CMLS, Cell. Mol. Life Sci. 57 (2000) 1207–1219
1420-682X/00/091207-13 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Regulation of transcription factors by protein degradation

J. M. P. Desterro, M. S. Rodriguez and R. T. Hay*

School of Biology, BMS Building, University of St. Andrews, St. Andrews, Fife KY16 9ST (United Kingdom), Fax + 44 1334 462595, e-mail: rth@st-and.ac.uk

Abstract. The level of transcription factors is tightly controlled by their rates of synthesis and degradation. Many critical factors are maintained at an appropriate level by targeted addition of ubiquitin and degradation via the proteasome. Whereas ubiquitination targets modified proteins for degradation, modification of substrates by the family of ubiquitin-like proteins does not target the proteins for degradation but can alter the stability and other properties of the modified proteins. Here we discuss the elaborate mechanisms that have evolved to allow specific recognition of substrates targeted for modification. Specific examples are discussed to illustrate the different mechanisms involved and the importance of regulated degradation in diseases such as cancer.

Key words. Transcription; protein degradation; ubiquitin; SUMO-1.

Introduction

Regulated eukaryotic gene transcription involves the assembly of an initiation complex at the core promoter region, and coordinated binding of multiple transcription factors and regulatory complexes to the promoter-enhancer region; many of these transcription factors are regulated by distinct signal transduction pathways. The activity of a particular transcription factor can be modulated in a number of different ways: by changing the rate of synthesis or degradation of the protein, by post-translational modification of the transcription factor or by altering its subcellular localisation. Proteolysis plays a crucial role in the regulation of a large number of transcription factors. A selective and programmed way of targeting many proteins for degradation, in eukaryotic cells, is carried out by the ubiquitin proteasome system. In contrast, modification of transcription factors by other ubiquitin-like proteins does not target the proteins for degradation but may alter their properties and change their susceptibility to degradation.

An increasing number of transcription factors have been shown to be substrates for modification by ubiquitin and ubiquitin-like proteins. Here we describe the mechanisms responsible for covalent modification of target proteins by ubiquitin-like proteins and discuss the biological consequences of modifications.

* Corresponding author.

The ubiquitin-proteasome system

Cellular proteins exist in a dynamic equilibrium. Their steady-state levels are maintained by a tightly controlled and highly regulated balance of synthesis and degradation. Protein degradation via ubiquitination represents a dynamic and coordinated mechanism which the cell employs to modulate regulatory factors.

Ubiquitin is a highly conserved 76-residue protein that exists in cells either free or covalently linked to other proteins. It was first isolated by Goldstein and co-workers from the thymus and was thought to be a thymic hormone [1]. However, in subsequent work it was found in all tissues and all eukaryotic organisms, and its ubiquitous distribution gave the protein its name. In addition to targeting damaged, misfolded or misassembled proteins, the ubiquitin system targets many cellular proteins, including transcription factors, cell growth modulators, signal transducers, cell cycle regulators, tumour suppressors, oncoproteins, short-lived enzymes, viral gene products and membrane polypeptide receptors for degradation [2]. With such a wide range of cellular targets, it is not surprising that the system is

involved in the regulation of many basic cellular processes such as cell cycle and division, differentiation and development, the response to stress and extracellular modulators, morphogenesis of neuronal networks, modulation of cell surface receptors, DNA repair, regulation of the immune and inflammatory responses and apoptosis. Degradation of a protein via the ubiquitin pathway involves two distinct and successive steps: covalent attachment of multiple ubiquitin molecules to the protein substrate, and degradation of the tagged protein by the 26S proteasome.

Ubiquitin addition

Ubiquitin conjugation is catalysed by an enzymatic cascade that begins with the ATP-dependent activation of the C-terminus of ubiquitin by the ubiquitin-activating enzyme (E1) leading to formation of a high-energy thioester linkage between the side chain of a cysteine residue in E1 and the C-terminal carboxyl group of the ubiquitin protein with release of AMP. In a transesterification reaction, the ubiquitin is transferred from the E1 to a conserved cysteine residue in a family of ubiquitin-conjugating enzymes (E2). Ubiquitin is then transferred from the E2 enzyme to the ultimate protein acceptor via an isopeptide linkage with the ε amino group of a lysine in the target protein. In many cases this final step requires the participation of ubiquitin protein ligase (E3), which may act either as the ultimate ubiquitin donor or in substrate recognition [2]. Proteins destined for degradation or processing via the 26S proteasome are coupled to multiple copies of ubiquitin by formation of further isopeptide bonds between additional ubiquitin molecules and lysine residues in previously conjugated ubiquitin (see fig. 1).

The structure of this system appears to be hierarchical: a single E1 carries out activation of ubiquitin required for all modifications. Several major species of E2 enzymes were characterised in mammalian cells, plants and yeast. E2s act in concert with E3s, which impart specificity to the process, and it appears that each E2 can act with one or more E3s. The mechanism of transfer of activated ubiquitin from a thioester intermediate to the amino group of a lysine acceptor in the target protein appears to differ in various types of E3s. In some cases, E3 accepts the activated ubiquitin from an E2 and binds it as a thioester intermediate prior to transfer to protein, whereas in others the E3 may help to transfer ubiquitin directly from E2 to a target protein by tightly binding to E2 and the protein substrate. In either case specificity derives from the high-fidelity protein-protein interactions between E3 and the substrate, but details of E3-substrate and E2-E3 interactions are limited. To date, four different classes of E3 ubiquitin ligases have been defined: Ubr1, the HECT (homologous to E6-AP C-terminus) domain family, the SCF (Skp1, Cullin, F-box) family and the APC (anaphase promoting complex)/cyclosome [2].

Posttranslational modifications such as phosphorylation of either the target protein or involved enzymes also play an important role in the recognition process.

An additional conjugation factor, named E4, was recently described as being involved in the multi-ubiquitin chain assembly [3]. Although E4 does not participate in the ubiquitin-enzyme thioester cascade and does not interact with substrate directly, it may be part of a large family of regulatory factors which together with E3s increase specificity of ubiquitination.

The 26S proteasome

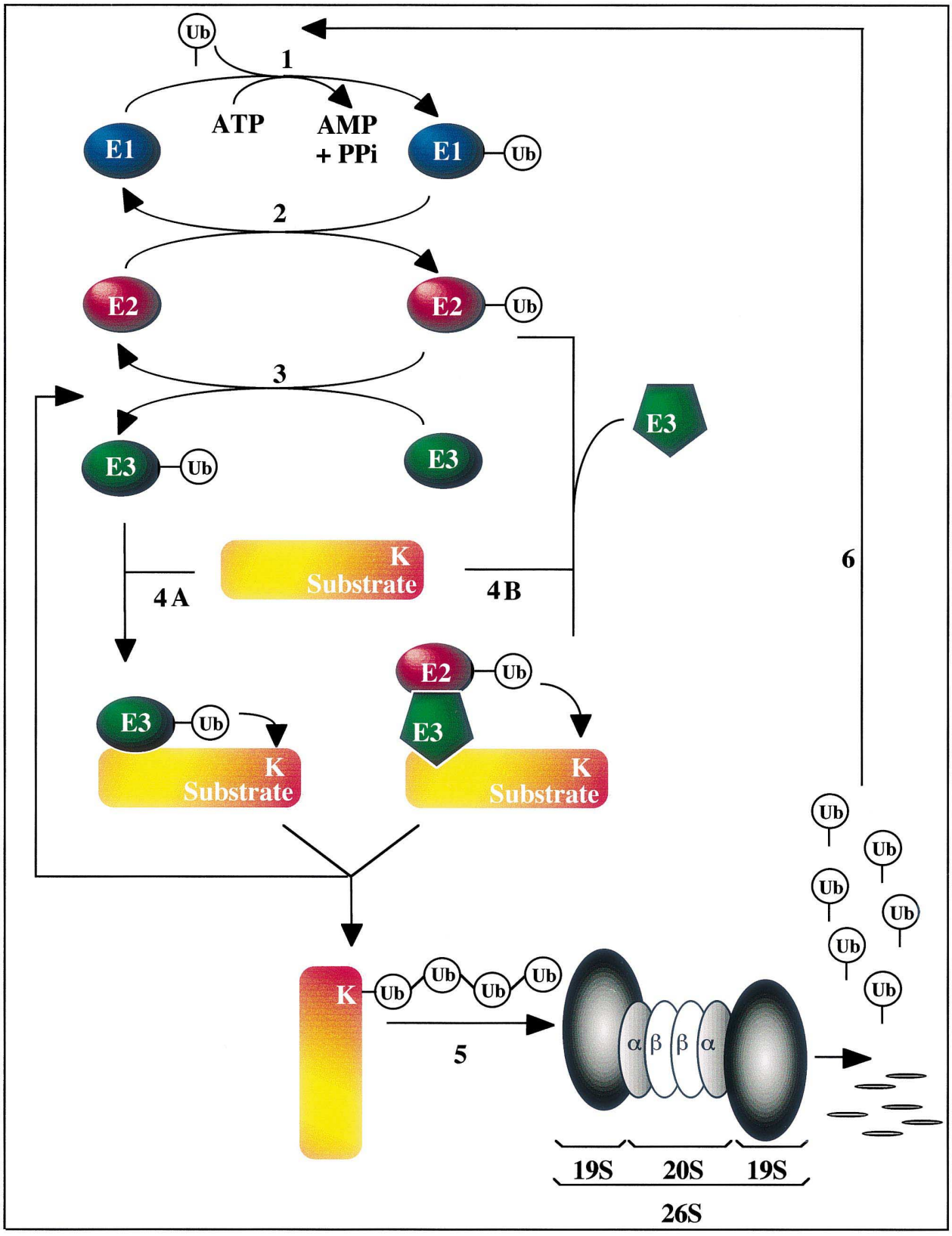
Once substrate proteins are conjugated to multiple copies of ubiquitin, they are directed to the 26S proteasome where the modified protein is degraded.

The proteasome is an ATP-dependent multicatalytic protease complex which recognises and specifically degrades cytosolic and nuclear proteins, most of which are ubiquitin tagged. It is composed of the 20S core catalytic complex flanked on both sides by the 19S regulatory complexes, which seem to be responsible for recognition and unfolding of the ubiquitin-tagged target protein.

The structure of the *Saccharomyces cerevisiae* 20S proteasome has been determined. It is composed of 14 pairs of different, but related, protein subunits which assemble into a single structure arranged into four seven-membered rings with the α-type subunits forming the two outer rings guarding an inner pair of catalytic β-type subunit rings [4].

The 19S complex is composed of at least 15 subunits, 6 of which are adenosine triphosphates (ATPases) and members of the AAA-ATPase family (ATPases associ-

Figure 1. The ubiquitin-proteasome degradation pathway. (1) Activation of ubiquitin (Ub) by the ubiquitin-activating enzyme—E1—in an ATP-dependent fashion. (2) Transfer of activated ubiquitin from E1 to the active cysteine residue of a member of the ubiquitin-conjugating enzymes—E2. (3, 4) Transfer of activated ubiquitin directly or indirectly from E2 to a protein substrate via ubiquitin protein ligase—E3. In some cases (4A), E3 accepts the activated ubiquitin from an E2 and binds it as a thioester intermediate prior to transfer to protein, whereas in others (4B) the E3 helps to transfer ubiquitin directly from E2 to a protein by tight binding of E2 and the protein substrate. Repeated conjugation of ubiquitin to lysine residues of formerly bound ubiquitin moieties leads to the formation of multiubiquitin chains. (5) Binding of the polyubiquitinated substrate to the ubiquitin receptor subunit in the 19S complex of the 26S proteasome and degradation of the substrate to short peptides by the 20S complex. (6) Recycling of ubiquitin via the action of isopeptidases.


Ub
1
ATP
AMP
+ PPi
E1
E1
Ub
2
E2
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3
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4 A
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5
α β β α
19S
20S
19S
26S

Fig. 1.

ated with a variety of cellular activities) [5]. The precise role of these ATPases, that is, the energy-dependent step in the degradation of proteins and the functions of most of the non-ATPase subunits are still unknown, but they may be involved in the recognition and unfolding of the target proteins, helping in their translocation into the 20S inner proteolytic complex.

An additional regulator that associates with the 20S proteasome in an ATP-independent fashion is the 11S complex. This complex is a ring-shaped hexamer, composed of alternating α and β subunits. It does not stimulate the degradation of proteins or of ubiquitin-protein conjugates but is γ-interferon inducible and is apparently involved in antigen processing [6]. Under the influence of γ-interferon there is no indication for an influence on the subunit composition of the 19S complex, but three proteolytically active β subunits of the 20S proteasome are replaced [7, 8]. The subcomponents of the 26S proteasome are dynamic structures, and it is likely that changes in the subunit composition reflect adaptation to current cellular conditions and influence protease specificity.

Proteasome inhibitors

Specific proteasome inhibitors have been developed, and they have become powerful research tools in probing the structure and function of the proteasome and ubiquitin pathway. Most of them act as pseudo-substrates that become linked covalently to the active-site hydroxyl groups in threonine of the β subunits, and as a result the chymotryptic and trypic-like activities are inactivated. Examples of these inhibitors most widely used are peptide aldehydes such as MG132, MG115, ALLN and natural products such as lactacystin [9]. In addition to being useful research tools, proteasome inhibitors also have potential use in the treatment of human diseases in which the ubiquitin proteolytic pathway is involved.

Deubiquitination enzymes

Targeting proteins for ubiquitin-mediated proteolysis is an irrevocable decision, and as such, the process needs to be highly specific and tightly regulated. This task is achieved by conjugation and deconjugation enzymes that act in a dynamic and coordinated mechanism.

Although the main regulation of this modification occurs at the level of the ubiquitin conjugation, deubiquitination, or removal of this modification, is being recognised as an important regulatory strategy. Deubiquitination enzymes (DUBs) are cysteine proteases that specifically hydrolyse ester, thiol ester and amido bonds to the carboxyl group of G76 of ubiquitin. This family of enzymes can be subclassified into at least two gene families that are structurally unrelated: the UCH (ubiquitin C-terminal hydrolase) family and the UBP (ubiquitin processing protease) family [10]. More than 60 full-length DUB sequences have been identified so far, and although little is known about their biological role, they are the largest family of enzymes in the ubiquitin system, suggesting that they may be involved in the recognition of different types of ubiquitin conjugates. These enzymes act at different levels in the ubiquitin pathway: in the generation of free ubiquitin by processing of linear precursor fusion proteins or branched-chain polyubiquitin; in removal of ubiquitin from ubiquitinated target protein, avoiding protein degradation by the 26S proteasome; and finally in clearing the proteasome of peptide remnants conjugated to ubiquitin chains [11].

Ubiquitin-like proteins

Ubiquitin modification of proteins provides an extremely versatile means of cellular regulation, but over the past few years a family of ubiquitin-like proteins has emerged that raise new and intriguing regulatory and mechanistic questions in cell biology. SUMO-1, also known as sentrin, GMP1, UBL1, PIC1 or Smt3 in yeast [12], is a member of this new family of ubiquitin-like proteins that is covalently linked to target proteins, many of which are transcription factors.

Several features of the ubiquitin pathway are conserved in the SUMO-1 conjugation pathway (fig. 2). Like ubiquitin, SUMO-1 is proteolytically processed to expose its mature C-terminus and is activated in an ATP-dependent manner by an enzyme composed of SAE1 and SAE2 [13–15] or Aos1p and Uba2p in yeast [16]. The activated SUMO-1 is then transferred to the active-site cysteine of the E2 equivalent Ubc9 [17–20], which can recognise the substrate and directly transfer the activated SUMO-1 to a lysine residue in the target protein in vitro [13, 15]. Analysis of the sites of SUMO-1 modification in multiple proteins indicates that a short motif, ΨKxE, represents the primary site of SUMO-1 modification [21–24]. As almost all identified targets for SUMO-1 modification are Ubc9 interacting proteins, it is likely that substrate specificity is achieved by Ubc9. The C-terminal region of Ubc9, which is thought to be involved in substrate binding [25], lies close to the catalytic site and favours the direct transfer of SUMO-1 to substrate proteins.

SUMO-1-specific proteases

The recently described SUMO-1-specific protease (Ulp1) from the yeast *Saccharomyces cerevisiae* is also a cysteine protease that is unrelated to the DUB enzymes but is rather similar to a class of viral proteases, the prototype of which is the protease coded by adenoviruses. Ulp1 is capable of both deconjugating SUMO-1 from modified proteins and of removing 4 amino acids from the C-terminus of the SUMO-1 primary translation product to generate the mature 97-amino-acid form. Like Ubc9, it is also required for progress through the yeast cell cycle. Database searching indicated that homologous enzymes are present in many eukaryotic species, and there may be many SUMO-1-specific proteases in human cells [26].

NF-κB/IκBα

Activation of NF-κB is mediated by signal-induced degradation of its inhibitor IκBα in the cytoplasm, which allows the active transcription factor to translocate into the nucleus and activate transcription of a large number of responsive genes (reviewed in [27]). As part of an autoregulatory loop, NF-κB induces efficient resynthesis of IκBα through activation of IκBα messenger RNA (mRNA) transcription [28, 29]. Newly synthesised IκBα accumulates transiently in the nucleus, where it is resistant to signal-induced degradation [30] and negatively regulates NF-κB-dependent transcription [31]. IκBα mediates transport of the NF-κB/IκBα complex back to the cytoplasm [32] in a nuclear export sequence (NES)-dependent process that is sensitive to leptomycin B (LMB) [30, 33]. IκBα, shuttling between the cytoplasm and the nucleus, regulates both the activation and inactivation of gene transcription through its association with NF-κB.

The signal transduction cascades that link cell surface events to NF-κB activation have been the subject of intense interest, and the IκB kinases IKKα (or IKK1) and IKKβ (or IKK2), which mediate signal-induced phosphorylation of IκBα on Ser 32 and Ser 36, have been identified [34]. Specific inhibition of the proteolytic activity of the proteasome prevents NF-κB activation and results in the accumulation of ubiquitinated forms of IκBα, indicating that IκBα is targeted for degradation by a phosphorylation-dependent ubiquitination process which occurs in two adjacent N-terminal lysine residues at positions 21 and 22 [35–38]. Polyubiquitinated IκBα is degraded by the 26S proteasome, which releases NF-κB into the nucleus, allowing transcription of responsive genes (fig. 3).

IκBα ubiquitination: the SCF$^{\beta TrCP}$ complex

Using different strategies, independent groups identified β-TrCP as a specific component of the IκBα ubiquitin ligase complex [39–43]. β-TrCP is a mammalian homolog of the *Drosophila* Slimb protein [44] and is an F-box/WD40 repeat protein that appears to act as the receptor for recruitment of phosphorylated substrates. Slimb negatively regulates both the Hedgehog and Wnt/Wingless pathways [44]. Human β-TrCP was isolated as a protein interacting with phosphorylated Vpu, a small human immunodeficiency virus (HIV)-encoded protein that interacts with CD4 in the endoplasmic reticulum and targets it for degradation [45]. β-TrCP also interacts directly with β-catenin, a component of the Wingless/Wnt pathway, in a phosphorylation-dependent manner [40, 46–48]. There is a common structural feature among IκBα, β-catenin and Vpu: phosphorylation occurs on two closely located serines at positions 32 and 36 (IκBα), 33 and 37 (β-catenin) and 52 and 56 (Vpu), suggesting that β-TrCP recognises a very similar motif, with a sequence consensus of DS*GΨXS*, where S* represents phosphoserine, Ψ a hydrophobic residue and X any amino acid. Further studies are required to determine whether any of the many proteins containing the above motif are also substrates of β-TrCP.

The first group that identified β-TrCP as a specific component of the IκB ubiquitin ligase took advantage of its high affinity for phosphorylated IκBα (pIκBα) and devised an immunoaffinity purification method to isolate the protein from HeLa cells [39]. Later studies showed that β-TrCP protein functions as the F-box

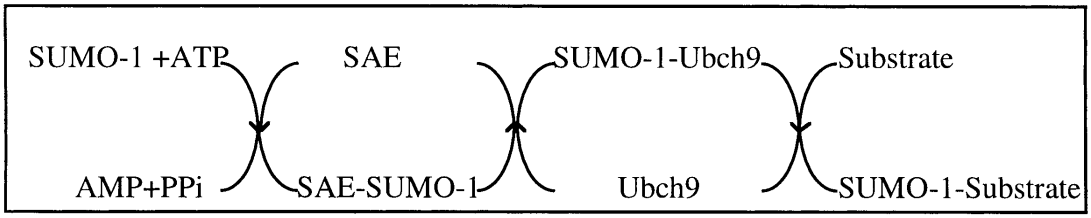

Figure 2. SUMO-1 conjugation pathway.

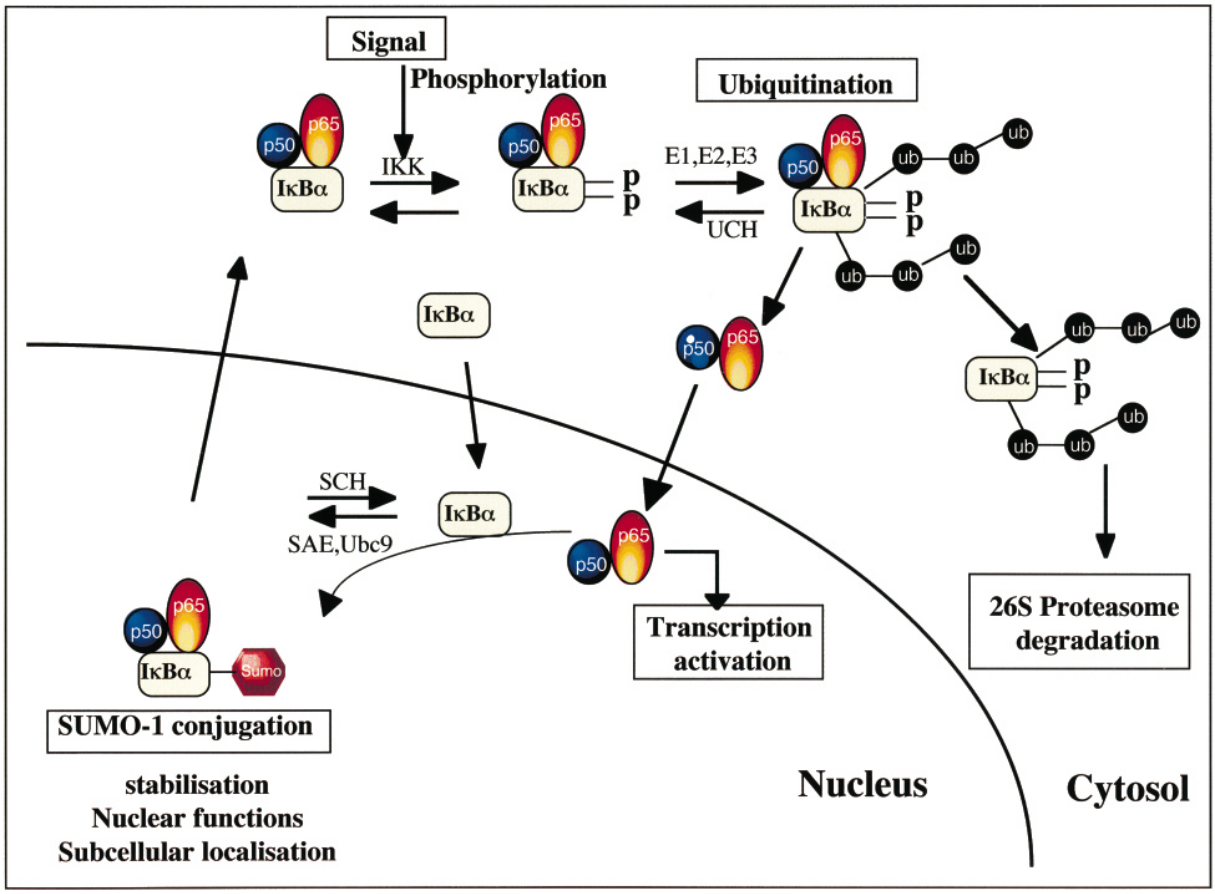

Figure 3. Fates of ubiquitin and SUMO-1-modified IκBα. See text for details. E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; UCH, ubiquitin C-terminal hydrolase; IKK, IκBα kinase; SAE, SUMO-1 activating enzyme; Ubc9, SUMO-1 conjugating enzyme; SCH, SUMO-1 C-terminal hydrolase.

protein of an SCF complex containing Skp1 and Cdc53/Cul1, which recognises pIκBα bound to NF-κB [40, 41] or a phosphorylated peptide substrate representing residues 20–43 of IκBα [43]. Whereas there is general agreement in the subunits that compose the $SCF^{\beta TrCP}$ complex, there is less agreement on the E2 involved in the polyubiquitination of IκBα. Many SCF complexes involved in cell cycle protein ubiquitination appear to utilise Cdc34 as the E2 for ubiquitination, and this is consistent with data indicating that Cdc34 is responsible for ubiquitination of pIκBα [43, 49]. However, in other studies in vitro ubiquitination of pIκBα was achieved using either UbcH5 [39, 41] or yeast extracts as a source of E2 activity [40]. The recently identified protein Rbx1/Roc1 is a novel subunit of $SCF^{\beta\text{-}TrCP}$ complex [49], and its role in the recruitment of Cdc34 to the SCF complexes strengthens the hypothesis that Cdc34 is the E2 required for the phosphorylation dependent ubiquitination of IκBα in vitro and raises the possibility of other factors being involved in the specificity of the SCF complexes. Nevertheless, it remains to be determined which E2 (or E2s) functions in the signal-induced ubiquitination of IκBα in vivo.

In β-TrCP, both the F-box and the WD domain are protein-protein interaction motifs. WD repeats at the C-terminus mediate binding to the substrate, and an F-box near the N-terminus is involved in interaction with Skp1p, which in turn is bound to Cdc53 (fig. 4). The β-TrCP F-box is necessary for IκBα ubiquitination in vitro, and an F-box deletion mutant of β-TrCP, which is still able to bind to pIκBα but fails to promote its ubiquitination in vitro, acts in vivo as a dominant-negative mutant, inhibiting the degradation of pIκBα and consequently NF-κB activation [39, 41, 50]. The F-box-deleted form of β-TrCP has the same effect on Vpu-mediated CD4 degradation [45] and blocks β-catenin degradation in human cells [46–48]. The $SCF^{\beta TrCP}$ is involved in the phosphorylation-dependent ubiquitin-mediated proteolysis of IκBα, β-catenin and CD4 (through HIV Vpu), suggesting that a single F-box

is capable of recognising different substrates with identical destruction motifs (fig. 4). Recently, it was demonstrated that modification of CDC53/Cul-1 by the small ubiquitin-like protein Nedd8 is required for $SCF^{\beta TrCP}$-dependent ubiquitination of phosphorylated IκBα [51].

IκBα SUMO-1 conjugation

IκBα is modified by SUMO-1 on lysine 21, which is also used for ubiquitin conjugation. Thus, SUMO-1-modified IκBα cannot be ubiquitinated and is resistant to proteasome mediated degradation. As a result, overexpression of SUMO-1 inhibits signal-induced activation of NF-κB-dependent transcription [21].

SUMO-1 acts antagonistically to ubiquitination: whereas multiubiquitination of IκBα targets the protein for destruction, SUMO-1 modification creates a pool of IκBα that is resistant to degradation (fig. 3). This function of SUMO is rather similar to that observed when mutations are introduced into ubiquitin in the lysine residues that are used for multiubiquitination. K29R and K48R mutants in ubiquitin generate proteins that can be conjugated to substrates but which cannot form multiubiquitin chains. As such, the modified proteins are resisitant to degradation [52].

p53

The p53 tumour suppressor plays a significant part in the response of the cell to genotoxic damage. The important role of p53 in maintenance of genome integrity is illustrated by the loss of p53 function in most human tumours and the high rate of tumour development in p53 knockout mice. p53 is a transcription factor that binds to specific sequences in the upstream region of many genes whose protein products regulate cell cycle progression and apoptosis. Thus, cells with damaged DNA do not proliferate, and the loss of this protective function allows the uncontrolled growth of cells containing oncogenic mutations [53].

The p53 tumour suppressor protein is regulated by ubiquitin-mediated proteasomal degradation [54]. In normal cells p53 is constitutively ubiquitinated by the Mdm2 ubiquitin ligase [55–58]. Exposure of cells to a range of different stress signals, including DNA damage, hypoxia and heat shock activates the p53 response,

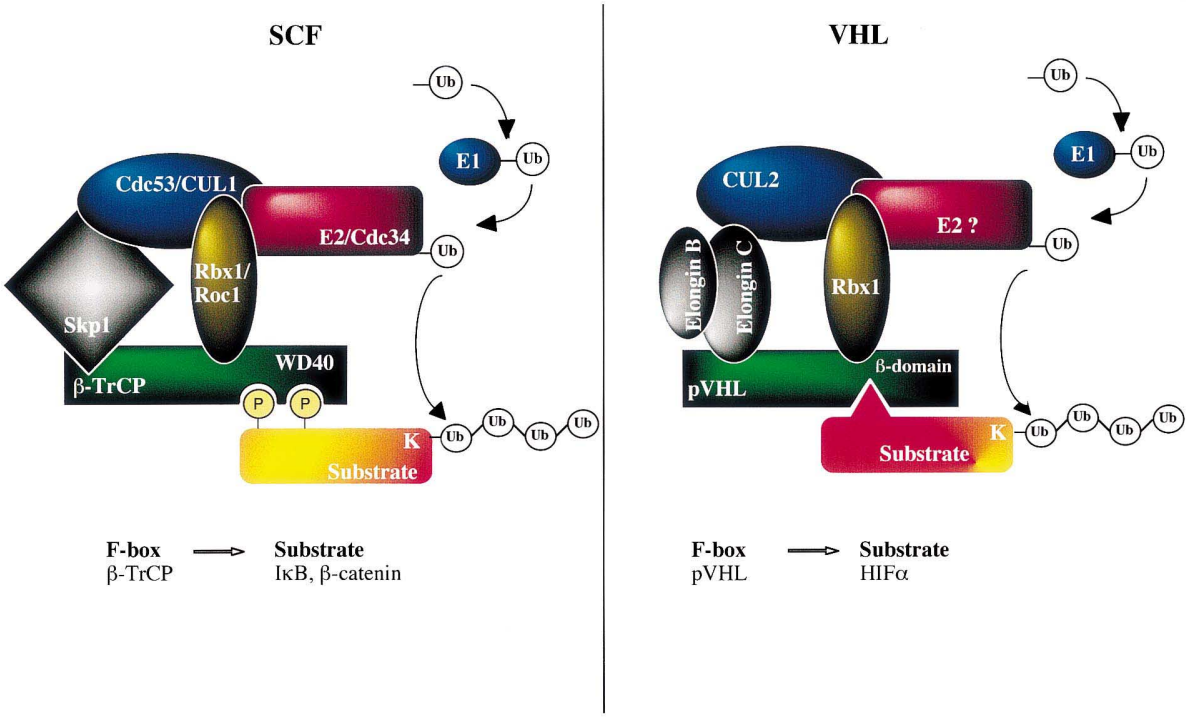

Figure 4. E3 ubiquitin ligase complexes. Similar overall architecture between the SCF and VHL complexes which participate in the ubiquitin-dependent degradation of many cellular proteins. Each complex is composed of a set of adapter proteins that recruit different binding partners through specific protein-protein interactions (see text for details). Complex subunits that share sequence similarity are shown in the same colour, and the respective substrates are indicated below. Rbx1 is the only component common to both the SCF and VHL complexes.

and p53 levels rise due to inhibition of this degradative pathway.

p53 is modified by SUMO-1 at a single site, K386 in the C-terminus of the protein [23, 59]. A role for SUMO-1 modification in p53 activation is consistent with the observation that overexpression of SUMO-1 leads to increased p53-dependent transcriptional activity. This increase is dependent on SUMO-1 modification of p53, as SUMO-1 overexpression does not influence the transcriptional activity of K386R p53. Although the site of SUMO-1 modification may represent a potential site of ubiquitination, mutation of K386R does not have a dramatic effect on ubiquitination in vitro. Whereas the C-terminal region is required for ubiquitination, it does not appear that a unique lysine is a target for mdm2, as no single lysine-to-arginine mutation of the six lysines in this region abolishes ubiquitination (M.S.R., J.M.P.D. and R.T.H., unpublished observations). Thus, although SUMO-1 modification of this region may not directly disrupt the interaction between p53 and mdm2, it could inhibit the ability of mdm2 to access the lysine residues in the C-terminus which are the substrates for ubiquitination. In addition to SUMO-1 modification, it has recently been reported that phosphorylation of p53 with DNA-dependent protein kinase reduces the substrate activity of p53 in an mdm2-dependent in vitro ubiquitination assay [60]. The tumour suppressor $p19^{ARF}$ blocks ubiquitination of p53, but in this case the target appears to be mdm2 rather than p53 [60]. A short region of $p19^{ARF}$ represented by a peptide containing residues 1–20 appears to interact directly with mdm2 and block its ability to act as a ubiquitin protein ligase [61]. Likewise, retinoblastoma protein (Rb) has also been shown to block mdm2-induced degradation of p53, and in this instance a trimeric, but inactive complex containing p53, Rb and mdm2 was detected [62]. As an alternative to effects on protein stability, SUMO-1 modification could increase the inherent transcriptional activity of p53. This could result from a change in protein structure which increases DNA binding activity, allows more efficient access to p53-dependent promoters embedded in repressive chromatin or has a greater capacity to recruit coactivators such as CBP/p300.

It is also possible that SUMO-1 modification alters the subcellular localisation of p53, as has been noted for other SUMO-1 modified proteins. SUMO-1 modified Ran GAP is recruited to the nuclear pore complex [63, 64], whereas SUMO-1 modified PML and SP100 are present in promyelocytic leukaemia protein (PML) oncogenic domains, or PODs [22, 65, 66]. IκBα is predominantly cytoplasmic but shuttles rapidly between the nucleus and the cytoplasm, and although the cellular localisation of SUMO-1-modified IκBα has not been determined, it is worth noting that IκBα, trapped in the nucleus by leptomycin B, is resistant to signal-induced degradation [30]. Mdm2 also shuttles between the nucleus and the cytoplasm [67], and blockage of nuclear export results in accumulation of p53 and mdm2 in the nucleus. Under these conditions p53 and mdm2 are located in subnuclear domains adjacent to the PODs [68], which are rich in SUMO-1-modified proteins. Like IκBα, nuclear p53 is resistant to degradation [69], and it is possible that SUMO-1 modification takes place when these proteins are imported into the nucleus. Thus, SUMO-1 modification could increase the nuclear content of p53 or alternatively concentrate the protein in distinct subnuclear domains that are active sites of transcription. Diverse stress stimuli activate the p53 response and are likely to act via separate signalling pathways with distinct activation mechanisms. SUMO-1 modification represents an additional route to p53 activation that may link the p53 response to other aspects of cellular metabolism.

AP-1

Activator protein 1 (AP-1) is a sequence-specific transcriptional activator composed of members of the Jun and Fos families [70]. They respond to a variety of extracellular stimuli by increasing the expression of genes implicated in cell cycle progression, differentiation, transformation and apoptosis [71, 72]. A 27-amino-acid N-terminal segment (30–57) of c-Jun denoted the δ domain mediates its degradation. Deletion of the δ domain abrogates ubiquitination and degradation of c-Jun [73]. v-Jun, the retroviral counterpart of c-Jun which lacks the δ domain, is not ubiquitinated in vivo. Phosphorylation of c-Jun by mitogen-activated protein kinase (MAPK)-type enzymes, such as the JNKs and ERKs on Ser 63, Ser73, Thr91 and Thr93, inhibits its ubiquitination and stabilises the protein [74], consequently increasing its DNA binding activity and transactivating potential [75, 76]. Although the role of ubiquitin in the targeting of c-Jun for degradation seems to be likely, proteasome-dependent degradation of c-Jun without prior ubiquitination has also been reported [77].

c-Fos is degraded by the ubiquitin proteasome system [78]. Ubiquitin-dependent degradation of c-Fos is increased in the presence of c-Jun [75, 79, 80] and further accelerated by MAPK, casein kinase II (CKII) and CDC2 kinase [79]. v-Fos, the retroviral counterpart of c-Fos, is resistant to c-Jun-mediated degradation [75]. A frameshift mutation that modifies the v-Fos C-terminal PEST region is likely to be responsible for its stability. Moreover, deletion of the PEST region of c-Fos renders the protein resistant to degradation [79]. The ubiquitin conjugation enzyme (E2) implicated in c-Fos ubiquiti-

nation has been identified as UbcH5 [80], although the identity of the 280-kDa homodimeric E3 ligase remains obscure. However, it has been suggested that the same E2-E3 complex could participate in the ubiquitination of c-Jun [80].

β-catenin/armadillo

It has recently been established that disruption of β-catenin degradation is a common event in a number of human cancers (reviewed in [81]). β-catenin is the mammalian homologue of the *Drosophila* gene *armadillo*, which is involved in determining segment polarity in the Wingless signalling pathway. The transcriptional activity of β-catenin is mediated via interactions with the T-cell-factor (TCF) family of transcription factors. β-catenin does not bind DNA alone, but contains transcriptional activation domains and functions by interacting with TCFs which are sequence-specific DNA-binding proteins [82]. Under normal circumstances β-catenin is found in a complex with the APC tumour suppressor gene that is mutated in familial adenomatous polyposis (FAP) and the serine-threonine kinase, glycogen synthase kinase (GSK3-β) [83, 84]. As a result β-catenin is constitutively phosphorylated on residues 533 and 537, which are part of the DS*GΨXS* consensus sequence. As discussed above, this sequence targets the protein for ubiquitination by the $SCF^{\beta TrCP}$ ubiquitin ligase complex (fig. 4) and ultimately proteasome-mediated degradation. This mechanism therefore ensures that β-catenin is maintained at low level in the cytoplasm of the cell, where it is transcriptionally silent. However, when signalling is initiated in the Wnt/Wingless pathway in *Drosophila* and *Xenopus* or when Akt/protein kinase B is activated, this leads to phosphorylation and inactivation of GSK3-β [85]. Consequently, β-catenin is not phosphorylated and is no longer a substrate for $SCF^{\beta TrCP}$-mediated ubiquitination and proteasomal degradation. As β-catenin levels rise, it is no longer retained in the cytoplasm by the APC complex, and instead interacts with TCF transcription factors. The TCF/β-catenin complex translocates to the nucleus [86], where it activates transcription from genes such as c-Myc [87] and cyclin D1 [88]. Mutations in genes which lead to β-catenin accumulation are detected in a wide variety of cancers [89, 90], thus emphasising the importance of the ubiquitin proteasome pathway in the development of disease.

Oxygen-dependent degradation of HIFα

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor involved in the response of cells to hypoxia. Genes activated by HIF-1 have critical roles in angiogenesis, apoptosis and energy metabolism [91–93]. Under normoxic conditions HIFα is constitutively degraded via the ubiquitin proteasome pathway [94]. However, when oxygen levels are reduced, degradation is blocked, the levels of HIFα rise and HIFα dependent transcription of hypoxia responsive genes is activated [95].

Constitutive activation of hypoxia-responsive genes is observed in a class of highly angiogenic tumours that are characterised by alterations in the von-Hippel-Lindau (VHL) tumour suppressor gene [96]. Although this hereditary cancer syndrome has been known for 100 years, it is only recently that the molecular basis for this disease has been established. The VHL protein (pVHL) is part of a multiprotein complex (fig. 4) that targets HIF1α for degradation in an oxygen-dependent fashion [97]. In cells lacking pVHL, HIF1α is not degraded, and the levels of the protein rise. The accumulated HIF1α translocates to the nucleus and drives transcription of genes involved in the formation of blood vessels, thus resulting in the angiogenic phenotype of the tumours. Although not yet demonstrated directly, it seems likely that the VHL-containing complex acts as an E3 ubiquitin protein ligase in a fashion analogous to the previously described SCFs. HIF1α is bound, in an oxygen-dependent means, by pVHL which is incorporated into a complex containing elongin C, elongin B, Cul2 and Rbx1. Structural analysis of the pVHL, elongin C and elongin B subcomplex indeed reveals that it shares structural features with SCF complexes [98]. pVHL contains two domains, one of which recognises the substrate, whereas the other is bound to elongin C and resembles an F-box. Elongin C in turn is structurally similar to Skp1 and interacts with Cul2 [99]. Also present in the multiprotein assembly is Rbx1 or Roc1, which is responsible for recruitment of the E2 ubiquitin conjugating enzymes to the SCF complex [100]. Rbx1 contains a RING finger which coordinates zinc and RING-finger-containing proteins seem to be present in all classes of E3 ubiquitin protein ligases. Thus, it appears that the loss of pVHL in angiogenic tumours leads to elevated transcription of angiogenic genes as a direct consequence of the failure to ubiquitinate and degrade HIF1α [101].

E2F

E2F transcription factors play a key role in cell proliferation control by regulating the expression of genes involved in progression through G1 and into the S phase of the cell cycle. The activity of E2F factors is regulated through association with Rb tumour suppressor protein. pRb forms a complex with E2F which results in the inhibition of E2F-mediated transactivaction. During

cell cycle progression, pRb can exist in the cell in both a phosphorylated and an unphosphorylated state, and only the underphosphorylated form of Rb is functional in E2F binding. E2F activity is further regulated through direct interactions with other factors, such as cyclin A, Sp1, histone deacetylase HADC1 or HADC2, p53 and the ubiquitin-proteasome pathway. E2F interacts with an F-box-containing protein, p45SKP2, which is the cell-cycle-regulated component of a ubiquitin protein ligase, SCF^{SKP2}. This interaction may be involved in targeting E2F for degradation by the ubiquitin-dependent pathway and downregulates E2F-1 activity [102].

Virus-induced degradation of p53

The importance of ubiquitin-proteasome-mediated degradation in the control of transcription is highlighted by the frequency with which viruses attack this system to disrupt cellular metabolism. As part of their replicative strategies viruses have evolved mechanisms to stop cells activating defence and apoptotic pathways and inducing the cell to activate survival responses. In many cases a cellular response to virus infection is to activate a p53-mediated suicide response. As discussed previously, this involves blocking mdm2-dependent ubiquitination of p53 such that proteasome-mediated degradation is inhibited, and transcriptionally active p53 accumulates. To counteract this response, viruses have themselves evolved strategies to induce degradation of p53 and thus abrogate the p53 response. Human papilloma virus types 16 and 18, which are associated with the development of cervical carcinoma, encode proteins which abrogate the p53 response. Products of the E6 gene bind p53 and recruit the ubiquitin protein ligase E6-AP, which leads to ubiquitination of p53 and its subsequent degradation [103, 104]. Similarly, in adenovirus-infected cells p53 degradation is induced by a combination of the E1b 55-kDa and E4 orf6 proteins, although details of this process have yet to be determined [105].

Paramyxovirus-induced degradaton of STAT transcription factors

Once cells are infected with viruses, they secrete interferons to activate antiviral defences in surrounding cells. The response of cells to interferons involves activation of JAK kinases and phosphorylation of STAT transcription factors. The phosphorylated STAT proteins dimerize and translocate to the nucleus, where they activate genes involved in the antiviral response [106]. Recently, it was demonstrated that paramyxoviruses block the interferon response by inducing degradation of specific STAT transcription factors. The V protein of Simian virus 5 targets STAT1 for degradation [107], whereas parainfluenza virus targets STAT2 for degradation [108]. Although studies with inhibitors have indicated that degradation is via the proteasome, the precise mechanism remains to be established.

Virus-induced degradation of PML

The HSV-1 Vmw110 immediate-early regulatory protein induces the proteasome-dependent degradation of several cellular proteins. Vmw110 associates with the acute promyelocytic leukaemia protein PML in subnuclear structures, which are disrupted within a few hours as a consequence of degradation of several SUMO-1-modified forms of PML in a proteasome-dependent fashion [109, 110]. Vmw110 also binds to centromeres and induces proteasome-dependent degradation of CENP-C, a centromeric protein. CENP-C degradation disrupts kinetochore structure and induces profound defects in mitotic events [111]. There are striking similarities between the interactions of Vmw110 with the PML bodies and centromeres, and one could speculate that SUMO-1 conjugation or deconjugation might have a role in both processes, creating a possible connection between these nuclear structures. The destruction of CENP-C may be important for the virus to prevent the cell from aborting the viral infection by inducing an apoptotic response.

Like HSV-1 Vmw110, the adenovirus E4 orf3 protein induces reorganisation of PML bodies in infected cells [112] and also appears to induce the loss of specific SUMO-1-modified forms of PML in the infected cell [113]. Whereas viruses often block apoptotic responses, many also induce a survival response by activating the transcription factor NF-κB. As discussed earlier, this is achieved by signal-induced degradation of the IκBα inhibition protein. A good example of this is provided by Epstein-Barr virus (EBV), in which the LMP protein induces the phosphorylation cascade that leads to IκBα degradation and NF-κB activation [114]. However, this example is far from unique, and many viruses utilise this route to maintain cells in a viable state for the progress of infection.

Conclusions

Although we have selected a rather limited number of examples for discussion, it is clear that common regulatory principles have emerged for precisely regulating the levels of transcription factors within the cell. Degradation is mediated by the ubiquitin-proteasome pathway, but this may be modulated by signal-induced phosphorylation, association with other proteins and modifica-

tion with ubiquitin-like proteins. All transcription factors must access the nucleus to perform their function, and it appears that in an increasing number of cases nuclear export of the transcription factor is coupled to degradation. The importance of appropriate, controlled protein degradation is illustrated by the many disease states in which this process is disrupted.

As we begin to appreciate the complexity of protein degradation, it is likely that new targets for the development of anticancer and antiinflammatory drugs will emerge.

Acknowledgements. Work in the author's laboratory was supported by the Medical Research Council and the Biotechnology and Biological Research Council.

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