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The crystal structure of APPL2 BAR-PH domain

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APPL (Adaptor protein containing PH domain, PTB domain and leucine zipper motif) is an adaptor protein with two isoforms APPL1 and APPL2. As a scaffold protein, the interaction of APPL with Akt2 [1], small GTPases [2], FSHR, and AdipoR [3] are associated with the roles in cell proliferation, cell cycle control, metabolism/insulin sensing [4], [5], relaying signal from endosome and transcription by activating AMPK related signaling pathway [6]. As a protein communication hub, APPL provdes a mechanism for spatial regulation of signaling transmission and processing. However, it has also been shown that APPL2 plays opposing functions from APPL1 and there have been emerging evidences that BAR-PH domain of APPL1 and APPL2 can interact with each other to form heterodimer *in vivo*.

The structure of APPL2 BAR-PH domain has been solved to resolution of 3.2 Å using X-ray crystallography. The dimer exhibits a canonical four-helical fold of conformation, just like the shape of crescent or banana, which is implicated in the assocication with curved membranes. The difference between APPL1 BP domain and APPL2 BP domain lays in the curvature of the crescents. The curvature radius increases to ~70 Å, in sharp contrast with ~55 Å of APPL1 BP dimer's [7]. It has been proposed that curvature radius of BAR family protein corresponds to specific type of endosome to which it binds. Also, the protein surface shows different electrostatic property. Thus it is reasonable to conclude that the structure of APPL2 BAR-PH domain may be implicated in alteration of endosome assocation specificity, also in the change of the profile of spatial regulation of signaling transmission.

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Regulation of an *Arabidopsis Thaliana* potassium channel

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Under abiotic stress, plant cells use calcium signaling pathways to activate certain ion channels and give a correct response. Potassium (K+) is an essential ion for plant development. Plants living under low K+ conditions often adapt their K+ uptake through a recently discovered calcium signaling pathway which mobilizes potassium channel in roots. Under K+ deficiency, a calcium sensor activates a kinase that in turn phosphorylates and activates the K+ channel. When K+ levels are restored, a phosphatase dephosphorylates and inactivates the channel.

We are carrying out structural studies (crystallographic and ITC experiments) with this K+ channel and its binding partners to understand at molecular level how K+ uptake is regulated under stress conditions.

Keywords: potassium channel, abiotic stress

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Structure-based mutagenesis studies of human type II Hsp40, Hdj1

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Hsp40 is a co-chaperone of Hsp70 that correctly folds polypeptides in non-native forms. Hsp40 is divided into two domains: the N-terminal DnaJ-like domain and the C-terminal domain. During protein refolding, DnaJ-like domain of about 8 kDa stimulates the ATPase activity of Hsp70. C-terminal domain of 21 kDa interacts with non-native polypeptides and also with the C-terminal region of Hsp70, and delivers the polypeptides to Hsp70. In this study aiming at elucidation of co-chaperone mechanism of Hsp40, we determined the crystal structures of the C-terminal domain of human type II Hsp40, Hdj1, complxed with the C-terminal octapeptide of human Hsp70, GPTIEEVD. Furthermore, we carried out mutagenesis studies on Hdj1 to identify the surface regions responsible for the cochaperone activity.

The crystal structure of the C-terminal domain of Hdj1 in complex with the octapeptide was refined at 1.9 Å resolution. Based on the refined structure, we designed Hdj1 single-point mutants at the peptide-binding sites. The Hdj1 mutants were expressed in *E. coli* and purified with cation-exchange chromatography. Cochaperone activities of wild-type Hdj1 and its mutants were measured using a luciferase refolding assay. Furthermore, the crystal structures of the mutants were determined.

C-terminal domain of Hdj1 is a twisted, horseshoe-shaped homo-dimer. The protomer consists of 11 β-strands and 3 α-helices and is folded into elongated globular domains I and II, and a C-terminal helix region related to dimerization. The octapeptides are located in two distinct sites of domain I, sites 1 and 2, of each protomer. In the site 1, the octapeptide forms an anti-parallel β-sheet with β2 strand of domain I. The negatively-charged side-chains of the octapeptide form salt bridges with the side-chains of Lys 181, Lys 182 and Lys 184 of Hdj1. The Ile side-chain of the octapeptide fits into the hydrophobic concaved surface. The site 1, therefore, is attributed to the recognition site toward Hsp70. The octapeptide in the site 2 forms an anti-parallel β-sheet with β4 strand of domain I. This site is located just behind the site 1. The negatively-charged side-chains of the octapeptide form salt bridges with the side-chains of Lys 213, Lys 217 and Lys 306 of Hdj1. The side-chain of Pro and Ile of the octapeptide are situated at the hydrophobic region which is flat and wider than the concave of the site 1. This region is suited for binding of the non-native polypeptides with hydrophobic side-chains bulkier than Ile. Hence, it is conceivable that the site 2 is the binding site toward non-native polypeptides. The point mutants at site 1 or site 2 are confirmed by determination of the structure that only one of the sites is be disrupted while the other site remains unchanged. These mutants reduced the cochaperone activity of