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Microscopy TODAY

Coming Events

2013

EMAG 2013
September 3–6, 2013
University of York, UK
emag-iop.org

FEMMS 2013
September 8–13, 2013
Lorne, Victoria, Australia
http://mcem.monash.edu.au/news-activities/femms-2013.html

Histotechnology
September 20–26, 2013
Providence, RI
www.nsh.org

CIASEM 2013
September 24–28, 2013
Cartagena, Columbia
ciasem2013.com/index_ing.html

Materials Science & Technology
October 27–31, 2013
Montreal, Canada
www.matscitech.org

Neuroscience
November 9–13, 2013
San Diego, CA
www.sfn.org/am2013

Cell Biology Annual Meeting
December 14–18, 2013
New Orleans, LA
www.ascb.org/meetings

2014

Microscopy & Microanalysis 2014
August 3–7, 2014
Hartford, CT
www.microscopy.org

2015

Microscopy & Microanalysis 2015
August 2–6, 2015
Portland, OR
www.microscopy.org

2016

Microscopy & Microanalysis 2016
July 24–28, 2016
Columbus, OH
www.microscopy.org

2017

Microscopy & Microanalysis 2017
July 23–27, 2017
St. Louis, MO
www.microscopy.org

2018

Microscopy & Microanalysis 2018
August 5–9, 2018
Baltimore, MD
www.microscopy.org

More Meetings and Courses
Check the complete calendar near the back of this magazine and in the MSA journal *Microscopy and Microanalysis*.

Carmichael's Concise Review

Nibbling at Membranes

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It has been known for decades that clathrin- and dynamin-mediated endocytosis is the major pathway for recycling the components of vesicle membranes after strong stimulation and high rates of exocytosis in secretory cells. This pathway occurs over tens of seconds to minutes after fusion of the secretory vesicle membrane with the plasma membrane. It resembles classical receptor-mediated endocytosis, but it has a trigger that is unique to secretion: the sudden appearance of the secretory vesicle membrane on the surface of the cell. However, the spatial localization, the relationship to individual fusion events, the nature of the cargo, and the timing and nature of nucleation events have been unknown. An elegant study by Mary Bittner, Rachel Aikman, and Ronald Holz has addressed these issues [1].

Bittner et al. used a combination of total internal reflection fluorescence microscopy of transiently expressed proteins and time-resolved quantitative confocal imaging of endogenous proteins on chromaffin cells from the bovine adrenal medulla. These are the cells that secrete epinephrine (also know as adrenaline) into the bloodstream. They demonstrated that (1) chromaffin vesicle membrane proteins remain as a distinct punctate entity in the plasma membrane for minutes after fusion, (2) clathrin and dynamin begin to accumulate within seconds at sites of fusion, (3) endogenous chromaffin vesicle membrane proteins are gradually removed from individual surface puncta by a “nibbling” mechanism (Figure 1) and appear intracellularly over a time course of minutes and are dependent on dynamin, and (4) stimulated endocytosis adds a new population of internalized vesicles distinct from those reflecting constituative endocytosis.

Dopamine-β-hydroxylase (DBH) is the enzyme that converts dopamine to norepinephrine (which in turn is converted to epinephrine by another enzyme). DBH is both a soluble protein within chromaffin vesicles and bound on the internal surface of the vesicle. After the vesicle membrane fuses with the plasma membrane, the membrane-bound DBH is exposed to the external environment.

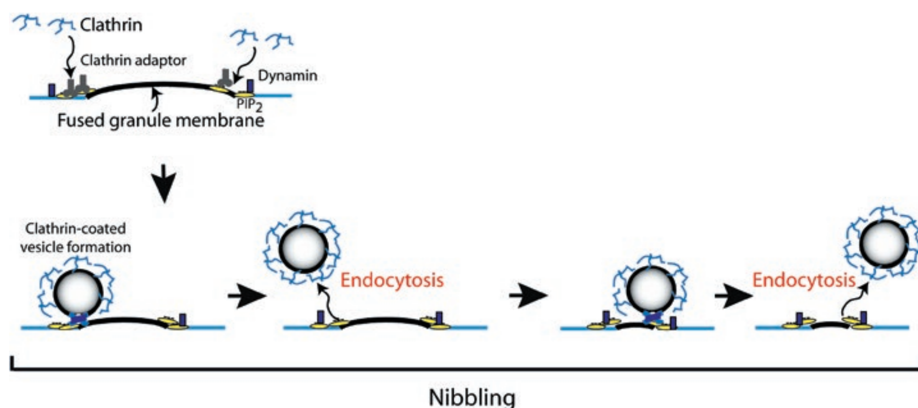

Figure 1: Model of a nibbling mechanism for clathrin-mediated endocytosis. Upon stimulation, the membrane of fused chromaffin vesicles inserts into the plasma membrane. Clathrin adapters are then rapidly recruited to sites of fusion by phosphatidylinositol-4,5-diphosphare (PIP_2) and vesicle membrane proteins (for example, synaptotagmin-1, VMAT2) with adapter binding motifs. Subsequent binding of clathrin permits the formation of clathrin-coated vesicles whose separation requires dynamin GTPase activity. Because the typical clathrin-coated vesicle (90 nm diameter, 0.025 square microns) is too small to internalize the entire patch of chromaffin vesicle membrane (0.282 square microns) as a single unit, the process is repeated (nibbled) and the patch is incrementally internalized.

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It was demonstrated with immunocytochemistry that DBH (and other vesicle membrane proteins) remained associated as puncta on the plasma membrane for many minutes after fusion with clathrin and dynamin joining the crowd, often within 20 seconds of fusion. Thus, the vesicle membrane itself is the likely nucleation site for the endocytosis that follows fusion. In a large series of well-controlled experiments, Bittner et al. showed, using quantitative techniques, that the vesicle membrane was internalized bit-by-bit over seconds to minutes, a process they dubbed "nibbling." The retrieval by nibbling was completely inhibited by the dynamin inhibitor dynogo4a.

A nibbling mechanism is a surprisingly logical explanation for the area mismatch between the clathrin-coated vesicles observed by electron microscopy and the fused vesicle membrane. The studies of Bittner et al. convincingly demonstrated that a nibbling mechanism is the major pathway for retrieval of the fused vesicle membrane from the cell surface after a strong stimulus to secrete.

References

[1] MA Bitner, RL Aikman, and RW Holz, *J Biol Chem* 288 (2013) 9177–88.

[2] The author gratefully acknowledges Drs. Mary Bittner and Ron Holz for reviewing this article.

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