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

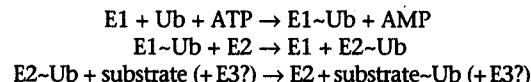
Raymond J. Deshaies

*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 years<sup>1</sup>. 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 enzyme<sup>4</sup>. 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 enzyme<sup>5</sup>. 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

**TABLE 1 – A PARTIAL LIST OF SHORT-LIVED PROTEINS DEGRADED BY UBIQUITIN-DEPENDENT PATHWAYS**

Protein	PO <sub>4</sub> ? <sup>a</sup>	Cts signals	Ubiquitin? <sup>b</sup>	Ubiquitin pathway	Physiological role of ubiquitin-mediated degradation	Refs
<i>Oncoproteins and tumour-suppressor proteins</i>						
c-Mos	Yes	2nd and 3rd codons	Yes	nd <sup>c</sup>	Prevent metaphase arrest in post-fertilization cell cycles?	
p53	Yes	nd	Yes	E6–E6-AP, hUBC4	Eliminate G1–S arrest or apoptosis in virally infected cells?	7–11
c-Jun	?	δ domain	Yes	nd	Maintain growth control	12
<i>Transcriptional proteins</i>						
NF-κB, p105	?	nd	Yes	nd	Produce active p50 subunit	13
IκB	Yes	nd	nd	nd	Signal-dependent activation of NF-κB	15–19
Mata2p	?	PEST <sup>d</sup>	Yes	UBC4–7	Allow rapid change in cell phenotype during mating-type switch?	
YAN	Yes	PEST	nd	nd	Allow cell to respond to inductive signals during differentiation?	23
<i>Cell-cycle proteins</i>						
Cln1,2,3p	Yes	PEST	Yes	CDC34	Regulate duration of G1 phase	28–31
cyclin A	?	D-box	Yes	CDC16/23/27?	Required for exit from mitosis	39
cyclin B	?	D-box	Yes	CDC16/23/27	Required for exit from mitosis	33–42
Cib5p	?	D-box <sup>e</sup>	nd	UBC9	?	41
NIMA	?	D-box? <sup>e</sup>	nd	nd	Required for exit from mitosis	45
p40 <sup>SIC1</sup>	Yes	PEST?	nd	CDC34/4/53	Required for exit from G1 phase	24–26
Far1p	Yes	nd	nd	CDC34?	Restrict pheromone-dependent cell-cycle arrest to G1 phase	32
CENPE	?	nd	nd	nd	?	46
<sup>a</sup> Yes <sup>a</sup> indicates that phosphorylation has either been proposed, or shown, to regulate degradation. <sup>b</sup> Yes <sup>b</sup> indicates that ubiquitinated forms of the protein have been detected. <sup>c</sup> Not determined. <sup>d</sup> Sequence rich in proline (P), glutamic and aspartic acid (E), serine (S) and threonine (T). <sup>e</sup> NIMA and Cib5p have destruction-box (D-box)-like sequences, but the effects of mutations in these sequences have not been reported.						

exit from mitosis in frog egg extracts<sup>6</sup>. Here, I review recent developments that illustrate the prominent role of ubiquitin-mediated protein degradation in the control of cell growth, gene expression and cell-cycle progression. Important principles have emerged from the recent studies, but there are still numerous gaps in our knowledge.

**Ubiquitin-mediated proteolysis and cancer**

Several oncoproteins and tumour suppressor proteins are rapidly degraded by ubiquitin-dependent pathways in animal cells (a partial list of ubiquitin-pathway substrates is presented in Table 1). Alterations in the rates of ubiquitin-dependent degradation may play important roles in the genesis of certain tumours. For example, the concentration of the p53 tumour suppressor protein is reduced dramatically in cells infected with oncogenic strains of human papillomaviruses (HPVs), even though the infected cells synthesize normal levels of p53 mRNA and protein<sup>7</sup>. The precipitous decline in p53 levels is triggered by physical association between p53 and the HPV-encoded E6 protein, which accelerates the turnover of p53. Reduced levels of p53 may deregulate both growth control and apoptosis in the

infected cells, ultimately culminating in malignancy. E6-dependent ubiquitination and degradation of p53 have been reconstituted *in vitro* with recombinant proteins<sup>7–9</sup>. As well as E6, efficient ubiquitination *in vitro* requires ubiquitin, E1 enzyme, either of two homologous E2 enzymes known as hUBC4 (Ref. 8) and UbcH5 (Ref. 10) and a cellular E6-binding protein known as E6-associated protein (E6-AP; see Fig. 1). E6-AP probably participates in a normal cellular ubiquitination pathway since E6-AP stimulates E6-independent ubiquitination of unidentified proteins in insect-cell lysates<sup>7</sup>. Thus, the role of E6 may be to help target E6-AP to p53. Since p53 is moderately unstable (half-life of ~25 min) even in cells that lack E6, it will be interesting to see whether there are cellular E6-like proteins that target E6-AP to p53 in uninfected cells.

In a striking departure from the model that has emerged from the study of artificial ubiquitination substrates, hUBC4 and UbcH5 do not transfer ubiquitin directly to p53. Rather, ubiquitin is successively transferred from hUBC4 or UbcH5 to E6-AP and then to p53 (Refs 8, 11). Although it is unclear whether this 'bucket brigade' mechanism will be a conserved feature of ubiquitination pathways, E6-AP is the