Healthcare industry BW

An RNA switch for protein mutations

RNA is a family of biological molecules with multiple roles, including the transmission of information and the catalysis of chemical reactions in a similar way to enzyme action. Ribozymes (ribonucleic acid enzymes) of this kind function for example within the ribosome where they link amino acids during protein synthesis. Professor Jörg Hartig from the University of Konstanz has developed a new ribozyme-based method that enables him to control the incorporation of specific amino acids during translation. The use of RNA switches, so-called riboswitches, have several advantages over traditional methods in controlling gene expression.

Proteins are normally characterised on the basis of mutant proteins which provide researchers with information about the role of individual amino acids for the function of the entire protein. Up until now, researchers had to introduce specific mutations into proteins and transfer each variant into a model organism in order to elucidate the function of a protein under investigation. Jörg Hartig, professor of biopolymer chemistry at the University of Konstanz, has now shown that there are easier ways of characterising proteins.

He has developed an RNA-based toolbox that enables him to exchange amino acids during translation when they are linked to each other in the ribosome, thereby affecting the composition of proteins on the post-transcriptional level. Hartig's tools are riboswitches that are able to control the expression of genes on the translational level. "Riboswitches are mRNA-based elements that regulate gene expression via binding of a specific ligand or effector. We can create protein variants without having to change the underlying gene sequence," Hartig explained.

Riboswitches are RNA elements that are encoded within the transcript (mRNA) they regulate; they are located directly upstream of the mRNA to be translated. Most known riboswitches occur in bacteria. They are not translated into amino acids and are generally divided into two parts, an aptamer domain and an expression platform. The aptamer domain is a short RNA segment that is able to form a special 3D structure and bind to a specific target molecule, tetracycline for example. Binding to the small molecule ligand leads to changes in the aptamer, resulting in structural changes in the expression platform, thereby regulating the expression of genes. For example, a conformational change in the expression platform might cover up the ribosome binding site, thereby preventing the translation of the respective gene.

Designer tRNA key to the exchange of amino acids

Prof. Jörg Hartig from the University of Konstanz has developed a new RNA tool with which he can control gene expression during translation.
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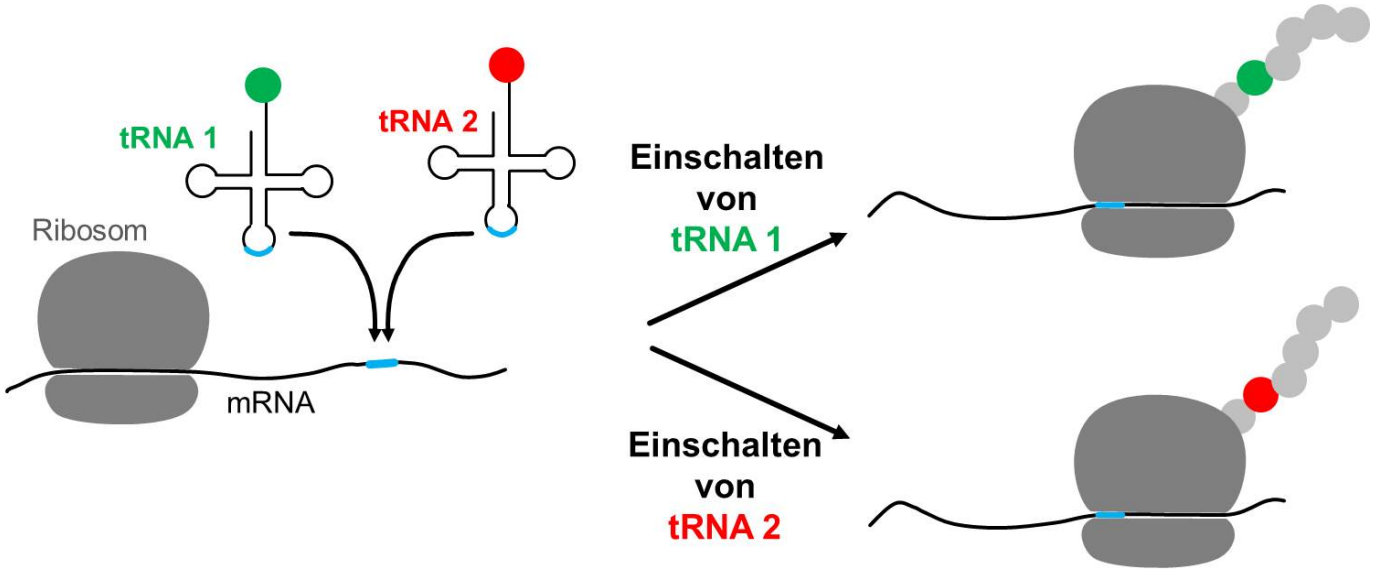
Jörg Hartig and his research group have come up with an even more sophisticated system and transferred the principle to tRNA molecules. “We have linked a ribozyme with a tRNA in such a way that interferes with the normal folding of the tRNA, thereby preventing the use of this particular

tRNA," Hartig explains. Induction of the riboswitch and hence cleavage of the tRNA leads to the separation of the ribozyme from the tRNA, which can then carry a specific amino acid to the ribosome.

"The tRNA is switched on upon the addition of a small effector molecule to the growth medium," Hartig further explains. The thus regulated tRNAs are so-called amber suppressor tRNAs that contain a mutation in the anti-codon loop that suppresses an amber stop codon (UAG) and thus reads through it, resulting in the incorporation of an amino acid in the growing protein chain. "With our tRNA switches we can determine whether translation stops at the stop codon or whether a specific amino acid is incorporated," said Hartig explaining how their system works.

The incorporation of specific amino acids in E. coli only requires a plasmid-encoded tRNA switch system consisting of an aptamer, a ribozyme and an amber suppressor tRNA. If several tRNA switches with different aptamer domains are used simultaneously, the addition of different effector molecules enables the researchers to control different tRNAs and hence control the incorporation of different amino acids. "We can synthesise different proteins by using different combinations of effector molecules: the addition of effector 1 enables the incorporation of amino acid 1, the addition of effector 2 enables the incorporation of amino acid 2, and so on. The addition of both effectors simultaneously leads to a mixture of different protein variants," said the biochemist. If no effector is added, then translation stops at the first stop codon, resulting in a shorter protein.



Schematic showing the post-transcriptional creation of protein variants by controlling designer tRNAs with RNA switches. © Hartig

Applications ranging from protein design to virus control

In general, RNA switches are not only interesting for biotechnological and synthetic biology applications that focus on the design of specific proteins. They also open up completely new gene regulation possibilities in systems where conventional approaches do not function adequately.

One example of this is the use of RNA switches for controlling gene expression in oncolytic viruses that specifically attack and kill tumour cells. It goes without saying that the efficient control of gene expression in such made-to-measure viruses is indispensable for safety reasons. However, conventional systems usually fail, as the required stoichiometry between transcription factors and the target sequences in the viral genome changes considerably during the viral replication cycles.

This problem does not occur when riboswitches are used, as these are part of the mRNA transcript. In other words, any RNA message that is being transcribed comes with its own on-/off-switch.

"In a joint research project with Dirk Nettelbeck from the DKFZ in Heidelberg we have been able to show that our RNA switches are perfect for controlling gene expression in viruses," said Hartig (see article entitled "Viruses with integrated gene switch").

Hartig believes that although their approach works with any other protein, it might not always work with the same efficiency due to sequence differences. Hartig's group is also working on the possibility of transferring the principle to eukaryotes. "We are optimistic that some time in the future our switches and underlying systems will enable us to control the incorporation of amino acids in higher organisms," predicts Hartig.

Further reading:

Markus Wieland, Jörg Hartig: Genregulatoren aus dem Baukasten – das Hammerhead-Ribozym; BIOspektrum 02/2010

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Article

16-Dec-2013
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BioLAGO

The article is part of the following dossiers

Chemical tools for biological applications

Universität
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