fusion partners that play important roles in this stem cell maintenance. The protein consists of a BTB domain, an intrinsically unfolded linker region and 9 zinc fingers. It is known that the BTB domain mediates the dimerization of the PLZF protein. Present theories suggest that a chromosomal fusion occurs with RARα and that PLZF dimer or oligomer binding causes the transcriptional repression responsible for APL. One hypothesis concerns the potential oligomerization of PLZF during DNA interaction, it is however not known which part of the protein is responsible for the protein:protein contacts.

The aim of the present SAXS study is to shed light on the dimerization and oligomerization, thus understanding the mechanisms of PLZF mediated DNA transcriptional repression. So far most studies have been concentrated on the BTB domain, since the unstructured linker region is inherently difficult to study. Using SAXS, we have the great advantage to work with proteins in solution, thus it was possible to collect data on the native-like condition of the linker region. The analysis however, is demanding and not much experience exists on data from intrinsically un-folded regions.

We have collected data on three PLZF constructs, one containing the BTB domain only, one consisting of the linker part only, and one spanning the BTB and linker part. The oligomerization process has been analyzed in a pH series spanning five pH values. Complementary analysis has been carried out using Dynamic Light Scattering and Circular Dichroism spectroscopy.

The fact that our data set is very extensive was very important for the interpretation of the data. We have successfully identified the involved species in the oligomerization process and have analysed the change of behavior of the intrinsically un-folded linker region during this process.

Keywords: SAXS, intrinsically un-folded, oligomerization

MS22.P04

OST1 structure provides the basis of regulation in response to stress in plants

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SNF1-related protein kinase 2s (SnRK2s) orchestrate the cellular stress responses by phosphorylation of transcription factors and ion channels [1]. SnRK2.6, also called Open Stomata 1 (OST1), is well characterized at molecular and physiological levels to control stomatal closure in response to abscisic acid (ABA) and osmotic stress [2], [3]. SnRK2s, protein phosphatases 2Cs (PP2Cs) and PYR/RCAR ABA receptors work together to decode environmental stress signals mediated by ABA [4], [5]. A balance between the PP2C mediated phosphorylation states of OST1 tunes the activity of the kinase [6], [7]. The structure of an inactive form of OST1 shows that the C-terminal regulatory domain of OST1 stabilizes the kinase into an unproductive conformation. Our data provide insights into the molecular activation/ inactivation mechanism of the kinase and also suggest a mechanism for the ABA dependent control of the phosphatase-binding and kinase activities of OST1.


Keywords: abiotic stress in plants, kinase, cell signaling transduction

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The crystal structure of an Arabidopsis thaliana C2 domain

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Calcium represents the most versatile ion in eukaryotic organisms. It is involved in nearly all aspects of cell development and participates in many regulatory processes [1]. The C2 domains act as a calcium dependent membrane targeting module of approximately 130 amino acids. These domains either form part of a protein or function as independent molecules [2]. We have studied a group of Arabidopsis thaliana C2 molecules of unknown function that may be involved in the recognition and delivery of protein molecules to cell membranes. A representative member of the group has been expressed, purified to homogeneity and crystallized. The crystal structure at 2.2 Å resolution shows that the molecule is a dimer that folds as a canonical C2 domain with the topology of those C2s forming part of phospholipases. The electron density map reveals two calcium ions per molecule. Isothermal titration calorimetry (ITC) was used to determine the calcium binding affinity in vitro, which was similar to those observed for other sensors that work at physiological calcium concentrations [3]. The joined analyses of the biophysical and crystallographic data suggest a model of calcium-dependent membrane insertion mechanism that will involve either a dimer dissociation or a strong rearrangement of the dimeric structure.


Keywords: protein, cell-signalling, membrane-targeting

MS22.P06

Mechanism of Signaling by a Receptor-Independent, Self-Activating G-Protein

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