The interface between the ubiquitin family and the DNA damage response

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The crucial role of ubiquitin signalling in genome-integrity maintenance was first recognized in 1987 by Stefan Jentsch and Alex Varshavsky, who showed that Rad6—the repair protein involved in DNA damage tolerance—is a ubiquitin-conjugating enzyme. Although this discovery inspired extensive research and led to the discovery of genome surveillance pathways that are fuelled by proteolytic and regulatory ubiquitylation and SUMOylation, it took more than two decades for these fields to meet at a dedicated interdisciplinary conference. This was rectified at an EMBO workshop held between 1 and 5 September on Red Island, Rovinj, Croatia.

The idea of bringing the fields of ubiquitin/SUMO research and the study of DNA damage responses together should be credited to Yossi Shiloh (Tel Aviv U., Israel) and Ivan Dikic (Goethe U., Frankfurt, Germany), who asked Helle Ulrich (Cancer Research UK, Clare Hall Laboratories) and Daniel Durocher (Samuel Lunenfeld Research Institute, Toronto, Canada) to join them in organizing the meeting.

The keynote speaker, Wade Harper (Harvard Medical School, MA, USA), reminded the audience that there are three areas in which the ubiquitin/SUMO and DNA damage response (DDR) systems interact: protein turnover (so far best understood in regulating cell-cycle checkpoints after DNA damage); protein recruitment to damaged chromosomes; and molecular switches (a dynamic research area focused on 'switching' repair pathways through alternating proliferating cell nuclear antigen (PCNA) ubiquitylation). Speakers at the meeting covered each of these areas, presenting both conceptual aspects and analytical approaches. Importantly, a large body of unpublished data was presented and provocative concepts were put forward, challenging some of the current models of the regulation of DDR pathways by ubiquitin/SUMO modifications.

This report does not summarize all of this work—which is expected to yield many exciting papers—but instead provides a personal reflection on emerging trends and the particular benefits of interdisciplinary

EMBO Workshop

The Interface between the Ubiquitin Family and the DNA Damage Response

September 1st - 5th, 2010
Red Island
Rovinj, Croatia

KEYNOTE SPEAKERS
Wade Harper
Harvard Medical School, Boston, USA

SPEAKERS

Arno Alpi, ¹	Roger A. Greenberg, ¹	Carol Prives, ¹
Jiri Bartak, ¹	Patrizia Hamele, ¹	Olga Rando, ¹
Dana Brantel, ¹	Ravindra K. Hoo, ¹	Yoshitaka Shibata, ¹
Janet Chen, ¹	Steve Jackson, ¹	Titia Sixma, ¹
Karlene Cheng, ¹	Stefan Jentsch, ¹	Agnes Smeyers-Verbeke, ¹
Yoon Dikic, ¹	Alan Lehman, ¹	Helle Ulrich, ¹
Yoshinori Dikic, ¹	Jill Lohman, ¹	Adrian Vekrellis, ¹
Daniel Durocher, ¹	Michaela Paganini, ¹	Wim Vermeulen, ¹
	Matthew Pines, ¹	Helen Wadden, ¹
		Shoubo Zhao, ¹

KEY TOPICS

- Ubiquitin: a dynamic research area
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approaches, as well as hot topics, recent advances and unresolved issues.

DNA damage tolerance was, unsurprisingly, among the most-discussed topics at the meeting. Ubiquitylation is one of the main determinants of the way in which cells respond to DNA lesions during DNA replication; monoubiquitylated PCNA triggers the recruitment of specialized DNA polymerases that can support error-prone trans-lesion synthesis, whereas polyubiquitylated PCNA supports an error-free pathway. A key conceptual advancement in this field resulted from work by Helle Ulrich (Clare Hall Laboratories, UK) and Stefan

Jentsch (MPI of Biochemistry, Martinsried/Munich, Germany), who independently discovered that these two branches of post-replication repair act not only in the S phase of the cell cycle, but also in the G2 phase (Daigaku *et al.*, 2010; Karras & Jentsch, 2010). These findings have profound implications; it is now clear that the DNA synthesis associated with post-replication repair is independent of genome replication.

An important remaining question is how cells regulate the switch between mono- and polyubiquitylated PCNA. An insight into this was provided by Titia Sixma (Netherlands Cancer Institute, Amsterdam) who showed that Rad18/Rad6—an E3/E2 complex required for PCNA monoubiquitylation—can limit the extension of ubiquitin chains.

Another area that received considerable attention at the meeting was the mechanism controlling the accumulation of repair and signalling proteins on chromatin in the vicinity of DNA breaks. This topic—referred to as the 'RNF8/RNF168 pathway'—is particularly important for several reasons. First, the number of talks and posters focusing on this pathway suggest that it is one of the most rapidly developing areas of research in this field. Second, many of these talks presented unexpected findings, including some which might even be considered paradigm-shifting. Third, this pathway illustrates the limitations of our understanding of why cells need to engage ubiquitin/SUMO signalling to maintain their genomic integrity.

As became clear at the meeting, the term 'RNF8/RNF168 pathway' is already outdated: in addition to RNF8 and RNF168—the first two members of this pathway that were identified—we now know of two additional E3 ubiquitin ligases (HERC2 and BRCA1), one E2 ubiquitin conjugating enzyme (UBC13), at least two ubiquitin hydrolases (BRCC36 and USP3) and two SUMO ligases (PIAS1 and PIAS4) that cooperate in this complex signalling cascade to facilitate the retention of repair factors at damaged chromosomes. It is thought that this list is still incomplete, and we learned at the meeting about some exciting insights into the structure and regulation of this pathway.

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Daniel Durocher (Samuel Lunenfeld Research Institute, Toronto, Canada) reported on an unorthodox non-catalytic step in DNA-damage-induced chromatin ubiquitylation (Nakada *et al*, 2010). He showed that OTUB1—a member of the ovarian tumour de-ubiquitylating enzymes (DUBs)—inhibits the ability of the RNF168/UBC13 holoenzyme to extend ubiquitin chains. OTUB1 does so in an unexpected way, by restraining isopeptide bond formation between the donor ubiquitin on charged UBC13 and the acceptor ubiquitin coordinated by UEV1. The identification of this mechanism is an exciting addition to our understanding of this pathway and it has implications for genome-instability syndromes. The Durocher lab showed that silencing of OTUB1 partly rescues accumulation of repair factors in ataxia telangiectasia mutated-deficient cells.

Although this study of OTUB1 supports the essential role of UBC13 in the DNA-damage-induced chromatin response—and thereby the role of Lys63-linked ubiquitin chains—other reports revealed that additional E2 ubiquitin-conjugating enzymes are probably involved. In fact, the spectrum of ubiquitin conjugates that are formed at damaged chromosomes seems to be one of the least-understood aspects of this pathway. This was discussed by Titia Sixma, who introduced a defined *in vitro* system for

measuring the ability of RNF8 and RNF168 to ubiquitylate histones in the context of assembled nucleosomes. The key, and potentially paradigm-shifting outcome of this work was that the view of the RNF8/RNF168 pathway as strictly linear and based solely on the Lys63 type of ubiquitin chains might need to be revised. Sixma's work also indicates that once the pathway is triggered, RNF8 and RNF168 can amplify the action of each other. It also seems increasingly likely that RNF8 triggers the pathway by ubiquitylating non-histone substrates and that such 'priming' chains might involve Lys48 ubiquitin conjugates. Experimental support for this was provided by Junjie Chen (M.D. Anderson Cancer Center, Houston, TX, USA) who reported that the three main E3 ligases involved in modifying damaged chromatin (RNF8, RNF168 and BRCA1) cooperate in generating distinct types of ubiquitin chain.

The idea of a complex 'ubiquitin landscape' at damaged chromosomes was supported by Roger Greenberg (U. Pennsylvania, Philadelphia, PA, USA). He showed that while RNF8/RNF168-mediated H2A ubiquitylation primarily acts to reversibly block the action of RNA polymerase II (RNAPII) within the damaged chromatin, the recruitment of repair factors to this compartment is mediated by targeting other (hitherto unknown) substrates of these E3 ligases (Shanbhag *et al*, 2010). An intriguing addition to this concept was provided by Matthew Weitzman (The Salk Institute, La Jolla, CA, USA), who showed that RNF8 and RNF168 are destabilized by the herpes simplex virus-1-encoded protein ICP0 to limit transcriptional silencing of viral genomes (Lilley *et al*, 2010).

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The spectrum of histones that are targeted for ubiquitylation after DNA damage clearly extends beyond H2A and its variants. As we learned from Yossi Shiloh, histone H2B is also targeted for mono-ubiquitylation at sites of DNA double-strand breaks (DSBs), and this seems to be important for timely accumulation of DSB repair proteins. Similar results were reported by Akihiro Kato of Kenshi Komatsu's laboratory (Kyoto U., Japan).

These reports provide important mechanistic insights into damage-induced chromatin ubiquitylation, but they also remind us how little we know about events downstream from these modifications. For example, the ubiquitin/SUMO conjugates must be 'decoded' by dedicated receptors in a highly controlled manner. The complexity of these conjugates and how they are decoded is a key challenge affecting all aspects of the ubiquitin/SUMO interface with the DDR. Informative examples that illustrate this issue were provided by Ivan Dikic and Alan D'Andrea (Dana-Farber Cancer Institute, Boston, MA, USA). The Dikic group has recently shown that the Werner helicase-interacting protein (WRNIP) accumulates in distinct nuclear foci and that this process depends on its UBZ domain—one of the many ubiquitin-binding domains known in proteins. Interestingly, when assayed *in vitro*, the WRNIP-derived UBZ binds to both mono- and polyubiquitin chains indiscriminately. This implies that the ubiquitin-mediated targeting of WRNIP to chromatin requires an additional regulatory layer.

Another key task for the field is to broaden the spectrum of known physiological substrates for the DDR-associated ubiquitin/SUMO modifiers

D'Andrea provided an intriguing parallel with the Fanconi anaemia pathway and the SUMO field, showing that UAF1—a USP1-interacting protein identified in his laboratory—contains SUMO-like domains that are crucial for 'navigating' the USP1-UAF1 complex to its substrates. The significance of this finding is linked to the specificity of sorting ubiquitin/SUMO regulators within the Fanconi anaemia pathway. However, it remains unclear why UAF1 binds to only a few substrates and what prevents it from being 'sequestered' by other proteins containing SUMO-binding motifs. It is thought that structural determinants might restrict use of ubiquitin/SUMO-binding domains, but these have not been identified yet.

Another key task for the field is to broaden the spectrum of known physiological substrates for the DDR-associated ubiquitin/SUMO modifiers. It was gratifying to see that many laboratories have been approaching this challenge from complementary angles and interesting substrates are being identified. Niels Mailand (Novo

Nordisk Foundation Center for Protein Research, Copenhagen, Denmark) reported that targets for SUMOylation include two DDR-associated ubiquitin ligases, RNF168 and HERC2. Yaron Galanty and Steve Jackson (U. Cambridge, UK) described how the SUMO E3 ligases PIAS1 and PIAS4 seem to control DNA DSB repair by homologous recombination and non-homologous end-joining, and how this might be achieved by the combined effects of SUMOylation and ubiquitylation events on DDR proteins. These are exciting examples of the interaction of SUMO and ubiquitin signalling activated by DNA-damage-induced chromatin alterations. It is likely that other substrates will be identified, but we still need to learn how the chromatin-associated ubiquitin/SUMO modifiers affect its main building blocks: histones. Kevin Hiom (U. Dundee, UK) provided an insight into this, he looked at the way in which BRCA1-mediated ubiquitylation of H2A affects chromatin folding and investigated its potential ability to generate a chromatin microenvironment that is permissive to DNA-end processing.

The interdisciplinary nature of this meeting was beneficial. The agendas of DDR meetings often include ubiquitin/SUMO-related issues; processes such as DNA damage tolerance, the cellular response to DNA crosslinks through the Fanconi anaemia pathway and protein accumulation at DSB-flanking chromatin cannot be discussed without alluding to ubiquitin/SUMO signalling. However, a broader picture inspired by related fields in which pathways are regulated in a similar way, has so far been missing.

One relevant field is cell-cycle regulation; a few years ago work from this area was common at DDR meetings, but it has been under-represented recently. It is not often acknowledged that ubiquitin/SUMO regulators that primarily evolved to control cell-cycle progression without genotoxic stress can affect the outcome of DNA damage.

An intriguing case highlighting the importance of communication between the DDR and the cell cycle was presented by Vishva Dixit (Genetech Inc., CA, USA), who identified DUB3 as a ubiquitin hydrolyse that binds to and removes ubiquitin chains from CDC25A—a phosphatase that regulates multiple cell-cycle transitions. This has implications for clinical oncology, because CDC25A is often stabilized—and thus overproduced—in breast cancer cells.

The Dixit group showed that this is due to overexpression of DUB3 (Pereg *et al*, 2010).

Contributions to this area were also provided by Wade Harper (Harvard Medical School, MA, USA), Michele Pagano (New York U. School of Medicine, NY, USA) and Matthias Peter (ETH Zürich, Switzerland). They reported on cullin-RING E3 ligases (CRLs), a large group of multi-subunit enzymes that are involved in regulating cell-cycle progression. Harper provided a fascinating insight into the molecular architecture of the CRLs *in vivo*. No other ubiquitin/SUMO machine has been analysed in such depth with the most sophisticated systems biology approaches—such as the AQUA quantitative proteomics developed by Stephen Gygi at Harvard Medical School, with whom the Harper group collaborated on this project. The concepts derived from this work, as well as the technology developed to conduct it, are an inspiration for further research to analyse the structure and function of E3 ligases specialized in DDR-associated events.

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Advanced (and in the DDR field unprecedented) technology was also introduced by Matthias Peter (ETH Zurich, Switzerland). He described a real-time cell-based screen combining siRNA microarrays and automated image segmentation to identify novel ubiquitin/SUMO regulators involved in regulating the length of the S phase. The image analysis software that was used in his study has just been released as an open resource (Held *et al*, 2010).

Michele Pagano contributed to the CRL lectures by showing that cyclin F—the founding member of the so-called F-box proteins that act as substrate-binding subunits of a subset of CRL enzymes—coordinates DNA repair and cell-cycle checkpoints, by regulating the available pool of deoxyribonucleotides.

One final interdisciplinary presentation from the transcription field provided inspiring parallels and revealed the true complexity of ubiquitin-regulated events. Jesper Svejstrup (Clare Hall Laboratories, UK) reminded us

that various chromosomal lesions impair not only DNA replication, but also gene transcription. Unlike the former, the latter ‘mishap’ cannot be resolved by damage bypass; instead, irreversibly stalled RNAPII must be degraded. Data from the Svejstrup laboratory revealed that degradation of stalled RNAPII is a complex process involving several internal ‘checkpoints’ (Harreman *et al*, 2009). RNAPII that is ‘destined to die’ must first be primed by RSP5, which generates Lys63-linked ubiquitin chains. These are then proofread and trimmed by the UBP2 ubiquitin hydrolase to form a single conjugated ubiquitin that is recognized by the elongin–cullin ubiquitin ligase complex generating Lys48-linked ubiquitin chains. Only when these steps are completed can RNAPII be degraded by the proteasome. This chain of events reminds us that cells literally ‘think twice’ before they destroy an important protein, and reinforces the notion that ubiquitin and SUMO signalling might be the most versatile post-translational modification of all.

The seemingly endless combinatorial complexity of the ubiquitin system is almost frightening, but it also suggests fascinating areas for further experimentation. As Alan D’Andrea pointed out, when he and Tony Huang wrote a review on this subject in 2006, there were only four examples of ubiquitin and SUMO regulation in DNA repair (Huang & D’Andrea, 2006). Just four years later, there are so many more exciting ubiquitin-controlled mechanisms in the DDR field, a trend that is likely to continue in the future.

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