Expanding horizons at Big Sky

Symposium on Ubiquitin and Signaling

Peter Kaiser¹* & Edward A. Fon²

¹University of California–Irvine, Irvine, California, USA and ²Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

The Keystone Symposium on Ubiquitin and Signaling took place between 4 and 9 February 2007, in Big Sky, Montana, USA, and was organized by B.A. Schulman, T. Hunter, M.W. Hochstrasser and C.A.P. Joazeiro.

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typically onto the ϵ -amino groups of lysine (Lys) residues. E3 Ub-ligases are frequently required to trigger conjugation and to confer substrate specificity (Fig 1; Herskho & Ciechanover, 1998). Since its discovery in the 1970s, ubiquitination has been equated with proteasome-mediated degradation because it is the main regulator of protein abundance in cells. However, other functions of ubiquitination, including protein transport and signalling, are now also well established. More than 25 years of research on ubiquitination has created a detailed understanding of Ub biology. Guided by this knowledge, progress in understanding Ubl modifications has been rapid. Nevertheless, understanding the function of Ubl modifications is still in its infancy and is limited to only a few of the more than ten Ubls that have been identified (Kerscher *et al*, 2006). What seems to be clear is that most Ubl modifications do not directly induce protein degradation but instead influence signalling functions. This Keystone Symposium on Ubiquitin and Signaling covered many aspects of Ub biology, and the function of SUMO and Nedd8.

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Introduction

Post-translational modifications of proteins control a wide range of biological phenomena. An array of inorganic and organic molecules can be covalently attached to proteins, including other proteins such as ubiquitin (Ub) and Ub-like proteins (Ubls), for example, the small ubiquitin-like modifier (SUMO) and Nedd8. The covalent attachment of Ub or Ubls to substrate proteins involves a series of transfer reactions that begin with the ATP-dependent activation of Ub or Ubls by E1, followed by their transfer to E2 and finally their conjugation to substrate proteins,

Keynote address

A. Goldberg (Boston, MA, USA) delivered the Cecile Pickart Memorial Lecture. He reflected on the tremendous advances that have occurred in the field and mused about the fundamental questions that still require further work. He discussed the relative promiscuity of many E3 ligases, such as muscle-specific RING finger (MuRF) and the chaperone-associated ligase carboxyl terminus of Hsp70-interacting protein (CHIP), which can function together with different E2 conjugating enzymes. However, Goldberg pointed out that little is known about what regulates the choice of E2 in a given cell at a given time (Fig 2). This choice can have significant consequences as different E2s assemble chains of Ub that are linked through different lysine residues, which have distinct functions (Fig 1). He presented *in vitro* studies showing that the E2 UbcH5 assembles chains that contain all possible types of linkages and forks, in which several Ub molecules are linked to adjacent lysines on the preceding Ub. It seems that substrates modified with such forked chains cannot be degraded by the proteasome and therefore cells must have developed mechanisms to prevent their formation. In fact, Goldberg's group recently found that the main cytosolic Ub-binding protein—the ubiquitin-interacting motif

¹Department of Biological Chemistry, School of Medicine, University of California–Irvine, 240D Med Sci I, Irvine, California 92697-1700, USA

²Centre for Neuronal Survival and Department of Neurology & Neurosurgery, Montreal Neurological Institute, McGill University, 3801 University Street, Montreal, Quebec H3A 2B4, Canada

*Corresponding author. Tel: +1 949 824 9442; Fax: +1 949 824 2688;

E-mail: pkaiser@uci.edu

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Fig 1 Ubiquitination. Ubiquitin (Ub) is activated by E1, transferred to E2s, which bind to E3 for conjugation of Ub predominantly onto lysine residues in substrates; E3s confer substrate specificity to the system. Additional rounds of Ub conjugation onto substrate-attached Ubs lead to the formation of Ub chains. Different lysines in Ub can be used for chain formation. Chains linked through lysine in position 48 (Lys 48) in Ub are the predominant proteasome-targeting signals *in vivo*. Lys 63-linked chains function in a proteasome-independent manner. The function of other chain topologies *in vivo* is unclear. Figure adapted with permission of BioMedCentral, from Kaiser & Huang, 2005.

(UIM)-containing protein S5a—can prevent branching and greatly stimulate proteasomal degradation *in vitro*. Such ubiquitin-binding domain (UBD)-containing proteins therefore seem to function as ‘Ub-chain chaperones’, which facilitate the degradation of proteins by 26S proteasomes *in vivo* or prevent their premature disassembly by deubiquitinating enzymes (DUBs). Conversely, Goldberg’s group found that Lys63-linked chains are good substrates for the proteasome *in vitro*, although *in vivo* studies favour a role for this modification in signalling and transport. This prompted Goldberg to hypothesize that cellular proteins protect such chains from degradation, and he cautioned how difficult it will be to extrapolate *in vitro* findings to the *in vivo* setting. He also discussed several new insights and remaining mysteries about the 26S proteasome, especially the long-standing questions about the roles of ATP in unfolding substrates and in opening the gated channel for their entry into the 20S particle. Finally, Goldberg discussed what happens ‘after the proteasome’—an area generally ignored by cell biologists and biochemists. Nearly all proteasome products are digested to amino acids in seconds, although some escape this fate and are trimmed by the newly discovered endoplasmic reticulum aminopeptidase 1 (ERAP1) to the 8–9 residue antigenic peptides that are presented to the immune system on surface major histocompatibility complex (MHC) molecules. He also noted that proteins containing long stretches of glutamine residues (polyQ), such as those responsible for neurodegenerative disorders including Huntington disease, are poorly cleaved by the proteasome. After their release from the proteasome, such polyQ peptides are digested in the cytosol by the puromycin-sensitive aminopeptidase. This finding points towards a potentially interesting therapeutic target in polyQ disease.

Functional genomics, proteomics and technological trends

Advances in genomics and proteomics are beginning to have an impact on Ub biology. With potentially more than 500 Ub-ligases that can pair with one or more of the 33 E2s (Fig 2), these global efforts are required to tackle the complexity of the Ub system. Model systems, and particularly budding yeast, have been crucial to discovering fundamental aspects of Ub biology; however, new technologies are starting to make similar questions tractable in mammalian systems and will undoubtedly lead to important findings. W. Harper (Cambridge, MA, USA) described the application of one of these powerful genomic tools that his laboratory and the group of S. Elledge (Boston, MA, USA) have developed. They used a small hairpin RNA (shRNA) library designed to target 900 genes in the Ub system combined with a visual screen to identify components in the spindle checkpoint pathway. Knockdown of the DUB ubiquitin-specific peptidase (Usp) 44 eliminated spindle checkpoint function by interfering with the regulation of the anaphase-promoting complex (APC). Specifically, Harper suggested that Usp44 deubiquitinates the APC activator Cdc20 to counteract disassembly of the Cdc20–Mad2 complex to prevent premature APC activation. The great potential of systematic knockdown screens to probe the Ub system has become evident from several exciting discoveries during the past few years from R. Bernards’ group (Amsterdam, the Netherlands). They used RNA interference libraries to knock down many of the DUBs in mammalian cells and identified (i) the cylindromatosis tumour-suppressor gene (CYLD) as a DUB that negatively regulates nuclear factor κ B (NF- κ B) signalling, (ii) Usp1 as a DUB for the Fanconi anaemia D2 protein and the replication factor proliferating cell nuclear antigen (PCNA), and (iii) the testis-specific Usp26 (Dub51) as a DUB for the androgen receptor.

Bernards also presented a strategy that used a genome-wide small interfering RNA (siRNA) library to identify genes involved in breast tumour cell resistance to the drug tamoxifen. The screen identified another DUB, Usp9X, which is an X-chromosome-linked orthologue of the *Drosophila* DUB *fat facets*.

The apparent involvement of DUBs in human disease has attracted the interest of pharmaceutical companies. DUBs are appealing targets for small molecule inhibition because they have a catalytic function and some substrate specificity (Nijman *et al*, 2005). By contrast, the potential drug targets in the ubiquitination cascade lack extensive substrate specificity (E1 and E2s) or, in the case of E3s, are difficult to inhibit with small molecules. F. Colland from Hybrigenics (Paris, France) described a high-throughput fluorescence resonance energy transfer (FRET)-based assay for linkage-specific Ub cleavage that was used to select chemical inhibitors for the human DUB USP8. A lead optimization programme identified compounds that selectively inhibit USP8 rather than other cysteine proteases, including other DUBs. B. Nicholson from Progenra (Malvern, PA, USA) presented a sensitive coupled enzymatic assay for the deconjugation of Ub or UbLs. The assay is based on the observation that the fusion of Ub, SUMO, interferon-stimulated gene (ISG15) or Nedd8 to the amino terminus of phospholipase A (PLA) inhibits its activity. Isopeptidase activity relieves PLA inhibition and can be monitored by a fluorescence-based PLA assay that is compatible with high-throughput formats and chemical library screens.

The tremendous advances in mass-spectrometry-based proteomics are beginning to allow system-level approaches to Ub and UbL biology (Kirkpatrick *et al*, 2005). In particular, quantitative mass spectrometry strategies—for example, stable isotope labelling with amino acids in cell culture (SILAC)—can be used to monitor dynamic changes of Ub and UbL modification on a proteome-wide scale. R. Hay (Dundee, UK) showed how effective the SILAC strategy can be to monitor changes in proteome-wide sumoylation profiles. He reported global changes in SUMO modification in response to heat shock. S. Gygi (Boston, MA, USA) presented his group's efforts to simultaneously measure the half-life of thousands of proteins. They analysed cell lysates separated on SDS-polyacrylamide gels from cells that were pulse-chase labelled with heavy-isotope-containing amino acids. The decrease in the heavy version of individual proteins over time was measured by mass spectrometry, which was then used to calculate the half-life of the protein. By using this approach, the stability of up to 3,000 proteins can be analysed in a single experiment. The majority of the detected proteins had relatively long half-lives, probably because of a detection bias towards abundant proteins, which are generally more stable. Mass spectrometry-based identification of proteasome or even E3 substrates is one approach to acquiring a global picture of the Ub–proteasome pathway. W. Kaelin (Boston, MA, USA) described a parallel genetic strategy using approximately 8,000 human open reading frames fused to a luciferase reporter. Luciferase itself is relatively stable but its degradation can be induced when fused to an unstable protein. Luciferase activity can therefore be used as an indirect measure of the stability of proteins encoded by the fused open reading frames. Kaelin's group tested the effect of Nedd8 inactivation on protein stability. Cullin-based Ub ligases depend on modification with Nedd8. A hamster cell line (ts41) that is temperature-sensitive for Nedd8 activation can therefore be used to potentially inactivate all cullin-dependent ligases by a simple temperature shift. Hundreds of open

Fig 2 Complexity of the mammalian ubiquitin conjugating system. A single E1 is thought to activate ubiquitin (Ub). At least 33 different E2s function as carriers of activated Ub and combine with more than 500 E3s for substrate-specific ubiquitination. The different combinations affect substrate selection and Ub-chain topology. Please note that evidence for a second E1 enzyme was presented at this meeting. Figure kindly provided by M. Petroski.

reading frames were identified as potential targets of cullin-based Ub ligases. Similar screens combined with specific ligase knock-downs could be a feasible approach to identify substrates for any given E3. Given the findings reported at this meeting, it is clear that global genomics and proteomics approaches are beginning to make important contributions to understanding Ub biology.

Imaging the ubiquitin–proteasome system

Several fluorescence-based degradation reporters have been developed that allow imaging of proteasome activity. Degradation reporters typically consist of a degron sequence or Ub fused to green fluorescent protein (GFP). The rate of degradation of these reporters is sensitive to changes in proteasome activity as well as to reduced ubiquitination. A proteasome reporter that is independent of ubiquitination has been designed by the fusion of a degron from ornithine decarboxylase (ODC) to GFP.

R. Kopito's group (Stanford, CA, USA) introduced GFP-based degradation reporters in cell models for Huntington disease and found an almost 90% reduction in proteasome function in Huntington disease cells. Because of the limitations in the use of artificial substrates of the Ub–proteasome system, such as the modified GFPu and ODC-GFP in animal models of Huntington disease, Kopito reported the development of a mass-spectrometry-based approach that allows quantitation of the levels of polyubiquitin chains with specific types of linkages from any tissue. By using this approach, they were able

to detect massive changes in Lys48-, Lys63- and Lys11-linked Ub chains in the brains of Huntington disease mice before the onset of neurological symptoms of the disease.

G. Patrick (San Diego, CA, USA) used live imaging of the GFPu degradation-reporter in primary hippocampal neurons and showed that manipulations that stimulate or inhibit synaptic activity resulted in rapid and transient changes in local proteasome activity in synaptic spines. The Ub–proteasome system is involved in various forms of synaptic plasticity such as long-term potentiation and long-term depression, which are believed to form the basis for learning and memory. Our understanding of the role of Ub in these processes is limited, but the importance of Ub in synaptic regulation is further underlined by results from Ub-profiling experiments reported by Patrick, who found that approximately 20% of synaptic brain proteins are ubiquitinated. The application of new mass spectrometry and imaging approaches to cultured neurons and brain preparations will undoubtedly facilitate future advances in this area.

Signalling

The dynamic regulation of signalling pathways and their crosstalk requires several post-translational modifications. Therefore, it is not surprising that ubiquitination and, although less well understood, Ubl modifications have a crucial role. One of the most stunning discoveries of the past few years in this area is the description of the signalling pathway of the plant hormone auxin. Auxins regulate many aspects of growth in plants but the identity of the auxin receptor has been elusive for more than 30 years. M. Estelle (Bloomington, IN, USA) described work from his group that led to the identification of the Ub–proteasome pathway as a crucial regulator of auxin signalling and culminated in the identification of the Ub ligase Skp1–cullin–F-box, transport inhibitor response 1 (SCF^{TIR1}) as the long-sought auxin receptor. SCF^{TIR1} induces ubiquitination and degradation of the transcriptional repressor proteins Aux and IAA, which activate specific gene expression programmes. Remarkably, auxins directly mediate the interaction of the Ub ligase with its substrate—that is, SCF^{TIR1} with Aux/IAA. N. Zheng (Seattle, WA, USA) described the structural basis for auxin-mediated ligase–substrate interaction. Surprisingly, the crystal structure solved by Zheng's group excluded allosteric effects and suggests that auxin acts as molecular glue, binding to both TIR1 and substrates. On the basis of these findings, Zheng proposed that, in principle, small molecules could be developed to target selected disease-causing proteins for ubiquitination and degradation.

F. Melchior (Göttingen, Germany) described a completely different signal and its effect on sumoylation. Melchior's group found that treatment of HeLa cells with physiologically relevant concentrations of hydrogen peroxide (1 mM) leads to a rapid and almost complete loss of SUMO1 and SUMO2 conjugates. They further showed that the underlying cause of this global inhibition of sumoylation is hydrogen-peroxide-induced crosslinking of SUMO–E1 (Aos1/Uba2) and SUMO–E2 (Ubc9) through their active-site cysteines. No evidence for crosslinking of the Ub–E1 under these conditions was detected. Melchior suggested that localized inhibition of SUMO conjugation involving reactive oxygen species could be an important mechanism regulating SUMO-dependent signalling pathways.

UBDs are frequently involved in signalling (Hicke *et al.*, 2005). Proteins with UBDs function as Ub receptors, linking ubiquitination to downstream signalling and transport machineries. The Ub

receptors themselves often become ubiquitinated, which might function as a switch, preventing their interaction in *trans* with other ubiquitinated proteins (Kirkin & Dikic, 2007). S. Polo (Milan, Italy) discussed 'coupled monoubiquitination' of epidermal growth factor receptor substrate 15 (Eps15), a prototypic Ub receptor, which requires binding between its UBD and monoubiquitin conjugated through a peptide bond to the E3 ligase Nedd4. This process might be mechanistically similar to Eps15 ubiquitination by parkin, described by E. Fon (Montreal, Quebec, Canada). However, instead of binding to a Ub moiety attached to the E3, the Eps15 UBD binds to the ubiquitin-like domain within the N-terminus of parkin. Expanding on the growing roles of UBDs, P. Kaiser (Irvine, CA, USA) discussed how Met4 contains a UBD that binds to ubiquitin in *cis*, thereby masking the degradation signal as well as preventing chain extension. Kaiser speculated that the UBD in Met4 might be a regulated switch that controls protein stability. S. Urbé (Liverpool, UK) described how another Ub receptor, signal transducing adaptor molecule (STAM), recruits two DUBs: associated molecule with the SH3-domain of STAM (AMSH) and ubiquitin-specific processing protease Y (UBPY). Her group has been studying how DUBs control the ubiquitination of cargo and the sorting machinery that directs proteins to the lumen of the multi-vesicular body, and subsequently to the lysosome for degradation. Urbé reported that AMSH and UBPY compete for the same binding site on STAM. However, AMSH preferentially removes Lys63-linked Ub, whereas UBPY removes both Lys48- and Lys63-linked chains. Furthermore, she found that AMSH knockdown enhances downregulation of the epidermal growth factor receptor; by contrast, UBPY knockdown reduces the rate of receptor downregulation and destabilizes STAM.

The many roles of Ub are well illustrated in the NF-κB pathway, in which several components are modified by Ub, resulting in proteasomal degradation for some, such as the inhibitor of NF-κB (IκB), and regulation of activity for others, such as TAB2/3 and NF-κB essential modulator (NEMO). M. Karin (San Diego, CA, USA) discussed how the Ub ligase ITCH is involved in c-Jun N-terminal kinase (JNK)–NF-κB crosstalk, which counterbalances pro-survival signalling with apoptosis. NF-κB induces the expression of the anti-apoptotic protein FLICE-like inhibitory protein (FLIP), which is ubiquitinated by ITCH and degraded by the proteasome. Karin's group has shown that JNK phosphorylates and activates ITCH. Therefore, the activity of JNK determines the stability of the anti-apoptotic protein FLIP, and the pro-survival activity of NF-κB depends on its ability to limit prolonged JNK activation. Y. Ben-Neriah (Jerusalem, Israel) discussed why commensal bacteria in the gut do not induce inflammatory signals. His group, in collaboration with E. Raz (San Diego, CA, USA), identified a specific role for cell polarity in NF-κB signalling in intestinal epithelial cells. Apical activation induced ubiquitination but not degradation of IκBa. Consequently, ubiquitinated IκBa accumulated in the cytoplasm and prevented NF-κB signalling.

How do proteins signal that they are terminally misfolded and can no longer be refolded by chaperones? T. Sommer (Berlin, Germany) addressed this question by studying the protein quality control pathway in the lumen of the endoplasmic reticulum in yeast. Sommer's group found that Yos9, an endoplasmic reticulum quality control lectin, binds to Hrd3, a component of the endoplasmic-reticulum-associated Ub ligase HRD (3-hydroxyl-3-methylglutaryl-coenzymeA reductase degradation). This physical interaction links protein quality control to the Ub pathway. Sommer proposed a

two-step recognition model for misfolded proteins in the lumen of the endoplasmic reticulum. First, the luminal part of Hrd3 recruits proteins with solvent-exposed hydrophobic regions, which are characteristic of misfolding or folding intermediates. Second, Yos9 binds specifically to proteins modified with eight mannosyl residues (Man_8). According to the Man_8 -timer model (Helenius & Aebi, 2001), the presence of Man_8 -oligosaccharides indicates that the time allotted for a protein to fold has expired. Therefore, according to Sommer, the HRD–Yos9 complex recognizes proteins with both Man_8 -structures and solvent-exposed hydrophobic regions, thereby distinguishing terminally misfolded proteins from folding intermediates.

Mechanism of ubiquitin transfer

Several decades have passed since the discovery of the E1–E2–E3 cascade; however, many fundamental questions remain about the mechanism of Ub transfer. How is directionality ensured? How are E2s activated by E3s? What triggers Ub transfer to substrates? How are chains assembled? Structural studies are beginning to provide some answers to these questions. Paradoxically, much of the insight has come from studies of SUMO and Nedd8, which have then been extrapolated to the Ub cascade. B. Schulman (Memphis, TN, USA) presented a switch-like mechanism that controls the E1–E2 relay for Nedd8. A series of structures solved by Schulman's group show a marked conformational change that reorients the Ub-fold-domain (UFD) of E1, which is the interaction site for E2. Similarly to Ub–E1, which has two Ub-binding sites, Nedd8–E1 binds to two Nedd8 molecules. In the double-loaded E1, the UFD is oriented for transfer of the thioester-bound Nedd8 from E1 to the active-site cysteine on E2. In the now single-loaded E1, the UFD rotates to a different position, which would cause E2 to clash with E1. The charged E2 is therefore released. Schulman proposes that this conformational switch ensures the irreversible flow of Nedd8 from E1 to E2. C. Lima (New York, NY, USA) discussed the mechanisms that underlie E2- and E3-mediated SUMO conjugation. In the Ub system, many E2s require E3s not only for substrate specificity but also for catalytic activation. By contrast, Ubc9, the SUMO–E2, does not solely depend on E3s for SUMO conjugation. Lima presented evidence that Ubc9 can exhibit specificity and efficiently induce the deprotonation of target lysines that are presented in the correct sequence context to initiate substrate sumoylation. Data was also presented for two non-RING (really interesting new gene) and RING-type E3 SUMO ligases. In addition to substrate-specific interactions, Lima presented evidence that E3s can enhance conjugation in a substrate-independent manner by organizing and activating the E2–SUMO complex for conjugation through contacts to both E2 and SUMO. V. Chau (Hershey, PA, USA) speculated on how RING E3s activate E2s. Chau presented structural studies indicating a conformational change in Ubc7 on binding to a RING domain. Inspired by Lima's results, Chau suggested that the RING-induced change in Ubc7 conformation promotes target lysine binding to activate Ub conjugation.

Not just lysine

The ϵ -amino group on lysine residues and the amino group at the N-terminus were long thought to be the exclusive sites of Ub attachment on substrates. Two talks in this meeting provided evidence for cysteine residues as ubiquitination sites. L. Coscoy (Berkeley, CA, USA) discussed the role of the Lys3 protein encoded by the Kaposi's

sarcoma virus in downregulating MHC class I molecules. Lys3 is a membrane-bound RING-type E3 ligase that ubiquitinates class I molecules leading to their endocytosis and degradation in the lysosome. Interestingly, Lys3 conjugated Ub onto a cysteine residue in the cytoplasmic tail of class I molecules. By contrast, the closely related Lys5 protein modified lysines. Coscoy presented data indicating that two motifs near the transmembrane domains of Lys3 and Lys5, together with the positioning of lysine and cysteine residues within the tail, can account for this difference. P. Lehner (Cambridge, UK) showed that Lys3 uses UbcH5 to conjugate the first Ub onto class I molecules. However, this is insufficient for downregulation, which requires subsequent elongation by Ubc13 that adds Lys63-linked chains. M. Hochstrasser (New Haven, CT, USA) discussed the autoregulation of Ubc7, the predominant E2 in the endoplasmic-reticulum-associated degradation pathway. Hochstrasser's group found that excess Ubc7 is targeted to the proteasome by a polyubiquitin chain assembled on the active-site cysteine. Whether ubiquitination on cysteines has a more general role is unclear; however, the labile nature of the cysteine–Ub conjugate and the reducing conditions generally used for analysis might have prevented its more frequent detection.

Surprises

There were several genuinely unexpected findings reported at the meeting. Kopito presented the phenotypes of UbB- and UbC-knockout mice. UbB and UbC are two stress-induced polyubiquitin genes. Knockout of UbC is embryonically lethal; by contrast, the UbB-knockout mice were viable but developed obesity, probably owing to their reduced activity and decreased energy expenditure. They were also resistant to leptin, a hormone that inhibits appetite. Furthermore, Ub levels were reduced in the hypothalamus—one of the main brain areas that controls appetite—whereas they were normal elsewhere in the brain. The hypothalamus also displayed evidence of neurodegeneration. The reason for the selective vulnerability of the hypothalamus to the loss of UbB probably reflects the uniquely high level of expression of UbB in this brain region.

The structure of the proteasome 19S regulatory particle remains elusive; however, R. Rosenzweig (Haifa, Israel) presented results of a collaborative study with M. Glickman (Haifa, Israel), M. Gaczynska and P. Osmulski (both San Antonio, TX, USA), which places the two subunits Rpn1 and Rpn2 on top of the 20S core particle, therefore extending the proteolytic channel. High-resolution atomic force microscopy studies showed the two doughnut-shaped Rpn1 and Rpn2 proteins sitting in the middle of the α -ring of the 20S particle. The dimensions of the doughnuts are consistent with their potential role in pore opening. Finally, Harper cast doubt on the dogma that a single E1 enzyme is responsible for the activation of Ub in mammals. A 'new E1', Uba6, functions with about one-third of the E2s. Most of them can also be charged by the 'old E1', but Harper's group identified one strictly Uba6-dependent E2, Use1.

Concluding remarks

Ub is now widely recognized as one of the crucial regulatory proteins in eukaryotes. The tremendous progress that has been made during the past years has led to a detailed insight into the mechanisms of ubiquitination and the biological processes it controls. Its involvement in human diseases has initiated the search for pharmaceuticals that modulate ubiquitination. New genomic and proteomic strategies are being applied to the Ub and Ubl systems. These

approaches seek a system-level understanding but are also generating new questions by unveiling the complexity of the pathways. For example, mass spectrometry strategies have revealed various Ub-chain topologies including mixed and branched chains, which could encode specific information. How these chains are synthesized, and how the signals are interpreted and transmitted will be subjects for future meetings. Although UbIs are being identified at a breathtaking pace, their functions in signalling, with the exception of SUMO, Nedd8 and ISG15, are less well understood. Many challenges therefore remain for the future and guarantee a vibrant field with exciting meetings in the years ahead.

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