Protein kinase dependent regulation of deoxyhypusination

mgr Paweł Kochanowski

Post-translational modifications (PTMs) are changes made to proteins during or after synthesis and most often involve covalent attachment of a functional group in an enzyme-mediated process. PTMs can change a variety of protein properties; they often regulate biological activity by inducing conformational changes, and some are essential for the formation of a fully functional protein. One type of PTM usually affects many different proteins and can often be generated by several enzyme proteins, but some of them are very unique.

Hypusination is a PTM that has been observed in only one protein – eukaryotic initiation factor 5A (eIF5A). It is a two-step process catalyzed by two enzymes, namely deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). The hypusine residue in eIF5A promotes translation elongation by facilitating peptide bond formation at ribosome stalling sites, e.g. polyproline stretches. This unique PTM is indispensable for translation and the lack of it is lethal to the developing mammalian embryo. Not surprisingly disturbances of hypusination lead to serious repercussions, for example mutations in the DHS coding gene cause global developmental delay. Moreover, increased expression of eIF5A has been observed in many tumours and DHS expression level has been correlated with poor prognosis in cancer patients.

Despite that, the regulation of hypusination remains elusive. So far the phosphorylation of DHS by casein kinase 2, multiple protein kinase C isoforms and mitogen-activated protein kinases ERK1/2 has been described. However, its role is still unclear. As DHS is the first and rate-limiting enzyme of this process, mechanisms affecting its activity are important for the whole process. Therefore, the main goal of this project is to characterize the regulation of the first step of hypusination by protein kinases that phosphorylate DHS or interact with it directly.

Within the proposed project, we aim to structurally and functionally characterize DHS – protein kinase interactions that may participate in the regulation of hypusination, and study them in great detail. Elucidation of the entirety of such regulatory mechanisms requires an integrative approach comprising biochemical/biophysical analyses, structural and cellular biology.

Therefore, we will first, biochemically characterize interactions of DHS with kinases, for which there were previous indications that they could phosphorylate DHS. Next, we will validate the presence of such phosphorylation events. We will also study the effects of the said interactions with protein kinases and phosphorylation events on the DHS catalytic activity. Finally, in order to verify whether the observed interactions are biologically relevant we will test them in a cellular model. For kinases whose interactions with DHS we will successfully verify, we will determine the atomic structures of their complexes with DHS using single-particle cryo-electron microscopy.

This study will return important data on the molecular interactions involved in the control of DHS activity and thus in the regulation of hypusination. Furthermore, given that the hypusination pathway and especially DHS have previously been proposed as a druggable target, this work may become a foundation for further translational studies.