

Dab2 Recruits EH Domain Proteins to Regulate Clathrin-Mediated Endocytosis

July 16, 2012

ME Arnegard

The clathrin-mediated endocytic pathway is a major route for endocytosis, the mechanism by which many essential substances are transported into cells, particularly large polar molecules. Membrane receptors compose some of the cargoes that are internalized via clathrin-mediated endocytosis (CME). During this process, clathrin and endocytic adaptors co-assemble to form clathrin-coated pits (CCP) in the membrane. The adaptor proteins bind to internalization signals in the cytoplasmic tails of specific receptors, which causes these cargoes to become tethered to clathrin and the cell membrane.

The tetramer AP2 is the prototypical clathrin adaptor. Its $\mu 2$ subunit recognizes both clathrin and a sorting signal within the cytoplasmic tail of the transferrin receptor (TfnR). The α and $\beta 2$ subunits of AP2 bind to numerous endocytic accessory proteins, including the scaffolding proteins Eps15 and Intersectin (Itsn), as well as proteins involved in sensing membrane curvature and a GTPase implicated in vesicle fission. Together, the AP2 subunits act as a nexus of protein-protein interactions that mediate the regulation of TfnR recruitment and the invagination and budding of CCP.

Another class of adaptor proteins consists of monomeric clathrin-associated sorting proteins, such as Dab2. This adaptor protein is known to contain a clathrin-binding motif and a motif that recognizes Dab2 cargoes like integrin $\beta 1$. Dab2 also contains NPF (asparagine proline phenylalanine) motifs that could possibly bind Eps homology (EH) domain-containing proteins, such as Eps15 and Itsn. These and other EH domain proteins possess binding sites for some of the same accessory proteins that are involved in AP2-mediated CCP invagination and budding, suggesting that the monomeric Dab2 protein might also accomplish these critical steps in CCP maturation. In the absence of experimental evidence, however, the mechanism by which Dab2 regulates CCP invagination and budding has remained a mystery.

Recently, first author Dr. Anjali Teckchandani, senior author Dr. Jonathan Cooper (both in the Division of Basic Sciences) and their collaborators demonstrated that Dab2 co-immunoprecipitates with Eps15 and Itsn in HEK-293 cells. Teckchandani *et al.* also showed that depletion of Eps15 and Itsn, by means of siRNA, inhibits endocytosis of both Dab2-dependent and Dab2-independent cargoes. The result was an increase in large clathrin-coated structures (CCS) on the surface of the cells. Next, the authors set out to rigorously test the role that EH domain proteins play in Dab2-mediated endocytosis, while controlling for the size distribution of CCS. To do so, they reconstituted Dab2-deficient HeLa cells with a mutated form of Dab2, in which the NPF motifs had been changed to NPV (asparagine proline valine), knocking out their ability to bind EH domain proteins. The clathrin structures that formed in these manipulated cells recruited integrin $\beta 1$ normally. However, they internalized the receptor less efficiently, demonstrating the importance of the Dab2-EH domain interaction for Dab2-dependent endocytosis. Although most of the clathrin structures were found to

contain both Dab2 and AP2, the authors showed that integrin $\beta 1$ and TfnR do not co-localize on the cell surface. Remarkably, TfnR endocytosis was unaffected in the Dab2-mutant cells.

Given these important new insights generated by the Cooper Lab, Teckchandani and co-authors propose an expanded model of the CME process (see figure). Their research suggests that endocytic adaptors need to be bound to their cargo in order to regulate EH domain proteins and drive efficient internalization. The authors also suggest an intriguing reason that the adaptors may need to bind both cargo and EH domain proteins for efficient CCP maturation: this requirement would ensure that the pits are 'fully loaded' before the CME process can continue beyond the endocytic checkpoint.

[Teckchandani A, Mulkearns EE, Randolph TW, Toida N, Cooper JA.](#) 2012. The clathrin adaptor Dab2 recruits EH domain scaffold proteins to regulate integrin $\beta 1$ endocytosis. *Molecular Biology of the Cell*, Epub ahead of print, doi:10.1091/mbc.E11-12-1007.

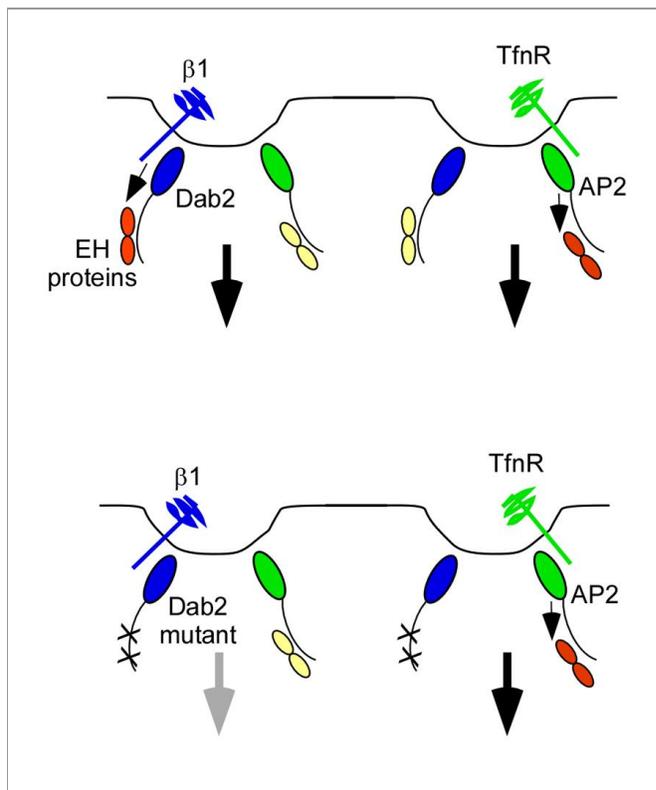


Image courtesy of Dr. Anjali Teckchandani

Model of clathrin-mediated endocytosis suggested by the authors' findings. Although the adaptor proteins Dab2 and AP2 are found together in most clathrin-coated structures (CCS), their receptors, integrin beta 1 and TfnR, do not localize to the same structures. Internalization of a clathrin structure is driven by adaptors being bound simultaneously to both their cognate receptors (blue or green) and to active EH domain proteins (orange). Inactive EH domains are shown in pale yellow. Small black arrows indicate regulation of EH domain proteins by the adaptor-receptor complexes. Large black arrows represent efficient internalization, whereas the large gray arrow represents reduced internalization. When cells are reconstituted with a Dab2 mutant that does not bind EH domain proteins, integrin beta 1 is not internalized efficiently. However, TfnR endocytosis proceeds normally because its adaptor, AP2, continues to bind EH domain proteins. The Ap2-EH domain complexes in CCS are thought to be unable to drive endocytosis of integrin beta 1-loaded pits because, in these cases, the complexes are not bound to receptors.