Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Modification of Proteins by Ubiquitin and Ubiquitin-Like Proteins

Oliver Kerscher,[1] Rachael Felberbaum,[2] and Mark Hochstrasser[1,*]

[1]Molecular Biophysics and Biochemistry, [2]Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520;
email: mark.hochstrasser@yale.edu

Annu. Rev. Cell Dev. Biol. 2006. 22:159–80

First published online as a Review in Advance on June 5, 2006

The *Annual Review of Cell and Developmental Biology* is online at http://cellbio.annualreviews.org

This article's doi:
10.1146/annurev.cellbio.22.010605.093503

Copyright © 2006 by Annual Reviews.
All rights reserved

1081-0706/06/1110-0159$20.00

*Corresponding author.

Key Words

sumoylation, neddylation, Ubl conjugation, proteasome, protein degradation

Abstract

Following the discovery of protein modification by the small, highly conserved ubiquitin polypeptide, a number of distinct ubiquitin-like proteins (Ubls) have been found to function as protein modifiers as well. These Ubls, which include SUMO, ISG15, Nedd8, and Atg8, function as critical regulators of many cellular processes, including transcription, DNA repair, signal transduction, autophagy, and cell-cycle control. A growing body of data also implicates the dysregulation of Ubl-substrate modification and mutations in the Ubl-conjugation machinery in the etiology and progression of a number of human diseases. The primary aim of this review is to summarize the latest developments in our understanding of the different Ubl-protein modification systems, including the shared and unique features of these related pathways.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Contents

INTRODUCTION 160
UBL ACTIVATION AND CONJUGATION TO SUBSTRATES 160
UBIQUITIN AND UBL POLYMERS 164
VARIATIONS ON UBL MODIFICATION: EASY AS E1, E2, E3? 165
Recruiting Substrates 165
Enzyme Sharing by Ubls 167
An Exception to the Universal E1-E2 Couple? 167
TAKING DIRECTIONS FROM UBIQUITIN AND UBLS 168
Ubiquitin and SUMO-Binding Domains 168
Ubls and Protein Complex Assembly 170
UBLS AND THEIR CONJUGATION MACHINERY: LOCALIZE TO REGULATE? 171
Subcellular Localization of Ubls 171
Localization of the Ubl-Conjugation Machinery 171
NEW FUNCTIONS FOR UBLS 172
SUMO and Ubiquitin in Some Unexpected Places 172
Ubl-Related Pathologies 173
CONCLUDING REMARKS 175

INTRODUCTION

The diversity and functional breadth of an organism's proteome can be greatly expanded by covalent posttranslational modifications. Examples of these modifications include small molecules such as phosphate, methyl, or acetyl groups. Entire proteins can also be attached covalently to protein substrates. The classic example of a protein that modifies other proteins is ubiquitin, a 76-residue polypeptide that is highly conserved among eukaryotes but is absent from bacteria and archaea. Among the many functions of ubiquitin, the best understood is the targeting of proteins for degradation by the proteasome. For this purpose, ubiquitin is attached to the substrate in the form of a polyubiquitin chain that is then recognized by specific receptors within the proteasome or by adaptor proteins that subsequently bind the proteasome (Elsasser et al. 2002, Young et al. 1998).

Ubiquitin: a 76-residue protein that is covalently attached to other proteins; it is the founding member of the Ubl protein family

Since the discovery of ubiquitin in the mid-1970s, an entire family of small proteins related to ubiquitin (called ubiquitin-like proteins, or Ubls) has been defined; new members are still being added (**Table 1**). Although not necessarily sharing high sequence similarity, the Ubls all possess essentially the same three-dimensional structure, the ubiquitin or β-grasp fold (Hochstrasser 2000). Moreover, for all Ubls whose covalent attachment to proteins has been experimentally demonstrated, the C-terminal residue of the Ubl is a glycine, and the carboxyl group of this glycine is the site of attachment to substrates. Lysine side chains are the most common target sites within substrate proteins, resulting in an amide (or isopeptide) bond between the Ubl and substrate.

UBL ACTIVATION AND CONJUGATION TO SUBSTRATES

All the Ubls appear to be attached to substrates via related enzymatic pathways (**Figure 1**). Ubiquitin and most of the Ubls are synthesized as inactive precursors that need to be processed at their C termini to expose the glycine carboxylate that is the site of substrate conjugation. Specific proteases called deubiquitinating enzymes (DUBs) are responsible for the processing in the case of ubiquitin, whereas Ubl-specific proteases (ULPs) process the other Ubls.

To be capable of substrate modification, the Ubl first must be activated (Haas et al. 1982). A specific activating enzyme (called E1, or E1-like enzyme) uses ATP to adenylate the Ubl modifier at its C terminus. The

high-energy mixed anhydride bond thus formed is quickly attacked by the sulfhydryl group of the E1 active-site cysteine, forming a high-energy thioester bond between the E1 and the Ubl and expelling AMP. From the E1, the modifier is passed to the active-site cysteine of an E2, or E2-like enzyme, also known as a Ub-conjugating or Ubl-conjugating enzyme. Finally, the modifier is conjugated to its substrate with the aid of an E3 protein ligase, resulting in the covalent isopeptide linkage of the modifier's C terminus to the ε-amino group of a lysine in the substrate.

Although lysines are the most frequent substrate sites for ubiquitin and Ubl attachment, conjugation can sometimes occur on other residues. More and more examples in which the free α-NH_2 group of an N-terminal residue of a protein is the primary ligation site are being unearthed (Ciechanover & Ben-Saadon 2004). Ubiquitin may also be conjugated to a substrate via a cysteine side chain. A recent study reported the apparent ubiquitin modification of a cysteine in the MHC class I heavy chain in a reaction catalyzed by a viral E3 called MIR1 (Cadwell & Coscoy 2005).

There are two main types of E3 for ubiquitin, the RING class and the HECT class. The RING E3s contain a subunit or domain with a RING motif, which coordinates a pair of zinc ions. RING E3s (and the structurally related but zinc-free U-box E3s) function at least in part as adaptors: They bind the ubiquitin-thioester-linked E2 and substrate protein simultaneously and position the substrate lysine nucleophile in close proximity to the reactive E2-Ubl thioester bond, facilitating transfer of the Ubl. Recent evidence suggests that the RING E3 also triggers subtle conformational changes in the bound E2, stimulating Ubl release from the E2 cysteine and transfer to substrate (Ozkan et al. 2005). Using the human ubiquitin E2 UbcH5b as a model, Ozkan et al. (2005) identified E2 residues that appear to be necessary for allosteric coupling between the E3-binding site and the distal E2 active site. Most telling were UbcH5b mutants that bound E3 and formed a thioester with ubiquitin with normal kinetics yet were defective in transfer of the E2-bound ubiquitin to substrate. The exact network of interactions necessary for communication between the spatially separate E3-binding and active sites of the E2 remains to be determined.

Catalysis of ubiquitin-substrate modification by the HECT E3s follows a mechanism distinct from that of the RING E3s. In HECT E3s, the ubiquitin is first transferred from the E2 to an active-site cysteine in the conserved HECT domain of the E3. The thioester-linked ubiquitin is then transferred to substrate. HECT domains have a bilobal architecture, with a large distance between the E2-binding site in the N-terminal lobe and the active-site cysteine in the C-terminal lobe. For the E3 to be catalytically active, both lobes of the molecule must be brought together, which may require movements of up to 50 Å (Ogunjimi et al. 2005). Notably, when mutations that restrict movement in the hinge between the two lobes in the HECT E3 are introduced, catalytic activity decreases markedly (Verdecia et al. 2003).

As shown in **Figure 1**, the E2 is at the center of a cascade of Ubl transfers linking activation of the Ubl by E1 to its eventual E3-catalyzed attachment to substrate. Interestingly, recent data indicate that an E2 cannot bind to E1 and E3 at the same time, meaning that after transfer of the Ubl from E1 to E2, the E1 must be released before the Ubl-thioester-linked E2 can bind a cognate E3 (Eletr et al. 2005, Huang et al. 2005). Specifically, competitive binding experiments of three human E2-E3 protein pairs reveal that the binding of E1 or E3 to E2 is mutually exclusive in all cases (Eletr et al. 2005). These pairs include both HECT and RING ubiquitin E3s as well as enzymes for Nedd8 conjugation. Ubiquitin or Ubl thioester formation on the E1 and E2 also alters their relative affinities for their cognate enzymes. When the ubiquitin E1 forms a thioester with ubiquitin, the E1 readily binds free, uncharged E2, but when the E1 is uncharged, it

Ubiquitin-like protein (Ubl): protein that contains the conserved ubiquitin fold and is conjugated to other proteins by a pathway similar to ubiquitin conjugation (see **Figure 1**)

DUB: deubiquitinating enzyme

ULP: Ubl-specific protease

E1: the activating enzyme that adenylates the C terminus of a Ubl, activating it for substrate conjugation

E2: the conjugating enzyme that receives a Ubl from its cognate E1 and transfers it either to a HECT E3 or substrate

E3: a Ubl-protein ligase that catalyzes transfer of the activated Ubl to a substrate group (usually a lysine side chain)

Nedd8/Rub1: a Ubl that is ~55% identical to ubiquitin and whose conjugation to certain ubiquitin E3s regulates their ligase activity

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Table 1 Ubl family of proteins

Modifier[a]	E1	E2	E3	Protease processing/cleavage?[b]	Function	Reference(s)
Ubiquitin	Uba1	Ubc1-8, -10, -11, Ubc13-Mms2	Many (primarily RING/U-box and HECT-domain proteins)	Yes (6 DUB classes: UBP, UCH, OTU/Cezanne, Ataxin-3/Josephin, MPN+/JAMM, and UL36USP)	Substrate degradation, localization, protein interactions, other	Aguilar & Wendland 2003, Amerik & Hochstrasser 2004
Ubl proteins						
Smt3/SUMO1-4	Uba2-Aos1	Ubc9	Siz1, Siz2, Mms21	Yes (Ulp1, Ulp2)	Substrate localization, protein interactions, other	Johnson 2004
Rub1/Nedd8	Uba3-Ula1	Ubc12	Dcn1	Yes (Yuh1, Cop9 signalosome)	Activation and destabilization of SCF complexes, transcriptional regulation of p53	Wu et al. 2005, Xirodimas et al. 2004
ISG15	UbeL1	UbcH8	Herc5, Efp	Yes (Ubp43)	May act in transcription and pre-mRNA splicing during IFN response; induced by IFN-α/β	Giannakopoulos et al. 2005, Zhao et al. 2005
Atg8	Atg7	Atg3	–	Yes (Atg4)	Autophagy, nutrient recycling in plants	Ohsumi 2001, Thompson et al. 2005
Atg12	Atg7	Atg10	–	No	Autophagy, nutrient recycling in plants	Ohsumi 2001, Thompson et al. 2005
Urm1	Uba4	–	–	No	Budding, nutrient sensing, oxidative-stress response	Goehring et al. 2003a,b

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

UFM1	Uba5	Ufc1	–	Yes	Function unknown; Uba5 is induced during the unfolded protein response	Komatsu et al. 2004
Fat10	–	–	–	No	Ubiquitin-independent substrate degradation; induced by IFN-γ and TNF-α	Hipp et al. 2005
FUBI/MNSFβ	–	–	–	Yes	May play a role in T cell activation	Nakamura & Tanigawa 2003
Hub1	–	–	–	No	Pre-mRNA splicing	Wilkinson et al. 2004
Suspected Ubls						
BUBL1, 2	–	–	–	–	Ciliate putative autoprocessed proteins	Dassa et al. 2004
Oligo(A) synthetase	–	–	–	–	Function unknown; Ubl is C-terminal portion of protein	Yamamoto et al. 1998
SF3a120	–	–	–	–	Function unknown; Ubl is C-terminal portion of protein	Kramer et al. 1995

[a]Yeast modifier names are given (listed first if a vertebrate ortholog is known and goes by a different name) if present in yeast; yeast names are also given for E1s, E2s, E3s, and Ubl-specific proteases, except for the ISG15 and UFM1 systems, which are not found in *S. cerevisiae*.
[b]When known, protease classes (in the case of ubiquitin) or names (for the other Ubls) are listed in parentheses.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

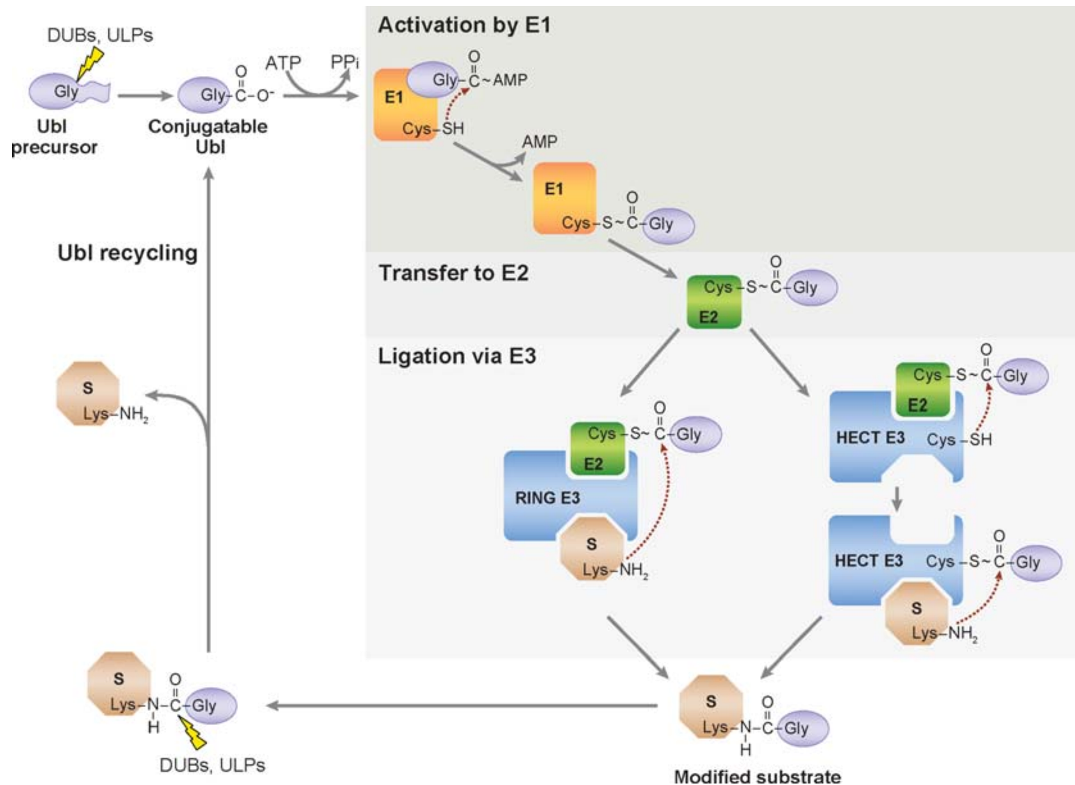

Figure 1

A generalized Ubl-conjugation pathway. Precursor Ubls (including ubiquitin) are processed by either DUBs (deubiquitinating enzymes) or ULPs (Ubl-specific proteases) to expose a C-terminal glycine in the mature Ubl. The processed Ubl is now conjugation competent and can be activated with ATP by E1, or Ubl-activating enzyme. The E1 adenylates the Ubl C-terminal carboxyl group, forming a high-energy Ubl-AMP intermediate (the "∼" represents a high-energy bond). This intermediate is attacked (indicated by the *dashed red arrow*) and covalently bound by the catalytic cysteine of the E1, creating a thioester linkage and releasing AMP. The Ubl is transferred to the catalytic cysteine of the E2, or Ubl-conjugating enzyme, via a transthiolation reaction. The Ubl can then be ligated to a substrate with the aid of an E3, or Ubl-protein ligase. The adaptor-like RING E3s catalyze modification by binding simultaneously the Ubl-E2 thioester complex and the substrate to be modified. This positions the amino group of a substrate lysine (the most common modification site) near the E2-Ubl thioester, catalyzing transfer of the Ubl to substrate. HECT E3s catalyze substrate ligation in two steps. First, the Ubl is transferred to a catalytic cysteine of the HECT E3 via transthiolation. Then, the E3-Ubl thioester complex transfers the Ubl to the substrate. The DUBs and ULPs can remove Ubls from substrates.

releases the loaded E2 (Hershko et al. 1983, Pickart and Rose 1985). In contrast, free E3s readily bind ubiquitin-loaded E2s and have much lower affinity for free E2s (for examples, see Kawakami et al. 2001, Siepmann et al. 2003). These differences in relative affinity help drive the vectorial transfer of activated Ubl between successive conjugation pathway enzymes and to the substrate.

UBIQUITIN AND UBL POLYMERS

Multiply-modified proteins can result either when ubiquitin or Ubl molecules are individually conjugated to different residues on a substrate or when they are attached to each other to form a chain that is conjugated to the substrate at a single site (or small number

of sites). By far the most fully documented example of such polymeric chains is for ubiquitin. Ubiquitin molecules in these chains are linked to one another in the same way in which they are usually linked to substrate proteins. In particular, the C terminus of the more distal ubiquitin molecule is attached to the previous ubiquitin molecule through an isopeptide bond with an ε-amino group of a Ubl lysine. Exactly how such chains are assembled is still not fully understood (Hochstrasser 2006b).

Ubiquitin has seven lysine residues, all of which are potentially involved in chain formation, although ubiquitin-Lys48 and -Lys63 are the best-characterized residues involved in polyubiquitylation (Haglund & Dikic 2005). Polyubiquitin chains of at least four Lys48-linked ubiquitin molecules can efficiently target a conjugated substrate to the proteasome for degradation (Thrower et al. 2000). Lys63-linked chains represent a distinct linkage topology and are involved in cellular processes such as DNA repair and signal transduction (Chan & Hill 2001). They typically do not target proteins to the proteasome. Functionally, both types of polyubiquitin chain attachment can be distinguished from monoubiquitylation, which is involved in processes such as endocytosis and transcriptional regulation (Haglund et al. 2003). Ubiquitin polymers comprising mixed linkages are also possible and have been observed both in vitro and in vivo, but their prevalence and significance in vivo remain unclear (Kirkpatrick et al. 2005, Saeki et al. 2004).

Other than ubiquitin, the only Ubls known to form chains are certain members of the SUMO family (**Table 1** and **Figure 2**). SUMO chains have been detected in vitro and in vivo in both yeast and mammalian cells; however, no function has yet been ascribed to them (Bylebyl et al. 2003). Interestingly, the major branch site for SUMO chains is in an N-terminal extension not shared with ubiquitin. Regarding other Ubls, a recent mass spectrometry study of ISG15 substrates hinted that ISG15 may conjugate multiple times to some proteins (Giannakopoulos et al. 2005). However, the study did not differentiate between single ISG15 molecules attached to several different lysines versus an ISG15 chain.

VARIATIONS ON UBL MODIFICATION: EASY AS E1, E2, E3?

Despite commonalities in the Ubls, considerable variation exists among the different Ubl conjugation pathways. This section highlights some of these differences.

Recruiting Substrates

As described above, a central function of the E3 ligase is to recognize and recruit a substrate that is to be modified by a Ubl. Many of the Ubl pathways, however, have either very few or no identified E3s. How then are substrates identified and recruited in these pathways?

Analyses of SUMO substrates have pointed to the existence of a sumoylation consensus motif, [I/V/L]-K-X-[D/E], where X is any amino acid (Johnson 2004). This sequence can bind directly to the E2 SUMO-conjugating enzyme, Ubc9 (Bernier-Villamor et al. 2002). Thus, this motif may serve as a signature sequence that identifies SUMO substrates to the conjugation machinery even without an E3. Indeed, in many cases, in vitro sumoylation reactions at high concentrations of SUMO E1 and E2 result in the correct targeting of the same lysine that normally is modified in vivo, without addition of a specific E3 (Johnson & Gupta 2001). Whether any substrates are sumoylated in vivo without an E3 is not known. Consensus modification sites in the substrates of other Ubls have not yet been identified, but very few substrate modification sites have been mapped for these Ubls.

The aforementioned sumoylation motif can often, but not always, predict which lysine in a substrate will be conjugated to SUMO. Sites not conforming to a sumoylation consensus motif also can be conjugated to

SUMO (small ubiquitin-like modifer): a Ubl that shares ~18% identity with ubiquitin and whose conjugation often alters the localization and interactions of its substrate with other proteins

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

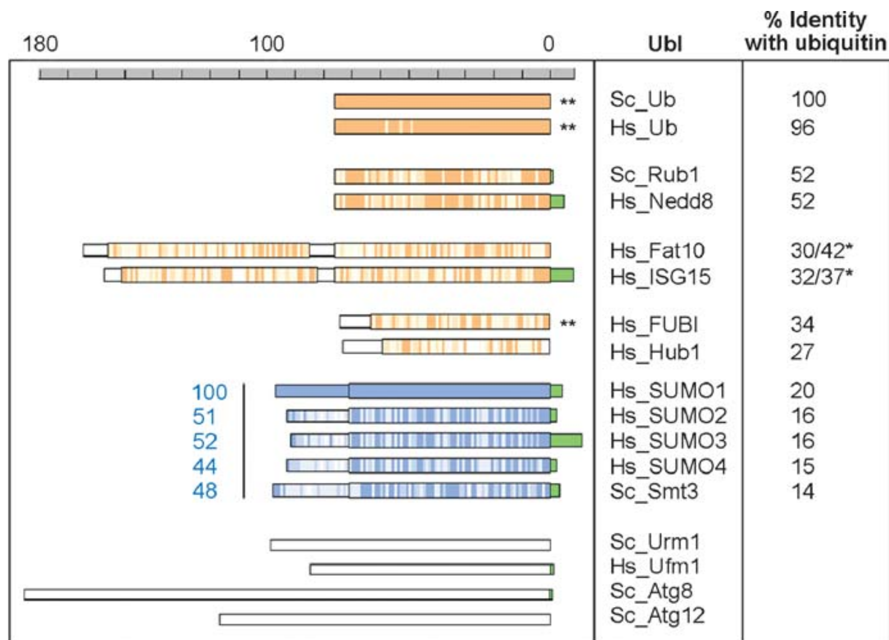

Figure 2

The eukaryotic Ubl protein family. The known Ubls from the yeast *Saccharomyces cerevisiae* (Sc) and humans (Hs) are depicted under the size scale at left (amino acid number, with mature C termini positioned at zero). The unprocessed C-terminal extensions of the precursors are depicted in green. Colored bars (closer to ubiquitin, *orange*; SUMO-related, *blue*) represent levels of amino acid conservation: dark bars, identical amino acid between yeast and human; light bars, conservative substitution; white bars, nonconservative substitution. The percent identity of each Ubl to yeast ubiquitin (Sc_Ub) is shown at right. Percent identity between individual human SUMO isoforms and yeast Smt3 is reported separately with blue numbers on the left. Significant amino acid conservation between ubiquitin and the Urm1, Ufm1, Atg8, and Atg12 Ubls is not detected. Atg8 and Atg12 show approximately 20% identity to each other (*not shown*). Percent identity between each of the two Ubl domains in Fat10 and ISG15 to Sc_Ub is indicated by the single asterisks. Ubiquitin and FUBI are also synthesized in precursor form, but their C-terminal extensions comprise either longer ribosomal peptides or, for a subset of ubiquitin-coding genes, additional ubiquitin units (indicated by the *double asterisks*). For these polyubiquitin precursors, the C-terminal ubiquitin has a single amino acid extension that is also cleaved off. Identity scores were generated using a Blosum62 matrix and best local alignment at **http://www.ch.embnet.org/software/LALIGN_form.html**. Size comparison and graphic pair scores were generated in Macaw 2.05 (Multiple Alignment Construction and Analysis Software, Greg Schuler/National Center for Biotechnology Information), using a Blosum62 matrix. Macaw graphics output was processed using Adobe Photoshop™ software.

SUMO, and not all sites conforming to the consensus motif are necessarily modified (Johnson 2004). Interestingly, regulatory factors can sometimes control which sites are utilized. For example, a phosphorylation-dependent sumoylation motif recently was described, suggesting that phosphorylation can promote the sumoylation of certain substrates such as heat-shock factors (Hietakangas et al. 2006). The structural context within which a lysine resides can also dictate whether or not it is sumoylated. Sumoylation of the ubiquitin-conjugating enzyme E2-25K occurs on a non-consensus lysine within an α-helical structure and not on neighboring lysines situated within consensus motifs (Pichler et al. 2005). Mutational studies with disordered peptides revealed that when the same sequences were

no longer constrained by a helical structure, sumoylation preferentially deviated to the consensus lysines. Therefore, factors beyond primary sequence also influence sites of modification.

Enzyme Sharing by Ubls

In recent years it has become apparent that not all Ubl-ligating enzymes are specific for a single Ubl. For instance, the two Ubls involved in autophagosome formation, Atg8 and Atg12, share a single E1, Atg7; however, each Ubl requires a distinct E2 (Ichimura et al. 2000). This remains the only known example in which a single E1 can activate two different Ubls. The ability of Atg7 to transfer Atg8 and Atg12 to distinct E2s suggests that these E2s bind to the E1-Ubl complex in ways that are productive only for transfer of the E2's cognate Ubl. The structural basis of this discrimination remains to be determined.

Conversely, two different E1s specific for different Ubls can share the same E2: UBEL1, the E1 for ISG15, and Uba1, the E1 for ubiquitin, both can use the UbcH8 E2 (Zhao et al. 2004). How is Ubl-substrate specificity maintained in this situation? The explanation may largely involve mass action. ISG15 and UBEL1 are strongly expressed only when cells are induced with interferon. Potentially, activated ISG15 is present under these conditions at levels sufficient to ensure that UbcH8 picks up a sizeable amount of it and, together with ISG15-specific E3s, transfers it to the appropriate targets. Recently, such interferon-induced ISG15 E3s, one a RING E3 and the other a HECT protein, have been reported, (Dastur et al. 2005, Zou & Zhang 2005). Interestingly, the newly identified ISG15 E3s also are able to function with ubiquitin. For the SUMO pathway, structural studies have uncovered direct contacts between an E3 and the SUMO protein (Reverter & Lima 2005, Song et al. 2005). ISG15-specific E3s also may be able to use direct ISG15 contacts to identify the cognate ISG15-E2 thioester even if the same E2 sometimes is linked to ubiquitin.

Other mechanisms controlling Ubl choice by an E3 are also possible. For instance, additional specificity factors may exist. An analogous specificity factor may be the Nub1L protein, which has been proposed to be an adaptor that delivers both Fat10 and Nedd8 conjugates to the proteasome for degradation (Hipp et al. 2005, Kamitani et al. 2001). Nub1L, however, binds Fat10 much more strongly than Nedd8 and thus preferentially promotes the degradation of Fat10-modified proteins (Hipp et al. 2004). A similar type of factor may allow discrimination between ISG15- and ubiquitin-bound E3s or E2-E3 complexes. Therefore, whether a substrate is modified by ubiquitin or a particular Ubl that shares the same E3 may be determined by the relative cellular levels of the activated modifiers or by additional specificity factors (or both).

Analogous to the mixed specificity of these ISG15/ubiquitin E3s, the mammalian Mdm2 protein is an E3 ligase that mediates both ubiquitylation and neddylation of the p53 tumor suppressor protein (Xirodimas et al. 2004). Interestingly, p53 can be ubiquitylated on six different lysines, whereas neddylation occurs at only three of these sites. Polyubiquitylation of p53 results in its proteasome-mediated degradation in the nucleus, whereas the monoubiquitylated form is exported from the nucleus (and therefore away from its transcriptional targets) but spared degradation (Boyd et al. 2000, Geyer et al. 2000). Similar to the negative effects of ubiquitin on p53 transcriptional activity, abrogation of p53 neddylation in tissue culture cells enhanced its transcriptional activity. Therefore, neddylation likely is also a negative regulator of p53 activity.

An Exception to the Universal E1-E2 Couple?

Conceivably, some Ubl-ligating enzymes may perform multiple steps in the activation and transfer of a single Ubl. For the eight Ubl-conjugation systems in which an E1 has been

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

identified (**Table 1**), all but one—the Urm1 system—is known to require a separate E2 protein. The transfer of Ubls from a cysteine side chain of an E1 to a cysteine on an E2 is not chemically necessary insofar as the Ubl C terminus is already activated when it is bound to the E1, and the transfer to E2 also yields an enzyme-Ubl thioester bond.

The Urm1 pathway may represent an exception to the E2 requirement in E1-catalyzed Ubl-protein conjugation. Urm1 shares significant sequence identity (~20%) with the bacterial proteins MoaD and ThiS, ubiquitin-fold proteins involved in molybdopterin and thiamin cofactor biosynthesis, respectively. The Urm1-activating enzyme, Uba4, is also related to the E1-like proteins, MoeB and ThiF, which act on MoaD and ThiS. As with activation of Ubls, MoaD and ThiS are activated through C-terminal adenylation. Although in MoaD and ThiS this activation leads to the attachment of a sulfur atom to their C termini rather than attachment of a substrate protein, the mechanistic and structural similarities to Ubl ligation are striking.

Urm1 ligation appears to be poised, mechanistically, between MoaD/ThiS activation and most Ubl-protein ligation mechanisms. A unique and conserved feature of the Urm1 E1 (Uba4) compared with the E1s for other Ubls is the presence of a rhodanese-homology domain (RHD) in the protein. Rhodanese and a number of RHD proteins are sulfurtransferases that form a persulfide (-S-S-H) on their active-site cysteine. Many MoeB family proteins, such as human MOCS3, have a similar domain organization, with an E1-like domain followed by an RHD. Based on these and other similarities, we previously proposed that thiocarboxylate formation in MoaD catalyzed by MoeB/MOCS3 is closely related mechanistically to Uba4-catalyzed Urm1 activation and transfer (Hochstrasser 2000, 2006b). In particular, we suggested that the Uba4 RHD forms a transient covalent intermediate with Urm1, which serves as the immediate donor to the substrate. In this scenario, the RHD functions as a kind of built-in E2, bypassing the requirement for a separate E2 enzyme. The RHD cysteine indeed is essential for Urm1-protein ligation in yeast (see Hochstrasser 2006a), but whether it functions in the way described here will require additional testing.

TAKING DIRECTIONS FROM UBIQUITIN AND UBLS

Posttranslational modification of proteins by Ubls is expected to modulate their function in the cell, primarily by altering their interactions with other macromolecules, thereby changing the tagged proteins' location, conformation, stability, or activity. Here, we discuss recent data that document some of the general ways in which the Ubls, including ubiquitin, direct these changes.

Ubiquitin and SUMO-Binding Domains

Attachment of a Ubl or Ubl chain to a protein creates a protein surface topography in the conjugate that is changed substantially from that of the unmodified protein, at least locally. Although in some cases Ubl modification may change the conformation of the tagged protein, the simpler possibility is that Ubl conjugation either links together a set of receptor-binding sites—in the Ubl and target protein—to enhance binding to another macromolecule or masks a receptor-binding site in the target protein to inhibit binding to another macromolecule. By this reasoning, enhanced binding of a Ubl-conjugated protein, relative to its unconjugated form, to another "receptor" protein implies the existence of a binding site for the Ubl in that other protein. Indeed, over the past decade, an extensive series of Ubl-binding domains has been described.

By far the most numerous and best-characterized Ubl-binding domains are those that recognize ubiquitin (Hicke et al. 2005). Generically termed ubiquitin-binding domains (UBDs), this large class of protein modules binds noncovalently to ubiquitin or

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

polyubiquitin chains. At least nine distinct structural subclasses of UBDs have been reported, and more can be expected. These domains are found in hundreds of proteins in the human proteome, and UBD-bearing proteins participate in a broad range of cellular processes, including protein ubiquitylation, deubiquitylation, and degradation (Hicke et al. 2005).

The first UBD was found in a regulatory subunit of the proteasome. Using Far-Western analysis with an ^{125}I-labeled polyubiquitin-protein substrate, Deveraux et al. (1994) identified a single subunit of the 19S regulatory complex called S5a (Rpn10 in yeast) that bound tightly to the radiolabeled substrate. Two related ~30-residue hydrophobic segments in S5a are responsible for the binding to polyubiquitin (Young et al. 1998). A subsequent bioinformatics analysis recognized a more general ~20-residue core related to the S5a ubiquitin-binding element, and this element was named the ubiquitin-interaction motif (Hofmann and Falquet 2001).

The most common UBD found to date is the ubiquitin-associated (UBA) domain (Hofmann and Bucher 1996). Interestingly, the role of this domain in ubiquitin binding first was elucidated for a set of proteins that also bind directly to the proteasome regulatory complex. These adaptor proteins appear to escort a subset of polyubiquitylated substrates to the proteasome. Adaptors, such as Rad23 and Dsk2, contain both a UBA domain, which binds polyubiquitylated substrates, and a ubiquitin-related domain that binds the proteasome's Rpn1/S2 subunit. Rpn1 is located in the base of the proteasome regulatory complex near the proteasomal ATPases that mediate the unfolding of substrates prior to their degradation (Elsasser et al. 2002). Rpn1 binds the ubiquitin-like domain of the adaptor proteins via a leucine-rich-repeat domain. Rpn1 may not be the only proteasome docking site of adaptor proteins. A recent study of the adaptor protein Nub1 revealed that it does not interact with the proteasome through its ubiquitin-like domain but rather via an uncharacterized element in its C terminus (Tanji et al. 2005). The exact Nub1 and proteasome sites that mediate this interaction have not yet been determined.

UBD: ubiquitin-binding domain

SBM: SUMO-binding motif

The identified UBDs are structurally diverse, yet the majority contact the same hydrophobic surface patch on ubiquitin, which centers on Ile44 (Hicke et al. 2005). Mutations of Ile44 or neighboring hydrophobic residues severely inhibit the ability of ubiquitin to function in vivo and in vitro. For example, substrates polyubiquitylated with mutant I44A ubiquitin fail to be degraded by the proteasome in vitro (Beal et al. 1996). Another shared feature among most of the UBDs is that the binding to a single ubiquitin is generally very weak; apparent dissociation constants range from ~2 μM to more than 0.5 mM. Despite this unimpressive affinity, mutational studies have made clear the physiological relevance of these weak binding interactions in many cases. Binding can be greatly enhanced either by polymerizing the ubiquitin signal or by combining weak binding to ubiquitin with additional weak binding sites in the tagged protein.

Much less is known about potential binding domains that are specific for other Ubls. An exception is the recent description of a SUMO-binding motif (SBM). The first hint of such a motif came from two-hybrid studies. Comparison of the identified SUMO-interacting proteins suggested a short common motif that was potentially important for SUMO binding; this was supported by mutagenesis experiments (Minty et al. 2000). Subsequent structural and mutational characterization of peptides that bind SUMO suggested the consensus sequence V/I-X-V/I-V/I, where X is any amino acid (Song et al. 2004). With as few as nine residues, these peptides are substantially smaller than the domains that typically bind ubiquitin (20–145 residues); nevertheless, these peptides form 1:1 complexes with SUMO that have dissociation constants between 5–10 μM, which is much tighter than the binding seen with

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

most of the ubiquitin-binding motifs discussed above. Yeast two-hybrid analysis of proteins able to bind noncovalently to yeast SUMO (Smt3) yielded an SBM characterized by a cluster of three to four aliphatic residues followed by a cluster of three to four negatively charged amino acids (Hannich et al. 2005), which is related to the SUMO-binding sequences documented in the two aforementioned mammalian studies (Minty et al. 2000, Song et al. 2004). There are almost certainly other SUMO-binding domains to be found that are distinct from the SBM, but it is striking that unrelated screens as different as the yeast and mammalian two-hybrid studies could yield such similar consensus sequences.

Recent structural analyses reveal that the mode of binding of the SBM to SUMO is very different from that of UBDs to ubiquitin (Reverter & Lima 2005, Song et al. 2005). The SBM peptides form a β strand that sits in a hydrophobic surface groove on SUMO and extends the SUMO β sheet; the ends of the SBM segments are acidic residues that interact with basic residues on the SUMO surface. Interestingly, the SBM β strand was bound to SUMO with opposite polarity in the two aforementioned studies. Deletion studies of SUMO E3 proteins lacking predicted SBMs suggest that the SBM-SUMO interaction is important for both the conjugation of SUMO to substrates as well as for the regulation of substrates that already have been sumoylated (Takahashi & Kikuchi 2005, Yang & Sharrocks 2005).

Although binding domains for Ubls other than ubiquitin and SUMO have yet to be identified, proteins containing sequences that match the consensus for UBDs but do not appear to bind ubiquitin have been described (Raasi et al. 2005). Perhaps these proteins bind other Ubls instead.

Ubls and Protein Complex Assembly

Besides modulating simple binary protein-protein interactions, Ubl modification of polypeptides that are part of large complexes may help orchestrate the assembly or disassembly of such complexes. The most frequently cited example that lends support to this hypothesis is the assembly of the promyelocytic leukemia nuclear bodies (PML bodies). The PML protein functions as a scaffold for the assembly of the PML bodies, which are subnuclear structures implicated in transcriptional regulation and DNA repair. The PML protein must be sumoylated for PML bodies to form and for a number of other sumoylated proteins to concentrate there as well (Zhong et al. 2000). By combining SUMO ligation sites and SBMs into the same polypeptides, networks of protein interactions can potentially be created (Seeler & Dejean 2001). In cells from patients with acute promyelocytic leukemia, the PML protein is fused to retinoic-acid receptor α, causing a loss of PML sumoylation and disrupting the PML bodies. Moreover, depletion of the SUMO3 paralog decreases the number and integrity of PML bodies found in HeLa cells (Fu et al. 2005). PML bodies may serve as a kind of molecular storage facility for transcription factors whose inventory is regulated by sumoylation.

Other Ubl modifications can also regulate the formation of multisubunit complexes. The Ubl Atg12 was first shown to conjugate to the Atg5 autophagy protein in yeast, and this modification since has been shown to be important for autophagy in multiple species (Mizushima et al. 2003, 1998; Thompson et al. 2005). Conjugation of Atg12 to Atg5 may stabilize their association with the protein Atg16 (Mizushima et al. 1999). Atg16 has a coiled-coil region through which it forms homo-oligomers, and it is thought that Atg16 cross-links multiple Atg12-Atg5 conjugates to form a large complex that localizes to autophagosome precursors and contributes to the development of autophagic isolation membranes into autophagosomes (Mizushima et al. 2003). Thus, just as SUMO seems to direct the formation of the PML bodies, the modification of Atg5 by Atg12 may help drive the formation of autophagic vesicles.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

UBLS AND THEIR CONJUGATION MACHINERY: LOCALIZE TO REGULATE?

One perplexing feature of Ubls such as SUMO and ISG15 is that they tend to modify only a very small fraction of any particular target protein. This places significant constraints on how the modification can regulate target protein function (see Hochstrasser 2006b, Johnson 2004). For instance, negative regulation of protein activity by Ubl attachment generally should not be effective under these circumstances. On the other hand, if the small fraction of modified protein were localized to some functionally unique cellular site, or if a transient modification were sufficient to switch the protein into a new state, inhibitory mechanisms could still operate. One way that such mechanisms may be enabled is by restricting spatially the Ubl and/or its conjugation machinery within the cell.

Subcellular Localization of Ubls

The subcellular distribution of a Ubl sometimes can provide useful clues about its functions and substrates. For example, in vertebrate cells, SUMO localizes to the nucleus and concentrates at nuclear pore complexes and in PML bodies (Johnson 2004). Localization of SUMO to PML bodies led to the testing of multiple PML body components for SUMO conjugation and binding, resulting in the current model noted above for PML body assembly through a network of covalent and noncovalent SUMO interactions among the different PML body components. Subcellular concentrations of Ubls may reflect hotspots of Ubl modification or sites at which abundant Ubl-modified proteins can bind and accumulate (or both). In directed and whole proteome-scale studies, the subcellular localization of specific Ubl conjugates, for example SUMO1- versus SUMO3-modified proteins, has helped to predict the functions for particular Ubl modification pathways (Manza et al. 2004, Vertegaal et al. 2004).

In addition, degenerative disorders such as Huntington's, Parkinson's, or Alzheimer's disease often are characterized by large protein aggregates in specific cell types and subcellular sites, and these aggregates are modified extensively by ubiquitin and in some cases SUMO. Studies correlating the degree of ubiquitin or Ubl modification of aggregate components with clinical severity suggest that these modifications may have a direct impact on disease progression (Arrasate et al. 2004, Neve 2003, Ross & Pickart 2004). For example, ubiquitin modification of the Huntingtin (Htt) protein correlates with reduced neurodegeneration, whereas SUMO modification of the same Htt lysines inhibits aggregate formation in cultured cells and exacerbates symptoms in a *Drosophila* model (Steffan et al. 2004). Sumoylation of Htt seems to increase Htt levels in cultured cells, so the enhanced toxicity in flies may also derive from an increase in Htt abundance. The data in general suggest that formation of large Htt aggregates protect against Htt-induced toxicity, but this remains to be clarified. Moreover, the exact impact of these different modifications on Htt level and function remains ill defined (but see below).

Localization of the Ubl-Conjugation Machinery

Some Ubl-modified substrates have an extremely circumscribed spatial distribution within the cell, raising the possibility that efficient Ubl attachment requires colocalization of components of the Ubl conjugation machinery to these sites. For example, the Ubl Atg8 is induced in response to starvation, whereupon it is conjugated to the lipid phosphatidylethanolamine as part of the autophagy pathway. Atg7, the E1 enzyme that activates Atg8 (and Atg12), is a cytosolic protein under normal growth conditions. However, when cells are starved, Atg7 becomes membrane bound, and a portion relocalizes to autophagocytic structures in the cell (Kim et al. 1999). Analogously, the SUMO E2 Ubc9

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

SCF: SKP1-CUL1-F-box protein E3

colocalizes with Rad51 on synaptonemal complexes of meiotic mouse chromosomes (Kovalenko et al. 1996). Although Rad51 may not be sumoylated, other interacting proteins such as Rad52 probably are (Ho et al. 2001). Finally, there are examples of membrane-associated ubiquitin E3s that localize to specific organelles such as the endoplasmic reticulum or trans-Golgi network, where misfolded proteins can be tagged for degradation (Lotz et al. 2004, Swanson et al. 2001).

Although cells can sometimes bring the Ubl machinery to substrates, at other times they can sequester it away from substrates. For example, the PML protein may aid in the stabilization of p53 in response to genotoxic stress by binding and sequestering the E3 ligase of p53, Mdm2, in the nucleolus, away from p53 (Bernardi et al. 2004).

An alternative to directing particular conjugating enzymes to different cellular sites would be for multiple isoforms of an enzyme to reside in distinct compartments. Precisely this type of functional specialization has occurred with the Fbw7 tumor suppressor protein. Fbw7, an F-box protein, is a substrate recognition subunit for the SCF (SKP1-CUL1-F-box protein) E3, a multisubunit ubiquitin ligase. F-box proteins share a sequence segment, the F-box, that binds them to the SKP1 subunit of the SCF complex, and they also contain distinct protein-protein interaction motifs that are used in substrate binding. Many, including Fbw7, recognize phosphorylated degradation signals (phosphodegrons) in their substrates. Fbw7 targets include the c-Myc growth regulator.

Alternative splicing generates three isoforms of Fbw7 (α, β, and γ). Welcker et al. (2004) recently showed that these isoforms reside in distinct subcellular sites: Fbw7α is predominantly nuclear, whereas Fbw7β is cytoplasmic, and Fbw7γ nucleolar. Localization is controlled by *cis*-acting sequences in the unique segment of each isoform. Interestingly, Fbw7γ colocalizes with the c-Myc protein in the nucleolus when the proteasome is inhibited. These and other data led to the suggestion that Fbw7γ-dependent degradation of c-Myc in the nucleolus keeps the growth-promoting activity of the oncoprotein in check.

NEW FUNCTIONS FOR UBLS

The functional consequences of ubiquitin and Ubl conjugation are slowly being deciphered, and the number of biological pathways known to be regulated by Ubl conjugation continues to grow. Here, we highlight a number of provocative examples of Ubl action. These serve both to emphasize how pervasive the ubiquitin/Ubl modification systems are in eukaryotic regulation and to point out how often we are surprised by novel findings, even in areas of Ubl biology in which our understanding seemed fairly mature.

SUMO and Ubiquitin in Some Unexpected Places

SUMO regulation of circadian rhythms. BMAL1 is a transcription factor that forms a heterodimer with the protein CLOCK, and together they form an integral part of the mammalian circadian oscillator. The BMAL1-CLOCK heterodimer controls the transcription of genes encoding circadian clock components. Unexpectedly, mouse BMAL1 is modified on a conserved lysine by SUMO, and the protein is sumoylated with a circadian rhythmicity in the liver that parallels its activation in other mouse tissues in which the clock is thought to operate (Cardone et al. 2005). Sumoylation requires CLOCK, and ectopic expression of a mutant sumoylation-defective BMAL1 in a cell culture model suggests that SUMO attachment is required for normal clock rhythmicity.

SUMO and gene silencing. In the fission yeast *Schizosaccharomyces pombe*, deletion of the nonessential SUMO-coding gene results in severe silencing defects of the *mat* mating-type locus and increased Lys4 methylation of histone H3, a hallmark of active chromatin.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

This suggests that SUMO is somehow involved in the establishment and/or maintenance of silent heterochromatin. Recently, Ubc9 (the SUMO E2) was shown to localize to heterochromatic loci and interact with the heterochromatin protein Swi6, the Swi6 paralog Chp2, and the methyltransferase Clr4, all of which are sumoylated (Shin et al. 2005). Expression of unsumoylatable mutants of Swi6 and Chp2 results in compromised silencing of the *mat* locus. These results suggest that sumoylation of specific proteins such as Swi6 mediates their ability to form and/or regulate heterochromatin.

Quantitative modification: opening ion channels by channel desumoylation. As noted above, for the vast majority of Ubl substrates, only a small fraction of the target protein is in the Ubl-modified state within the cell. SUMO-protein conjugates, for example, almost always follow this generalization. Recently, however, several examples of proteins that are nearly quantitatively modified by SUMO have been reported. One is the vaccinia virus A40R early protein (Palacios et al. 2005). A40R sumoylation is required for its localization to ER viral replication sites. Mutation of the A40R sumoylation site causes the protein to self-associate and aggregate into long rods. Thus, SUMO attachment to A40R appears to block its interaction with other proteins, likely another copy of A40R. A second potential example of quantitative sumoylation is the plasma membrane K2P1 potassium leak channel (Rajan et al. 2005). Human K2P1 channels do not exhibit any measurable activity when expressed in frog oocytes or in COS7 cells. Further analysis, however, suggested that the cause of this inactivity is the complete sumoylation of K2P1 on a single lysine residue (K274). Either mutation of K274 to glutamate or overexpression of the SUMO protease SENP1 (hULP1) led to the appearance of a pH-sensitive potassium conductance. If these data can be confirmed, particularly for an endogenously expressed K2P1 channel, the system should be well suited for a detailed mechanistic analysis of how SUMO conjugation could alter the physical properties of a target protein.

Auxin signaling and ubiquitin in plants. Auxin, a derivative of tryptophan, is a key plant hormone (phytohormone) that is required for almost every aspect of plant growth and development (for a review, see Woodward & Bartel 2005). Plant cells contain genes that are turned on or off in response to auxin signaling. Auxin binds to soluble cellular receptors, which somehow trigger a signaling pathway that ends in the regulated transcription of auxin-responsive genes. A complex of transcription factors, called Arf and Aux/IAA proteins, binds DNA and represses the transcription of the auxin-responsive genes. Notably, the Aux/IAA proteins are regulated by ubiquitin-mediated destruction; in particular, auxin signaling leads to their degradation, allowing transcription to commence.

Aux/IAA proteins are ubiquitylated by a version of the SCF ubiquitin ligase that utilizes the TIR1 F-box protein as its substrate receptor. When TIR1 binds Aux/IAA proteins, they are polyubiquitylated by SCF^{TIR1} and degraded by the proteasome. Remarkably, it was recently discovered that the TIR1 SCF subunit also directly binds auxin (Dharmasiri et al. 2005, Kepinski & Leyser 2005), and TIR1 along with three TIR1-related proteins (AFB1-3) appear to be the only soluble auxin-binding factors present in plant extracts. TIR1 and AFB association with Aux/IAA proteins is auxin dependent, so in response to an auxin signal, they are ubiquitylated and degraded. The discovery that a ubiquitin ligase subunit is the long-sought auxin receptor provides a key missing piece to the mechanistic explanation of the far-ranging effects of auxin on plant growth and development.

Ubl-Related Pathologies

As mentioned above, many neurodegenerative disorders are characterized by the accumulation of intracellular proteinaceous

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

aggregates. The formation of these inclusions may involve the dysregulation of various Ubls as well as components of the ubiquitin-proteasome system. Disease progression, as discussed below, seems to be modulated by a complex interplay of different Ubl-protein modifications.

Huntington's disease and Ubls. Mutant Htt contains N-terminal polyglutamine expansions of varying lengths that have been linked to the formation of intranuclear aggregates, a hallmark of Huntington's disease (see above). Although the precise role of normal Htt in the cell is not clear, mutant Htt somehow interferes with basic cellular functions such as transcription, signaling, transport, and endocytosis and ultimately leads to neuronal degeneration (reviewed by Landles & Bates 2004). As discussed above, pathogenic mutant Htt can be either ubiquitylated or sumoylated. The latter modification renders the protein more soluble, more abundant, and apparently more toxic (Steffan et al. 2004). In the *Drosophila* model for Huntingon's, genetically lowering SUMO levels, in contrast to lowering ubiquitin levels, reduces neurodegeneration in flies expressing the pathogenic Htt. Reduced sumoylation of Htt may facilitate aggregation of Htt into inclusions that limit its toxicity or may allow enhanced Htt degradation (or both).

A provocative recent study suggests that one role of ubiquitin may be to provide a physical link between Htt protein aggregates and the autophagocytic machinery where the aggregates can be degraded (Bjorkoy et al. 2005). Autophagy is a degradative mechanism in which a double-membraned vesicle is formed around sections of cytoplasm; the resulting autophagosome then fuses with the lysosome, and the autophagosome's contents are hydrolyzed. A key intermediary between the autophagocytic machinery and Htt appears to be the p62 protein (also called sequestosome 1). This protein has a ubiquitin-binding UBA domain and is known to associate with polyubiquitylated Htt aggregates. Surprisingly, the same protein is also closely associated with an important autophagic factor, light chain 3 (LC3). LC3 is orthologous to the yeast Ubl Atg8; both proteins are C-terminally conjugated to phospholipid, which leads to a tight association with the developing autophagosome membrane. By bridging polyubiquitylated Htt aggregates to the initiating membrane of the autophagosome, p62 may drive the selective engulfment of the aggregates by autophagosomes so that the potentially toxic proteins can be cleared from the cell. It is striking that at least three different Ubls (SUMO, LC3, and ubiquitin) directly impact Huntington's disease progression. Whereas SUMO has the potential to increase the levels of toxic forms of Htt, ubiquitin and LC3 help remove aggregated Htt from the cell.

This example of ubiquitin, SUMO, and LC3 interplay demonstrates how "cross-talk" between Ubls can impact important physiological processes. Several additional examples have come to light in recent years (for a review of SUMO and ubiquitin cross-talk, see Ulrich 2005). A particularly intricate case, discussed briefly above, involves the regulation of the p53 tumor suppressor by its modification by SUMO, monoubiquitin, polyubiquitin, and Nedd8.

Alzheimer's disease: a role for SUMO chains? Alzheimer's disease, a form of progressive dementia, is caused by the degeneration of specific brain regions. This process is closely linked to the accumulation of aggregated fragments of the amyloid precursor protein (APP). The transmembrane APP is processed by several different proteases ("secretases"), and when certain cleavages predominate, the resulting peptides, or Aβ fragments, tend to aggregate into amyloid fibers. Overexpression of SUMO2 in tissue culture cells cotransfected with the APP gene suppresses Aβ fragment accumulation (Li et al. 2003). Interestingly, overexpression of a mutant SUMO2 that cannot form polySUMO chains (Tatham et al. 2001) has the opposite

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

effect. Although the same study failed to detect SUMO2-modified APP, a proteomic analysis of SUMO1-modified proteins identified APP as a possible SUMO1 target (Gocke et al. 2005). However, SUMO1 cannot form polySUMO chains. Hence, the exact mechanism by which SUMO2 suppresses toxic Aβ processing and accumulation remains unclear.

CONCLUDING REMARKS

As should be evident from the above survey, ubiquitin and Ubl modification of proteins represents a highly versatile means of regulating protein function. Nature has made widespread the use of such conjugation through the elaboration of multiple variants of the same basic enzymatic mechanism. On the order of a dozen or so Ubls have been documented to date, and for eight of these, at least one enzyme in the pathway for substrate conjugation has been identified. Ubiquitin itself can attach to proteins in the form of polymers of different topology, and these topological variants impart differences in function as well. The fundamental E1-E2 couple, which probably arose very early in the evolution of the ubiquitin system from more ancient sulfur transfer pathways, has been supplemented with an array of specificity factors (E3s) in some of the pathways, especially the ubiquitin pathway. Deconjugating enzymes have turned ubiquitin and many of the Ubls into dynamic modifiers whose attachments are tightly regulated both spatially and temporally. The basic biochemical consequence of protein modification by ubiquitin or Ubls usually is a change in the target's association with other proteins. This can occur by both direct and indirect mechanisms and can either stimulate or inhibit particular protein-protein interactions. Given the intricacy of the ubiquitin/Ubl system, research into its functions and mechanisms should continue to yield novel insights into cell regulation.

SUMMARY POINTS

1. The different ubiquitin-like proteins (Ubls) are activated and conjugated to substrates by similar biochemical mechanisms.
2. All the structurally characterized Ubls share the ubiquitin or β-grasp fold, even when their primary sequences are not detectably similar.
3. Ubl-protein conjugation is subject to many kinds of controls, including regulation of the accessibility and activity of conjugating enzymes, control of deconjugating enzyme activity, and posttranslational modification of substrates by other covalent groups, such as other Ubls.
4. The ubiquitin and Ubl activation mechanisms are related to biosynthetic sulfur transfer by sulfurtransferases, suggesting an evolutionary derivation of the Ubl-protein conjugation pathways from a more ancient sulfurtransferase system.
5. Dysregulation of Ubl-protein modification contributes to the progression and severity of various diseases, such as cancer and neurodegenerative disorders.

ACKNOWLEDGMENTS

We thank Alaron Lewis, Mary Kroetz, and Stefan Kreft for comments on the manuscript. Work from our group is supported by grants from the NIH (GM46904 and GM53756 to M.H. and training grant GM07223-31 to R.F.). O.K. and R.F. contributed equally to this review.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

LITERATURE CITED

Aguilar RC, Wendland B. 2003. Ubiquitin: not just for proteasomes anymore. *Curr. Opin. Cell Biol.* 15:184–90

Amerik AY, Hochstrasser M. 2004. Mechanism and function of deubiquitinating enzymes. *Biochim. Biophys. Acta* 1695:189–207

Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. 2004. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431:805–10

Beal R, Deveraux Q, Xia G, Rechsteiner M, Pickart C. 1996. Surface hydrophobic residues of multiubiquitin chains essential for proteolytic targeting. *Proc. Natl. Acad. Sci. USA* 93:861–66

Bernardi R, Scaglioni PP, Bergmann S, Horn HF, Vousden KH, Pandolfi PP. 2004. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nat. Cell Biol.* 6:665–72

Bernier-Villamor V, Sampson DA, Matunis MJ, Lima CD. 2002. Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. *Cell* 108:345–56

Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, et al. 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J. Cell Biol.* 171:603–14

An analysis that suggests that ubiquitin and ubiquitin-binding proteins link intracellular protein aggregates, such as those found in Huntington's disease, and the autophagocytic machinery.

Boyd SD, Tsai KY, Jacks T. 2000. An intact HDM2 RING-finger domain is required for nuclear exclusion of p53. *Nat. Cell Biol.* 2:563–68

Bylebyl GR, Belichenko I, Johnson ES. 2003. The SUMO isopeptidase Ulp2 prevents accumulation of SUMO chains in yeast. *J. Biol. Chem.* 278:44113–20

Cadwell K, Coscoy L. 2005. Ubiquitination on nonlysine residues by a viral E3 ubiquitin ligase. *Science* 309:127–30

This paper suggests that ubiquitylation of the MHC class I heavy chain by the viral E3 ligase MIR1 can occur on a cysteine residue.

Cardone L, Hirayama J, Giordano F, Tamaru T, Palvimo JJ, Sassone-Corsi P. 2005. Circadian clock control by SUMOylation of BMAL1. *Science* 309:1390–94

Chan NL, Hill CP. 2001. Defining polyubiquitin chain topology. *Nat. Struct. Biol.* 8:650–52

Ciechanover A, Ben-Saadon R. 2004. N-terminal ubiquitination: more protein substrates join in. *Trends Cell Biol.* 14:103–6

Dassa B, Yanai I, Pietrokovski S. 2004. New type of polyubiquitin-like genes with intein-like autoprocessing domains. *Trends Genet.* 20:538–42

Dastur A, Beaudenon S, Kelley M, Krug RM, Huibregtse JM. 2005. Herc5, an interferon-induced HECT E3 enzyme, is required for conjugation of ISG15 in human cells. *J. Biol. Chem.* 281:4334–38

Deveraux Q, Ustrell V, Pickart C, Rechsteiner M. 1994. A 26 S protease subunit that binds ubiquitin conjugates. *J. Biol. Chem.* 269:7059–61

Dharmasiri N, Dharmasiri S, Estelle M. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* 435:441–45

This research group, along with Kempinski & Leyser (2005), discovered that Tir1, an SCF F-box protein, is a receptor for the plant phytohormone auxin. This suggests that hormonal signals in plants are directly sensed and transduced by specific ubiquitin-protein ligases.

Eletr ZM, Huang DT, Duda DM, Schulman BA, Kuhlman B. 2005. E2 conjugating enzymes must disengage from their E1 enzymes before E3-dependent ubiquitin and ubiquitin-like transfer. *Nat. Struct. Mol. Biol.* 12:933–34

Elsasser S, Gali RR, Schwickart M, Larsen CN, Leggett DS, et al. 2002. Proteasome subunit Rpn1 binds ubiquitin-like protein domains. *Nat. Cell Biol.* 4:725–30

Fu C, Ahmed K, Ding H, Ding X, Lan J, et al. 2005. Stabilization of PML nuclear localization by conjugation and oligomerization of SUMO-3. *Oncogene* 24:5401–13

Geyer RK, Yu ZK, Maki CG. 2000. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nat. Cell Biol.* 2:569–73

Giannakopoulos NV, Luo JK, Papov V, Zou W, Lenschow DJ, et al. 2005. Proteomic identification of proteins conjugated to ISG15 in mouse and human cells. *Biochem. Biophys. Res. Commun.* 336:496–506

Gocke CB, Yu H, Kang J. 2005. Systematic identification and analysis of mammalian small ubiquitin-like modifier substrates. *J. Biol. Chem.* 280:5004–12

Goehring AS, Rivers DM, Sprague GFJ. 2003a. Attachment of the ubiquitin-related protein Urm1p to the antioxidant protein Ahp1p. *Eukaryot. Cell* 2:930–36

Goehring AS, Rivers DM, Sprague GFJ. 2003b. Urmylation: a ubiquitin-like pathway that functions during invasive growth and budding in yeast. *Mol. Biol. Cell* 14:4329–41

Haas AL, Warms JV, Hershko A, Rose IA. 1982. Ubiquitin-activating enzyme. Mechanism and role in protein-ubiquitin conjugation. *J. Biol. Chem.* 257:2543–48

Haglund K, Di Fiore PP, Dikic I. 2003. Distinct monoubiquitin signals in receptor endocytosis. *Trends Biochem. Sci.* 28:598–603

Haglund K, Dikic I. 2005. Ubiquitylation and cell signaling. *EMBO J.* 24:3353–59

Hannich JT, Lewis A, Kroetz MB, Li SJ, Heide H, et al. 2005. Defining the SUMO-modified proteome by multiple approaches in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 280:4102–10

Hershko A, Heller H, Elias S, Ciechanover A. 1983. Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *J. Biol. Chem.* 258:8206–14

Hicke L, Schubert HL, Hill CP. 2005. Ubiquitin-binding domains. *Nat. Rev. Mol. Cell Biol.* 6:610–21

Hietakangas V, Anckar J, Blomster HA, Fujimoto M, Palvimo JJ, et al. 2006. PDSM, a motif for phosphorylation-dependent SUMO modification. *Proc. Natl. Acad. Sci. USA* 103:45–50

The authors of this study identify a phosphorylation-dependant sumoylation motif.

Hipp MS, Kalveram B, Raasi S, Groettrup M, Schmidtke G. 2005. FAT10, a ubiquitin-independent signal for proteasomal degradation. *Mol. Cell Biol.* 25:3483–91

Hipp MS, Raasi S, Groettrup M, Schmidtke G. 2004. NEDD8 ultimate buster-1L interacts with the ubiquitin-like protein FAT10 and accelerates its degradation. *J. Biol. Chem.* 279:16503–10

Ho JC, Warr NJ, Shimizu H, Watts FZ. 2001. SUMO modification of Rad22, the *Schizosaccharomyces pombe* homologue of the recombination protein Rad52. *Nucleic Acids Res.* 29:4179–86

Hochstrasser M. 2000. Evolution and function of ubiquitin-like protein-conjugation systems. *Nat. Cell Biol.* 2:E153–57

Hochstrasser M. 2006a. Biochemical functions of ubiquitin and ubiquitin-like protein conjugation. In *Protein Degradation (Vol. 2): The Ubiquitin-Proteasome System*, ed. RJ Mayer, A Ciechanover, M Rechsteiner, pp. 249–78. Weinheim: Wiley-VCH Verlag

Hochstrasser M. 2006b. Lingering mysteries of ubiquitin-chain assembly. *Cell* 124:27–34

Hofmann K, Bucher P. 1996. The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. *Trends Biochem. Sci.* 21:172–73

A bioinformatic analysis that identified the UBA domain.

Hofmann K, Falquet L. 2001. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. *Trends Biochem. Sci.* 26:347–50

Huang DT, Paydar A, Zhuang M, Waddell MB, Holton JM, Schulman BA. 2005. Structural basis for recruitment of Ubc12 by an E2 binding domain in NEDD8's E1. *Mol. Cell* 17:341–50

Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, et al. 2000. A ubiquitin-like system mediates protein lipidation. *Nature* 408:488–92

Johnson ES. 2004. Protein modification by SUMO. *Annu. Rev. Biochem.* 73:355–82

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Johnson ES, Gupta AA. 2001. An E3-like factor that promotes SUMO conjugation to the yeast septins. *Cell* 106:735–44

Kamitani T, Kito K, Fukuda-Kamitani T, Yeh ET. 2001. Targeting of NEDD8 and its conjugates for proteasomal degradation by NUB1. *J. Biol. Chem.* 276:46655–60

Kawakami T, Chiba T, Suzuki T, Iwai K, Yamanaka K, et al. 2001. NEDD8 recruits E2-ubiquitin to SCF E3 ligase. *EMBO J.* 20:4003–12

Kepinski S, Leyser O. 2005. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* 435:446–51

Kim J, Dalton VM, Eggerton KP, Scott SV, Klionsky DJ. 1999. Apg7p/Cvt2p is required for the cytoplasm-to-vacuole targeting, macroautophagy, and peroxisome degradation pathways. *Mol. Biol. Cell* 10:1337–51

Kirkpatrick DS, Denison C, Gygi SP. 2005. Weighing in on ubiquitin: the expanding role of mass-spectrometry-based proteomics. *Nat. Cell Biol.* 7:750–57

Komatsu M, Chiba T, Tatsumi K, Iemura S, Tanida I, et al. 2004. A novel protein-conjugating system for Ufm1, a ubiquitin-fold modifier. *EMBO J.* 23:1977–86

Kovalenko OV, Plug AW, Haaf T, Gonda DK, Ashley T, et al. 1996. Mammalian ubiquitin-conjugating enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complexes. *Proc. Natl. Acad. Sci. USA* 93:2958–63

Kramer A, Mulhauser F, Wersig C, Groning K, Bilbe G. 1995. Mammalian splicing factor SF3a120 represents a new member of the SURP family of proteins and is homologous to the essential splicing factor PRP21p of *Saccharomyces cerevisiae*. *RNA* 1:260–72

Landles C, Bates GP. 2004. Huntingtin and the molecular pathogenesis of Huntington's disease. *EMBO Rep.* 5:958–63

Li Y, Wang H, Wang S, Quon D, Liu YW, Cordell B. 2003. Positive and negative regulation of APP amyloidogenesis by sumoylation. *Proc. Natl. Acad. Sci. USA* 100:259–64

Lotz K, Pyrowolakis G, Jentsch S. 2004. BRUCE, a giant E2/E3 ubiquitin ligase and inhibitor of apoptosis protein of the trans-Golgi network, is required for normal placenta development and mouse survival. *Mol. Cell Biol.* 24:9339–50

Manza LL, Codreanu SG, Stamer SL, Smith DL, Wells KS, et al. 2004. Global shifts in protein sumoylation in response to electrophile and oxidative stress. *Chem. Res. Toxicol.* 17:1706–15

Minty A, Dumont X, Kaghad M, Caput D. 2000. Covalent modification of p73α by SUMO-1. Two-hybrid screening with p73 identifies novel SUMO-1-interacting proteins and a SUMO-1 interaction motif. *J. Biol. Chem.* 275:36316–23

Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, et al. 2003. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J. Cell Sci.* 116:1679–88

Mizushima N, Noda T, Ohsumi Y. 1999. Apg16p is required for the function of the Apg12p-Apg5p conjugate in the yeast autophagy pathway. *EMBO J.* 18:3888–96

Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, et al. 1998. A protein conjugation system essential for autophagy. *Nature* 395:395–98

Nakamura M, Tanigawa Y. 2003. Characterization of ubiquitin-like polypeptide acceptor protein, a novel proapoptotic member of the Bcl2 family. *Eur. J. Biochem.* 270:4052–58

Neve RL. 2003. A new wrestler in the battle between α- and β-secretases for cleavage of APP. *Trends Neurosci.* 26:461–63

Ogunjimi AA, Briant DJ, Pece-Barbara N, Le Roy C, Di Guglielmo GM, et al. 2005. Regulation of Smurf2 ubiquitin ligase activity by anchoring the E2 to the HECT domain. *Mol. Cell* 19:297–308

Ohsumi Y. 2001. Molecular dissection of autophagy: two ubiquitin-like systems. *Nat. Rev. Mol. Cell Biol.* 2:211–16

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Ozkan E, Yu H, Deisenhofer J. 2005. Mechanistic insight into the allosteric activation of a ubiquitin-conjugating enzyme by RING-type ubiquitin ligases. *Proc. Natl. Acad. Sci. USA* 102:18890–95

Palacios S, Perez LH, Welsch S, Schleich S, Chmielarska K, et al. 2005. Quantitative SUMO-1 modification of a vaccinia virus protein is required for its specific localization and prevents its self-association. *Mol. Biol. Cell* 16:2822–35

Pichler A, Knipscheer P, Oberhofer E, van Dijk WJ, Korner R, et al. 2005. SUMO modification of the ubiquitin-conjugating enzyme E2-25K. *Nat. Struct. Mol. Biol.* 12:264–69

Pickart CM, Rose IA. 1985. Functional heterogeneity of ubiquitin carrier proteins. *J. Biol. Chem.* 260:1573–81

Raasi S, Varadan R, Fushman D, Pickart CM. 2005. Diverse polyubiquitin interaction properties of ubiquitin-associated domains. *Nat. Struct. Mol. Biol.* 12:708–14

Rajan S, Plant LD, Rabin ML, Butler MH, Goldstein SA. 2005. Sumoylation silences the plasma membrane leak K^+ channel K2P1. *Cell* 121:37–47

Reverter D, Lima CD. 2005. Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. *Nature* 435:687–92

Ross CA, Pickart CM. 2004. The ubiquitin-proteasome pathway in Parkinson's disease and other neurodegenerative diseases. *Trends Cell Biol.* 14:703–11

Saeki Y, Tayama Y, Toh-e A, Yokosawa H. 2004. Definitive evidence for Ufd2-catalyzed elongation of the ubiquitin chain through Lys48 linkage. *Biochem. Biophys. Res. Commun.* 320:840–45

Seeler JS, Dejean A. 2001. SUMO: of branched proteins and nuclear bodies. *Oncogene* 20:7243–49

Shin JA, Choi ES, Kim HS, Ho JC, Watts FZ, et al. 2005. SUMO modification is involved in the maintenance of heterochromatin stability in fission yeast. *Mol. Cell* 19:817–28

Siepmann TJ, Bohnsack RN, Tokgoz Z, Baboshina OV, Haas AL. 2003. Protein interactions within the N-end rule ubiquitin ligation pathway. *J. Biol. Chem.* 278:9448–57

Song J, Durrin LK, Wilkinson TA, Krontiris TG, Chen Y. 2004. Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. *Proc. Natl. Acad. Sci. USA* 101:14373–78

Song J, Zhang Z, Hu W, Chen Y. 2005. Small ubiquitin-like modifier (SUMO) recognition of a SUMO binding motif: a reversal of the bound orientation. *J. Biol. Chem.* 280:40122–29

Steffan JS, Agrawal N, Pallos J, Rockabrand E, Trotman LC, et al. 2004. SUMO modification of Huntingtin and Huntington's disease pathology. *Science* 304:100–4

Swanson R, Locher M, Hochstrasser M. 2001. A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matα2 repressor degradation. *Genes Dev.* 15:2660–74

Takahashi Y, Kikuchi Y. 2005. Yeast PIAS-type Ull1/Siz1 is composed of SUMO ligase and regulatory domains. *J. Biol. Chem.* 280:35822–28

Tanji K, Tanaka T, Kamitani T. 2005. Interaction of NUB1 with the proteasome subunit S5a. *Biochem. Biophys. Res. Commun.* 337:116–20

Tatham MH, Jaffray E, Vaughan OA, Desterro JM, Botting CH, et al. 2001. Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J. Biol. Chem.* 276:35368–74

Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD. 2005. Autophagic nutrient recycling in *Arabidopsis* directed by the ATG8 and ATG12 conjugation pathways. *Plant Physiol.* 138:2097–110

A study that indicates a role for RING E3s in allosterically activating E2 rather than just functioning as an adaptor between substrate and E2.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. 2000. Recognition of the polyubiquitin proteolytic signal. *EMBO J.* 19:94–102

Ulrich HD. 2005. Mutual interactions between the SUMO and ubiquitin systems: a plea of no contest. *Trends Cell Biol.* 15:525–32

Verdecia MA, Joazeiro CA, Wells NJ, Ferrer JL, Bowman ME, et al. 2003. Conformational flexibility underlies ubiquitin ligation mediated by the WWP1 HECT domain E3 ligase. *Mol. Cell* 11:249–59

Vertegaal AC, Ogg SC, Jaffray E, Rodriguez MS, Hay RT, et al. 2004. A proteomic study of SUMO-2 target proteins. *J. Biol. Chem.* 279:33791–98

Welcker M, Orian A, Grim JA, Eisenman RN, Clurman BE. 2004. A nucleolar isoform of the Fbw7 ubiquitin ligase regulates c-Myc and cell size. *Curr. Biol.* 14:1852–57

Wilkinson CR, Dittmar GA, Ohi MD, Uetz P, Jones N, Finley D. 2004. Ubiquitin-like protein Hub1 is required for pre-mRNA splicing and localization of an essential splicing factor in fission yeast. *Curr. Biol.* 14:2283–88

Woodward AW, Bartel B. 2005. Auxin: regulation, action, and interaction. *Ann. Bot. (London)* 95:707–35

Wu JT, Lin HC, Hu YC, Chien CT. 2005. Neddylation and deneddylation regulate Cul1 and Cul3 protein accumulation. *Nat. Cell Biol.* 7:1014–20

Xirodimas DP, Saville MK, Bourdon JC, Hay RT, Lane DP. 2004. Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. *Cell* 118:83–97

Yamamoto A, Iwata A, Koh Y, Kawai S, Murayama S, et al. 1998. Two types of chicken 2′,5′-oligoadenylate synthetase mRNA derived from alleles at a single locus. *Biochim. Biophys. Acta* 1395:181–91

Yang SH, Sharrocks AD. 2005. PIASx acts as an Elk-1 coactivator by facilitating derepression. *EMBO J.* 24:2161–71

Young P, Deveraux Q, Beal RE, Pickart CM, Rechsteiner M. 1998. Characterization of two polyubiquitin binding sites in the 26 S protease subunit 5a. *J. Biol. Chem.* 273:5461–67

Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, et al. 2004. The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-α/β-induced ubiquitin-like protein. *Proc. Natl. Acad. Sci. USA* 101:7578–82

Zhao C, Denison C, Huibregtse JM, Gygi S, Krug RM. 2005. Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. *Proc. Natl. Acad. Sci. USA* 102:10200–5

Zhong S, Muller S, Ronchetti S, Freemont PS, Dejean A, Pandolfi PP. 2000. Role of SUMO-1-modified PML in nuclear body formation. *Blood* 95:748–52

Zou W, Zhang DE. 2005. Interferon-inducible ubiqitin E3 ligase EFP also functions as an ISG15 E3 ligase. *J. Biol. Chem.* 281:3989–94

This paper shows that different isoforms of an SCF ubiquitin ligase subunit, Fbw7, reside in different cellular compartments and that this is linked to the substrate specificity of the corresponding SCF ligases.

This work shows that Mdm2, a p53-ubiquitin ligase, also neddylates the p53 transcription factor. Hence, a single ligase can catalyze attachment of different Ubls to the same protein.

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

Annual Review of Cell and Developmental Biology

Volume 22, 2006

Contents

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.

From Nuclear Transfer to Nuclear Reprogramming: The Reversal of Cell Differentiation
J.B. Gurdon 1

How Does Voltage Open an Ion Channel?
Francesco Tombola, Medha M. Pathak, and Ehud Y. Isacoff 23

Cellulose Synthesis in Higher Plants
Chris Somerville 53

Mitochondrial Fusion and Fission in Mammals
David C. Chan 79

Agrobacterium tumefaciens and Plant Cell Interactions and Activities Required for Interkingdom Macromolecular Transfer
Colleen A. McCullen and Andrew N. Binns 101

Cholesterol Sensing, Trafficking, and Esterification
Ta-Yuan Chang, Catherine C.Y. Chang, Nobutaka Ohgami, and Yoshio Yamauchi 129

Modification of Proteins by Ubiquitin and Ubiquitin-Like Proteins
Oliver Kerscher, Rachael Felberbaum, and Mark Hochstrasser 159

Endocytosis, Endosome Trafficking, and the Regulation of *Drosophila* Development
Janice A. Fischer, Suk Ho Eun, and Benjamin T. Doolan 181

Tight Junctions and Cell Polarity
Kunyoo Shin, Vanessa C. Fogg, and Ben Margolis 207

In Vivo Migration: A Germ Cell Perspective
Prabhat S. Kunwar, Daria E. Siekhaus, and Ruth Lehmann 237

Neural Crest Stem and Progenitor Cells
Jennifer F. Crane and Paul A. Trainor 267

Of Extracellular Matrix, Scaffolds, and Signaling: Tissue Architecture Regulates Development, Homeostasis, and Cancer
Celeste M. Nelson and Mina J. Bissell 287

Intrinsic Regulators of Pancreatic β-Cell Proliferation
Jeremy J. Heit, Satyajit K. Karnik, and Seung K. Kim 311

Epidermal Stem Cells of the Skin
Cédric Blanpain and Elaine Fuchs 339

The Molecular Diversity of Glycosaminoglycans Shapes Animal Development
Hannes E. Bülow and Oliver Hobert 375

Recognition and Signaling by Toll-Like Receptors
A. Phillip West, Anna Alicia Koblansky, and Sankar Ghosh 409

The Formation of TGN-to-Plasma-Membrane Transport Carriers
Frédéric Bard and Vivek Malhotra 439

Iron-Sulfur Protein Biogenesis in Eukaryotes: Components and Mechanisms
Roland Lill and Ulrich Mühlenhoff 457

Intracellular Signaling by the Unfolded Protein Response
Sebastián Bernales, Feroz R. Papa, and Peter Walter 487

The Cellular Basis of Kidney Development
Gregory R. Dressler 509

Telomeres: Cancer to Human Aging
Sheila A. Stewart and Robert A. Weinberg 531

The Interferon-Inducible GTPases
Sascha Martens and Jonathan Howard 559

What Mouse Mutants Teach Us About Extracellular Matrix Function
A. Aszódi, Kyle R. Legate, I. Nakchbandi, and R. Fässler 591

Caspase-Dependent Cell Death in *Drosophila*
Bruce A. Hay and Ming Guo 623

Regulation of Commissural Axon Pathfinding by Slit and its Robo Receptors
Barry J. Dickson and Giorgio F. Gilestro 651

Blood Cells and Blood Cell Development in the Animal Kingdom
Volker Hartenstein 677

Axonal Wiring in the Mouse Olfactory System
Peter Mombaerts 713

Annu. Rev. Cell Dev. Biol. 2006.22:159-180. Downloaded from arjournals.annualreviews.org
by Yale University SOCIAL SCIENCE LIBRARY on 03/05/07. For personal use only.