NOVEL FUNCTION OF RNASE P PROTEIN: C5 PROTEIN IS ESSENTIAL FOR MAINTAINING STABILITY OF M1 RNA IN ESCHERICHIA COLI

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INTRODUCTION. RNase P is a processing enzyme involved in 5′ processing of tRNAs by removing the 5′ leader sequence. The Escherichia coli RNase P holoenzyme is composed of two subunits: a large RNA subunit (M1 RNA, 377 nucleotides), and a small basic protein (C5 protein, 119 amino acids). Previous studies of RNase P have focused on the roles of M1 RNA and C5 protein as a ribozyme and a cofactor in RNase P catalysis, respectively (1,2). However, in vivo functions of each subunit remain to be fully elucidated. Here we examined the role of C5 protein in maintaining the in vivo stability of M1 RNA.

METHODS. In vivo stability of M1 RNA was determined in cells lacking C5 protein for the formation of the RNase P holoenzyme. Total cellular RNAs were prepared at intervals after the addition of rifampicin and they were subjected to northern analysis.

RESULTS. Overexpression of M1 RNA previously showed its apparent degradation in the cell (3). This degradation might be due to the absence of C5 protein for overexpressed M1 RNA. To determine whether C5 protein would be involved in M1 RNA stability, we set an experimental strategy for making M1 RNA naked in the cell. By overexpressing truncated M1 RNAs in the cells, we depleted C5 protein to otherwise be assembled into RNase P. We constructed C5 protein-interacting derivatives by deleting P8/9 or P12, and a non-interacting derivative by deleting P3. Overexpression of P8/9- or P12-deletion derivatives reduced the M1 RNA stability, but P3-deletion derivative did not. The reduced M1 RNA stability was recovered by overexpressing C5 protein. We also examined the M1 RNA stability in a temperature-sensitive C5 protein mutant strain. M1 RNA was rapidly degraded in this mutant strain at the nonpermissive temperature, suggesting that functional C5 protein is essential for M1 RNA stability. Collectively, our data demonstrate that the C5 protein functions to maintain the in vivo stability of the M1 RNA.

DISCUSSION. Our study shows that the C5 protein functions as a regulator of M1 RNA biogenesis besides the well-known functions as a cofactor in the RNase P catalytic reaction. From our present and previous findings (4), we propose the strategies of E. coli cells to maintain C5 protein as a stable RNA. An primary rnpB transcript, pM1 RNA, is unstable with a half-life of about 5 min. Processing at the 3′ end increases the stability of M1 RNA, but not much with a half-life of about 10 min. Finally, the processed product, M1 RNA, binds to C5 protein and forms the RNase P holoenzyme to become a stable RNA with a half-life of about 60 min (Fig. 1).
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**REFERENCES.**