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

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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.

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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 forms 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 C2 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.

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

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In animals, heterotrimeric guanine nucleotide–binding protein (G protein) signaling is initiated by G protein–coupled receptors (GPCRs), which activate G protein subunits. By contrast, the plant *Arabidopsis thaliana* lacks canonical GPCRs. Its G protein subunit (AtGPA1) is self-activating in the absence of any receptor or guanine nucleotide exchange factor and features both faster binding of GTP and slower GTP hydrolysis when compared to mammalian G{alpha} proteins.

Receptor-independent G proteins must have a fundamentally different mechanism for the control of guanine nucleotide exchange, and thus for G protein activation. To decipher that mechanism, we have combined crystallographic, biophysical, molecular dynamics and mutagenesis studies [1].

We show that AtGPA1, like animal G proteins, contains a Ras-like domain that is homologous to 'small' G proteins and an {alpha}-helical domain of less defined function. The guanine nucleotide-binding site is located in a cleft between the two domains. In sharp contrast to animal G proteins, however, our structure revealed pronounced disorder in the {alpha}-helical domain.

Subsequent molecular dynamics simulations showed strong anti-correlated movements between the helical domain and the Ras domain, indicating frequent dissociation. Within the helical domain, {alpha}A and {alpha}B helices exhibited the greatest structural fluctuations. These pronounced movements correlate with the high intrinsic activity of AtGPA1.

Exchanging the {alpha}-helical domains between AtGPA1 and animal G{alpha}i1, which is not self-activating, conferred kinetic and stability features of one G{alpha} to its counterpart. Using such chimeric constructs, we demonstrate that the AtGPA1 helical domain is necessary for self-activation, and that the {alpha}A helix within it is sufficient to confer self-activation to an animal G protein subunit.

Our study reveals the structural basis of the mechanism for G protein activation in Arabidopsis based on the intrinsic mobility of the AtGPA1 {alpha}-helical domain. GPCRs are thought to activate G proteins by a distinctly different mechanism that involves the {alpha}5 helix of the Ras domain and the G{beta}{gamma} dimer to accelerate the dissociation of the guanine nucleotide. However, recently, the {alpha}-helical domain of a mammalian G{alpha} was implicated in conferring specificity of the interaction of G{alpha} with an effector of G{beta}{gamma}, the G protein-activated potassium channel [2]. Thus the mechanism for nucleotide exchange found in the plant G protein may be partially retained in animal G proteins.

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Comparison of the structures and peptide binding specificities of the BRCT domains of MDC1 and BRCA1

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The tandem BRCT domains of BRCA1 and MDC1 facilitate protein signalling at DNA damage foci through specific interactions with serine-phosphorylated protein partners. The MDC1 BRCT binds pSer-Gln-Glu-Tyr-COO^- at the C-terminus of the histone variant, γH2AX, via direct recognition of the C-terminal carboxylate, while BRCA1 recognizes pSer-X-X-Phe motifs either at C-terminal or internal sites within target proteins. Using fluorescence polarization binding assays, we show that while both BRCTs prefer a free main chain carboxylate at the +3 position, this preference is much more pronounced in MDC1. Crystal structures of BRCA1 and MDC1 bound to tetrapeptide substrates reveal differences in the environment of conserved arginines (Arg1699 in BRCA1, Arg1933 in MDC1) that determine the relative affinity for peptides with $–COO^-$ vs $–CO\text{-}NH_2$ termini. A mutation in MDC1 that induces a more BRCA1-like conformation relaxes the binding specificity, allowing the mutant to bind phospho-peptides lacking a $–COO^-$ terminus.

Keywords: DNA damage response, BRCT domains, X-ray crystallography

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Structural and functional studies of potential cancer targets

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The tetraspanin CD151 is implicated in the regulation of cancer invasion and metastasis by initiating signaling events. CD151 is expressed in various cell types, including epidermal basal cells, epithelial cells, skeletal, smooth and cardiac muscle, endothelial cells, platelets and Schwann cells. Higher levels of CD151 are also associated with poor prognosis in lung and prostate cancer [1] and overexpression of CD151 promotes metastasis in colon carcinoma and fibrosarcoma cells [2]. CD151 has been successfully expressed and purified to facilitate structural and functional studies.

The Siah (Seven in Absentia Homologue) family of proteins functions as ubiquitin ligases for specific intracellular targets. Siah proteins have been implicated in the ubiquitylation and degradation of a range of proteins, including the PHD family of proteins [3]. Under hypoxia, Siah proteins are up-regulated and target PHD for degradation, leading to an increase expression of HIF-1f^{ς}, the major transcription factor controlling hypoxic and angiogenic responses [4]. We have previously determined the crystal structure of the substrate-binding domain of Siah, in apo and peptide-bound states, to moderate resolution [5], [6]. We now have a much higher resolution structure that will enable a fragment-based approach for the discovery of Siah inhibitors.

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