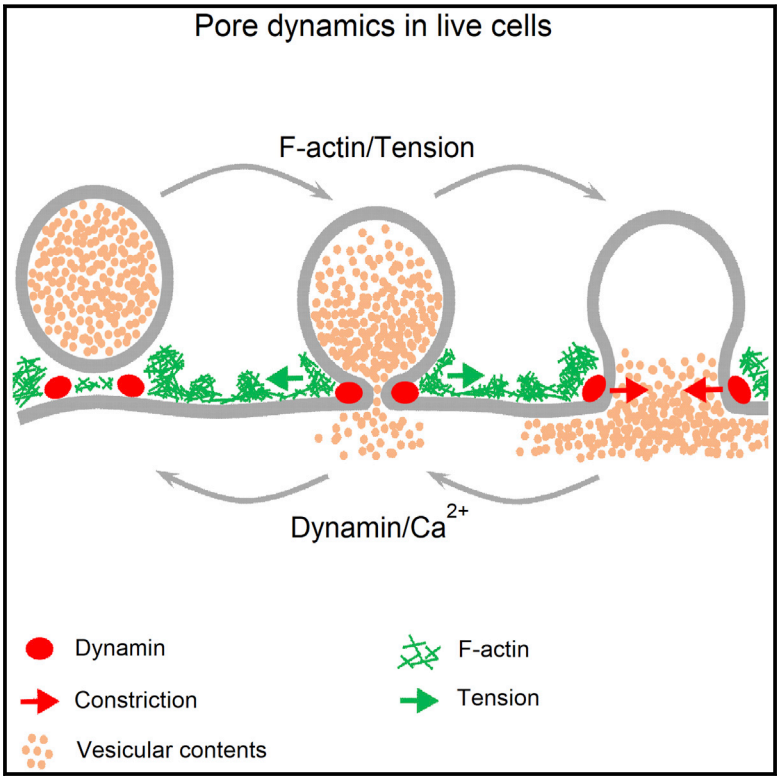
Article

Cell

Visualization of Membrane Pore in Live Cells Reveals a Dynamic-Pore Theory Governing Fusion and Endocytosis

Graphical Abstract



Highlights

- Visualizing 0–490 nm pore opening, expansion, constriction, and closure in live cell
- Competition between expansion and constriction generates diverse pore dynamics
- Actin/tension expand the pore, and calcium/dynamin constrict the pore up to 490 nm
- Establishment of a dynamic-pore theory controlling fusion and fission efficiency

Authors

Wonchul Shin, Lihao Ge, Gianvito Arpino, ..., Peter J. Wen, Hsueh-Cheng Chiang, Ling-Gang Wu

Correspondence

wul@ninds.nih.gov

In Brief

The missing live-cell evidence proving the fusion pore hypothesis reveals metastable pores that are two orders of magnitude larger than previously thought and can constrict and close instantly or slowly.

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Visualization of Membrane Pore in Live Cells Reveals a Dynamic-Pore Theory Governing Fusion and Endocytosis

Wonchul Shin,[1] Lihao Ge,[1] Gianvito Arpino,[1] Seth A. Villarreal,[1] Edaeni Hamid,[1] Huisheng Liu,[1,2] Wei-Dong Zhao,[1,3] Peter J. Wen,[1] Hsueh-Cheng Chiang,[1,4] and Ling-Gang Wu[1,5,*]

[1]National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

[2]Present address: Beijing Advanced Innovation Center for Precision Medicine, Beihang University, Beijing, China

[3]Present address: Department of Developmental Cell Biology, China Medical University, Shenyang, China

[4]Present address: Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan

[5]Lead Contact

*Correspondence: wul@ninds.nih.gov

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SUMMARY

Fusion is thought to open a pore to release vesicular cargoes vital for many biological processes, including exocytosis, intracellular trafficking, fertilization, and viral entry. However, fusion pores have not been observed and thus proved in live cells. Its regulatory mechanisms and functions remain poorly understood. With super-resolution STED microscopy, we observed dynamic fusion pore behaviors in live (neuroendocrine) cells, including opening, expansion, constriction, and closure, where pore size may vary between 0 and 490 nm within 26 milliseconds to seconds (vesicle size: 180–720 nm). These pore dynamics crucially determine the efficiency of vesicular cargo release and vesicle retrieval. They are generated by competition between pore expansion and constriction. Pharmacology and mutation experiments suggest that expansion and constriction are mediated by F-actin-dependent membrane tension and calcium/dynamin, respectively. These findings provide the missing live-cell evidence, proving the fusion-pore hypothesis, and establish a live-cell dynamic-pore theory accounting for fusion, fission, and their regulation.

INTRODUCTION

Fusion and fission, which mediate many biological processes, such as exocytosis, endocytosis, intracellular trafficking, cell division, fertilization, and viral entry, are thought to involve a membrane pore for releasing vesicular contents and for membrane scission (Lindau and Alvarez de Toledo, 2003; Chernomordik and Kozlov, 2008; Saheki and De Camilli, 2012; Alabi and Tsien, 2013; Wu et al., 2014; Antonny et al., 2016; Chang et al., 2017). Four lines of evidence collected in the last half of the century support this view. First, electron microscopy (EM) at synapses shows pore-like structures thought to reflect fusion or fission (Ceccarelli et al., 1973; Heuser and Reese, 1981; Miller and Heuser, 1984; Koenig and Ikeda, 1989; Shupliakov et al., 1997; Watanabe et al., 2013). However, using EM, it is difficult to determine whether the structure is for fusion, fission, on the way to expansion or closure, or formed by unknown mechanisms. Second, postsynaptic or amperometric current time course, which reflects transmitter release, diffusion, and postsynaptic or amperometric current generation, may imply fusion pore dynamics (Chow et al., 1992; Albillos et al., 1997; Wang et al., 2003; Alabi and Tsien, 2013; Li et al., 2016). Third, fluorescently tagged vesicular proteins, lipids, or quantum dots loaded into vesicles are released with different kinetics, implying different fusion pore dynamics (Aravanis et al., 2003; Taraska et al., 2003; Zhang et al., 2009). Different sizes of fluorescent dyes can be differentially loaded into vesicles, implying different fusion or fission pore sizes (Takahashi et al., 2002; Vardjan et al., 2007). A vesicle-like cavity is sometimes observed after content release in PC12 cells, implying a fusion pore that does not collapse (Taraska et al., 2003). Fourth, conductance measurements may estimate $< \sim 5$ nm fusion or fission pore for $< \sim 1$ μm vesicles (Albillos et al., 1997; Klyachko and Jackson, 2002; He et al., 2006, 2009). However, this estimate assumes a cylindrical geometry with a constant length, solution conductance, and membrane conductance while pore size changes. These assumptions are not verified, and pores $> \sim 5$ nm are usually beyond the conductance measurement limit.

The above studies lead to a widely held view, referred to here as the "metastable narrow-pore theory," that fusion forms a narrow pore that either closes rapidly to limit the speed and the extent of releasing vesicular cargoes (kiss-and-run) or expands irreversibly until flattened (full collapse) to promote release, and fission requires forming a narrow pore wrapped by dynamin or dynamin-like proteins for membrane scission (Lindau and Alvarez de Toledo, 2003; Saheki and De Camilli, 2012; Alabi and Tsien, 2013; Wu et al., 2014; Antonny et al., 2016; Chang et al., 2017). Pore regulation under this framework is thought to determine fusion and fission efficiency. However, fusion or fission pores have not been directly observed and thus proved in any live cells. Tools for direct pore observation in live cells are needed to prove the fusion/fission pore hypothesis, various hypothesized pore behaviors, and the underlying mechanisms and functions.