Integrating Actin Assembly and Endocytosis

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The extent to which the many different pathways of endocytosis share underlying molecular mechanisms is currently unknown. In this issue of Developmental Cell, Yarar et al. (2007) report that SNX9, a protein that binds phosphatidylinositides, dynamin, and N-WASP, coordinates actin assembly with several distinct endocytic processes.

A large number of accessory or adaptor proteins important for endocytosis have been identified in the past decade, among them members of the sorting nexin (SNX) family, identified by their PX domains. Most PX domains bind phosphatidylinositides, either phosphatidylinositol(3)phosphate or phosphatidylinositol(4,5)bisphosphate (PIP2). The latter is critically important for membrane traffic, signal transduction, and modulating the actin cytoskeleton. Recently SNX9 has been shown to be required for efficient clathrin-mediated endocytosis (Soulet et al., 2005). SNX9 can bind N-WASP (Worby et al., 2001), an important activator of actin polymerization, and dynamin 2 (Lundmark and Carlsson, 2003), a large GTPase required for both clathrin-mediated and certain non-clathrin-mediated endocytic processes. The key question for proteins like SNX9 is whether their disparate binding partners indicate that they have multiple, independent functions that they might coordinate, or whether they integrate those partners into a single function or pathway.

The role of actin in endocytosis is somewhat controversial, as actin polymerization has been reported to be required in many endocytic pathways but either can be replaced, or is not essential, for the basic membrane deformations required to invaginate membrane and bud off an endocytic carrier. In an in vitro assay of actin polymerization stimulated by N-WASP and Arp2/3, SNX9 accelerated actin polymerization into a dense dendritic network. Liposomes containing PI(2)P accelerated actin polymerization only if they were preincubated. When added together at the beginning of an actin polymerization assay, they had no effect, even if the assay was run almost as long as the preincubation period. This suggests that oligomers of SNX9 were not forming during the polymerization assay. Binding to N-WASP either inhibited the oligomerization of SNX9 dimers or interfered with binding of the dimers to PI(2)P liposomes. If these observations can be extrapolated to the situation in vivo, they suggest that an ordered assembly reaction is required for actin polymerization on endocytic membranes in which PI(2)P is generated and bound by SNX9 before N-WASP is recruited. If so, there must be some as yet unknown mechanism to prevent SNX9 and N-WASP from binding prematurely in the cytoplasm.

Yarar and colleagues suggest that the role for actin assembly in endocytosis is to generate force for the purpose of the shape changes required to generate an endocytic carrier. Chief
among these would be polymerizing actin at the neck of a budding vesicle or tubule and forcing it away from the plasma membrane. However, actin polymerization is not universally observed during the budding of clathrin vesicles, and actin depolymerization does not shut down all of endocytosis. A simple explanation for the lack of a uniform requirement for actin for the formation of endocytic vesicles might be that cells have developed multiple mechanisms to form and fission off endocytic carriers. The requirement for a mechanism using actin might be dependent upon whether or not actin and actin regulatory proteins like N-WASP are locally abundant. One can imagine that in locations (such as the adherent plasma membrane) where a dense cortical network is in the way of membrane deformation and movement, a rearrangement of the actin cytoskeleton might be necessary and has been adapted to move membrane carriers through the obstruction by using its own components. At other locations where an actin cytoskeleton is less abundant, a mechanism using actin might not be required. Since the actin cytoskeleton is dynamic and sensitive to growth states, the requirement for actin in endocytosis could vary for the same cell type under different growth conditions, and might even vary at different locations in the same cell.

REFERENCES


Ubiquitin Ligation without a Ligase

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Classically, ubiquitination requires three enzymes acting in sequence: E1, E2, and E3. E3 ubiquitin ligases typically provide substrate specificity. An article in Molecular Cell (Hoeller et al., 2007) now describes the E3-independent monoubiquitination of certain proteins. The mechanism has interesting parallels to SUMO ligation.

Ubiquitin covalently modifies other proteins, either as ubiquitin chains (polyubiquitination) or single ubiquitin moieties (monoubiquitination) (Kerscher et al., 2006). Attachment of ubiquitin to a protein enhances its interaction with ubiquitin receptors containing ubiquitin-binding domains (UBDs). The consequences of such interactions depend on their timing and cellular location and on the type of ubiquitin modification. Generally, a lysine in the substrate is coupled to the C-terminal glycine of ubiquitin by an amide (isopeptide) bond. This requires prior activation of the ubiquitin C terminus by E1, after which the ubiquitin is passed to an active site cysteine side chain in an E2, creating a thioester-linked E2-ubiquitin complex. An additional factor, the E3 ubiquitin ligase, is usually necessary for efficient ubiquitin transfer from E2 to substrate. E3 enzymes contain substrate- and E2-binding domains and may activate ubiquitin transfer by the E2.

One area of cell regulation that makes extensive use of monoubiquitination is endocytosis (Mukhopadhyay and Riezman, 2007). Ubiquitin attachment to plasma membrane proteins usually causes their downregulation by stimulating endocytosis and trafficking to the lysosome for degradation. Additionally, multiple endocytic adaptor proteins contain UBDs, which allow them to modulate membrane receptor trafficking by binding to the ubiquitinated receptor or to other endocytic factors that have been ubiquitinated. UBDs come in a variety of structural flavors but have in common the ability to bind ubiquitin noncovalently, usually with fairly low affinity (Harper and Schulman, 2006). Interestingly, many of the monoubiquitinated endocytosis factors also bear a UBD. Covalent monoubiquitination of these proteins depends on the ability of the UBD to engage ubiquitin