Poster Presentation

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Structural basis for the recognition of Eps15 by the SGIP1 μ homology domain

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Clathrin-mediated endocytosis (CME) is a process for eukaryotic cells to internalize extracellular molecules. FCHo1 and FCHo2 are involved in the initial clathrin assembly step of CME [1, 2]. These proteins contain the lipid-binding EFC/F-BAR domain at the N-terminus [3], and the μ homology domain (μHD) at the C-terminus. The μHDs of these proteins interact with a region rich in a repeated sequence motif, Asp-Pro-Phe (DPF), of an endocytic scaffold protein, Eps15. Eps15 contains fifteen DPF motifs. SGIP1 is also involved in CME and contains the μHD highly homologous to those of FCHo1/2 at the C-terminus, which also interacts with Eps15. The μHDs of these proteins share weak amino-acid sequence homology with the μ subunits of the adaptor protein complexes, such as AP-2, which links cargo proteins and clathrin in CME. To investigate the mechanism of Eps15 recognition by the FCHo1/FCHo2/SGIP1 μHDs, we first identified the minimal Eps15 fragment retaining the ability to interact with the μHD by the interaction studies using the SGIP1 μHD. We found that two Eps15-derived 11-residue peptides each containing two DPF motifs connected by a short 2—3 residue linker interact with the SGIP1 μHD with modest affinities (Kd = 15—20 μM). In contrast, peptides containing only one DPF motif did not bind to the μHD. Thus, the SGIP1 μHD requires two DPF motifs for binding. Moreover, the structures of the SGIP1 μHD in complex with the peptides containing two DPF motifs revealed that the SGIP1 μHD extensively recognizes the two adjacent tandem DPF motifs, while not contacting the flanking residues, consistent with the interaction studies. This mode of recognition is distinct from that of the Eps15 DPF motif recognition by the AP-2 appendage domains, providing the rationale of the recruitment of Eps15 by FCHo1/2 to the nascent endocytic sites, while allowing the FCHo1/2-bound Eps15 to recruit AP-2 complex with the different parts of the Eps15 DPF repeat region for clathrin assembly.

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