„Role of small RNA-GraL, in the gene expression of Escherichia coli""
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Small RNAs are molecules involved in the expression of multiple genes in bacterial cells. They have multiple different modes of action, but commonly they work by direct interaction with their target mRNA. To work correctly, many of them require the Hfq protein. sRNA influence bacterial response to oxidative stress, membrane stress or iron deficiency. They are also involved in control of such processes as: virulence, carbon metabolism or *quorum sensing*. GraL is a small RNA encoded in the leader sequence of *Escherichia coli greA* gene and can possibly act *in cis* and *in trans*. It is produced as a result of an early termination of the *greA* transcript. GreA protein is a transcriptional factor, that increase transcription fidelity and prevent pauses of the RNA polymerase transcription elongation complex. The *greA* gene is autoregulated- overproduction of GreA protein *in trans* causes a decrease in *greA* gene expression.

The aim of this work was to elucidate GraL's role in a bacterial cell. Fusions of 13 putative GraL targets with the β-galactosidase gene were constructed. Changes in their activity at different cellular concentrations of GraL were examined. The most probable molecular target of GraL turned out to be the *nudE* gene, which encodes a Nudix hydrolase. However, the β-galactosidase activity of the *nudE-lacZ* fusion also changes in response to changes in the GreA level. To confirm GraL- *nudE* mRNA interactions, *in vitro* EMSA assays were conducted. GraL binds to Hfq, but is easily displaced by *nudE* mRNA from such a complex. In conclusion, *nudE* gene expression is most probably controlled by both GraL and GreA.

Another step in this work was to elucidatie GraL's role *in cis*. Using transcriptional gene fusions of selected fragments of *greA*'s promoter and leader sequences with the β-galactosidase gene, it was determined that the GraL sequence is necessary and sufficient for *greA* gene expression autoregulation, i.e it is independent of the promoter sequence. Moreover, for this *in cis* GraL activity the Hfq protein is not required. Additionally, using Northern blots, level of GraL was determined depending on different GreA cellular concentrations. It was revealed that the GreA protein is necessary for GraL production, which level increases when GreA is overproduced *in trans*. That implies that the *greA* gene expression is controlled at the GraL termination step. Further experiments solidified the idea that the GraL terminator sequence plays a major role in *greA* gene autoregulation, however, secondary structure of the terminator is also important.

Additional search for factors altering *greA* expression was conducted. It was executed by construction of random kanamycin transposon genomic libraries. Results suggest that *greA*/GraL expression is affected by disturbance in the expression of the genes involved in cell membrane metabolism, cell envelope stress response, NAD metabolism and cell redox potential.