MS13 Hot structures in biology

Chairs: Mariusz Jaskolski, Udo Heinemann

MS13-P1 How Bacteria Make Rocket Fuel: Structure and Mechanism of Hydrazine Synthase

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The last few decades have seen major changes in our understanding of the global biological nitrogen cycle, largely due to the discovery of the *anammox* pathway. This *an*aerobic *amm*onium *ox*idation pathway constitutes a shortcut in the nitrogen cycle that allows bacteria to directly combine nitrite (NO_2^-) with ammonium (NH_4^+), resulting in nitrogen gas (N_2) and water (H_2O), and which yields energy for the cell. It has been estimated that anammox is responsible for up to 50% of the nitrogen gas production in some ecosystems. Moreover, the process is increasingly being used in energy-efficient waste water treatment plants.

Interestingly, the anammox pathway relies on the highly reactive intermediate hydrazine ($H_2N\text{-}NH_2$), known to most people only as rocket fuel. The synthesis of hydrazine constitutes the first step in the formation of the highly stable triple bond in nitrogen gas, and is performed by the enigmatic hydrazine synthase complex.

We have studied hydrazine synthase with an array of biophysical methods: X-ray crystallography, solution small-angle X-ray scattering, analytical ultracentrifugation and EPR spectroscopy. We find that the hydrazine synthase multienzyme complex forms crescent-shaped heterohexamers with two distinct active sites connected by a tunnel. The active sites contain entirely novel features and together, the data suggest a mechanism for biological hydrazine synthesis that relies on hydroxylamine as an intermediate.

Keywords: protein crystallography

MS13-P2 MCPIP3 (ZC3H12C) Regulates the Innate Immune Response by Acting as a Ribonuclease.

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During inflammation, different innate pattern recognition receptors like Toll-like receptors (TLR) and RIG-1 like receptors sense the infection, and then the inflammatory response is orchestrated by pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 which ultimately result in elimination of pathogens (1). Cytokine levels are tightly controlled at transcriptional and post-transcriptional level by various proteins, because a cytokine storm could lead to immunodeficiency and autoimmune disorders (2).

The MCPIP (macrophage chemotactic protein-induced proteins) family (also known as ZC3H12 family) of proteins are ribonucleases containing a CCCH-type zinc finger. These proteins play an important role in innate immune response by regulating cytokine levels through targeting their mRNAs (3). MCPIP1 (Regnase1) has recently been shown to recognize specific stem-loop structures in the 3' untranslated region (UTR) of translationally active mRNAs for IL6 and other immunological factors and lead to their degradation by utilizing the helicase activity of UPF1 (4).

MCPIP3 (ZC3H12C) is another member of the family which has also been reported to be involved in cytokine regulation by mRNA degradation. Moreover, it is highly expressed in precursor B-lymphocytes and is also involved in lymphocyte differentiation, as MCPIP3 knock-out mice show severe defects in lymphocytes. A crystal structure for the RNase domain of ZC3H12C has been determined at a resolution of 1.9 Å which shows a Rossmann-fold like structure with an Mg^{2+} ion bound in the catalytic site. Initial RNase assays show that the protein variants used in structure analysis are catalytically active.

Overall, we are trying to understand the structure-function relation of MCPIP3 which will enable us to understand how it recognizes its target mRNA and how it coordinates with other family members in order to regulate the lymphocyte maturation and inflammatory responses.

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Keywords: Macrophage activation, Innate immune response, B-lymphocyte differentiation, CCCH Zinc-finger, Ribonuclease