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Thursday, March 17, Session I

L1

STRUCTURAL BASIS OF KATANIN FUNCTION AT MICROTUBULE MINUS END

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Katanin plays a crucial role in severe human diseases such as tauophaties or microcephaly [1-2]. It is a microtubule-severing enzyme that has the unique capacity to catalyse the removal of tubulin dimers from the interior of the microtubule lattice and thereby cut microtubules (MT) into short fragments. It is necessary for meiotic spindle assembly, determination of mitotic spindle length, severing at microtubule crossovers, and cell motility [3-4]. Katanin is known for decades; however, structural information is missing and the role of interaction partners in the regulation of katanin function is not fully understood.

Here we performed a detailed biophysical, structural and functional characterization of katanin alone and in complex with two binding partners, ASPM (abnormal spindle-like microcephaly-associated protein) and CAMSAP (calmodulin-regulated spectrin-associated protein). We identified minimal regions of CAMSAP and ASPM necessary and sufficient for the interaction with katanin. We characterized the complexes biophysically using mainly sedimentation velocity analytical centrifugation and solved the crystal structures of katanin, and the katanin/CAMSAP, katanin/ASPM complexes. Our work revealed that CAMSAP and ASPM compete for the same binding site on katanin and functional analysis showed that katanin/ASPM and katanin/CAMSAP form MT minus-end binding complexes, that play major roles in regulating MT dynamics.

L2

STRUCTURE-FUNCTION STUDY ON PLANT ALDEHYDE DEHYDROGENASE FROM MOSS *PHYSCOMITRELLA PATENS*

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Aldehyde dehydrogenases (ALDHs) comprise a protein superfamily of $NAD(P)^+$-dependent enzymes (EC 1.2.1). The superfamily of plant ALDHs currently contains 13 distinct families. In recent years we kinetically and structurally characterized NAD^+-dependent ALDH10 family members from pea (*Pisum sativum*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), ALDH7 family members from maize and pea and ALDH2 family members from maize. The ALDH10 isoforms have been independently shown to oxidize various aminoaldehydes and thus they have been also called aminoaldehyde dehydrogenases or betaine aldehyde dehydrogenases. ALDH7 (EC 1.2.1.31) is also known as a piperideine-6-carboxylate (P6C) dehydrogenase or antiquitin and is primarily involved in the metabolism of lysine. Plant ALDH2 family (EC 1.2.1.3) comprises isoforms catalyzing the oxidation of ethanol-derived acetaldehyde to acetate as well as of other aliphatic and aromatic aldehydes. Some ALDHs from family 2 were originally identified as genes restoring fertility in plants (called RF2A and RF2B), which have the ability to

suppress the male-sterile phenotype and restore the production of pollen in maize.

In this work we analyzed enzyme kinetics of ALDH2, ALDH10 and ALDH21 family members from the non-vascular moss *Physcomitrella patens*, which is a model organism for studies on plant evolution. A single ALDH21 gene is restricted to primitive terrestrial plants including mosses *P. patens, Tortula ruralis* and the spikemoss *Selaginella moellendorffii* while it is absent in higher plants. Crystal structures of the ALDH21 apoform and the complexes with the coenzyme and product were solved up to 2.15 Ĺ resolution and revealed the importance of several arginine and tyrosine residues for substrate and coenzyme binding. In particular, Arg 228 functions as a gate to the coenzyme binding site and seals the site upon coenzyme binding.

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L3

LECTINS WITH BETA-PROPELLER FOLD IN OPPORTUNISTIC PATHOGENS

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Lectins are carbohydrate binding proteins of non-immune origin that play role in various biological processes, such as cell-cell interaction, biofilm formation or host-pathogen recognition. They differ in carbohydrate specificity as well as in affinity, which can be further increased by presence of several binding sites per lectin molecule causing so called avidity effect. Lectins are ubiquitous in nature. They were found in bacteria, plants, fungi and animals including human being. Attention is paid especially to lectins from pathogenic organisms (*Pseudomonas aeruginosa*, *Chromobacterium violaceum*, *Aspergillus fumigatus*, *Photorhabdus asymbiotica*, etc.), where they frequently assist host-pathogen recognition and take part in early stage of infection.

From the structural point of view, a few dozens of lectin folds have been already described and there is no reason to take this amount as final. An interesting group of lectins are lectins possessing 6 or 7 bladed beta-propeller fold. In this case, the blades are formed by tandem repeats, where binding sites are located in between blades. This not only allows for high affinity through multivalency effect, but also for increased variability in ligand specificity since slight variations in binding site composition is frequently observed. Structural data based on X-ray diffraction are evaluated in order to determine the real number of active binding sites and their fine specificity, as this knowledge is essential for treatment (e.g. anti-adhesive therapy) or for biotechnological applications.

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L4

STRUCTURAL AND BINDING PROPERTIES OF A QUADRUPLE EPITOPE REGULATING TAU PROTEIN OLIGOMERIZATION

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Polymerization of tau protein and the deposition of insoluble tau lesions highly correlate with the cognitive decline in Alzheimer's disease and related tauopathies [1]. The most probable nuclei of tau protein aggregation are the hexapeptides VQIINK, VQIVYK and other two segments, each localized in one of the microtubule-binding repeats of tau [2, 3]. Recently we have described an anti-tau monoclonal antibody, DC8E8, which effectively blocked tau-tau interaction by binding four highly homologous epitopes in the immediate vicinity of aggregation-promoting hexapeptides [4]. The sequence of DC8E8 epitope served as a base for the construction of an active vaccine, which is currently under clinical development [5].

In the present study we aimed to answer the questions about the sequence and structure requirements of DC8E8-based immunomodulation. We have determined the kinetics of DC8E8 binding to each of its four binding sites on the tau protein molecule. Further, we have crystallized DC8E8 Fab fragment and its complexes with several tau peptides and solved the structure by X-ray crystallography. Finally, we validated our structural findings by alanine-scanning mutagenesis of DC8E8 paratope residues to confirm antibody contact sites. Obtained results allowed mechanistic insights into DC8E8 inhibitory activity.

1. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del Tredici K, et al. J. Neuropathol. Exp. Neurol. 2012, 71, 362-381.
2. Novak M, Kabat J & Wischik CM EMBO J. 1993, 12, 365-370.
3. von Bergen M, Friedhoff P, Biernat J, Heberle J, Mandelkow EM & Mandelkow E Proc. Natl. Acad. Sci. U. S. A. 2000, 97, 5129-5134.
4. Kontsekova E, Zilka N, Kovacech B, Skrabana R & Novak M Alzheimers Res. Ther. 2014, 6, 45.
5. Kontsekova E, Zilka N, Kovacech B, Novak P & Novak M Alzheimers Res. Ther. 2014, 6, 44.

Thursday, March 17, Session II

L5

ASYMMETRIC CELL DIVISION DURING SPORULATION IN *BACILLUS SUBTILIS*

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Bacillus subtilis is a Gram-positive microorganism which is able to differentiate during process called sporulation. A hallmark of sporulation in *B. subtilis* is the polar cell division. As occurs during vegetative cell division, the tubulin-like GTPase FtsZ forms a ring-like structure at mid-cell. At the onset of sporulation, however, the division apparatus is not assembled at this site, instead the Z-ring migrates from mid-cell on a spiral trajectory to the two cell poles in a process that depends on sporulation-specific overexpression of *ftsAZ* and the presence of SpoIIE. SpoIIE colocalizes with the polar Z-rings. One of the Z-rings matures into the sporulation septum while the other dissolves. Asymmetric cell division otherwise appears to involve the same set of proteins as constitute the divisome during vegetative cell division. However, the resulting sporulation septum is much thinner. Interestingly, SpoIIE is the only sporulation-specific protein whose deletion or mutation causes changes in the ultrastructure of the asymmetric septum. *spoIIE* null mutants are defective in sporulation and at low frequency give rise to aberrantly thick asymmetric septa [1]. Accompanying these morphological changes is a coordinated programme of differential gene expression, involving intercellular signalling processes, that leads to the activation of the RNA polymerase sigma factors, ^{F} and ^{G} in the forespore and ^{E} and ^{K} in the mother cell [2].

SpoIIE from *B. subtilis* is an 827 residue protein that consists of three regions. It has 10 putative membrane-spanning segments (region I) at its amino terminus and a PP2C-type phosphatase domain (region III) at its C-terminus. The central region II is required for localisation of SpoIIE to the divisome and its reported interaction with FtsZ. The structure of the PP2C phosphatase domain of SpoIIE was already solved [3]. In contrast, the structure of N-terminal two-thirds of SpoIIE and the character of its interactions with partner proteins are unknown. We re-