# Regarding Clathrin-Coated Pits and Endocytosis, Bigger Isn't Better

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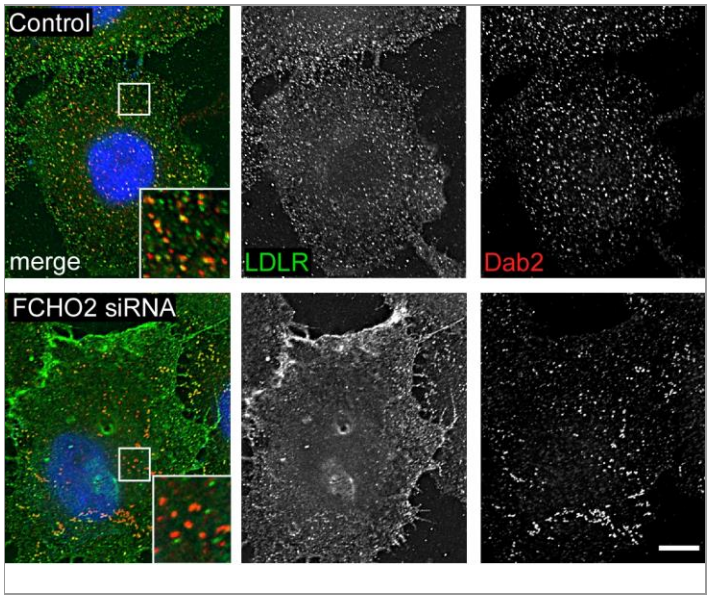
Endocytosis is a primary means by which nutrients, cellular surface receptors (e.g., signaling and adhesion molecules) and even other cells are brought into a cell. Multiple endocytic pathways exist, including caveolar endocytosis, phagocytosis, pinocytosis and clathrin-mediated endocytosis (CME). CME requires adaptor proteins to mediate the recruitment of specific receptors to clathrin-coated pits. The primary adaptor protein for CME, AP2, employs different domains to coordinate the binding of membrane phospholipids, receptors, clathrin and accessory proteins that close and detach the clathrin-coated pit from the plasma membrane. AP2 also binds other adaptor proteins, such as ARH, a low-density lipoprotein receptor (LDLR) adaptor protein, thus expanding the cargo that can be directly or indirectly internalized via AP2.

In 2006, former graduate student Dr. Meghan Maurer and advisor Dr. Jonathan Cooper of the Basic Sciences Division found that LDLR could be endocytosed via an alternate pathway in human fibroblasts that utilizes the clathrin-associated adaptor protein, Disabled-2 (Dab2). However, unlike ARH, Dab2 can efficiently recruit LDLR into clathrin-coated pits in the absence of AP2. To understand how this Dab2-dependent LDLR endocytosis pathway operates, current graduate student Erin Mulkearns and Jon Cooper initiated a focused search for accessory proteins that assist Dab2-directed endocytosis. Using mass spectrometry to identify proteins that specifically associate with Dab2 in HeLa cells, they detected a novel Dab2-interacting protein, FCHO2, which is an accessory protein that binds to and manipulates membranes. Mulkearns showed that both FCHO2 and AP2 interact with the same region of Dab2. Moreover, the Dab2/FCHO2 interaction was required for efficient LDLR internalization when AP2 was depleted from HeLa cells. The authors also found that when FCHO2 expression was downregulated, clathrin-coated structures decreased in number, but increased in size, and LDLR internalization was reduced by 50 percent. However, efficient endocytosis of LDLR by both AP2 and Dab2 pathways could be rescued if low temperatures were employed to slow clathrin-coated pit budding and promote LDLR accumulation in FCHO2-depleted clathrin-coated structures. This novel finding suggests that FCHO2 regulates the size of clathrin-coated structures and their ability to trap receptors, both of which are essential aspects of CME.

Mulkearns EE, Cooper JA. 2012. FCHO2 organizes clathrin-coated structures and interacts with Dab2 for LDLR endocytosis.*Molecular Biology of the Cell*, Epub ahead of print, doi: 10.1091/mbc.E11-09-081

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In HeLa cells that stably express a labeled form of LDL receptor (LDLR, green stain), Dab2 (red) recruits LDLR into clathrin-coated structures (yellow, merge). However, in cells treated with siRNA against FCHO2 mRNA to downregulate FCHO2 expression (bottom panels), clathrin-coated structures are fewer and larger in size, and LDLR recruitment into these structures is less efficient, thus reducing the amount of LDLR/Dab2 co-staining. (Scale bar is 10 micrometers.)