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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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Keywords: G-proteins, GPCR, signaling

MS22.P07

Acta Cryst. (2011) A**67,** C343

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

MS22.P08

Acta Cryst. (2011) A**67,** C343

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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Keywords: crystallography, anticancer, drug