinger, P. Régnier, and E. Hajnsdorf, “The poly(A) binding protein Hfq protects RNA from RNase E and exoribonucleolytic degrada-
tion,” Nucleic Acids Research, vol. 31, no. 24, pp. 7302–7310, 2003.
[12] Y. Ikeda, M. Yagi, T. Morita, and H. Aiba, “Hfq binding at RhlB-recognition region of RNase E is crucial for the rapid degradation of target mRNAs mediated by sRNAs in Escherichia coli,” Molecular Microbiology, vol. 79, no. 2, pp. 419–432, 2011.
[13] N. de Lay and S. Gottesman, “Role of polynucleotide phosphorylase in sRNA function in Escherichia coli,” RNA, vol. 17, no. 6, pp. 1172–1189, 2011.
[14] J. M. Andrade, V. Pobre, A. M. Matos, and C. M. Arraiano, “The crucial role of PNIPase in the degradation of small RNAs that are not associated with Hfq,” RNA, vol. 18, no. 4, pp. 844–855, 2012.
[15] P.-H. Lin and S. Lin-Chao, “RhlB helicase rather than enolase is the β-subunit of the Escherichia coli polynucleotide phospho-
rylase (PNPase)-exoribonucleolytic complex,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 46, pp. 16590–16595, 2005.
[16] A. Fender, J. Elf, K. Hampel, B. Zimmermann, and E. G. H. Wagner, “RNAs actively cycle on the Sm-like protein Hfq,” Genes and Development, vol. 24, no. 23, pp. 2621–2626, 2010.
[17] K. Moon and S. Gottesman, “Competition among Hfq-binding small RNAs in Escherichia coli,” Molecular Microbiology, vol. 82, no. 6, pp. 1545–1562, 2011.
[18] A. J. Carpousis, “The Escherichia coli RNA degradosome: structure, function and relationship in other ribonucleolytic multienzyme complexes,” Biochemical Society Transactions, vol. 30, no. 2, pp. 150–155, 2002.
[19] G.-G. Liou, H.-Y. Chang, C.-S. Lin, and S. Lin-Chao, “Dead box RhlB RNA helicase physically associates with exoribonuclease PNPase to degrade double-stranded RNA independent of the degradosome-assembling region of RNase E,” Journal of Biological Chemistry, vol. 277, no. 43, pp. 41157–41162, 2002.
[20] E. Massé and S. Gottesman, “A small RNA regulates the expression of genes involved in iron metabolism in Escherichia coli,” Proceedings of the National Academy of Sciences of the United States of America, vol. 99, no. 7, pp. 4620–4625, 2002.
[21] T. A. Geissmann and D. Touati, “Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator,” The EMBO Journal, vol. 23, no. 2, pp. 396–405, 2004.
[22] G. Padalou-Brauch, R. Hershberg, M. Elgrably-Weiss et al., “Small RNAs encoded within genetic islands of Salmonella typhimurium show host-induced expression and role in virulence,” Nucleic Acids Research, vol. 36, no. 6, pp. 1913–1927, 2008.
[23] Z. Deng, X. Meng, S. Su et al., “Two sRNA RyhB homologs from Yersinia pestis biovar microtus expressed in vivo have differential Hfq-dependent stability,” Research in Microbiology, vol. 163, pp. 413–418, 2012.
[24] D. Zhou, Z. Tong, Y. Song et al., “Genetics of metabolic variations between Yersinia pestis biovars and the proposal of a new biovar, microtus,” Journal of Bacteriology, vol. 186, no. 15, pp. 5147–5152, 2004.
[25] J. Geng, Y. Song, L. Yang et al., “Involvement of the post-transcriptional regulator Hfq in Yersinia pestis virulence,” PLoS ONE, vol. 4, no. 7, Article ID e6213, 2009.
[26] A. Leroy, N. F. Vanzo, S. Sousa, M. Dreyfus, and A. J. Carpousis, “Function in Escherichia coli of the non-catalytic part of RNase E: role in the degradation of ribosome-free mRNA,” Molecular Microbiology, vol. 45, no. 5, pp. 1231–1243, 2002.
[27] B. M. Beckmann, A. Grünweller, M. H. W. Weber, and R. K. Hartmann, "Northern blot detection of endogenous small RNAs (∼14 nt) in bacterial total RNA extracts," *Nucleic Acids Research*, vol. 38, no. 14, p. e147, 2010.

[28] D. Drider and C. Condon, "The continuing story of endoribonuclease III," *Journal of Molecular Microbiology and Biotechnology*, vol. 8, no. 4, pp. 195–200, 2005.

[29] C. Portier, L. Dondon, M. Grunberg-Manago, and P. Régnier, "The first step in the functional inactivation of the Escherichia coli polynucleotide phosphorylase messenger is a ribonuclease III processing at the 5’ end," *The EMBO Journal*, vol. 6, no. 7, pp. 2165–2170, 1987.

[30] S. C. Viegas, I. J. Silva, M. Saramago, S. Domingues, and C. M. Arraiano, "Regulation of the small regulatory RNA MicA by ribonuclease III: a target-dependent pathway," *Nucleic Acids Research*, vol. 39, no. 7, pp. 2918–2930, 2011.

[31] V. R. Kaberdin, D. Singh, and S. Lin-Chao, "Composition and conservation of the mRNA-degrading machinery in bacteria," *Journal of Biomedical Science*, vol. 18, no. 1, article 23, 2011.

[32] I. J. Silva, M. Saramago, C. Dressaire, S. Domingues, S. C. Viegas, and C. M. Arraiano, "Importance and key events of prokaryotic RNA decay: the ultimate fate of an RNA molecule," *Wiley Interdisciplinary Reviews*, vol. 2, no. 6, pp. 818–836, 2011.

[33] L. Dominguez-Malfavon, L. D. Islas, B. F. Luisi, R. Garcia-Villegas, and J. Garcia-Mena, "The assembly and distribution in vivo of the Escherichia coli RNA degradosome," *Biochimie*, vol. 95, no. 11, pp. 2034–2041, 2013.

[34] S. Lin-Chao, N.-T. Chiou, and G. Schuster, "The PNPase, exosome and RNA helicases as the building components of evolutionarily-conserved RNA degradation machines," *Journal of Biomedical Science*, vol. 14, no. 4, pp. 523–532, 2007.