20 WEST, M. A., BRETSCHER, M. S. and WATTS, C. (1989) *J. Cell Biol.* 109, 2731–2739
21 SANDVIG, K. and VAN DEURS, B. (1990) *J. Biol. Chem.* 265, 6382–6388
22 KELLER, H. U. (1990) *J. Cell. Physiol.* 145, 465–471
23 RIDLEY, A. J., PATERSON, H. F., JOHNSTON, C. L., DIEKMANN, D. and HALL, A. (1992) *Cell* 70, 401–410
24 MICHIELS, F., HABETS, G. G. M., STAM, J. C., VAN DER KAMMEN, R. A. and COLLARD, J. G. (1995) *Nature* 375, 338–340
25 HALL, A. (1994) *Annu. Rev. Cell Biol.* 10, 31–54
26 RIDLEY, A. J. (1994) *BioEssays* 16, 321–327
27 KELLER, H. U. and NIGGLI, V. (1994) *J. Cell. Physiol.* 161, 526–536
28 HASEGAWA, T. (1993) *J. Cell Biol.* 120, 1439–1448
29 LAVOIE, J. N., HICKEY, E., WEBER, L. A. and LANDRY, J. (1993) *J. Biol. Chem.* 268, 24210–24214
30 ZHUANG, Y-X., CRAGOE, E. J., SHAIKEWITZ, T., GLASER, L. and CASSEL, D. (1984) *Biochemistry* 23, 4481–4488
31 SANDVIG, K., OLSNES, S., PETERSEN, O. W. and VAN DEURS, B. (1987) *J. Cell Biol.* 105, 679–689
32 HEUSER, J. (1989) *J. Cell Biol.* 108, 855–864
33 DESJARDINS, M., HUBER, L. A., PARTON, R. G. and GRIFFITHS, G. (1994) *J. Cell Biol.* 124, 677–688
34 HARTWELL, L. H. and WEINERT, T. A. (1989) *Science* 246, 629–634
35 SWANSON, J. A., YIRINEC, B. D. and SILVERSTEIN, S. C. (1985) *J. Cell Biol.* 100, 851–859
36 STEINMAN, R. M. (1991) *Annu. Rev. Immunol.* 9, 271–296
37 SALLUSTO, F. and LANZAVECCHIA, A. (1994) *J. Exp. Med.* 179, 1109–1118
38 SCHMID, S. L. and JACKSON, M. R. (1994) *Nature* 369, 103–104
39 JIANG, W. *et al.* (1995) *Nature* 375, 151–155
40 FRANCIS, C. L., RYAN, T. A., JONES, B. D., SMITH, S. J. and FALKOW, S. (1993) *Nature* 364, 639–642
41 ALPUCHE-ARANDA, C. M., RACOOSIN, E. L., SWANSON, J. A. and MILLER, S. I. (1994) *J. Exp. Med.* 179, 601–608

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

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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[1]. 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:

$$E1 + Ub + ATP \rightarrow E1\sim Ub + AMP$$
$$E1\sim Ub + E2 \rightarrow E1 + E2\sim Ub$$
$$E2\sim Ub + \text{substrate } (+E3?) \rightarrow E2 + \text{substrate}\sim Ub\ (+E3?)$$

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 [hect-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[4]. 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[5]. 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