Posters

Protein Structure and Conformation II

1024-Pos Board B1

Revealing Activation Mechanism of Alk2 Kinase Mutations in Fibrodysplasia Ossificans Progressiva (FOP)

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Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder resulting in transformation of soft tissue into episodic bone formation. Most of patients die around age 40 and no effective therapy is available. The causative genetic mutations have been identified in the intracellular glycine-serine-rich (GS) domain and kinase domain of Activin-like kinase-2 (ALK2, also known as ACVR1), a type I receptor of bone morphogenetic protein (BMP)[1]. Cumulative studies support that these mutations abnormally activate BMP signaling in a ligand- dependent or independent manner. The molecular mechanisms underlying FOP remains unclear. To test a long-standing hypothesis that FOP mutations reducing the ALK2 interaction with the negative regulator FKBP12 protein, we performed extensive molecular dynamic studies of ALK2 systems. Six systems were simulated in explicit solvent for at least 300 nanoseconds each: $ALK2^{WT}$, $ALK2^{Q207E}$, $ALK2^{R206H}$, and $ALK2^{R206H+}$ (with protonated histidine), in complex with FKBP12, as well as phosphorylated ALK2 GS-domain with and without FKBP12.Our results show that both the phosphorylated and mutated ALK2 break a major inhibitory Arg375-Asp354 salt-bridge between A-loop and DLG motif, which initiates A-loop conformation change from DLG-in toward DLG-out. But in wild-type ALK2, this salt-bridge remains intact throughout 300 ns trajectory. Furthermore, the dissociation of FKBP12 triggers the formation of a Glu248-Lys235 salt-bridge between αC helix and β3 strand. Altogether, we conclude that the R206H promotes dissociation of FKBP from ALK2 and triggers αC helix swing toward the catalytic domain, which indicates the kinase shifting towards active conformation. Interestingly, Q207E model can trigger the formation of Glu248-Lys235 salt bridge (αC swing) without the FKBP dissociation. This finding well explains the different responses to inhibitory FKBP12 among FOP mutations in the reported luciferase assay for ALK2 activity[2].

1. Hum Mutat **30**:379
2. J Biol Chem **287**:36990

1025-Pos Board B2

A Comparative Study of Gamma Subunits of A.Thaliana and O.Sativa

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Heterotrimeric G-proteins are composed of α, β and γ subunits that have roles as molecular switches to turn on intracellular trafficking by external stimuli. They are involved in multiple developmental processes ranging from early seedling development, organ shape determination to hormone perception and ion-channel regulation.

Although the activation mechanism is well understood in animals, recent studies show that the intrinsic signaling and network mechanism in plants have noticeable differences. Unlike animals, plants lack of the canonical G-protein-coupled receptors, thus G protein α subunit is self-activating. Future studies are expected to reveal more components of the heterotrimeric G-protein signal transduction pathways and to identify the mechanisms by which G-proteins regulate plant phenotypic and developmental plasticity. In the present study, we aimed to investigate the activation mechanism of G proteins in *Arabidopsis thaliana* and rice (*Oryza sativa Indica)*. Their respective gamma subunits (AGG2) and (RGG2) were heterologously expressed in *E.Coli*, followed by purification, biochemical and structural characterization. AGG2 has disordered structure, which is stabilized by dimerization in the absence of the natural partner, the gamma subunit, under fully reducing conditions. Small angle X-ray solution scattering and thermal stability data show that the AGG2 dimer has an elongated and flexible structure. Recently RGG2 has also been cloned and expressed in *E. coli* with the aim of purifying the recombinant protein for biochemical and biophysical characterization. Results on comparative analyses of structural features of AGG2 and RGG2 will be presented and discussed in the context of their respective function(s).*

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1026-Pos Board B3

Structural and Functional Basis of Alternative ESCRT-0 Protein Complexes

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Early endosomes represent the first sorting station for vesicular ubiquitylated cargo. Cargo transport is mediated by the endosomal sorting complex required for transport (ESCRT) machinery. Similar to the structural organization of ESCRT-0 proteins, alternative ESCRT-0 (alt-ESCRT-0) proteins, such as Tollip and Tom1, also present multiple ubiquitin-binding domains, including the C2 and CUE (Tollip) and VHS and GAT (Tom1) domains. Tollip localizes the Tollip-Tom1 complex at endosomal compartments by association with phosphatidylinositol 3-phosphate (PtdIns(3)P) through its central C2 domain. Tom1, through its GAT domain, is recruited to endosomes by binding to Tollip's Tom1-binding domain (TBD) through an unknown mechanism. Our NMR data revealed that Tollip TBD is a natively unfolded domain that partially folds at its N-terminus when bound to the first two helices of the Tom1 GAT domain through high affinity hydrophobic contacts. Furthermore, this association abrogates binding of Tollip to PtdIns(3)P by additionally targeting its C2 domain. Binding of the Tollip C2 domain is mediated by the third helix of the Tom1 GAT domain. We also show that the Tom1 GAT domain is able to bind ubiquitin and PtdIns(3)P at overlapping sites, albeit with modest affinity. We propose that association with Tom1 favors Tollip's release from endosomal membranes, allowing Tollip to commit to cargo trafficking. More recently, Tom1 has been shown to associate to signaling endosomal PtdIns(5)P through its VHS domain delaying epidermal growth factor receptor degradation in a bacterial infection model. We found that PtdIns(5)P binding of the Tom1 VHS domain is in the moderately fast-exchange regime at the NMR timescale. We propose that an incremental change in PtdIns(5)P intracellular levels sequesters Tom1 in endosomal cargo-free membrane domains, keeping the protein from its cargo sorting function.

1027-Pos Board B4

Insights into the Autoinhibition Mechanism of the Tiam1 Guanine Nucleotide Exchange Factor

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The T-cell lymphoma invasion and metastasis 1 (Tiam1) is a Dbl-family guanine nucleotide exchange factor (GEF) specific for the Rho-family GTPase Rac1. Mutations or aberrant regulation of Tiam1 have been implicated in oncogenic transformation of cells and linked to several kinds of invasive and metastatic forms of cancer. In addition to the C-terminal catalytic Dbl-homology (DH) and pleckstrin homology (PH) bi-domain (DH-PHc), Tiam1 contains several N-terminal protein-protein interaction domains (PHn-CC-Ex, RBD and PDZ) that are thought to contribute to the regulation of its GEF activity through inter-domain interactions. The mechanism for how the Tiam1 GEF function is regulated, however, remains poorly understood. Here, we show that truncation of N-terminal domains activates Tiam1 catalytic GEF function, while addition of the PHn-CC-Ex domain inhibited the catalytic activity of the DH-PHc domain in vitro. In addition, enzymatic kinetics experiments revealed that auto-inhibition of Tiam1 GEF function occurs through a competitive inhibition mechanism whereby the N-terminus decreases the substrate (GTPase Rac1) binding affinity, but does not change the maximum velocity of catalytic activity. We will also present ongoing single-molecule total internal reflection fluorescence (TIRF) data that characterize the kinetics of binding between Rac1 and the activated DH-PHc and inhibited PHn-PHc fragments. Finally, we derived a structural model for the Tiam1 PHn-PHc fragment based on small-angle X-ray scattering (SAXS) data. The model reveals an auto-inhibited conformation in which the N-terminal PHn-CC-Ex domain and linker regions block access of Rac1 to the catalytic bi-domain. Together, these data elucidate the biochemical and structural mechanisms for Tiam1 GEF regulation.

1028-Pos Board B5

Structures of Human Phosphofructokinase-1 and Atomic Basis of Cancer-Associated Mutations

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Phosphofructokinase-1 (PFK1), the 'gatekeeper' of glycolysis, catalyzes the committed step of the glycolytic pathway by converting fructose-6-phosphate