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Functional Interaction between Small Noncoding RNAs in *Escherichia coli*

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Small noncoding RNAs (sRNAs) with a variety of regulatory functions have been found from bacteria to mammals in recent years. In *Escherichia coli*, about 100 sRNA molecules have been identified and expression of some sRNAs is involved in the responses to certain stress conditions. Although studies have attempted to characterize the role of sRNAs in *E. coli*, biological functions of most sRNAs are still unknown. In this study, we tried to identify functional relations between 6S RNA and RygC RNA in *E. coli*. The *rygC* gene is located downstream of the *ssrS* gene encoding 6S RNA and *in vitro* transcription of *ssrS* extends to *rygC.* 6S RNA bind to RNA polymerase (RNAP) σ^{70}-holoenzyme (E^{70}) and reduces its activity, making it possible to alter the utilization of E^{70} to σ^{S}-holoenzyme (E^{S}) in the stationary phase of growth. Synthesis of 6S RNA highly increases during stationary phase. 6S RNA not only represses transcription from some σ^{70}-dependent promoters but also activates certain σ^{S}-dependent promoters. The highly conserved 6S RNA structure resembles DNA templates in an open promoter complex so that it might bind E^{70} by mimicking DNA promoters. Actually it has shown that 6S RNA itself can act as a template for transcription of specific small RNA molecules. The presence of precursor 6S RNA molecules, with six to eight additional bases at the 5' terminus, was previously described, indicating that 6S RNA is derived from a larger primary transcript. In addition, 6S RNA can be synthesized as a transcript containing an open reading frame (*ygfA*) and the RNA gene *rygC* at the 3' end. Given that 6S RNA functions as a regulator of the modulation for E^{70} activity, biogenesis of 6S RNA should be regulated according to the cellular requirements for E^{70} activity. However, the mechanisms underlying this regulation remain obscure, mainly due to the lack of knowledge of 6S RNA biosynthesis.

To further understand the regulatory circuit of 6S RNA biosynthesis, we have characterized the biogenesis of 6S RNA. We found that 6S RNA is transcribed from two tandem promoters, designated P1 and P2, which are proximal and distal to the mature 6S RNA sequence, respectively. P1 is a canonical σ^{70}-dependent promoter, whilst P2 is both a σ^{70}- and a σ^{S}-dependent promoter. Hence, transcription of

6S RNA can be regulated by switching σ factors for the formation of specific RNAP holoenzymes, in response to environmental signals. We also found that the transcripts that are generated by these two promoters are differentially processed at their 5' ends by RNase E and RNase G. The P2 transcript is processed exclusively by RNase E, whereas the P1 transcript is processed by both RNase E and RNase G. The 5' processing of the P1 transcript is also far less rapid when compared to the long precursor. Hence, the two primary transcripts differentially contribute to the synthesis of *E. coli* 6S RNA via different processing enzymes and with differing efficiencies. As a result of this biosynthetic pathway, the generation of 6S RNA can be regulated via the coupled action of sigma factors and endoribonucleases.

RygC was previously identified as about 140-nt RNA, which is encoded by *rygC* located in the downstream region of *ssrS-ygfA*. Interestingly, RygC RNA shows a significant sequence similarity with other four sRNAs, RygD, RygE, RyeC, and RyeD, whose genes reside on different chromosome loci. RygC is highly expressed during stationary phase like 6S RNA. We identified a proximal *rygC* promoter through *in vitro* and *in vivo* transcriptional analyses. Therefore, RygC can be synthesized both by extensions from 6S RNA transcription and by transcription from the proximal promoter. The proximal promoter initiates transcription by both E^{70} and E^{S}. We found the presence of two RygC molecules of 141 nt and 109 nt in the cell. It is likely that the 109-nt RNA is derived from the 141-nt RNA by processing of 3′ end. The *rygC*-knockout strain did not show any mutant phenotype, but we found that RygC-overexpressing cells enter the stationary phase earlier than the control cells. This growth defect was restored by co-overexpression of 6S RNA. Proteomic analysis showed that the RygC overexpression causes the increase of PspA, a phage shock protein, in the cell. The northern analysis and *lacZ*-transcriptional fusion analysis showed that the cellular increase of PspA results from transcriptional activation of the *pspA* operon. This transcriptional activation requires PspF, a previously known transcription factor for *pspA* transcription. We found that co-overexpression of 6S RNA nullified the increase of the *pspA* expression. Collectively, our results suggest that function of RygC is reciprocally related to that of 6S RNA. Recently it has been shown that the 6S RNA mutation leads to activation of *pspF* transcription, which consequently results in increase of *pspA* expression. The fact that *pspA* expression is increased by either 6S RNA mutation or RygC overexpression also provides additional evidence for the reciprocal relationship of functions between 6S RNA and RygC.

References

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