MS10-P3 **Structural studies of sacsin.** Guennadi Kozlov,[a] Alexey Y. Denisov,[a] Jean-François Trempe,[a] Harshit Pande,[a] Martine Girard,[b] Marie-Josée Dicaire,[c] Peter S. McPherson,[b] Bernard Brais,[c] Kalle Gehring,[a] *[a]Department of Biochemistry, Groupe de recherche axé sur la structure des protéines, McGill University, Montréal, Canada, [b]Centre for Neuronal Survival and Department of Neurology & Neurosurgery, Montreal Neurological Institute, Montréal, Canada, [c]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, Canada*
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Sacsin is a 520 kDa protein involved in an early onset neurodegenerative disease Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) prevalent in the Charlevoix-Saguenay-Lac-Saint-Jean region of Quebec. Structural information about sacsin should help in clarifying the functional role of the protein and its involvement in neurodegeneration. The very C-terminus of the protein contains a Higher Eukaryotes and Prokaryotes Nucleotide-binding (HEPN) domain of unknown function. We determined a high-resolution 1.9 Å crystal structure of the HEPN domain from human sacsin [1]. The structure is made of five helices in an antiparallel arrangement with a long loop between helices α4 and α5 containing several short structured segments. Importantly, the HEPN domain forms a stable dimer in the crystal with a large buried surface formed by helices α1 and α2 and the α4-α5 loop. Multi-angle light scattering and NMR self-diffusion experiments confirmed that the sacsin HEPN domain also forms a dimer in solution. The structure explains why the N4549D mutation causes disease in some ARSACS patients. The mutation leads to electrostatic repulsion near dimerization interface, destabilizing the dimer and resulting in insoluble protein. The HEPN structure contains a large positively charged cavity at the dimer interface that is optimized for binding negatively charged ligands. Isothermal titration calorimetry and NMR titrations showed that this surface binds nucleotides with low micromolar affinity, though the identity of physiological ligand is currently unclear. Recently, we crystallized and solved the structure of the N-terminal ubiquitin-like (UBL) domain from human sacsin at 2.1 Å resolution. Unexpectedly, the structure shows a swapped dimer with the swapping mediated by exchange of first β-strands of each protomer. Ongoing experiments aim to address the physiological relevance of swapped dimer observed in the crystal. The study provides important steps towards better understanding of structure/function relationships of sacsin and its involvement in neurodegeneration.

[1] Kozlov, G., Denisov, A. Y., Girard, M., Dicaire, M.-J., Hamlin, J., McPherson, P. S., Brais, B. & Gehring, K. (2011). *J. Biol. Chem.* **286**, 20407-20412.

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MS10-P4 **HP0315 from *Helicobacter pylori* as a VapD protein with an endoribonuclease activity.** Ae-Ran Kwon,[a] *[a]Department of Herbal Skin Care, College of Herbal Bio-Industry, Daegu Haany University, Korea*
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VapD-like virulence-associated proteins have been found in many organisms, but little is known about this protein family including the 3D structure of these proteins. Recently, a relationship between the Cas2 family of ribonucleases associated with the CRISPR system of microbial immunity and VapD was suggested [1]. Here, we show for the first time the structure of a member of the VapD family and present a relationship of VapD with Cas2 family and toxin-antitoxin (TA) systems. The crystal structure of HP0315 from *Helicobacter pylori* was solved at a resolution of 2.8 Å. The structure of HP0315, which has a modified ferredoxin-like fold, is very similar to that of the Cas2 family. Like Cas2 proteins, HP0315 shows endoribonuclease activity. HP0315-cleaved mRNA, mainly before A and G nucleotides preferentially, which means that HP0315 has purine-specific endoribonuclease activity. Mutagenesis studies of HP0315 revealed that D7, L13, S43 and D76 residues are important for RNase activity, in contrast, to the Cas2 family. HP0315 is arranged as an operon with HP0316, which was found to be an antitoxin-related protein. However, HP0315 is not a component of the TA system. Thus, HP0315 may be an evolutionary intermediate which does not belong to either the Cas2 family or TA system.

[1] Beloglazova, N., Brown, G., Zimmerman, M.D., Proudfoot, M., Makarova, K.S., Kudritska, M., Kochinyan, S., Wang, S., Chruszcz, M. & Minor, W. (2008). *J. Biol. Chem.*, **283**, 20361-20371.

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