The enzymes in ubiquitin-like post-translational modifications

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SUMMARY **Ubiquitin and at least ten ubiquitin-like proteins are important post-translational modifiers that regulate nearly every aspect of cellular function. These modifications require several chemical reactions that are catalyzed by at least three enzymes. Significant progress has been made in the structure-function analysis of these enzymes. This review describes new advancements in an understanding of the mechanisms of the enzymes catalyzing ubiquitin-like modifications, and highlights the important problems that remain to be addressed.**

Key Words: SUMO, ubiquitin, enzymes, X-ray crystallography, NMR spectroscopy

Introduction

The discovery that ubiquitin can conjugate to target proteins to regulate their cellular life spans and functions has revolutionized our understanding of eukaryotic regulation (*1*,*2*). Ubiquitin belongs to a family of at least ten homologous protein modifiers that conjugate to cellular target proteins using a similar biochemical mechanism (*3*,*4*). Post-translational modifications with these ubiquitin-like modifiers regulate nearly every aspect of cellular functions including immune response, viral and bacterial infection, gene transcription, RNA processing, DNA-repair, cell cycle progression, and intracellular trafficking.

Ubiquitin-like modifications are different from other post-translational modifications in that they require multiple enzymes to catalyze several sequential reactions (*5*). The chemical reactions leading to ubiquitination are adenylation, thioester formation, transesterification and isopeptide bond formation. Catalysis is strictly necessary for the adenylation step; the other reactions can occur in the absence of enzymes, but at much slower rates. For example, an intein-based method for protein ligation *in vitro*, which proceeds via similar chemistry as ubiquitination, can take as long as overnight to complete at room temperature (*6*). Other intracellular processes, such as protein lipidation and non-ribosomal peptide synthesis also use similar, catalyzed chemical mechanisms (*7*,*8*). The enzymes in all of these processes dramatically accelerate the reactions by mechanisms that are not yet well understood.

The ubiquitin-like modifications universally require at least four types of enzymes referred to generally as isopeptidase, E1 (activation enzyme), E2 (conjugation enzyme) and E3 (ligase) (Figure 1). A ubiquitin-like protein (Ublp) is usually synthesized as a precursor, which is matured by an **isopeptidase** to remove C-terminal residues and expose the Gly-Gly motif. Conjugation of a Ublp to target proteins then begins with **E1**, which catalyzes the adenylation of Ublp's C-terminal COOH group. The adenylated Ublp binds E1 non-covalently, and a thioester bond is formed between the SH group of a Cys residue on E1 and the C-terminal -COOH group of Ublp. Ublp is then transferred to a conjugation enzyme **E2**, where it forms a thioester bond with the -SH group of the catalytic Cys residue of the E2. In the final step, Ublp is attached to target proteins by the formation of an isopeptide bond between its C-terminal -COOH group and the ε-amino group of a Lys residue on the target protein. This step generally requires **E3 ligase**, although additional protein factors referred to as **E4** may also be involved in poly-ubiquitination of some proteins (*9*). The E1, E2 and E3 enzymes are commonly involved in nearly all ubiquitin-like modifications. Isopeptidases also remove ubiquitin-like proteins from modified targets, and thus regulate the levels of these modifications.

The ubiquitin-like post-translational modifications are dependent on protein-protein interactions between the enzymes, protein substrates, and Ublp to accomplish each step of the reactions leading to the modifications. The protein associations in these processes are dynamic, and a stable complex of all components does not exist (*10*). Characterizing the molecular mechanism

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of these multi-enzymatic processes is important to our understanding of how multi-protein machineries carry out macromolecular chemistry. This review will focus on the recent structural and enzymological studies of the enzymes in ubiquitin-like post-translational modifications.

The E1 enzyme and the transfer of Ublp from E1 to E2

A single and unique E1 enzyme is responsible for activating each ubiquitin-like modification (*11*). Both the ubiquitin and SUMO E1 enzymes are essential genes in yeast (*12,13*). The three-dimensional structures of the NEDD8 and SUMO E1 enzymes as well as several of their complexes have been solved (*14,15*). The structure of a small domain of ubiquitin E1 has also been solved (*16*). These E1 enzymes contain regions that resemble the bacterial proteins ThioS and MoeB (*17-20*). Both the SUMO and NEDD8 E1 enzymes are tight heterodimers of two polypeptides, which are homologous to the N-terminal and C-terminal portions of the ubiquitin E1, respectively. The overall structure of E1 contains three domains (Figure 2A). One domain contains the ATP-binding site and catalyzes the adenylation of Ublp. Another domain contains the catalytic Cys residue which forms a covalent thioester bond with the C-terminus of a Ublp. The third domain has a three-dimensional fold similar to that of ubiquitin in the absence of any sequence similarity, and is known as the Ubl domain.

Recently structural studies using both X-ray crystallography and NMR spectroscopy have greatly advanced our understanding of the mechanism of Ublp's transfer from E1 to E2. The Ubl domain, Cys domain and adenylation domain of E1 all participate in recruiting E2 for the transfer of Ublp from E1 to E2 (*21,22*) (Figure 2C). The Ubl domain has the highest affinity for E2 among the three E1 domains (*23*). It also has the flexibility to undergo a large scale rotation to properly position E2 (Figures 2B and 2C) (*22*). In addition, our NMR study has shown that the SUMO E2 has an intrinsic affinity for the Cys domain of its cognate E1 (Figure 2D) (*21*). The affinity between the E2 and the Cys domain of E1 is not high, but is important for the guided translocation of E2 to the catalytic Cys residue of E1 and for properly positioning the E2 for the transfer of SUMO from E1 to E2. The affinity between E2 and the adenylation domain is also expected to be weak, based on the small contact interface. The multiple low affinity binding sites on E1 for E2 provide an effective high affinity between the two enzymes to ensure efficient catalysis at low protein concentrations. At the same time, the low affinity of each site allows rapid protein association and dissociation for efficient catalysis.

The mechanism of how an Ublp translocates from the adenylation active site to catalytic Cys on E1 remains unclear. The structure of NEDD8 E1 in complex with NEDD8 and ATP, and the structure of SUMO E1 in complex with SUMO-1 and ATP have shown similar features of how the adenylated Ublps bind to their cognate E1 (*14,15*). The C-termini of both NEDD8 and SUMO are buried deeply within their cognate E1 enzymes near the adenylation active site. However, this site is distal (approximately 30 Å away) from the Cys residues with which it forms the thioester bonds in the subsequent step of the conjugation pathway (Figure 2E). The structures raise the question of how Ublp transfers with high efficiency and specificity between the two catalytic active sites of E1.

The E2 enzyme and its recognition of substrates and Ublp

Multiple E2 enzymes have been identified for ubiquitination of different target proteins (*1*), but only one specific E2 appears to be required by each of the ubiquitin-like proteins NEDD8 (*24*) and ISG15 (*25*). Additionally, a single E2 called Ubc9 serves all SUMO paralogues (*26*). The E2 enzymes are of variable sizes, but they all contain a core catalytic domain of approximately 150 amino acid residues (Figure 3). Some E2s consist of just the core catalytic domain, whereas others contain N- and/or C-terminal extensions of variable lengths beyond their core catalytic domains (*27*). There are also E2-like proteins involved in ubiquitin-dependent processes that lack the catalytic Cys residues and are catalytically inactive, such as the Tsg101 UEV domain and Mms2 (*28,29*).

The core catalytic domains of E2 enzymes have a highly conserved three-dimensional structure, with the biggest differences manifested in two surface loops of variable lengths (*30-33*). One of the variable loop insertions in E2 is located adjacent to the catalytic Cys residue (Figure 3). Mutations in this loop of Ubc9 affected the transfer of SUMO from E1 to E2 (*34*). Enzyme kinetic analysis indicates that this loop is also important for substrate recognition by the E2. A conserved Asn residue near the active site Cys has been identified as the catalytic residue that stabilizes the transition state oxyanion during the transfer of Ublp from E2 to target proteins (*35*).

Characterization of the SUMO modification pathway has provided a clear indication of direct substrate-E2 interaction. A consensus sequence, ψKxE (where ψ represents a bulky hydrophobic residue, K is a Lys, x is any amino acid, and E is a Glu), occurs in most SUMO-1 substrate proteins (*36*), although modifications at non-consensus sequences may occur much less frequently (*37*). This consensus amino acid sequence in substrates is a Ubc9 binding motif, and binds specifically to a region of Ubc9 near the active site Cys93 (Figure 3) (*38,39*). The substrate-binding surface

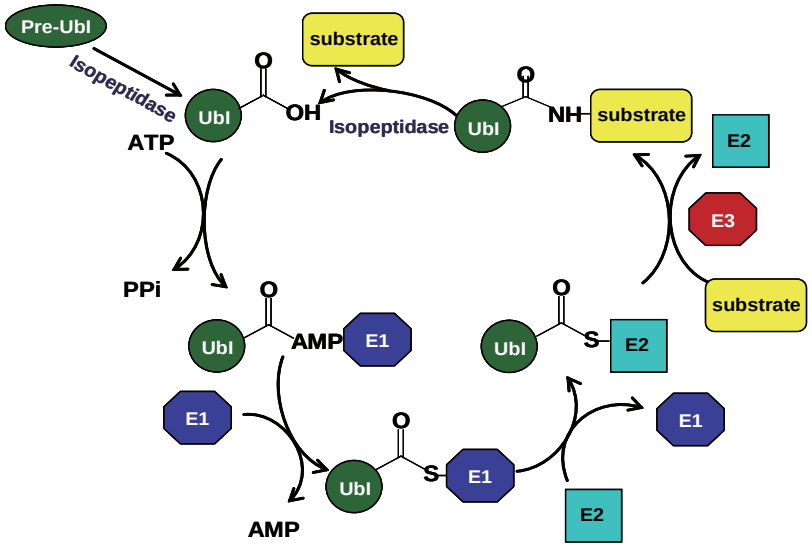

Figure 1. Schematic diagram of the chemical reactions involved in ubiquitin-like modifications.

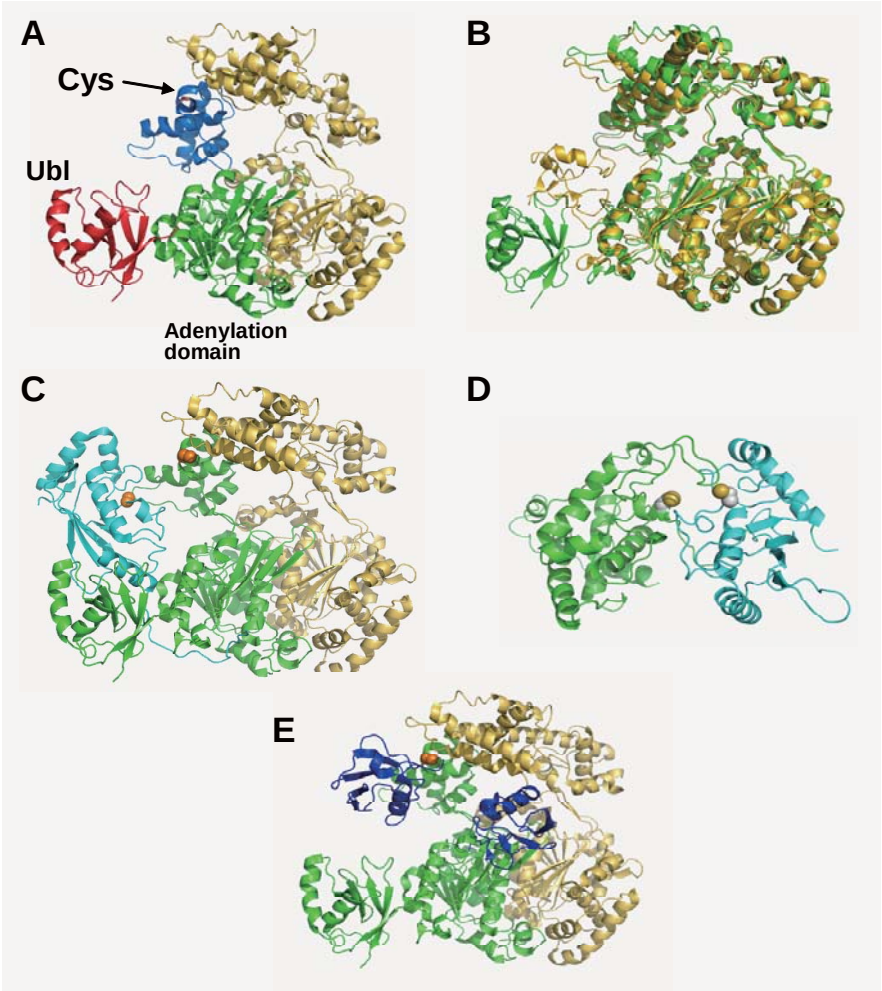

Figure 2. Summary of structural mechanism of E1. (A) The structure of NEDD8 E1 with different domains color as red, blue and green corresponding to the Ubl, Cys, and adenylation domains, respectively. The catalytic Cys is indicated in orange on the Cys domain. The APPBP1 subunit of the E1, which does not contain any catalytic active site, is indicated in gold. (B) Superimposed E1 structures from two different complexes to demonstrate the flexibility of the Ubl domain. (C) The complex of E2 and E1. E2 is colored in light blue. The two subunits of the NEDD8 E1 are colored with green and gold, respectively. The catalytic Cys residues of both E1 and E2 are shown with their sidechains in orange. (D) The interaction of the SUMO E2 (Ubc9, in blue) with the Cys domain of the SUMO E1 (green). The catalytic Cys residues of both enzymes are shown with their sidechains. (E) The structure of NEDD8 E1 in complex with two NEDD8, one non-covalently bond to the adenylation active site with the C-terminus deeply buried inside, and another NEDD8 covalently bond to the catalytic Cys. The two catalytic sites on E1 are distal from each other.

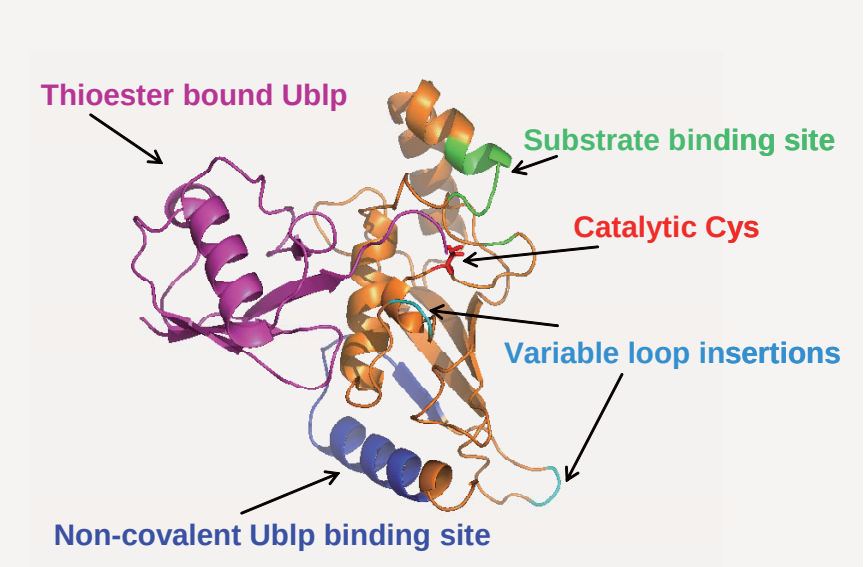

Figure 3. Summary of current knowledge of key functional sites of E2. The structure of Ubc9, shown in orange, is a representative of the conserved catalytic core structure of E2. The different functional sites are indicated in the figure. The thioester bound Ublp is shown in a position analogous to that in an ubiquitin-E2 thioester.

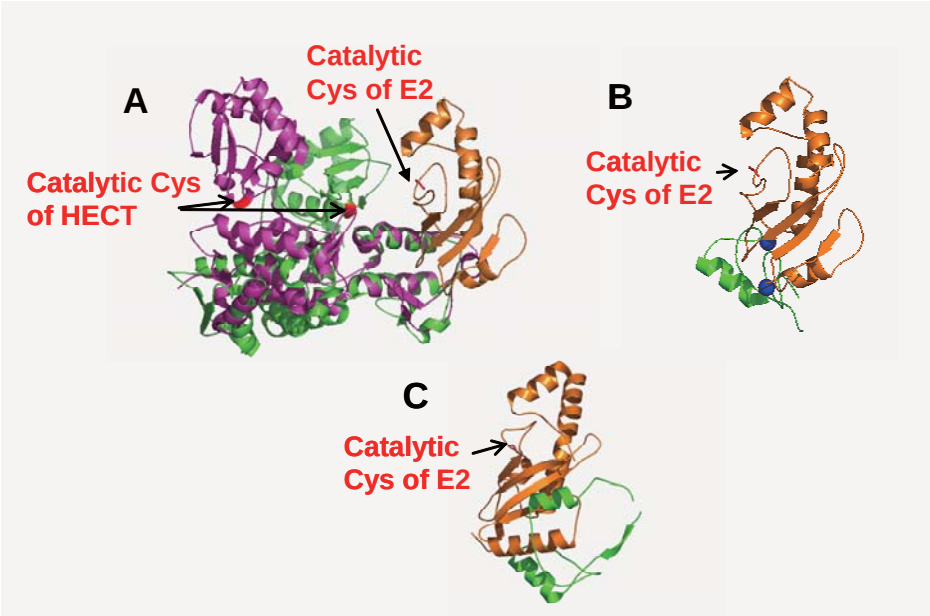

Figure 4. E3 structures and regulation. (A) The interaction between E2 and HECT motif. E2 is shown in orange and one HECT domain is shown in green. Another HECT domain is also shown in magenta in order to illustrate the conformational flexibility of HECT domains. The catalytic Cys residues of HECT domains are indicated in red. (B) The interaction between E2 and the RING motif. E2 is shown in orange and the RING domain is shown in green. (C) A complex between RanBP2 (green) and Ubc9 (orange).

has been previously shown to include residues that demonstrate significant dynamics on the microsecond to millisecond time scale (*40*). The conformational flexibility in E2 appears to be conserved, because the region of Ubc7 that is equivalent to Ubc9's substrate binding site also has one of the largest structural variations between crystal structures of the same protein determined from different complexes (*41,42*). The Lys residue where the modification occurs on substrates is found in a groove formed by the backbone atoms of residues Asp127, Pro128, Ala129 and the sidechain atoms of residues Asp127 and Tyr87 of Ubc9. In this position, the ε-amino group of the substrate Lys is 2.6 Å away from the Sγ atom of Ubc9's Cys93. Among the four residues that form important contacts with the Lys at the modification site, only the Pro and Ala are relatively conserved in E2 enzymes catalyzing ubiquitination. It remains to be established that a similar substrate binding mechanism is involved in the ubiquitination pathway; however, ubiquitination sites of target proteins do not have a consensus sequence. The direct interaction of substrates and Ubc9 may account for the lack of HECT-domain containing E3s in SUMO modifications.

Ubiquitin E2 enzymes have been shown to form homo and heterodimers. For example, Ubc3 (CDC34)

forms a heterodimer with the DNA repair related Ubc2 (Rad6) (*43*) and also forms a homodimer facilitated by the formation of ubiquitin thioester (*44*). These dimers are required for the degradation of many key regulators of cell cycle progression through the proteosome pathway. For example, a Ubc6 and Ubc7 heterodimer is responsible for the turnover of Matα2 transcription factor in yeast (*45*). Dimerization of E2s may be responsible for the formation of poly-ubiquitin chains in some cases; however, dimerization does not appear to be a general requirement for poly-ubiquitination. For example, Ubc5 does not appear to form homo- or hetero-dimers *in vitro* or *in vivo* (*40*), but Ubc5 can catalyze the formation of poly-ubiquitin chains (*45*).

Ublp-E2 thioester is an important intermediate in ubiquitin-like conjugation. Biochemical studies have shown that different ubiquitin-E2 thioester have different stabilities (*46*). Several structural characterizations of ubiquitin-E2 thioester covalent complexes have been carried out using NMR methods. In one study, the catalytic Cys residue was mutated to a Ser to produce an Ubc2b-ubiquitin oxyester, which has a more stable covalent linkage than thioester, and the oxyester was purified for NMR characterization (*47*). In other studies, productive thioesters were made in the NMR tube by the addition of E1 and ATP (*48,49*). These studies have shown that thioester bound ubiquitin contacts the surface of E2 that is centered on the second α-helix, which is adjacent to the active site Cys (Figure 3). The binding interface on ubiquitin is located on the β-sheet. The first study also demonstrated that the oxyester-bonded ubiquitin has linewidths that are similar to that of the free protein, and this suggests that ubiquitin moves somewhat independently from the covalently bound E2.

A specific and conserved non-covalent interaction between Ublp and E2 has been observed across the ubiquitin-like proteins (*23,47,49-51*). The non-covalent interaction between SUMO-1 and Ubc9 involves interfaces that are structurally conserved in Ublps and E2 enzymes. The binding site on Ubc9 covers the N-terminal helix, following β-strand, and the loop between them (Figure 3), and the binding surface on SUMO-1 is located on the main β-sheet (*50*). This interaction is conserved across SUMO paralogues, and is both enthalpically and entropically driven (*26*). Similar interactions have been observed between an E2 and ubiquitin, between the E2-like protein Mms2 and ubiquitin (*47,49,51*). However, the ubiquitin-like domains of the NEDD8 and SUMO E1 enzymes bind to their E2 in a similar, but clearly distinct manner (*23,52*). The functions of the non-covalent Ublp-E2 interaction are unclear. Mutations in human Ubc9 that disrupt the interaction with SUMO were shown to significantly reduce the transfer rate of SUMO-1 from E1 to E2 without affecting the transfer of SUMO from E2 to substrates (*26*). Similar amino acid substitutions on ubiquitin E2 hindered its catalysis of poly-ubiquitin chains (*53*). Further studies are necessary in order to understand the role of Ublp-E2 interaction.

The E3 and E4 enzymes

E3 ligases have received the most attention among the three enzymes involved in ubiquitin-like modifications, because many E3s are key players in essential cellular processes, and/or are prominent oncogenes and tumor suppressor genes. For example, one of the proteins frequently mutated in breast and ovarian cancers, BRCA1, has E3 ligase activities for both ubiquitination and sumoylation (*54*). The SCF (Skp1-Cullin-F-box protein) complexes and APC (anaphase-promoting complex), which are critical for cell-cycle progression, are ubiquitin E3 ligases (*55-58*). The host ubiquitin-like modification systems can be hijacked for pathogen infections. For example, the human papilloma virus (HPV) encoded E6 protein and E6-AP (E6 associated protein) form a complex that functions as an E3 ligase to reduce the level of the tumor suppressor protein p53 in HPV positive tumors (*59,60*). Knowledge about E3 ligases has enabled the development of research tools. For example, over-expression of specific ubiquitin E3 in cells can achieve specific and sustained knockout of only a subset of a target protein (*61*). Most E3s that catalyze ubiquitin-like modifications contain either the HECT or the RING domain (*62*). Both the HECT and RING domains are E2 recognition motifs (*41,42,63-65*).

HECT containing E3s receive ubiquitin from E2 to form thioester bonds with ubiquitin before transferring it to substrate proteins. The three-dimensional structures of several HECT domains in complex with E2s have been determined by X-ray crystallography (*42,66,67*). The HECT domain contains three subdomains; the N-terminal subdomain contacts E2 directly, the C-terminal subdomain contains the catalytic Cys residue, and the middle subdomain separates the two (Figure 4A). The linkers connecting the different subdomains have considerable flexibility, which allows the subdomains to move quite independently. Such domain movement can change the distance between the catalytic Cys residues on HECT and on E2 from 40 Å apart to close enough that the transesterification reaction can happen efficiently (Figure 4A). There is likely a general base to stabilize the transition state in which the HECT thioester bond is broken and the substrate isopeptide bond is formed. However, such a residue has not been identified in the HECT motif.

The RING-containing E3s constitute the largest family of E3 and can be divided into two sub-families, Zn-binding RING and Zn-independent RING-like fold formed by the U-box motif (*65,68*). RING motifs bind to the same site of E2 as the HECT domain despite the absence of sequence and structural similarity to the HECT domain (Figure 4B). Some U-box containing

proteins have been shown to function as E4s, which recognize mono-ubiquitinated proteins and catalyze the formation of long poly-ubiquitin chains (*9*). RING-containing E3s do not form thioester intermediates with ubiquitin, but bind to both E2 and substrate proteins. Both E3 recognition of substrate proteins and E2 enzymes are critical for catalyzing substrate modifications. For example, E3s in the N-end rule pathways recognize specific N-terminal residues of their target proteins (*2*). Most SUMO E3 enzymes contain RING domains and belong to the Siz/PIAS family of proteins (*69-72*). These SUMO E3s are similar to RING containing ubiquitin E3s in that they use the RING domains for binding E2 and also contain separate domains for binding target proteins.

Biochemical and structural studies have not provided a clear understanding of how RING-containing E3 ligases activate the transfer of Ublp from E2 to substrates. Because substrate and E2 binding activities are both required for the function of most of these ligases, the RING-containing E3s are thought of as adaptors that bring substrate and E2 together. Structural studies of a SCF complex indicate that the substrate and E2 binding sites on this E3 are surprisingly distal (as far apart as 50 Å) (*41,42,63,73-76*). Such a wide distance between the substrate and E2 was contrary to the efficiency of the E3 (*77*). In one study, a flexible linker that was engineered in the Cullin protein of a SCF complex destroyed the E3's enzymatic activity, indicating that the rigidity of SCF complexes is important for their ligase activity (*63*). This is clearly different from the catalytic mechanism employed by HECT domains. Additionally, there are increasing biochemical data contradicting the theory that these E3s are merely adaptors. For example, it has been shown that a small subunit in APC containing only the RING domain, but not the substrate binding domain, could enhance APC specific ubiquitination (*78,79*). Another study also showed that simply bringing an E2 and a substrate close together by fusing a substrate protein to the C-terminus of an E2 was not sufficient to bypass E3 to activate ubiquitination of the substrate protein (*80*). It has also been shown that E3 interacts with E2 differently from interacting with E2-ubiquitin thioester (*81,82*). However, the structural details of the interaction between E2-ubiquitin thioester and an E3 have not been characterized.

Another mystery in the E3 mechanism is the way in which poly-ubiquitin chains are formed. Poly-ubiquitin chains can form on E2 first and are then transferred to substrates, or ubiquitin is added one by one from an E2 to substrate proteins (*83*). Thus, the E3 catalyzed reactions have substrate "flexibility", which is either the target protein or the ubiquitin moiety of the ubiquitinated target protein. On the other hand, the reaction has clear "specificity"; in most cases, the poly-ubiquitin chains are formed on a very specific Lys residue (*e.g.* Lys48 or Lys63) on ubiquitin, instead of randomly on any Lys residue. The substrate flexibility and specificity in ubiquitination is distinct from most well characterized enzymatic reactions. In some cases, an E3 ligase only catalyzes mono-ubiquitination, and then an E4 takes over to add poly-ubiquitin chains to target proteins (*9*). However, in most *in vitro* reactions, poly-ubiquitination readily occurs with the addition of E3 and in the absence of E4.

Compared to the E1 and E2 enzymes, the E3 enzymes are much less conserved. For example, a SUMO E3 RanBP2 (also known as Nup358) (*84*) do not contain HECT, RING or U-box domains (*84*). Unlike other characterized E3s, RanBP2 does not form detectable non-covalent complexes with target proteins, nor does it form thioester bonds with SUMO-1 (*84*). The three-dimensional structure of the region of RanBP2 that has the E3 ligase function in complex with Ubc9 and sumoylated RanGAP1 has been solved by X-ray crystallography (*85*), which shows that the interaction between Ubc9 and RanBP2 is distinct from previously characterized E2-E3 interactions (Figure 4C). In this case, the E2-E3 interaction involves a surface of E2 that is different from that involved in binding the HECT or RING containing E3s (*86*). In general, it is not clear how the structurally distinct RING-containing E3s and RanBP2 achieve similar functions in the absence of sequence and structural conservation.

An interesting finding from the RanBP2-related studies is that the interaction between SUMO and E3 is critical for the E3's ligase activity (*85*). The M-IR2 domain of RanBP2, which binds specifically to SUMO-1 and not to SUMO-2, can catalyze SUMO-1 but not SUMO-2 modifications (*86*). This is the first E3 ligase in which the interaction between an E3 ligase and the ubiquitin-like protein has been shown to be important for the ligase activity, although it has been known for more than 20 years that some ubiquitin E3 ligases bind ubiquitin non-covalently (*10*).

Enzyme kinetic analysis of the conjugation process

Quantitative enzyme kinetic analysis is necessary to provide insights into reaction mechanisms that would be difficult to obtain otherwise. Quantitative enzyme kinetic approaches have been developed to examine every step of the reactions that lead to ubiquitin-like modifications. An elegant quantitative framework has been developed to characterize each step of E1 catalyzed reactions (*87*). Steady-state methods can be used to obtain enzyme kinetic information for the E2 and E3 enzymes (*39,82*). These approaches take advantage of the fact that in a reaction requiring multiple enzymes, kinetic information on a particular enzyme can be extracted when this enzyme limits the overall reaction rate. For example, in sumoylation reactions, the conditions can be set so that E2 limits the rate of the

overall reactions. Therefore, the kinetic parameters obtained reflect the properties of E2 (*39*). A non-steady-state kinetic approach (*34*) was also developed based on the transfer experiments pioneered in studies of the ubiquitin modification pathway (*10*). The advantage of the non-steady-state kinetic analysis is that one can extract individual kinetic constants, such as on-rate and off-rate of the enzyme-substrate association, and the catalytic rate constant in order to gain detailed insights into each step of the reaction. Fluorescence labeled protein substrates are extremely useful for evaluating the reaction rates (*88*), particularly in quantitating the heterogeneous products of ubiquitination reactions (*89*).

The deconjugation enzyme

The deconjugation enzymes in the ubiquitin and homologous pathways have two functions: to activate the Ublp and to remove Ublp from target proteins (*90*). As mentioned above, the first step in Ublp activation is the cleavage of the C-terminal residues by deconjugation enzymes to expose the Gly-Gly motif. The deconjugation enzymes are also important in regulating Ublp's modifications by removing these modifications.

The deconjugation enzymes can be classified into different families: the ubiquitin C-terminal hydrolases (UCH) that remove small peptides from the C-terminus of ubiquitin (*91*), ubiquitin-specific processing proteases (UBP) that remove mono- and poly-ubiquitin modifications (*92*), the ubiquitin-like proteases (ULP) that act on SUMO and NEDD8 (*93-96*), the JAMM motif-containing metalloproteases that act on ubiquitin and NEDD8 (*97*), and members of the ovarian tumor (OTU) superfamily (*98*). The different families of deconjugation enzymes do not share significant sequence similarities and overall folds. Except for the JAMM motif-containing metalloprotease family, the other four families of deconjugation enzymes have conserved geometry of the catalytic triad formed by a His, an Asn and a Cys (*99-104*). They belong to the cysteine protease superfamily. Therefore, inhibitors of the cysteine proteases can also inhibit these deconjugation enzymes. The JAMM motif-containing proteases have diverse functions, including deubiquitination and degradation by the 26S proteasome, and deubiquitination of proteins at the endosome (*105*).

The deconjugation enzymes present excellent targets for developing research tools and therapeutics because they are critical for regulating these post-translational modifications, and thus directly interfere with related biological functions. For example, the deubiquitination activity of CYLD, which encodes an UBP and has UBP activity, has been shown to play a role in regulating the activity of NF-κB (*106*). Mutations in CYLD are linked to familial cylindromatosis, which is an autosomal dominant predisposition to tumors of skin appendages. Most cancer associated mutations of CYLD result in truncations or frameshift alterations of the UBP domain. Herpes virus associated ubiquitin-specific protease (HAUSP or USP7) is responsible for deubiquitination of ubiquitinated p53 and promotion of p53 dependent cell growth arrest and apoptosis (*107*). Dysfunction in UCH has been associated with neurodegeneration and cancer (*108*). In the human genome, there are many uncharacterized proteins that resemble the sequences of deconjugation enzymes. Their functions as deconjugation enzymes for ubiquitin or ubiquitin-like modifications remain to be established.

Interplay between different post-translational modifications

Ubiquitin-like modifications are regulated by each other and by other post-translational modifications. For example, the ubiquitin-like protein NEDD8 modifies the cullin family of proteins, which are subunits of the SCF family of ubiquitin E3 ligases (*55*). Sumoylation and ubiquitination sometimes occur at the same Lys residue and antagonize each other (*109*). The oncogene MDM2, which contains a RING motif, has the ligase activity for both NEDD8 and ubiquitin modifications of the tumor suppressor protein p53 (*110*). Phosphorylation and dephosphorylation of target proteins can regulate the interactions of target proteins with E3 ligases (reviewed in (*62*)). Sumoylation of a transcription factor or histone triggers recruitment of histone deacetylases (HDAC) to remove histone acetylation and thus leads to transcriptional repression (*111,112*). Ubiquitination of histone H2B regulates its methylation and leads to gene silencing (*113*).

Concluding remarks

Significant progress has been made in elucidating the molecular mechanism of the enzymes involved in ubiquitin and ubiquitin-like modifications. Ubiquitin-like modifications are similar to other intracellular macromolecular chemical reactions, such as DNA repair, gene transcription, and RNA processing, in that they require multiple proteins to catalyze multiple reactions. The common theme in these processes is that the protein-protein interactions often involve multiple, medium to low affinity binding sites. The multi-valent, medium to low affinity interactions allow for the rapid turn over of the enzymes and the efficient catalysis at low enzyme and substrate concentrations. Similarly, low affinity, multi-valent protein-protein interactions are also common in Ublp-dependent protein-protein interactions. Ubiquitin-like proteins provide additional docking sites for protein-protein interactions. However, the affinities of ubiquitin-binding motifs for ubiquitin or the SUMO-binding motif for SUMO were not high with the K_d in

the 10-100 μM range (*114*). Despite the low affinities, these interactions play critical roles in the presence of other low or medium affinity interactions, and offer a mechanism to turn protein-protein interactions on and off quickly by conjugation and deconjugation of Ublps. Ubiquitin-like modifications are important in nearly every aspect of cellular function. Therefore, understanding the mechanism of the enzymes catalyzing these modifications will lead to the development of strategies to manipulate them for developing research tools and novel therapeutic approaches.

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