In the family with ubiquitin

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The Cold Spring Harbor meeting on 'The Ubiquitin Family', held in May 2011, brought together scientists from a wide range of fields under the umbrella of ubiquitin and ubiquitin-like protein structure, function and regulation.

Clouds gathered as the ubiquitin community convened for its biennial Cold Spring Harbor meeting, organized by J. Wade Harper (Harvard Medical School, USA), Ron Hay (U. Dundee, UK), Ron Kopito (Stanford U., USA) and Brenda Schulman (St Jude Children's Research Hospital, USA). Nevertheless, the warm and cheerful atmosphere among the participants more than counteracted the awful weather, and the rain allowed participants to concentrate entirely on a few days of excellent science in the ever-expanding ubiquitin field. It was a well-attended meeting, with representation from almost all the major labs in the field. The range of topics discussed reflected this diversity and included the enzymology of the ubiquitin-proteasome system (UPS), DNA repair, host-pathogen interactions, vesicle transport and cancer therapy, to name a few. In general, however, there was a lot of biochemistry and not so much cell biology, which led people to joke about the fact that mice—and even photos of cells—were scarce until the last two sessions.

A major theme of the meeting was targeting the UPS for drug development, either to inhibit or enhance its function. Ray Deshaies (Caltech, USA)—one of the keynote speakers—talked about inhibiting the UPS, as did Mike Tyers (U. Edinburgh, UK) and Neil Bence (Millennium Pharmaceuticals, USA). The lab of Alexei Kisselev (Dartmouth Medical School, USA), Millennium Pharmaceuticals and Progenra Inc. (USA) all presented posters on their research into inhibitors of the UPS. Ray noted that there is an enormous 'green field' opportunity in this area, as there are over 1,000 potential drug targets in the UPS and only one drug has been approved for clinical use so far—the proteasome inhibitor

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bortezomib, manufactured by Millennium. He discussed ongoing efforts to target the JAMM-domain metalloproteases CSN5 and RPN11, as well as the p97 ATPase (Chou *et al*, 2011). Tyers described the development of an inhibitor for the human ubiquitin-conjugating enzyme CDC34 (Ceccarelli *et al*, 2011), and Neil Bence elaborated on Millennium's success in inhibiting the ubiquitin-activating enzymes UBA1 and UBA6. Dan Finley (Harvard Medical School), conversely, discussed research aimed at enhancing the activity of the UPS, which could also be effective against a variety of protein-aggregation diseases. His lab, together with that of Randy

King (Harvard Medical School), recently developed a small-molecule inhibitor of the proteasome-associated deubiquitinating enzyme USP14 that enhances cellular resistance to oxidative stress and the degradation of proteins involved in neurodegeneration (Lee *et al*, 2010).

A range of structure-based studies delved into the specificity and mechanism of action of ubiquitin E2s and E3s, SUMOylated protein recognition and deubiquitinating enzyme (DUB) regulation. Rachel Klevit (U. Washington, USA) discussed the specificity of ubiquitin E2s for RING-domain in comparison with HECT-domain E3s, with RING-in-between-RING (RBR) E3s behaving as RING-HECT hybrids. She showed that RBR E3s bind to the ubiquitin-conjugating enzyme UBCH7 through their RING1 domain and transfer ubiquitin to a crucial cysteine residue on RING2, hence the proposed similarity between the RING2 of RBRs and HECT E3s (Wenzel *et al*, 2011).

Eric Fischer from Nicolas Thomä's group (Friedrich Miescher Institute for Biomedical Research, Germany) presented structural studies on the role of E3 ubiquitin-ligases in the DNA damage response and, more specifically, in nucleotide excision repair. By solving the structure of DDB1/DDB2/CUL4/RBX1 bound to damaged DNA, the Thomä group identified the constraints through which the E3 ligase operates in the vicinity of the damaged site. Chris Lima (Sloan-Kettering Institute, USA) discussed the bimodular recognition of SUMO-conjugated substrates. By using proliferating cell nuclear antigen (PCNA) as a model SUMO substrate, he showed through biochemical and structural analyses that distinct PCNA and SUMO interaction motifs in Srs2 contribute to specificity in substrate recognition of SUMO-PCNA.

DUB regulation by non-substrate binding partners and by phosphorylation was the focus of the talks by Ning Zheng (U. Washington, USA) and Andrea Cochran (Genentech, USA), respectively. Within the SAGA complex, the yeast DUB Ubp8 is known to form a complex with Sgf11, and Zheng showed that this interacting protein controls both the substrate specificity and catalytic activity of Ubp8. Studies presented by Cochran identified DUBA as an ovarian-tumour-class DUB, the phosphorylation of which is required for interaction with the carboxy-terminal tail of ubiquitin and for catalytic activity. Although phosphorylation alone does not affect the overall structure of DUBA, it leads to the formation of a productive enzyme-substrate complex upon substrate binding.

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The poster sessions maintained the focus on DUBs by revealing new substrates that implicate DUBs in the regulation of receptor transport, gene transcription, endoplasmic-reticulum-associated degradation, circadian control, ribosome biogenesis and the biogenesis of spliceosomal nuclear ribonucleoproteins. An ongoing theme—and the topic of many posters—is the way ubiquitin chains of specific linkage are assembled and recognized. David Komander (MRC Laboratory of Molecular Biology, UK) described a fluorescence resonance energy transfer (FRET)-based assay that monitors the conformation of diubiquitin of different linkages at a single-molecule level. The data indicate that Lys48-linked chains can exist in a half-closed conformation, and DUBs could open the structure to facilitate catalysis.

Proteases that target ubiquitin-like molecules were also discussed at the meeting. Klaus-Peter Knobeloch (U. Freiburg, Germany) talked about UBP43 (USP18), a protease that deconjugates ISG15 from modified substrates. Inactivation of the enzymatic activity of UBP43 in a knock-in mouse model enhances ISGylation and resistance to vaccinia virus infection, but does not cause the brain abnormalities or interferon hypersensitivity that has

been seen previously in complete knockouts. These results show that USP18 has protease-dependent and -independent functions *in vivo*. Maria Masucci (Karolinska Institute, Sweden) introduced the dual-specificity viral protease BPLF1, which hydrolyses both ubiquitin and NEDD8 conjugates (Gastaldello *et al*, 2010), and Frauke Melchior (Center for Molecular Biology, Heidelberg, Germany) described a new SUMO protease—belonging to a family that were previously thought to be ubiquitin-specific proteases—that localizes to Cajal bodies and is required for normal cell proliferation.

Mapping of ubiquitin and ubiquitin-like protein-modification sites at the proteome level, using a diglycine antibody in combination with mass spectrometry, was pioneered by Steve Gygi and Wade Harper's lab (Harvard Medical School) and presented by Eric Bennett, a postdoc in the Harper lab. As judged from discussions at several posters, this strategy is soon to be used by other groups. Thus, a more comprehensive map of ubiquitin-like protein modification is expected in the near future.

Different strategies to identify E3 ubiquitin ligase substrates were discussed. Keiichi Nakayama (Kyushu U., Japan) presented a particularly memorable strategy, known as DiPIUS (differential proteomics-based identification of ubiquitylation substrates). His lab used quantitative mass spectrometry to determine the relative abundance of interacting proteins for wild-type and mutant forms of various E3 ligases. Nakayama demonstrated that this approach could be applied successfully to identify new substrates for cullin-RING E3 ligases (CRLs) by using F-box protein mutants defective in binding to the core CRL complex. FBW7 seems to be the F-box protein *du jour*. In addition to the identification of OASIS, BBF2H7 and KLF5 as new SCF^{FBW7} substrates, a poster from the Nakayama lab described the phenotypes of various tissue-specific deletions of FBW7, which led to either deregulated proliferation or abnormal differentiation, associated with accumulation of c-Myc or Notch, respectively. Bruce Clurman (Fred Hutchinson Cancer Research Center, USA) talked about the mechanism of tumour suppression by SCF^{FBW7} and described a new mouse model that recapitulates human colon cancer in mice. Wenyi Wei (Beth Israel Deaconess Medical Center, USA) discussed FBW7-mediated degradation of MCL1; loss or mutation of

FBW7 in many tumours—including T-cell acute lymphoblastic leukaemia—leads to MCL1 accumulation, which is the basis for resistance of tumours to some chemotherapeutics (Inuzuka *et al*, 2011; Wertz *et al*, 2011).

Continuing on the cancer theme, Vishva Dixit (Genentech)—the other keynote speaker—discussed the role of the E3 ligase substrate adaptor COP1 as a tumour suppressor that negatively regulates ETS transcription factors. One of them, ETV1, is frequently translocated in prostate cancer, creating a truncated protein that cannot be regulated by COP1. In addition, COP1 downregulation promotes prostatic epithelial-cell proliferation and tumorigenesis (Vitari *et al*, 2011).

One intriguing topic that was covered at the meeting was the possible cross-talk between the NEDD8 and ubiquitin pathways. The talks by Roland Hjerpe, from the group of Thimo Kurz (The Scottish Institute for Cell Signalling, U. Dundee, UK) and Dimitris Xirodimas (Molecular Biology Research Center, Montpellier, France) presented data showing that NEDD8 can be conjugated to non-cullin substrates by enzymes of the ubiquitin pathway. Although the functional significance of this phenomenon is unclear, this cross-talk operates under diverse stress conditions and possibly in neuronal development, as presented by Damian Refojo (MPI of Psychiatry, Munich, Germany). On a related topic, Gwenael Rabut (Institute of Genetics and Development, Rennes, France) showed that the budding yeast cullin Rtt101 can be ubiquitinated at the NEDDylation site, leading to a similar enhancement of cullin-RING ligase function *in vivo*.

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The role of ubiquitination in membrane dynamics was another topic of particular interest. Michael Rape (U. California Berkeley, USA) gave a striking talk on the role of ubiquitin in the control of COPII vesicle size and function. Starting from a small-interfering RNA screen for E3 ligases required for embryonic stem-cell division, his lab found that a Cul3 ubiquitin ligase modifies a COPII coat protein. This in turn increases the size of COPII vesicles and promotes collagen

export, which is essential for maintaining the stem-cell niche. Rape has teamed up with Randy Schekman (U. California, Berkeley, USA) on this and apparently solved, at least in part, a long-standing conundrum in the transport field: how large, cargo-transporting vesicles are made.

Continuing the theme of ubiquitin and membranes were Mickael Cohen (CNRS, Paris, France) and Mafalda Escobar-Henriques (Cologne U., Germany), who discussed the way that ubiquitination regulates mitofusins and mitochondrial outer membrane (MOM) fusion. Although ubiquitination of the yeast mitofusin Fzo1 clearly involves the F-box protein Mdm30, the mechanism of its degradation is still debated. The two proposed models agree that Fzo1 ubiquitination and degradation are regulated by its GTPase domain and take place at the mitochondrial post-tethering step to facilitate MOM fusion (Cohen *et al*, 2011; Anton *et al*, 2011). Yien Che Tsai from Alan Weissman's laboratory (National Cancer Institute, USA) showed that DNA damage induces phosphorylation of the mitofusin MFN2 (the human homologue of Fzo1), thereby increasing MFN2 association with the E3 ligase and promoting its ubiquitin-dependent degradation.

Regarding the role of ubiquitin in autophagy, Ivan Dikic (Goethe U., Frankfurt, Germany) showed that optineurin (OPTN) is a xenophagy receptor regulated by phosphorylation, which acts as a regulatory cue to promote selective clearance

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of *Salmonella enterica* (Wild *et al*, 2011). OPTN recruits TBK1 kinase to the surface of cytosolic, ubiquitinated bacteria where TBK1 becomes active and, in turn, phosphorylates OPTN, thereby increasing its affinity for the ubiquitin-like modifier LC3, which is conjugated to autophagosomal membranes. A similar interaction between phosphorylation and the initiation of autophagy was presented by Gen Matsumoto from the Nukina lab (RIKEN Brain Science Institute, Japan). He showed that CK2 phosphorylates p62 in the ubiquitin-binding domain and that a phosphorylation-mimicking mutant has increased affinity for Lys63 and Lys48 polyubiquitin chains *in vitro*. Thus, phosphorylation of autophagy receptors emerges as a new way to regulate ubiquitin-selective autophagy.

In all, it was a very productive meeting of an increasingly large community. Such small meetings in this field are a luxury that might be in danger of extinction; as ubiquitin and ubiquitin-related modifiers are tied to more processes, it becomes difficult to cover them all at one time. As with protein phosphorylation, the community might ultimately be structured around common functions rather than around the modification *per se*. One conclusion is that protein

modification with ubiquitin and ubiquitin-like proteins has a central role in cellular physiology, and understanding the way in which it is regulated and affects different signalling pathways will enable us to harness the system against disease and infection. Judging by the speed of progress in this field, the next Cold Spring Harbor meeting, due to take place 14–18 May 2013, on ubiquitin is surely something to look forward to.

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