IMPRS on Multiscale Biosystems

Project description

Title: **The role of thiolated tRNAS on translation efficiency in *Escherichia coli***

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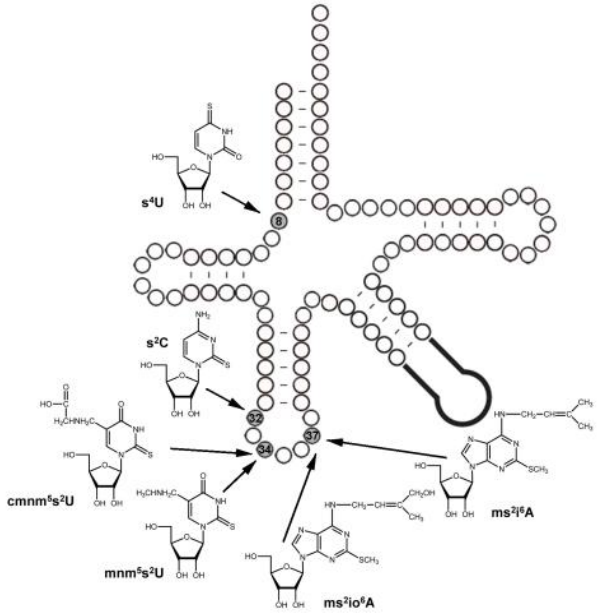
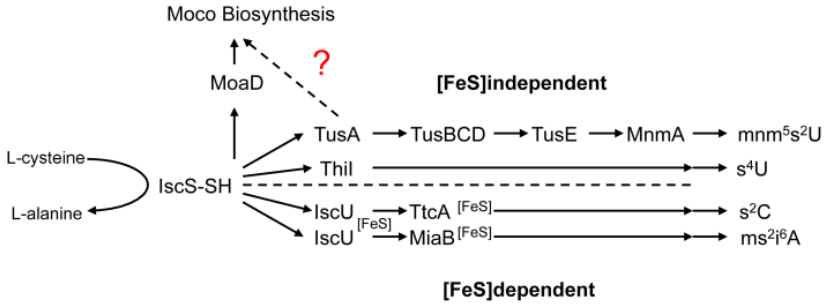
In collaboration with: Dr Angelo Valleriani

Project description: Sulfur is an important element in all living cells, incorporated into proteins not only in form of cysteine and methionine, but also as iron sulfur clusters (FeS), sulfur-containing cofactors and vitamins, and into RNA through a variety of modifications. Sulfur is delivered to these various biosynthetic pathways by complex processes, involving successive transfer of sulfur as persulfide between multiple proteins. One sulfur modification includes the 2-thiouridine formation at the wobble position 34 of tRNA $tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$ in *Escherichia coli*. So far, little is known about the function of this modification. It has been proposed that thiomodified $tRNA^{Lys}$ confers efficient ribosome binding and 2-thiomodified $tRNA^{Glu}$ is required for specific recognition by glutaminyl-tRNA synthetase. This implies that the 2-thiomodification of uridine 34 plays a critical role in the decoding mechanism. Also, a slower growth phenotype was reported for *E. coli* cells with impairment in genes for tRNA thiolation. In *E. coli*, the modification 2-thiouridine of 5-methylaminomethyl-2-thiouridine (mnm^5s^2U) was shown to be catalyzed by TusA, TusB, TusC, TusD, TusE, MnmA and IscS. The initial sulfur mobilization step is catalyzed by IscS forming a protein-bound persulfide after conversion of L-cysteine to L-alanine. The TusA protein was identified to function as a sulfur mediator from IscS for the synthesis of 2-thiouridine, however, it is also involved in other sulfurtransferring pathways. TusA transfers the sulfur to TusD in the TusBCD complex, which then interacts with TusE. MnmA is the final sulfur accepting protein which binds and adenylates the tRNA and makes it thus susceptible for sulfur.





In this project, we want to analyze the role of tRNA thiolation for *E. coli* further. The respective candidate is expected to experimentally analyze the levels of tRNA in *E. coli* in respect to growth conditions and external factors like oxidative stress. The protocol for the quantification of thiolated tRNA is established in the laboratory. Furthermore, in defined *in vitro* systems we will first observe and quantify the enzymatic dynamics that involves different competing pathways of tRNA thiolation. In a second step, we will use a purified *in vitro* translation system to analyze the role of thiolated tRNAs on the translation efficiency by determining changes in the rate of translation. Also, the role of thiolated tRNAs on programmed ribosomal frameshifting will be investigated. The detection of changes in the total pool of thiolated tRNAs in the cell will shed light on its importance for translation and cell viability, which is also important for eukaryotes. Thus, *E. coli* will be used as a model system for the adaptation of the role of thiolated tRNA in higher eukaryotes. The experiments will be conceived in collaboration with Dr Valleriani, who will then use the data to establish a predictive mathematical model of translational control via tRNA thiolation.

Required background: Biochemistry, molecular biology and protein biochemistry methods, cloning, site directed mutagenesis, purification of DNA and RNA

Paper to read before the interview: Maynard ND, Macklin DN, Kirkegaard K, Covert MW (2012) *Competing pathways control host resistance to virus via tRNA modification and programmed ribosomal frameshifting*, Mol Sys. Biol. **8: 567**

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