Control of RNA Stability for Gene Function

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There are many RNA molecules in *Escherichia coli*. Most of them are ribosomal RNAs and constitute about 80% of total cellular RNA. tRNA ranks second with 15% and mRNA constitutes only about 5%. It is generally known that rRNA and tRNA are stable, while mRNAs are unstable. However, stabilities of mRNAs differ one another with half-lives of less than 1 min to more than 15 min. Therefore, control of RNA stability is directly related to gene function. RNA stability depends on how much RNA molecules escape from degradation. Some key steps for RNA degradation have been suggested, which include RNase E cleavage (in degradosome), poly(A)-dependent degradation, removal of pyrophosphate at the 5' end, and interaction with small regulatory RNA. However, there is still a controversy on the key steps for RNA degradation. We took an advantage of the fact that M1 RNA is a metabolically stable RNA in order to more systematically evaluate the key steps. We questioned why stable RNAs are stable in the cell and how they escape from degradation. To answer the question, we used M1 RNA as a model RNA molecule. M1 RNA is a catalytic component of RNase P. RNase P is a tRNA processing enzyme that cleave 5' extra sequence of precursor tRNA to generate the mature 5' end of tRNA. RNase P is a ribonucleoprotein composed of M1 RNA as a catalytic component and C5 protein as a protein cofactor. Although M1 RNA itself cleaves precursor tRNAs without C5 protein *in vitro*, both M1 RNA and C5 protein are essential for cell viability. *In vitro* and *in vivo* studies indicate that the major primary transcript from the *rnpB* gene is the one initiating form the nearest promoter, P-1, and terminating at the first terminator, T1. This primary transcript, named precursor M1 RNA (pM1 RNA), carries an extra stretch of 36 nucleotides at the 3′ end. These extra nucleotides are removed by a 3′ processing event. This reaction is initiated with cleavage by RNase E. However, the biological significance of this reaction in bacterial metabolism remained obscure. We set out to determine why cells need 3′ processing of M1 RNA. We analyzed bacterial strains carrying mutations in the *rne*-dependent site of their *rnpB* genes and show that the 3′ processing of M1 RNA is also essential for cell viability. Furthermore, we demonstrate that pM1 RNA undergoes poly(A)-dependent degradation if it is not processed at the 3′ end. Hence, 3′ processing of M1 RNA provides a functional mechanism for the protection of the primary transcript against degradation [1]. In addition, since this 3′ processing reaction is initiated by RNase E, this enzyme therefore plays a role in the conversion of unstable pM1 RNA molecules into a stable form. In addition to pM1 RNA, large *rnpB*-containing transcripts are produced from unknown upstream promoters. However, it was unclear how these large transcripts contribute to M1 RNA biosynthesis. To examine their biological relevance to M1 RNA biosynthesis, we constructed a model

upstream transcript, upRNA, and analyzed its cellular metabolism. We found that upRNA was primarily degraded rather than processed to M1 RNA in the cell and that this degradation occurred in RNase E-dependent manner. The *in vitro* cleavage assay with RNase E showed that the M1 RNA structural sequence in upRNA was much more vulnerable to the enzyme than the sequence in pM1 RNA [2]. Considering that RNase E is a processing enzyme involved in 3′ end formation of M1 RNA, our results imply that this enzyme plays a dual role in processing and degradation to achieve tight control of M1 RNA biosynthesis. Meanwhile, C5 protein functions as a cofactor in the RNase P reaction. It stabilizes the catalytically active conformation of M1 RNA and modulates substrate specificity in RNase P reaction. We examined whether C5 protein plays a role in maintaining metabolic stability of M1 RNA. The sequestration of C5 protein available for M1 RNA binding reduced M1 RNA stability *in vivo*, and its reduced stability was recovered via overexpression of C5 protein. In addition, M1 RNA was rapidly degraded in a temperature-sensitive C5 protein mutant strain at non-permissive temperatures [3]. These results demonstrate that C5 protein also plays an essential role in stabilizing M1 RNA in the cell.

References

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