

Meeting report

Ubiquitin junction, what's your function?

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A report on the Ubiquitin and Intracellular Protein Degradation FASEB summer conference, Saxtons River, USA, 23-28 June 2001.

The modification of proteins by polyubiquitin is now well known as an elaborate signal that targets substrates for rapid degradation by the 26S proteasome, a multiprotein compartmentalized protease particle that contains clusters of proteases within its barrel-shaped cavity. It has become apparent, however, that ubiquitin signals a host of other processes, as illustrated during the recent FASEB summer conference 'Ubiquitin and Intracellular Protein Degradation'. In addition to covering new findings about conventional ubiquitin-mediated proteasomal degradation, the meeting greatly expanded on other aspects of the ubiquitin system, including endoplasmic reticulum (ER)-associated degradation, monoubiquitination, ubiquitin-like proteins, and the growing significance of ubiquitin in human disease.

Ubiquitin ligases and proteasomal degradation

Ubiquitin, a 76 amino-acid protein that is highly conserved among eukaryotes, is covalently bound to proteins through a series of reactions catalyzed by three now-famous enzymes, named E1, E2 and E3. These enzymes activate and transfer ubiquitin down a series of thiol-ester linkages, culminating in covalent attachment of ubiquitin via an isopeptide bond to a lysine residue of the substrate protein. E3 enzymes, or ubiquitin ligases, mediate the critical step of substrate recruitment and have been subject to much recent scrutiny.

Polyubiquitination typically targets proteins for rapid proteasomal degradation, as in the case of the mammalian E3 enzyme SCF^{Skp2} and its substrate, the p27^{Kip1} cyclin-dependent-kinase inhibitor. SCF (Skp1/Cullin/F-box protein) complexes are multi-protein ubiquitin ligases in

which the variable F-box protein component recruits different substrates to the core complex. Avram Hershko (Technion-Israel Institute of Technology, Haifa, Israel) kicked off the meeting by reporting the identification of Cks1, previously known as a subunit of cyclin dependent kinases, as a protein required for p27 ubiquitination; it probably acts by bridging the F-box protein Skp2 to phosphorylated p27. Michele Pagano (New York University Medical Center, USA) continued coverage of p27 by showing that p27 polyubiquitination is dependent both on cell adhesion, as a result of the adhesion-dependent expression of Skp2, and on phosphorylation of the tumor suppressor protein Rb.

Identification of novel E3 enzymes has provided insights into many biological processes. Alfred Goldberg (Harvard Medical School, Boston, USA) shed light on the enigmatic process of muscle atrophy by identifying Atrogin-1, an F-box protein that interacts with other SCF components and is induced in muscle during diverse physiological and disease states in which muscle atrophy occurs. As well as diverse F-box proteins, a variety of SCF-like complexes are assembled on different cullin family members. Philip Branton's group (McGill University, Montreal, Canada) discovered that VACM-1 is a cullin protein involved in degradation of the p53 tumor suppressor protein in response to adenovirus infection. Another interesting finding was made by Mark Hochstrasser (Yale University, New Haven, USA) who, after a long search, identified Doa10, a transmembrane E3 enzyme that targets the yeast mating transcription factor $\alpha 2$ for proteasomal degradation.

The capture of ubiquitinated substrates by the 26S proteasome remains a critical, but poorly understood, aspect of the ubiquitin system. Chou-Chi Li (National Cancer Institute, Frederick, USA) provided evidence that VCP (homologous to Cdc48 in *Saccharomyces cerevisiae*) is a mammalian chaperone for the proteasome. VCP and Cdc48, which bind to both the proteasome and the polyubiquitin chains of

substrates, are required for degradation of multiple ubiquitinated proteins. The 26S proteasome consists of a central 20S proteolytic barrel with 19S regulatory caps on either end that mediate binding, unfolding, and translocation of substrates into the 20S barrel. The 19S cap consists of two subcomplexes: a subcomplex that is distal to the 20S barrel and contains the known polyubiquitin binding protein Rpn10, and a subcomplex that is in contact with the pore of the 20S barrel and consists of ATPase subunits. Cecile Pickart (Johns Hopkins University, Baltimore, USA) reported that a 19S ATPase subunit interacts with polyubiquitin chains, potentially linking substrate binding to processes close to the axial pore of the 20S proteasome.

George DeMartino (University of Texas Southwestern Medical Center, Dallas, USA) and Richard Vierstra (University of Wisconsin, Madison, USA) characterized the effects of loss of individual 19S subunits using two different systems: by RNA interference (RNAi) in *Drosophila* cells and deletion analysis in *Arabidopsis*, respectively. Although both procedures caused compensatory increases in other 19S components, phenotypic defects were nevertheless noticeable in *Arabidopsis*, despite the existence of multiple genes for most 19S subunits. Thus, Vierstra found that 19S caps of specific isoform compositions appear to mediate distinct regulatory pathways. Another type of proteasomal cap is the 11S regulator, a potential mediator of peptide exit from the 20S barrel. Christopher Hill (University of Utah, Salt Lake City, USA) showed exciting movies based on crystallography, demonstrating that the 11S regulator PA26 causes conformational shifts in the carboxy-terminal tails of 20S α subunits to open the axial pore.

Form and function in ubiquitin modification

Polyubiquitin chains are commonly formed through linkages between the carboxyl termini and lysine 48 residues of successive molecules of ubiquitin, attached to internal lysines of the substrate as an isopeptide linkage. There are exceptions to this paradigm, however. Aaron Ciechanover (Technion-Israel Institute of Technology, Haifa, Israel) has discovered three proteins, the muscle differentiation factor MyoD and the viral oncoproteins LMP1 and E7, that are targeted for proteasomal degradation upon ubiquitination at their very amino termini, independent of lysines within each substrate. Zhijian Chen (University of Texas Southwestern Medical Center, Dallas, USA) showed that an alternate linkage via lysine 63, formed by the E2 heterodimer Ubc13/Mms2, signals a non-proteasomal function. The E3 enzyme TRAF6 targets itself for this form of ubiquitination, leading to the proteasome-independent activation of kinase cascades that lead to transactivation of the transcription factors NF- κ B and c-Jun, respectively. Andrew VanDenmark (Johns Hopkins University, Baltimore, USA) and Michael Ellison (University of Alberta, Edmonton, Canada) reported crystal structures of Ubc13/Mms2 that provided

insights into the mechanism of lysine-63-linked ubiquitin chain formation.

Polyubiquitination is also critical in protein quality control, where it helps to eliminate improperly processed or misfolded proteins from the ER, in a process called ER-associated degradation (ERAD). Defective proteins are transported back to the cytosolic side of the ER membrane, targeted by the novel E3-containing complex Hrd1 (Der3)/Hrd3, and ubiquitinated by the E2 enzyme Ubc7, thereby being targeted for 26S proteasomal degradation, as reported for the yeast system by Dieter Wolf (University of Stuttgart, Germany) and Randolph Hampton (University of California, San Diego, USA). Hampton added an extra twist to the ERAD system by reporting that the active ER enzyme, HMG-CoA reductase, is degraded by the same pathway. In another elegant ER-associated study, Stephan Jentsch (Max Planck Institute for Biochemistry, Munich, Germany) presented a system that transcriptionally controls the synthesis of unsaturated fatty acids. In response to altered fatty-acid composition, the ER-bound inactive precursor of the Spt23 transcription factor is polyubiquitinated and proteasomally processed to a soluble form. This then translocates to the nucleus to activate transcription of OLE1, which encodes a fatty acid desaturase.

Monoubiquitination can target proteins to the lysosome, either by directing endocytosis of cell-surface receptors or by sorting newly synthesized hydrolases from the Golgi to their resident lysosomal compartment. To identify ubiquitin-binding endocytic proteins, Linda Hicke (Northwestern University, Evanston, USA) screened yeast proteins with known ubiquitin-binding motifs and identified epsins and Ede1, proteins involved in clathrin-coated endocytosis, and Vps27, a late endosomal protein, as potential substrate receptors. Scott Emr (University of California, San Diego, USA) characterized other Vps proteins, specifically the ESCORT-I (Vps23/28/37) complex, by tracking vesicular transport of substrates tagged with green fluorescent protein (GFP) from the Golgi or cell surface to the lysosome. The ESCORT-I complex appears to mediate ubiquitin-dependent cargo selection at the late endosome.

Ubiquitin-like proteins

Ubiquitin-like proteins (ULPs) covalently attach to substrates using a conjugation pathway analogous to that of ubiquitin; but these modifiers usually require processing at the carboxyl terminus and conjugate only as monomers. ULPs fulfill numerous different functions. The Nedd8 ULP modifies the cullin subunit of SCF complexes and appears to promote ubiquitination by recruiting E2 enzymes. Tomoki Chiba (Tokyo Metropolitan Institute of Medical Science, Japan) showed that deletion of UBA3, the catalytic subunit of the Nedd8 E1 enzyme, causes embryonic lethality in mice, consistent with the important role of Nedd8 in plants and

fission yeast (but, surprisingly, not in budding yeast). As discussed by Ray Deshaies (California Institute of Technology, Pasadena, USA), the Cop9 signalosome, a large complex with structural similarity to the 19S regulatory cap, has an associated 'de-Neddylation' activity, which may be linked to the signalosome by the conserved protein Jab1.

The ULP Sumo (small ubiquitin-like modifier) has varied substrates and functions. As an example, it modifies and stabilizes Mdm2, a ubiquitin ligase for p53. Ze'ev Ronai (Mount Sinai School of Medicine, New York, USA) showed that both phosphorylation of Mdm2 by the p38 mitogen-activated protein (MAP) kinase and binding of Mdm2 by p19^{ARF} impair recruitment of the Sumo E2 enzyme, Ubc9, to Mdm2. Yoshinori Ohsumi (National Institute for Basic Biology, Okazaki, Japan) reported findings on Apg8, an intriguing new ULP that modifies phosphatidylethanolamine, and presented data on the conjugation pathways for Apg8 and another ULP, Apg12, both of which are required for autophagy in yeast.

Ubiquitin and human disease

With the many critical roles that ubiquitin plays in the cell, it is not surprising that the ubiquitin system is increasingly implicated in human disease. Ron Kopito (Stanford University, USA) showed that a number of neurodegenerative diseases lead to the formation of protein aggregates that impair proteasomal degradation. Similarly, Martin Rechsteiner (University of Utah, Salt Lake City, USA) showed that REG γ , an 11S regulator in brain tissue, decreases the ability of the proteasome to digest extended polyglutamine tracts, which are a hallmark of Huntington's disease. Jon Huibregtse (University of Texas, Austin, USA) found that the E6 viral oncoprotein acts as an adaptor for the E6AP E3 ligase to target two substrate proteins that are required for cell polarity, Scribble and Discs Large. The PDZ domains in Scribble, and Discs Large mediate interactions with E6 and may account for E6-mediated carcinogenesis independent of p53. Finally, Alan D'Andrea (Dana Farber Cancer Institute, Boston, USA) reported that a failure to monoubiquitinate the FANCD2 protein may account for DNA-damage sensitivity in Fanconi anemia, because unmodified FANCD2 is unable to localize to nuclear foci in association with the tumor suppressor protein BRCA-1.

In summary, this meeting underscored the emerging theme that ubiquitin modification can engender multiple different outcomes depending on the biological context. Unlike the lyrics of the *Schoolhouse Rock* ditty *Conjunction Junction*, 'ubiquitin junction, what's your function?' is a complex question that researchers in many different fields will be studying for years to come.