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Analysis of the Role of *E. coli* RNA-binding Protein Hfq in the Regulatory Mechanism of Gene Expression

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Research aims

The endoribonuclease RNase E, encoded by an essential gene *rne*, plays a major role in cellular RNA metabolism, i.e., maturation of functional RNAs such as rRNA and tRNA, degradation of many mRNAs and processing of *ftsZ* mRNA encoding essential cell division protein FtsZ. RNase E function is somehow regulated by the RNA binding protein Hfq. In this study, we investigate the role of Hfq in intracellular RNA metabolism catalyzed by RNase E.

Methods

E. coli K-12 strain HAT100 [F^- *ara* Δ(*lac-pro*) *thi zce-726::Tn10*] and its derivatives HAT103 (the same as HAT100 but *rne-1*), and HAT113 (the same as HAT100 but *rne-1 hfq10::cat*) were used in this study. Fully grown *E. coli* cultures were serially diluted with 0.85% NaCl and spotted onto L agar plates. Plates were incubated at 30°C, 40.5°C and 42°C. Total cellular RNA was isolated and analyzed by the modified agarose gel electrophoresis and Northern hybridization. FtsZ protein was detected by using polyclonal anti-FtsZ antiserum (mouse) as first antibody and alkaline phosphatase-conjugated anti-mouse IgG antibody as second antibody.

Results

The temperature-sensitive *rne-1* mutant strain HAT103 showed temperature-sensitive growth at 40.5°C or higher . Unexpectedly, colony-forming ability of HAT103 was suppressed by introduction of the *hfq::cat* mutation, almost completely at 40.5°C and partially even at 42°C (Figure). This suggests that Hfq protein is involved in essential reaction(s) catalyzed by RNase E.

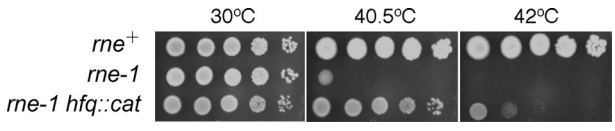

Figure. Suppression of temperature-sensitivity of *rne-1* mutant by *hfq::cat* mutation

RNase E is responsible for maturation of functional RNAs. We therefore examined effects of the *hfq::cat* mutation on the maturation of 16S rRNA, 5S rRNA and tRNA. In the rne^+ control, the processing of these RNA molecules occurred completely. In the *rne-1* strain, precursor molecules of 16S rRNA, 5S rRNA and $tRNA^{Phe}$ were detected at 40.5°C and it became accumulated more at 42°C. Introduction of *hfq::cat* mutation showed almost no effect on the maturation of these RNA molecules. These results showed that *hfq::cat* did not recover the maturation of 16S rRNA, 5S rRNA and $tRNA^{Phe}$ affected by *rne-1* mutation.

We then analyzed *ftsZ* transcripts in rne^+, *rne-1*, and *rne-1 hfq::cat* strains by Northern hybridization. Unprocessed precursor transcripts were detected in the *rne-1* mutant strain at both 40.5°C and 42°C. Introduction of *hfq::cat* mutation did not recover the processing of *ftsZ* mRNA, as in the cases of the maturation of 16S rRNA, 5S rRNA and $tRNA^{Phe}$. We also examined the synthesis of FtsZ by Western blotting using anti-FtsZ antibody. In the *rne-1* mutant strain, the amount of FtsZ protein decreased remarkably at 40.5°C and 42°C as reported previously. In the *rne-1 hfq::cat* mutant strain, it was recovered to the almost similar levels of wild-type cells. These results indicate that *hfq::cat* mutation recovered translation of FtsZ protein from unprocessed precursor transcripts without recovering processing of *ftsZ* mRNA.

Conclusion

We found that the temperature-sensitive growth of the *rne-1* mutant strain was suppressed by introduction of the *hfq::cat* mutation. The *hfq::cat* mutation did not affect the maturation of functional RNAs such as 16S rRNA, 5S rRNA and tRNA, and the processing of *fstZ* mRNA, but recovered the synthesis of FtsZ protein form unprocessed precursor mRNA. These results suggest that inviability of RNase E deficiency is due to a decreased level of the cell division protein FtsZ. The post-transcriptional regulation by RNase and RNA-binding protein demonstrated in this study could be applied for the protein expression systems in combination with the conventional transcriptional regulations.