NIH Award from the National Institute of Diabetes and Digestive and Kidney Diseases

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- **Project:** Regulation of Endocytosis by Ubiquitin and Ubiquitin-Binding Proteins
- **Start Date:** September 1, 2009
- **Total Award Amount:** $366,019

**How the results of this project will benefit society:**
The research in this grant will define how plasma membrane proteins are regulated by removal from the cell surface by endocytosis, the process in which a substance gains entry into a cell without passing through the cell membrane. The small protein ubiquitin plays an important role in this process and disruption of ubiquitin-dependent endocytosis results in or is linked to many diseases, including hypertension, inflammation, immune diseases, multiple cancers, viral infections and bacterial toxin entry into cells. Endocytosis regulated by ubiquitin is also essential for developmental processes in complex organisms, such as cell fate specification and development of the nervous system.

**The problem the project is trying to solve:**
Ubiquitin and ubiquitin-binding proteins play a critical role in the regulation of protein activity at the surface of eukaryotic cells by controlling the internalization of proteins into the endocytic pathway. Dysfunction of ubiquitin-dependent endocytosis results in or is linked to many diseases, including hypertension, inflammation, immune diseases, multiple cancers, viral infections and bacterial toxin entry into cells. Ubiquitin-dependent endocytosis is also essential for developmental processes in complex organisms, such as cell fate specification and development of the nervous system. Specific proteins that are regulated by ubiquitin-dependent endocytosis include leptin receptors, growth factor receptors, glucose transporters, glutamate receptors, ion channels, the aquaporin-2 water channel and the anthrax toxin receptor.

One of the roles of ubiquitin in the internalization of membrane protein cargo is to serve as an internalization signal; another is to regulate the endocytic machinery. Several components of the endocytic machinery are modified with monoubiquitin and many of these ubiquitinated endocytic proteins also bind to ubiquitin noncovalently through ubiquitin-binding domains (UBDs). We have identified proteins required for the internalization step of endocytosis in the yeast Saccharomyces cerevisiae that bind to directly to ubiquitin.

Based on our recent studies with these proteins, we hypothesize that ubiquitin-UBD interactions regulate the assembly of the internalization machinery into dynamic protein networks at sites of endocytosis on the plasma membrane. Because UBDs are found in many proteins with diverse functions, the regulation of protein complex assembly by ubiquitin-UBD interactions is also likely to occur in other basic cell biological processes.

**How this project will work:**
To define how ubiquitin and UBDs regulate the endocytic machinery, we will use three proteins that act at distinct stages of receptor internalization as paradigms — epsin, Ede1 and Sla1. All of these proteins and their mammalian homologues bind to ubiquitin and are likely ubiquitinated by Nedd4/Rsp5 ubiquitin ligases. Using biochemical assays for protein-protein and protein-lipid interactions, and the physiological analysis of yeast mutants, we will test the role of ubiquitin-binding and ubiquitination of Ent2, Ede1 and Sla1 in endocytosis, specifically during the formation of primary endocytic vesicles and organization of cortical actin at endocytic sites.

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