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Abstract

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Grant Number:	1R01DK053257-01
PI Name:	HICKE, LINDA A.
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PI Title:	ASSISTANT PROFESSOR
Project Title:	UBIQUITIN-DEPENDENT ENDOCYTOSIS OF SIGNALING RECEPTORS

Abstract: DESCRIPTION: Down-regulation of cell surface receptors is essential for appropriate responses to the environment. The mechanism of this process is still incompletely understood. Recent data indicates that several receptors become ubiquitinated following stimulation by ligand and this process has been implicated in receptor down regulation. In her postdoctoral research Dr. Hicke found that the alpha pheromone receptor in Saccharomyces cerevisiae (Ste2p) is multiply phosphorylated and ubiquitinated after receptor stimulation, and that these steps are required for endocytosis and trafficking to the vacuole. She identified a short region at the carboxy terminal tail that was both necessary and sufficient for this pathway. Dr. Hicke seeks to understand the details of this process. Inherent throughout this work is the issue of how this pathway is separable from degradation by the proteasome, since this is the classical function of ubiquitin. First Dr. Hicke will study the ubiquitination of Ste2p per se. She will determine if plasma membrane localization is required for ubiquitination by placing her signal on a cytosolic protein. She will determine if Ste2p is subjected to multiple monoubiquitinations or polyubiquitination. She will also determine in experiments involving ubiquitin chimeras whether ubiquitin at the cell surface is sufficient for endocytosis, and use in vitro mutagenesis to map regions in ubiquitin important for endocytosis. In the second specific aim Dr. Hicke will reconstitute phosphorylation and ubiquitination of Ste2p in a yeast permeabilized cell system. She will purify the responsible kinase as well as known proteins involved with ubiquitin transfer. This will allow her an assay to find other important factors, if they exist, for these steps. In the third aim Dr. Hicke will establish selections and screens to find proteins involved in ubiquitin-mediated endocytosis. Three screens are proposed. One utilizes a potassium channel-Ste2p fusion, the second is a blue-white screen with a Ste2p-beta-galactosidase chimera, and the last involves the continued ability to mate in the absence of synthesis of new Ste2p. Interesting proteins that develop from these screens will be further studied. Lastly, the relationship of ubiquitin-mediated endocytosis and degradation to pathways involving other endocytosis signals will be studied by indirect immunofluorescence.

Thesaurus Terms:
biological signal transduction, receptor expression, receptor mediated endocytosis, ubiquitin

cell membrane, phosphorylation, potassium channel, proteasome, protein kinase
Saccharomyces cerevisiae, cell free system, immunofluorescence technique

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