Travels with ubiquitin: from protein degradation to DNA repair

» I watched out for emerging fields in eukaryotic biology and for people who ask the most interesting questions. «

Uncertain whether to follow full steam my interests in geology or perhaps biology, I entered university in Berlin with the strong desire to become a researcher. Although my geologist's hammer has still its firm place in the trunk of my car, I never regretted my decision to focus on biology. In particular the precision and elegance of genetics attracted me, and this fascination still drives my research. Finishing my PhD studies on the genetics of DNA methylation in bacteria with Thomas Trautner at the Max Planck Institute for Molecular Genetics in Berlin, I watched out for emerging fields in eukaryotic biology and for people who ask the most interesting questions. Browsing through *Cell*, I was struck by two back-to-back papers from Alex Varshavsky, a brilliant researcher who was at that time at the MIT, USA. In these two, now classical papers Alex and colleagues reported that protein modification by ubiquitin ('ubiquitylation') is not only important for the elimination of abnormal proteins, but also for viability and cell cycle progression. This suggested to me that ubiquitin has much more in store than being merely a 'garbage controller'. At this time, Alex was already famous for his original research in several other areas, and also the imaginative methods he invented, for example a technique that is now called chromatin immunoprecipitation (ChIP).

A few months later, in 1985, equipped with a stipend from the Deutsche Forschungsgemeinschaft (DFG) and a small folder, which contained all published work of this new and exciting field, I started my postdoctoral work in Alex's lab. After periods of frustration and failed experiments, I finally decided to clone the genes that encode ubiquitin-activating and -conjugating enzymes. Hundred litres of yeast culture, enzyme purification, cyanogen bromide cleavage, Edman degradation and phage library screens finally brought success, and I named my genes *UBA1* (ubiquitin-activating enzyme, E1) and *UBCs* (ubiquitin-conjugating enzymes, E2s). One of my *UBCs* turned out to encode the cell cycle protein Cdc34; another encoded the DNA repair protein Rad6 (Jentsch et al, 1987). In particular Rad6 was quite famous already at this time because an entire DNA repair pathway carries its name. Cdc34 got fame a bit later when it turned out that this enzyme is the conjugating enzyme for SCF and related cullin-based E3 ubiquitin ligases.

Largely through the pioneering work of Avram Hershko, Aaron Ciechanover and Irving Rose, who received the Nobel Prize in chemistry for the discovery of ubiquitin-mediated protein degradation, ubiquitin was generally considered as the protein with the 'license to kill'. However, my findings indicated for the first time that ubiquitylation may play 'non-proteolytic' roles as well, and that one function is to promote DNA repair. Indeed, 'non-proteolytic' ubiquitin is now appreciated to be as important as 'proteolytic', since it not only mediates a plethora of DNA repair functions but also protein sorting, cell signalling and gene expression (Fig 1).

From protein degradation...

Heading back to Germany in 1988, I found a splendid position as an independent group leader at the renowned Friedrich Miescher Laboratory of the Max Planck Society in the small historical

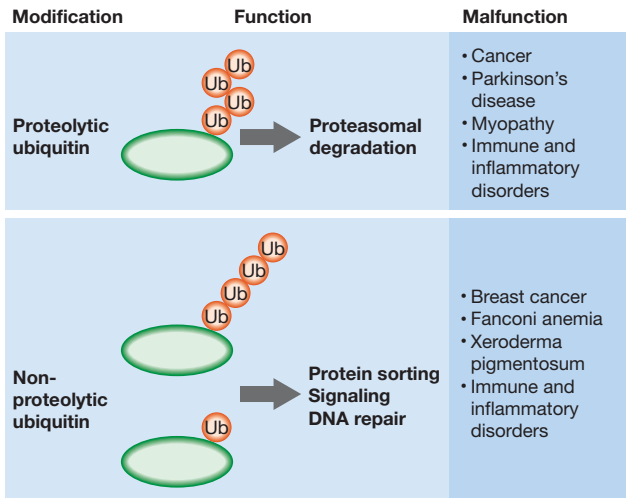

Figure 1. 'Proteolytic' and 'non-proteolytic' ubiquitin modifications in health and disease. Proteins modified by specific (*e.g.* lysine-48-linked) poly-ubiquitin chains (red) are normally targeted to degradation. 'Non-proteolytic' ubiquitin modifications are mono-ubiquitin and a non-canonical (lysine-63-linked) poly-ubiquitin chain. Malfunctions of the respective pathways can cause numerous human diseases of which selected ones are listed.

university town of Tübingen. Being independent for the first time is a challenge, but there's nothing more exciting than setting up your first own lab! I quickly had a fantastic small group, and our 'Big Plan' was to clone all genes of the ubiquitin pathway. A bit too ambitious as we now know, but we cloned almost all *UBC* genes from yeast and also cloned the first *UBC* genes from man, mouse, flies and worms (which we termed *UbcH1*, *UbcM1*, *UbcD1*, *etc.*). Yeast genetics showed again all its legendary 'awesome powers': we found enzymes that mediate bulk protein degradation and cadmium tolerance, and an endoplasmic reticulation (ER)-bound enzyme that acts on ER-resident proteins. This pathway, now known as ER-associated degradation (ERAD), was previously believed to function inside of the ER. However, our studies indicated for the first time that proteins of the ER are rather degraded in the cytosol (Sommer & Jentsch, 1993). ERAD became a hot topic because of its medical importance, *e.g.* for cystic fibrosis, and many dedicated factors were discovered afterwards. Also in the early 1990s, several labs had shown that large particles, coined 'proteasomes', can degrade ubiquitylated substrates *in vitro*, but whether they also do so *in vivo* was controversial at this time. However, using the blazing speed of yeast genetics, we demonstrated quickly that proteasome mutants indeed stabilize otherwise short-lived ubiquitin conjugates.

...Via signal transduction...

After five exciting years in Tübingen, I became full professor for cell biology at the Center for Molecular Biology of Heidelberg University (ZMBH), which played a model role in Germany as a faculty-spanning research centre. I feel blessed as I always had a lab full of fantastic and interacting people, and Heidelberg was certainly no exception. There, we identified a new ubiquitin-like modifier, Rub1/Nedd8 and all its enzymes as well as its cullin target (Liakopoulos et al, 1998). We added protein biochemistry to our mainstay genetics and discovered a previously unknown activity we termed 'E4', which acts on mono-ubiquitylated proteins and promotes poly-ubiquitin chain elongation in conjunction with E1–E3 (Koegl et al, 1999). After a brief period of skepticism, our concept of stepwise ubiquitylation caused indeed a paradigm change, as many pathways are now known to require first mono-ubiquitylation for one function, and subsequent poly-ubiquitylation for another function. Examples can be found for proteins involved in cell cycle control, transcription, signal transduction and DNA repair.

> *» At that time, the ubiquitin field was already one of the hottest on the market. «*

At that time, the ubiquitin field was already one of the hottest on the market, and it was difficult to find an issue of a leading journal that had no article on ubiquitin within its pages. But things got 'worse': several ubiquitin-like proteins (UBLs) were discovered, including SUMO, Rub1/Nedd8 and two UBLs that control autophagy. Even more confusing, 'cross-talks' between different UBLs were described, and Cecile Pickart and others showed that ubiquitin could be assembled into poly-ubiquitin chains in more than one way to promote different functions.

After a few years working in Heidelberg, I received an offer from the Max Planck Society to head a department at the Max Planck Institute of Biochemistry in Martinsried near Munich. We moved in 1998, and shortly after we discovered an intriguing signal transduction pathway, which turned into a scientific gold mine. We identified two yeast homologues of the popular mammalian transcription factor NFκB as substrates of a specific ubiquitin ligase we run after. However, in yeast, these two factors, Spt23 and Mga2, are anchored to the ER membrane and seem to sense its lipid composition. In the absence of unsaturated fatty acids, their C-terminal membrane anchors are degraded by proteasome-dependent ERAD, thereby setting free their tightly folded and degradation-resistant N-terminal domains. These then enter the nucleus and drive transcription of *OLE1*, encoding fatty acid desaturase (Hoppe et al, 2000). We realized that mobilization of the cleaved transcription factors from the ER membrane also requires a conserved chaperone known as Cdc48 or p97 (Rape et al, 2001). We found that Cdc48/p97 preferentially binds ubiquitylated proteins, 'segregates' them from their environment, and often delivers them to the proteasome (Rape et al, 2001; Richly et al, 2005). This interesting enzyme is now in the limelight of ubiquitin research, as it seems to regulate many functions of medical importance: from ERAD in the cytosol to DNA repair in the nucleus.

...To DNA repair

SUMO, ubiquitin's mysterious cousin, caught our interest as well. One SUMO substrate we purified is to blame for a major shift in our scientific directions. This protein, proliferating cell nuclear antigen (PCNA), is a processivity co-factor of DNA polymerases, but also couples DNA synthesis to many replication-linked functions. My talented students found that PCNA is not only SUMO modified ('SUMOylated') but also ubiquitylated. Indeed, PCNA can be alternatively modified on the very same residue by either SUMO, a single ubiquitin moiety or by a specific (lysine63-linked) poly-ubiquitin chain (Hoege et al, 2002). I was particularly pleased when we found out that the key enzyme involved in PCNA ubiquitylation is Rad6, the protein I isolated during my postdoctoral studies. These three PCNA modifications mediate three different functions, and hence we called it 'The PCNA Switch' (Fig 2). This switch is important in all eukaryotes, including us humans, for flawless duplication of the genome, but paradoxically also for generating mutations. Damaged DNA, *e.g.* damaged by UV light, poses major

> *» The PCNA switch is important for flawless duplication of the genome, but paradoxically also for generating mutations. «*

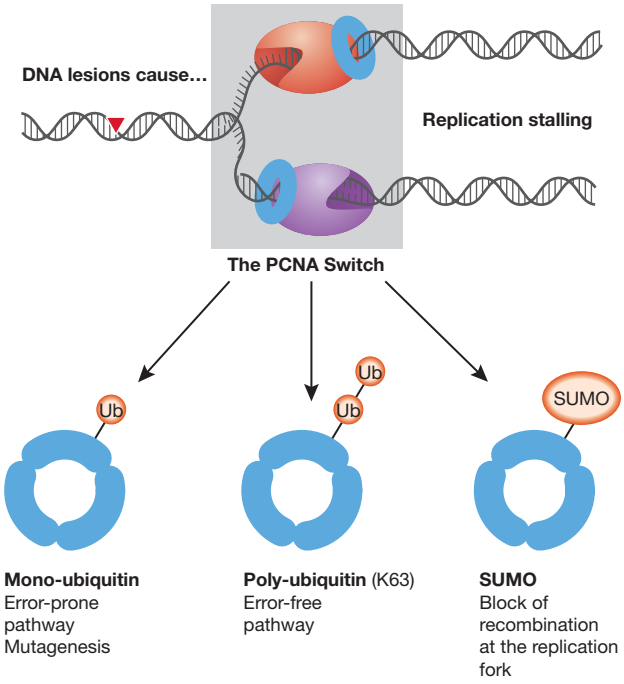

Figure 2. The PCNA switch.
Damaged DNA, *e.g.* by UV light, can interfere with replication, which can cause chromosome breakage and cancer. The PCNA switch controls three modes of DNA damage tolerance *via* mono-ubiquitylation, poly-ubiquitylation and SUMOylation. Notably, the switch controls mutagenesis, which causes altered or defective genes, but also supports evolution of species.

obstacles during replication. This situation can lead to a complete replication stop, but also to broken chromosomes and in humans to cancer. However, this situation is usually prevented through activation of the 'PCNA switch' (Hoege et al, 2002; Karras & Jentsch, 2010). PCNA mono-ubiquitylation recruits dedicated 'translesion' DNA polymerases that can operate even on damaged DNA. However, these enzymes are 'sloppy', *i.e.* they can make errors and thereby produce mutations. PCNA poly-ubiquitylation, on the other hand, activates another pathway, which seems more complex but does not make errors or create mutations. Finally, PCNA SUMOylation blocks a third, more dangerous rescue pathway (Pfander et al, 2005). The 'PCNA switch' thus decides which DNA damage tolerance pathway is used. Although the 'error-free' pathway seems to be the most reasonable, the 'error-prone' mechanism, which transforms, *e.g.* UV light-induced DNA lesions into mutations, is elementary for altering genes by mutagenesis. This pathway is beneficial, *e.g.* for generating antibody diversity through IgG gene hypermutation, and, last but not least, elementary for the evolution of species.

As the name 'ubiquitin' suggests, ubiquitylation is widespread and new and exciting functions connected to this protein are discovered every year. Thus, perhaps not surprisingly, many human diseases—from breast cancer, Fanconi anemia, *Xeroderma pigmentosum* to Parkinson's disease—are caused by malfunctions of the pathway. Factors of the ubiquitin pathway are hence in the focus of new drug design, and the same can be expected for proteins involved in SUMOylation. Besides all this, what makes ubiquitin and SUMO research so special and particularly fun is that one can sail from one research area to the next, landing on new unexplored grounds, but still with the firm belief that new excitement is on the horizon.

The author declares that he has no conflict of interest.

Stefan Jentsch

Stefan Jentsch is awarded the 2011 Louis-Jeantet Prize for Medicine for his work on small protein modifiers. He is a Director at the Max Planck Institute of Biochemistry, Martinsried, Germany.

FONDATION LOUIS-JEANTET

Department of Molecular Cell Biology,
Max Planck Institute of Biochemistry,
Martinsried, Munich, Germany
E-mail: jentsch@biochem.mpg.de

References

Hoege C et al (2002) Nature 419: 135-141
Hoppe T et al (2000) Cell 102: 577-586
Jentsch S et al (1987) Nature 329: 131-134
Karras GI et al (2010) Cell 141: 255-267
Koegl M et al (1999) Cell 96: 635-644
Liakopoulos D et al (1998) Embo J 17: 2208-2214
Pfander B et al (2005) Nature 436: 428-433
Rape M et al (2001) Cell 107: 667-677
Richly H et al (2005) Cell 120: 73-84
Sommer T et al (1993) Nature 365: 176-179

DOI 10.1002/emmm.201000116

