Crystal Structure of the EFC/F-BAR Domain -Mechanism of Membrane Invagination in Endocytosis-

Endocytosis is a fundamental process for eukaryotic cells to take up molecules from the extracellular space. One of the well-characterized endocytic pathways is clathrin-mediated endocytosis (CME), which is involved in various physiological processes, such as receptor internalization, somatic nutrient uptake, and synaptic vesicle recycling [1]. CME proceeds sequentially with the help of at least twenty different proteins (Fig. 1). Some of the endocytic proteins, such as epsin, amphiphysin, endophilin, and dynamin, directly bind and deform phospholipid membranes into tubules *in vitro* [2]. Their membrane deformation activities are believed to play an important role in the dynamic morphological changes of the plasma membrane in different steps of CME. These proteins utilize several mechanisms for membrane deformation, as shown in Fig. 1 (inset).

Pombe Cdc15 homology (PCH) proteins are involved in various actin-based physiological processes, including CME. They contain a recently identified membrane deformation module, the extended FER-CIP4 homology (EFC) domain, also known as the FCH and BAR (F-BAR) domain [3,4]. The EFC/F-BAR domain is responsible for the induction of tubular membrane invaginations by PCH proteins and tubulates the lipid membrane *in vitro*. The EFC domain shares a weak sequence homology with the BAR domain and induces tubular membranes with diameters several times larger than those of membranes induced by the BAR domain.

To elucidate the mechanism underlying membrane tubulation by the EFC domain and its role in CME, we determined the crystal structures of the EFC domains of human formin-binding protein 17 (FBP17) and Cdc42-interacting protein 4 (CIP4), using the X-ray diffraction data collected at the RIKEN Structural Genomics Beamline I (**BL26B1**) at SPring-8 and the Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory [5].

The structures of the EFC domains of FBP17 and CIP4 revealed an elongated gently curved helical bundle dimer (Fig. 2(a)). Structural and mutational analyses showed that the positively charged concave surface of the EFC domain interacts with the negatively charged phospholipid bilayer by electrostatic interactions, leading to membrane tubulation (Fig. 2(b)). The structure of the EFC domain was similar in topology to that of the BAR domain (Fig. 2(a)). However, the overall structures of these domains differ in length and curvature. The diameter of a tubular membrane that fits the concave surface of the EFC domain was several times larger than that of the BAR domain (Fig. 2(a)). Therefore, the difference in curvature between the two domains correlates well with the fact that the diameters of the EFC-induced tubular membranes are several times larger than those of the BAR-induced membranes.

Interestingly, the EFC dimers of FBP17 and CIP4 formed filaments by end-to-end interactions in the crystals. Introduction of mutations into the residues involved in filament formation abolished the membrane tubulation activity of the EFC domain, suggesting the importance of filament formation in tubulation. The EFC filament seems to be flexible and able to bend at the dimer-dimer interfaces to form spiral/ring-like EFC filaments. On the basis of on these facts, we constructed a mechanistic model of membrane tubulation induced by the EFC domain, in which the curved EFC filaments drive tubulation (Fig. 3(a)). This model was later verified by direct observation of tubular membranes covered with EFC filaments by phase contrast cryo-transmission electron microscopy.

Because the biochemical and structural data strongly

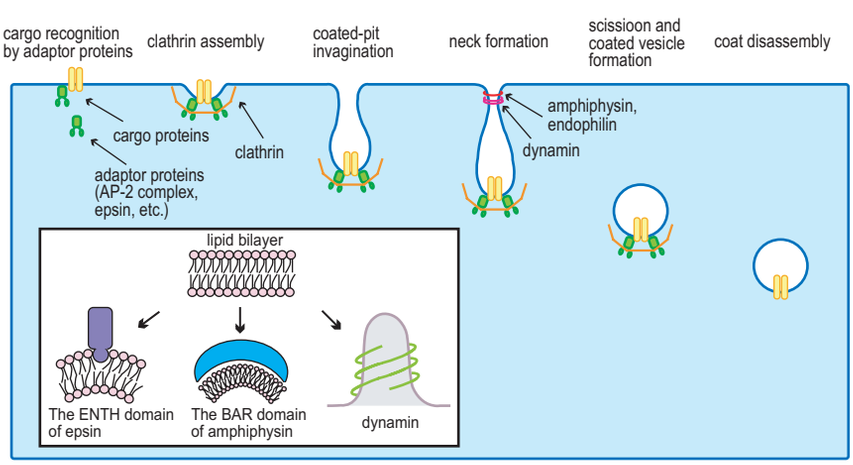

Fig. 1. Schematic diagram of coordinated vesicle formation in CME. Proteins involved in CME are indicated. Short descriptions of the different steps of CME are presented. (inset) Schematic diagram showing mechanisms of membrane deformation by proteins involved in CME.

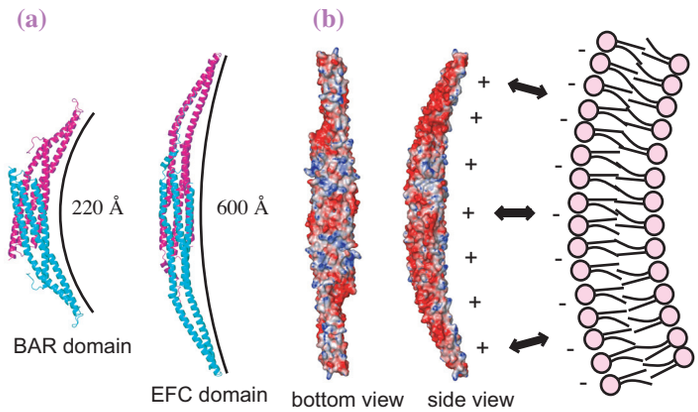

Fig. 2. **(a)** Ribbon diagrams of dimers of BAR domain (left) and EFC domain (right). The structure of the amphiphysin BAR domain and that of the CIP4 EFC domain are shown as representative structures. One molecule of each dimer is colored magenta and the second molecule is colored cyan. The diameter of tubular membranes that fit the concave surfaces of the BAR domain and that of the EFC domain are indicated. **(b)** Electrostatic potential surfaces of EFC-domain dimer with blue indicating positive charges and red negative charge. The bottom view (concave side, left) and the side view (right) are shown. The side view of the EFC domain is accompanied by a cartoon showing its interaction with the phospholipid membrane. The "+" and "–" symbols indicate positive and negative charges on the surfaces of the EFC domain and the phospholipid bilayer, respectively.

suggest that the EFC domain induces tubular membranes with clearly larger diameters than the BAR-induced membranes, we speculated that the EFC domain and the BAR domain may function in distinct steps of CME. The diameters of hemispherical coated pits formed in the early step of CME are typically more than ~700 Å, whereas that of the narrow neck formed at the foot of the coated pits in the late step of CME is ~200 Å. Because the EFC domain induces tubular membranes with more than ~650 Å diameters, it is plausible that the EFC domain plays a role in the invagination step between the initial clathrin assembly step and the neck formation step (Fig. 3(b)). This hypothesis was tested by the real-time imaging of fluorescence-labeled clathrin and FBP17 during CME [5]. It was shown that FBP17 co-localized with clathrin for a period corresponding to that of the invagination step of CME.

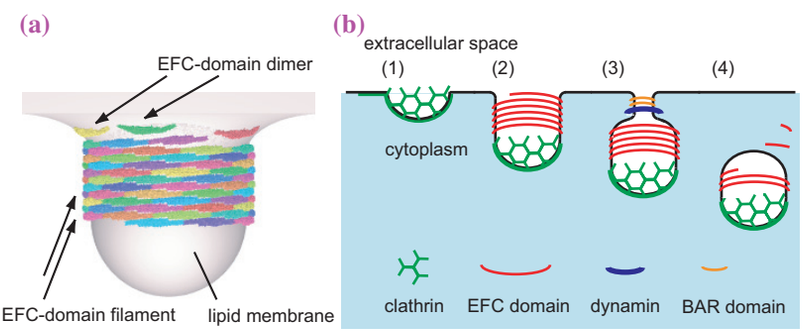

Fig. 3. **(a)** Model for membrane tubulation induced by EFC domain. Each EFC-domain dimer is colored differently. Incoming EFC dimers join end-to-end into a spiral-like filament at the foot of the nascent tubular membrane to drive continuous membrane tubulation. **(b)** Model for coordinated vesicle formation in CME. (1) Clathrin assembly, (2) coated pit invagination, (3) neck formation, and (4) scission steps are shown.

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