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Mechanisms of RNA Silencing Mediated by Hfq-binding Small RNAs in *Escherichia coli*

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Regulation of gene expression by small RNAs (sRNAs) has been known for many years in *Escherichia coli* since the serendipitous discovery of MicF, which is involved in the down-regulation of the expression of *ompF*. The characterization of several additional sRNAs such as DsrA and OxyS, also found fortuitously, along with the identification of a large number of sRNAs by systematic searches, have shown that sRNAs are widely involved in the regulation of gene expression primarily at post-transcriptional levels. Although the biological functions of many of the newly identified sRNAs remain to be elucidated, it is becoming clear that a major class of *E. coli* sRNAs bind to an RNA chaperone Hfq, and act by imperfect base pairing to regulate mRNA function under specific stress conditions. I will discuss on the mechanisms of action of Hfq-binding sRNAs focusing on newly emerging features that have been uncovered by the study on one particular sRNA, SgrS that down-regulates target *ptsG* mRNA under a metabolic stress.

SgrS is induced in response to accumulation of glucose-phosphate resulting in destabilization of the *ptsG* mRNA encoding the glucose transporter $IICB^{Glc}$ in an RNase E-dependent fashion. The destabilization of *ptsG* mRNA is dependent on Hfq and the C-terminal scaffold region of RNase E. An important discovery derived from the study of SgrS is that Hfq associates with RNase E through its scaffold region, and SgrS associates with RNase E through Hfq. In other words, RNase E forms a ribonucleoprotein complex with SgrS through Hfq. The RNase E-based ribonucleoprotein complex containing SgrS and Hfq is distinct from the conventional RNA degradosome consisting of RNase E, PNPase, RhlB RNA helicase, and a glycolytic enzyme enolase. The physical association of SgrS/Hfq with RNase E explains for how the functional cooperation of SgrS-Hfq and RNase E is achieved.

The synthesis of $IICB^{Glc}$ can be efficiently prevented by SgrS even in cells expressing the C-terminally truncated RNase E in which the destabilization the *ptsG* mRNA no longer occurs. Thus, translational repression occurs without the RNase E-dependent degradation of target mRNAs, indicating that SgrS down-regulates the $IICB^{Glc}$ expression primarily by inhibiting translation, and the RNase E-dependent

rapid degradation of *ptsG* mRNA is not necessary for the *ptsG* down-regulation. The physiological relevance of the RNase E-dependent rapid degradation of target mRNAs would be to make gene silencing irreversible and to ensure an effective turnover of mRNAs by eliminating translationally inactive mRNAs.

A systematic mutational study established that crucial base pairs for SgrS action are confined to the 6-nt region overlapping the Shine-Dalgarno (SD) sequence of *ptsG* mRNA. In particular, two single mutations in either *ptsG* mRNA or SgrS disrupting a G-C base pair within this short sequence completely eliminated SgrS regulation, and compensatory mutations restored it. Correspondingly, the duplex formation *in vitro* was eliminated by a single mutation that disrupted the C-G base pair within the crucial region, and was restored by the compensatory mutation. Thus, the six base pairs overlapping the *ptsG* SD sequence among the predicted base pairs are important for SgrS action. The requirement for base pairing between SgrS and *ptsG* at a short sequence overlapping the *ptsG* SD sequence is consistent with the view that SgrS competes with and prevents the binding of 16S rRNA of the ribosome resulting in translational inhibition.

The duplex formation between the *ptsG* mRNA and SgrS proceeds slowly without Hfq but very rapidly in the presence of Hfq *in vitro*. The rapid association of SgrS with the *ptsG* mRNA in the presence of Hfq would be essential for SgrS to compete with the ribosome entry to the *ptsG* mRNA. In addition, we have successfully reconstituted the *ptsG* silencing by SgrS/Hfq in by using PURESYSTEM.

Another intriguing finding is that that the region corresponding to the first two transmembrane stretches of *ptsG* is required for the rapid degradation of *ptsG* mRNA, suggesting that that localization of target mRNA at the membrane is an important factor SgrS action. The membrane localization of *ptsG* mRNA somehow reduces the efficiency of ribosome loading for subsequent rounds of translation. This can help SgrS RNA to act efficiently on the *ptsG* mRNA, by competing with ribosome entry, resulting in the RNase E-dependent degradation of the *ptsG* mRNA. In other words, translational status (efficiency of ribosome loading) is an important factor that affects sRNA action on target mRNAs.

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