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Red light to elucidate the regulatory mechanisms of gene expression

The interaction of proteins and RNA is a crucial factor in the regulation of gene expression. These protein-RNA interactions can be specifically controlled and investigated inside the cell using proteins with customised chemical functions. In his doctoral thesis, chemist Moritz Schmidt from the University of Konstanz addresses the possibility of conveying new functions to proteins by introducing non-natural amino acids. He has developed a technique that uses red light to cross-link proteins with RNA.

The properties of a cell depend on the expression levels of their genes. The expression levels of the genes are responsible for the fact that even genetically identical cells, i.e. liver cells and nerve cells of one and the same organism, differ in many properties. Protein-RNA interactions play a major role in the transcription, maturation, transport, translation and degradation of RNA and are therefore a key factor in the regulation of gene expression. “The bonds between proteins and RNA are not permanent but temporary and are forged in the cell under specific conditions. Isolating RNA-protein complexes for in-depth investigations is therefore impossible,” said Schmidt.

After outstanding results in his bachelor’s degree in life sciences at the University of Konstanz, Schmidt was accepted onto the university’s fast-track programme enabling him to start his doctoral thesis without having to do a master’s thesis. He now works in a research group led by Dr. Summerer in the Department of Chemistry and is specifically focussed on methods for expressing proteins with customised, non-natural properties in living cells with the objective of modifying their biological function. For example, he successfully developed a method that enables the covalent linkage of a protein to its RNA binding partner. This method can be used to gather information on the interaction of RNAs and proteins under physiological conditions.

Synthetic, noncanonical amino acids can be used in place of canonical amino acids to improve the biophysical properties of proteins. Noncanonical amino acids differ from the 20 canonical amino acids in that their codons are allocated to encode an amino acid which is not among the 20 standard amino acids occurring in DNA and RNA. The artificial expansion of the genetic code also enables the incorporation of noncanonical amino acids into proteins through ribosomes in which case a special transfer RNA (tRNA) in the ribosome replaces the stop codon of the mRNA with an unnatural amino acid. The respective aminoacyl tRNA synthetase enzyme attaches the noncanonical amino acid to the tRNA, which then acts as adapter molecule and guides the amino acid to the correct site (a stop codon). Moritz Schmidt also uses this method for the evolution of proteins with specific functions. “During translation, a non-natural amino acid is incorporated into the protein in place of a stop codon,” Schmidt explains.

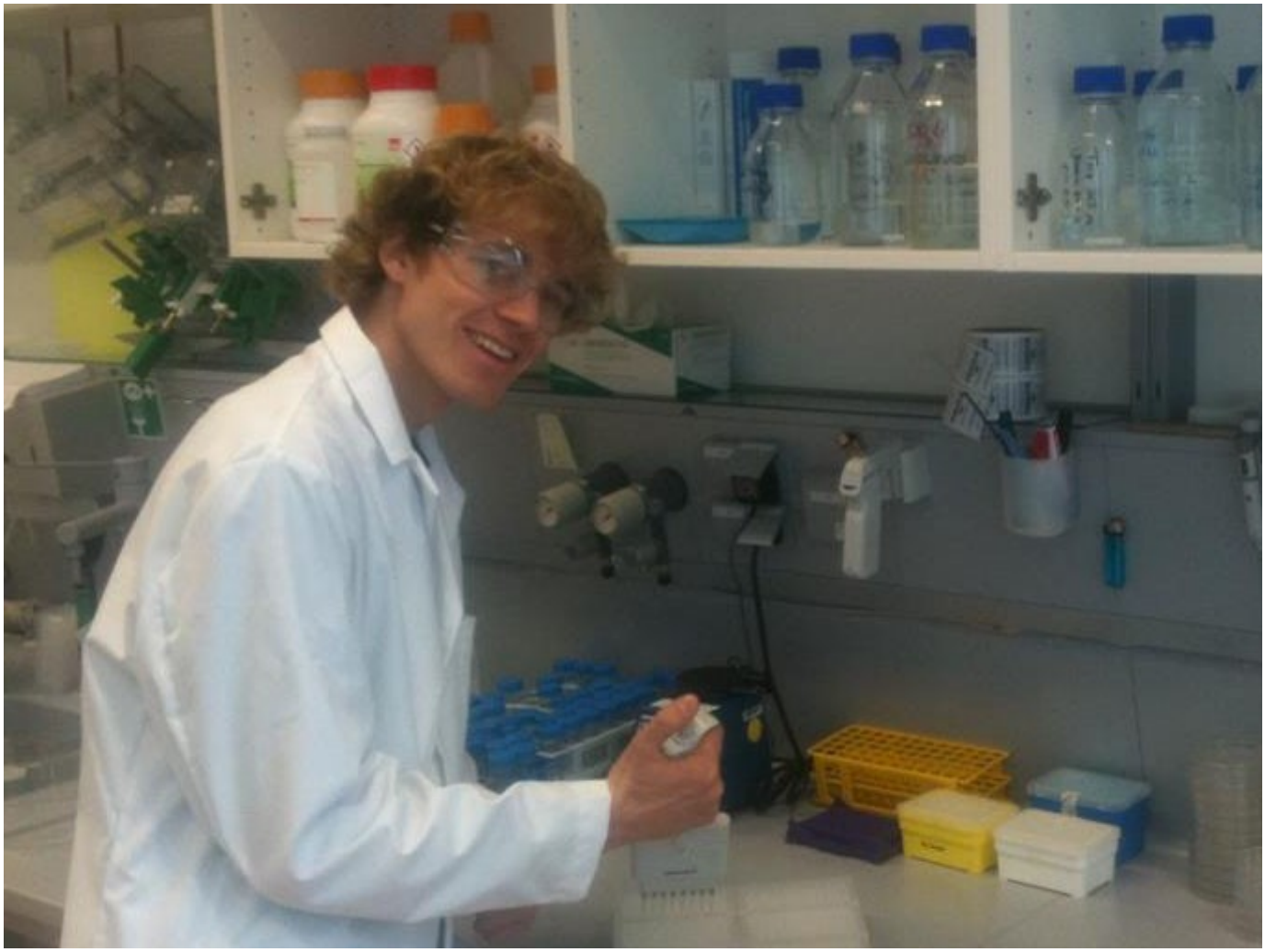
Moritz J. Schmidt has received a Hoechst Doctoral Student Scholarship for his outstanding achievements in the life sciences.

Customised tools for targeted protein design

The site-specific incorporation of a noncanonical amino acid depends on the aforementioned translation components that are introduced into the cell or into the entire organism. The process requires a special aminoacyl tRNA synthetase enzyme which is specific for a particular amino acid and attaches the accurate amino acid to the corresponding tRNA. “Therefore, the incorporation of noncanonical amino acids requires an aminoacyl tRNA synthetase-tRNA pair that recognises and processes the artificial amino acids as substrate and which does not cross-react with the translation components that are present in the cell,” Schmidt explains.

"The synthetase-tRNA pair used comes from an organism that is evolutionarily rather distant from the experimental organism used. This ensures that the synthetase enzymes do not recognise other amino acids and that the tRNA is not used by another cellular synthetase as substrate," Schmidt says. In order to find a synthetase enzyme that is able to selectively recognise a new non-natural amino acid, the researchers screen huge synthetase libraries containing up to one billion mutants. Suitable synthetases are selected using a reporter construct (e.g. antibiotics resistance) that links the ability to suppress a stop codon with the cell's ability to survive in the presence of an antibiotic.

Red light is used to control protein-RNA cross-linking



Moritz J. Schmidt's doctoral thesis deals with the development of systems that enable the simple expression of proteins and peptides with customised chemical functions in living cells.

Red light is used to control the temporally and spatially correct cross-linking of a protein with an RNA. "Red light excites a light-activated substance, a so-called photosensitiser, which is added prior to illumination with red light. The excited sensitiser subsequently leads to the conversion (i.e. oxidation) of the amino acid into a reactive species, which then serves as photo-cross-linker," said Schmidt explaining the principle. In its oxidised state, the amino acid preferentially forms stable, covalent bonds with nucleobases in its vicinity. This leads to the fixation of transient interactions, which can then be identified and investigated more effectively. In principle, the method can also be used to control the expression of genes and to study the consequences of such a deactivation.

Medical and pharmaceutical applications are possible

Most of the genetically encoded photo-cross-linkers are used for the investigation of protein-protein interactions. The photochemistries used are based on illumination with UV light, which has a reduced penetration depth and is therefore rather unsuitable for application in tissue. In addition, UV light can lead to DNA damage and other negative effects. “Red light does not damage cells, and also has a high penetration depth. This makes it better suited for applying to tissues,” said Schmidt.

In modern medicine, red light and special photosensitisers are already used for photodynamic therapies for treating tumours and other tissue alterations (e.g. formation of new blood vessels) with light and special photosensitisers. Schmidt’s research has the potential to contribute to the development of new protein-based drugs for photodynamic therapies. “I think it would be worth exploring whether red light can also be used effectively for the development of pharmaceuticals that target specific nucleic acids,” said Schmitz highlighting other potential application possibilities of red light.

Original publication: Red-Light-Controlled Protein-RNA Crosslinking with a Genetically Encoded Furan. M. Sc. Moritz J. Schmidt and Dr. Daniel Summerer, Angewandte Chemie International Edition, Volume 52, Issue 17, pages 4690–4693, April 22, 2013

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Article

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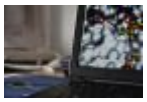

Molecular design made to measure and the requirements

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