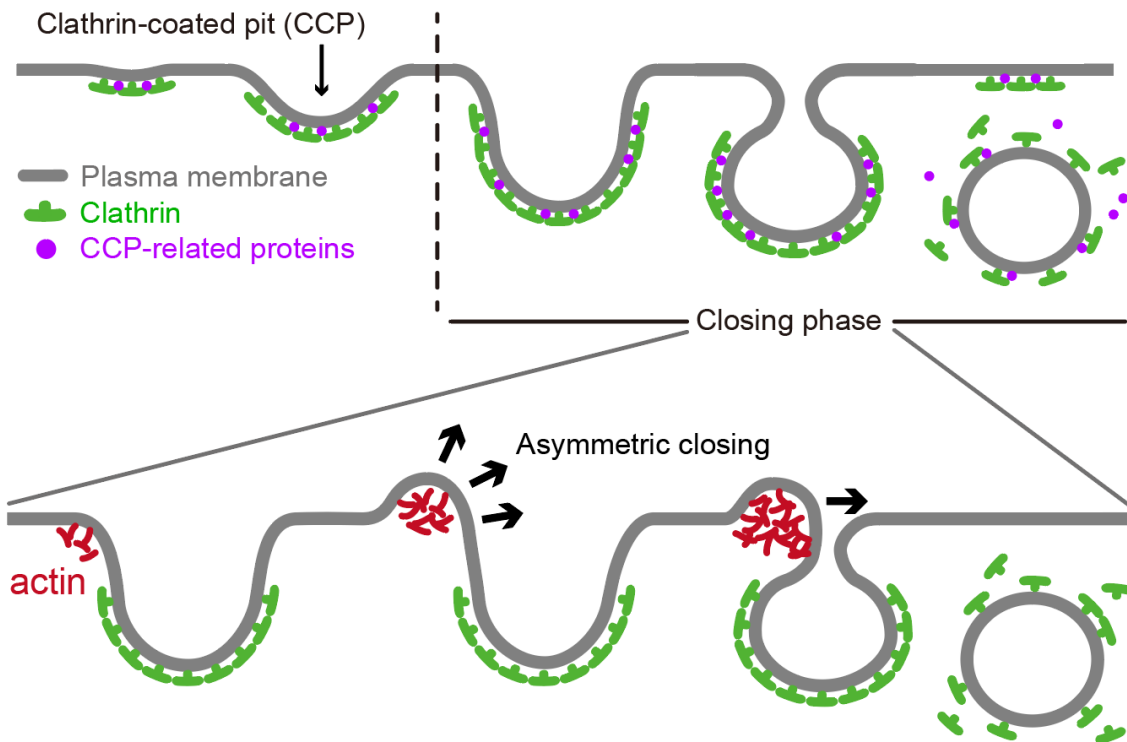


Correlative imaging of high-speed atomic force microscopy and fluorescence microscopy revealed asymmetric closing process of endocytosis

Presenter and the first author: Yiming Yu and Shige H. Yoshimura (Kyoto University, Japan)

Background



Clathrin-mediated endocytosis (CME) plays a critical role in regulating signal transduction from the extracellular environment into intracellular space. Recent studies focusing

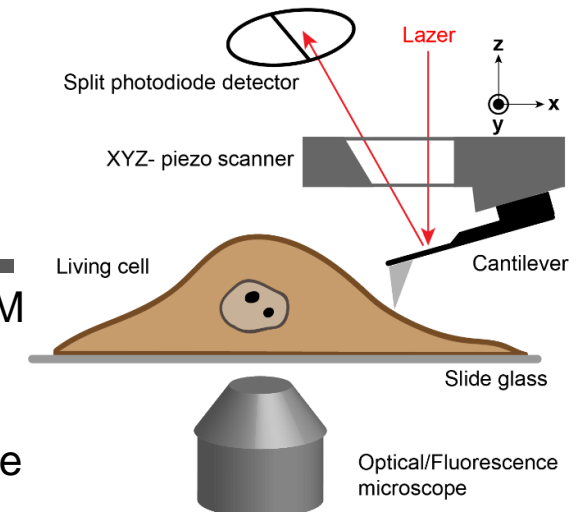
on the morphological changes of the plasma membrane during CME identified a unique actin-dependent and asymmetric membrane protrusion near the clathrin-coated pit (CCP) before the complete closing of the pit that gradually covered the pit area.

Aim

1. Elucidate the molecular mechanisms of asymmetric closing.
2. Investigate the biological significance of asymmetric CCP closing.

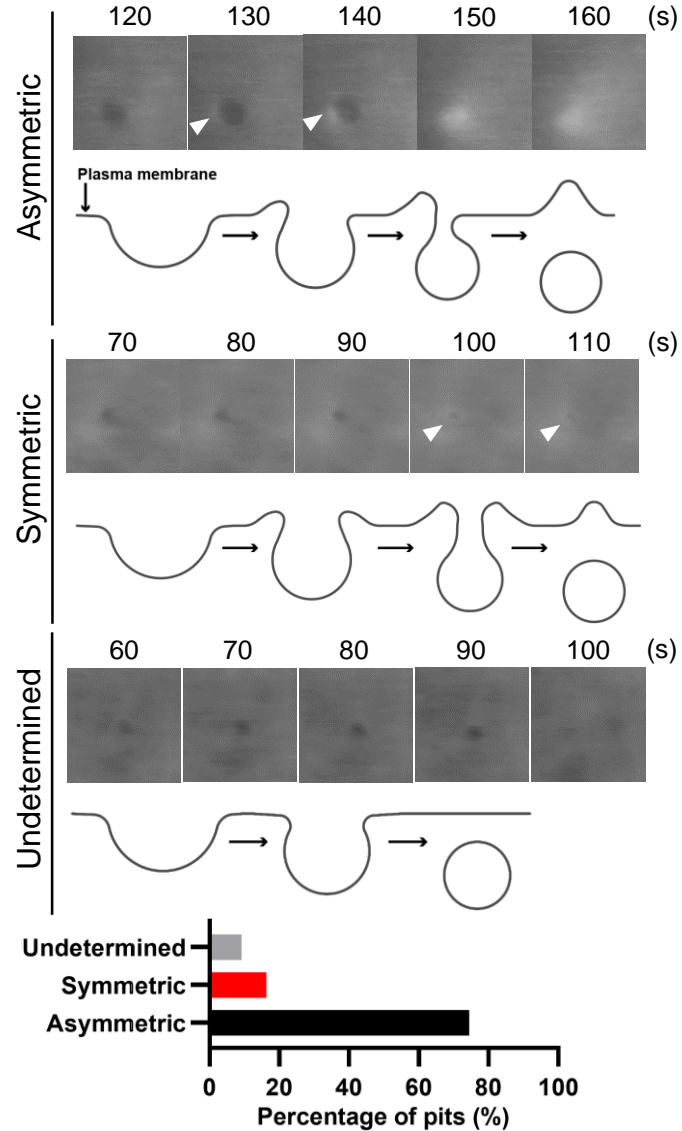
Method

Correlative imaging of HS-AFM and confocal laser scanning microscopy was used to observe the plasma membrane of live mammalian cells.

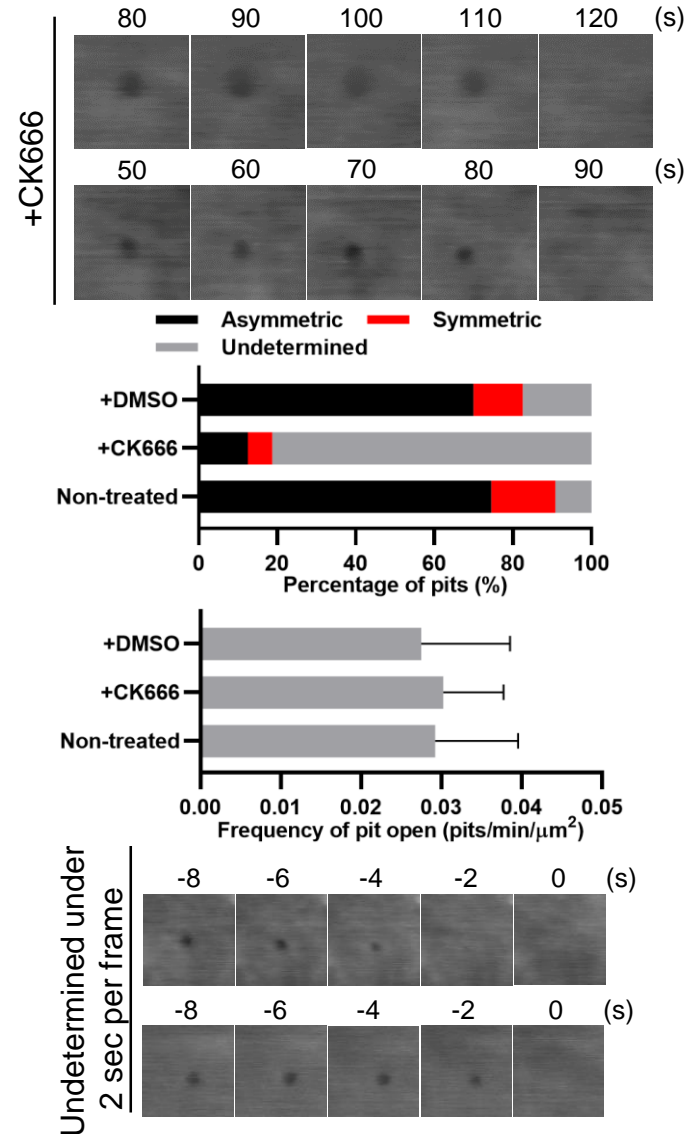


Results

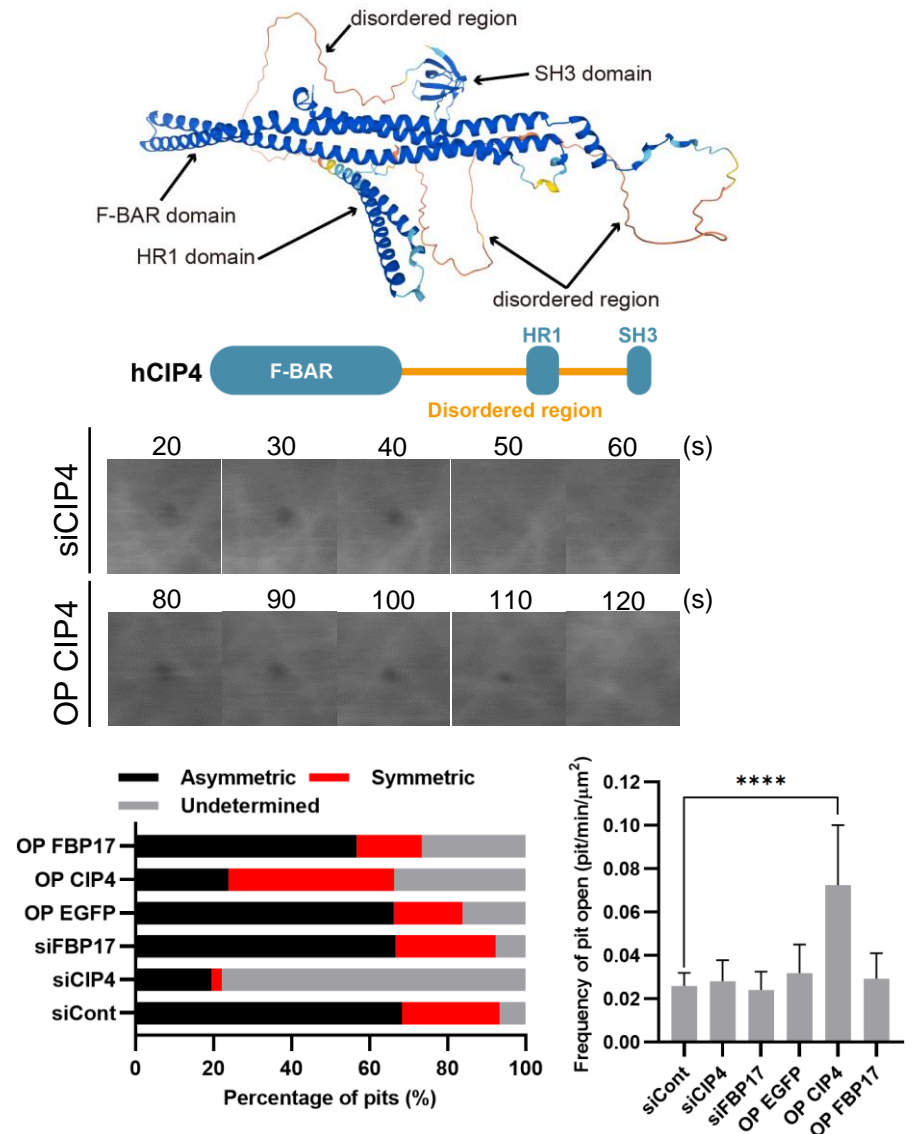
Most CCPs close asymmetrically



Asymmetric closing is actin-dependent



Strong assembly of BAR-domain protein is necessary for the asymmetric closing



Conclusions

1. The asymmetric closing is the dominant closing pattern of CCP at the closing step of CME.
2. Different closing patterns were manipulated by different molecular mechanisms.
3. Actin nucleation signaling is necessary to both asymmetric and symmetric closing.
4. Endogenous amount of BAR domain proteins may regulate the closing pattern of CCP.

References

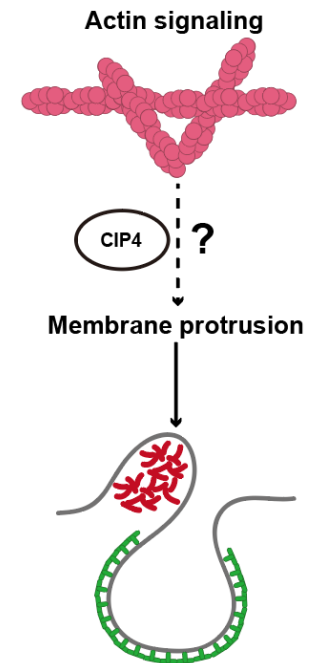
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yoshimura@lif.kyoto-u.ac.jp
yu.yiming.86e@st.kyoto-u.ac.jp



Questions to be answered by future experiment

1. Which part of the CIP4 is responsible for its function in mediating the formation of membrane protrusion?
2. How does the amount of CIP4 connect to the symmetry of membrane protrusion?
3. How does CIP4 cooperate actin signaling to the CCP area?
4. What is the biological significance of asymmetric closing of CCP?



Laboratory of Plasma Membrane and Nuclear Signaling
Sakyo-ku Yoshida-Konoe, Kyoto 606-8501, Japan
Faculty of Medicine Campus, South Campus
Reserach Building for Integrated Life Sciences
(Building G)