Structural basis for key protein–protein interactions of deoxyhypusine hydroxylase

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Post-translational modifications of proteins are processes involving changes to the chemical structure of proteins after they have been synthesized by ribosomes during translation. They are often limited to the enzyme-mediated attachment of additional simple chemical groups to the amino acid residues that make up the protein, for example, a phosphate group or a hydroxyl group. These modifications significantly alter the chemical and biological properties of proteins, and are often necessary for a protein to fulfil its specific function in the cell.

While simple post-translational modifications are oftentimes not exclusive to any specific protein and can be introduced by a variety of enzymes, there are also unique post-translational modifications that occur only for a selected, single protein and involve dedicated enzymatic machinery. Such modifications usually result in the formation of a more complex chemical group. One such modification is **hypusination**, which has been found in only one protein to date – eukaryotic translation initiation factor 5A (eIF5A). This protein harbours hypusine in its structure – an amino acid residue modified by hypusination. By binding to the ribosome, eIF5A facilitates the formation of bonds between problematic amino acid pairs during protein synthesis, for example proline–proline. eIF5A is crucial for normal development, and any defects in the hypusination pathway are either lethal or lead to serious disorders.

Hypusination is carried out in two successive steps by enzymes: deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DHH). Although the mechanism of hypusination has been partially elucidated, the molecular basis of its second step, as well as the interactions of DHH with other proteins, remain poorly characterized.

The main goal of this project is to complement the knowledge of how DHH binds to its most important molecular partner – deoxyhypusinated eIF5A, a transient product in the hypusination pathway – and how it carries out the enzymatic reaction. DHH requires the presence of two iron ions in its structure – it is therefore equally important to investigate how these ions, supplied by another protein, PCBP1, are introduced into DHH. The final part of the project is an attempt to discover previously unrecognized interactions of DHH with other proteins, which may be important for the regulation of the hypusination.

Within our research, in addition to advanced biochemical and biophysical techniques, we will use experimental structural biology methods – X-ray crystallography and cryo-electron microscopy, which will enable us to determine the structure of DHH complexes with other proteins, as well as mass spectrometry, which will allow the high-throughput identification of unknown proteins interacting with DHH.

We believe that the project will provide numerous new insights into DHH and thus render a more complete picture of hypusination. Since hypusination and its defects are linked to excessive cell divisions leading to tumour progression, as well as to other diseases such as diabetes, malaria or viral infections, the enzymes that carry it out are clinically relevant and have been identified as potential targets for new drugs.