The mother of all endocytosis

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The mother of all endocytosis

Massive endocytosis is initiated by a series of steps that involve a sudden influx of calcium ions, changes in mitochondria, and modification of surface proteins by lipids. A better understanding of this process could lead to new approaches to reducing the tissue damage that is caused by heart attacks.

DAVID E CLAPHAM

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Image
Micrograph of a patch clamp experiment with a BHK cell (the circular structure at the bottom of the image)
Insight

exocytosis or endocytosis (Neher and Marty, 1982). Now, in a refreshingly original study, Donald Hilgemann and co-workers at UTSW and Cornell—Michael Fine, Maurine Linder, Benjamin Jennings and Mei-Jung Lin—have used a combination of capacitance recording and other techniques to study the mechanism responsible for the massive endocytosis that occurs after a sudden influx of calcium ions into cells called BHK fibroblasts (Hilgemann et al., 2013).

Massive endocytosis is preceded by depolarization of the inner membrane of the mitochondria and/or the formation of pores called permeability transition pores: both of these processes allow coenzyme A (a small molecule i.e., required for the oxidation of pyruvate and fatty acids) to move from inside the mitochondria to the cytoplasm of the cell. Experimentally, this is accomplished by various mitochondrial insults, such as increasing the concentration of calcium ions inside the cell or poisoning the electron transport chain. Inhibition of calcium uptake blocks massive endocytosis.

The key finding is that the movement of coenzyme A (CoA) into the cytoplasm leads to the synthesis of acyl-CoA, which acts as a substrate for an enzyme, DHHC5, that transfers the fatty acid group called palmitoyl to proteins and thus anchors these proteins to the plasma membrane. In short, mitochondrial damage and the increased availability of substrates for DHHC5 unleash the proteins that initiate endocytosis. One such protein, flotillin, was previously shown to be palmitoylated by DHHC5 (Li et al., 2012). However, proteins are not the only participants in massive endocytosis: cholesterol and PIP2 (a phospholipid i.e., found in the cell membrane) can also induce massive endocytosis minutes after the sudden increase in the concentration of calcium ions has subsided (Lariccia et al., 2011).

In the second paper, Hilgemann, Lin, Fine and other co-workers—Jui-Yun Lu and Sandra Hoffman (both UTSW) and Gary Frazier (University of Texas at Dallas)—investigate reperfusion injury, the damage caused when blood supply returns to a tissue after a period of ischemia, in cardiac muscle cells (Lin et al., 2013; Figure 1). Reoxygenation after anoxia, such as occurs during a heart attack, results in massive endocytosis. Using protocols that raise the levels of calcium or acyl-CoA inside the cell, the UTSW team infers that opening the permeability transition pores, activating protein kinase C, and triggering a calcium-dependent mechanism can all induce massive endocytosis. However, massive endocytosis is strongly inhibited in mice lacking DHHC5, resulting in significantly preserved right ventricular contractile function. Finally, Lin et al. show that a diverse range of protein targets undergo palmitoylation (that is, attachment of the fatty acid group, palmitoyl) after reperfusion injury. This most consequential finding of a reduction of massive endocytosis and the preservation of contractility in DHHC5 knockout mice suggests a potential new approach to ameliorate the problem of reperfusion injury.

Although Hilgemann and co-workers tip their hat to the possibility that massive endocytosis can be an ongoing process, the hypothesis mainly rests on dire cellular 

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sort of large cytoplasmic calcium entry is widely associated with fast massive exocytosis, presumably required for the resealing of membranes (McNeil and Steinhardt, 2003). The link between initial exocytosis followed by endocytosis via vesicles larger than coated vesicles was described in pituitary cells (Thomas et al., 1994). As in those cells, exocytosis in fibroblasts occurs in the first few seconds after a large calcium increase in fibroblasts and is followed by massive endocytosis, but the initial exocytosis is not observed in cardiac muscle cells (Lin et al., 2013). This suggests that these cells lack the pool of vesicles used in the fast exocytosis phase. The identities of these exocytic or endocytic vesicles are not established.

Other questions remain. Is massive endocytosis an evolutionarily primitive form of endocytosis—that separates protein- and cholesterol-rich membranes? Does coenzyme A evoke a regulatory network linking mitochondria to numerous cytoplasmic processes? The understanding of massive endocytosis would be solidified by live tissue imaging of acyl-CoA levels or DHHC5 activity, but these will require the invention of new indicator methods.

One mystery is the role of protein kinase C in massive endocytosis. Since this enzyme phosphorylates phospholemman (a small membrane protein i.e., involved in ion transport), the question arises as to the functional consequence of phosphorylation before or after palmitoylation. Comparison of all palmitoylated proteins before and after massive endocytosis, and the examination of potential newly formed membrane phases, will help illuminate why such massive endocytosis occurs. Perhaps even more important is to follow the fate of such cells over longer durations—that is, is massive endocytosis simply a cellular death throes, or is it a useful adaptation?

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David E Clapham is in the Howard Hughes Medical Institute, Department of Cardiology, Boston Children’s Hospital, Boston, United States and the Department of Neurobiology, Harvard Medical School, Boston, United States
dclapham@enders.tch.harvard.edu

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