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Make it or break it: the role of ubiquitin- dependent proteolysis in cellular regulation

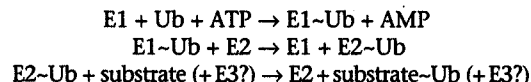
Raymond J. Deshaies

Effective regulation of the concentration of a protein in the cell requires rapid protein degradation. Until recently, it was widely believed that intracellular proteolysis was largely confined to the turnover of damaged, or otherwise abnormal, proteins. Recently, however, the role of protein degradation in cellular regulation has gained centre stage, and ubiquitin/proteasome-dependent proteolysis has been shown to play a key role in processes as diverse as embryonic development, transcription and the cell cycle.

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Although several proteolytic pathways coexist in the cytosol of eukaryotic cells, the majority of cytosolic proteolysis is catalysed by the ubiquitin-dependent 26S proteasome pathway. The biochemistry and genetics of the ubiquitin-proteasome pathway have been well reviewed in recent years¹. To briefly recap,

the proteasome degrades proteins that contain covalently linked multiubiquitin chains. Ubiquitin is attached to proteins in a multistep process as diagrammed below:



First, ubiquitin is attached via its C-terminus to the ubiquitin activating (E1) enzyme. Activated ubiquitin is then attached covalently to ubiquitin-conjugating (E2) enzymes. Most cells contain a single E1, but there are at least 12 genes in yeast that encode E2 enzymes. In many cases, ubiquitin can be transferred directly from a charged E2 to a substrate protein. Nevertheless, many physiological ubiquitination events may require the activity of a ubiquitin ligase, referred to as E3. Two different classes of E3 enzyme have been identified [hct-domain proteins (Ref. 2) and Ubr1p (Ref. 3)] and, as they are not homologous to one another, there may be other, unrecognized, E3s in the cell. Most ubiquitin conjugates are rapidly degraded to completion by the 26S proteasome. However, some ubiquitin conjugates are sufficiently stable to accumulate, suggesting that ubiquitination may regulate protein function by multiple mechanisms.

Three important discoveries during the 1980s indicated that protein ubiquitination plays a significant role in cellular regulation. First, temperature-sensitive mutant (*ts*) mammalian cell lines with defects in progression through the cell cycle were shown to have thermolabile E1 enzyme⁴. A specific role for protein ubiquitination in cell-cycle control was confirmed subsequently by the discovery that *CDC34*, which is required for progression through the G1-S transition in *Saccharomyces cerevisiae*, encodes an E2 enzyme⁵. The key event triggering the acknowledgement of protein degradation as a vital regulatory mechanism was the discovery that ubiquitin-dependent destruction of cyclin B is essential for the