EMBO reports

The seven wonders of ubiquitin: a multi-interview

Personal insights into the ubiquitin field

PARTICIPANTS

Ivan Dikic, Professor and Director of Institute of Biochemistry II at the Goethe University and Director of the Buchmann Institute for Molecular Life Sciences (BMLS), Germany
Wade Harper, Professor of Cell Biology, Harvard Medical School, USA
Ron Hay, Chair of Molecular Biology, University of Dundee, UK
Thomas Langer, Professor at the Institute for Genetics, University of Cologne, Germany
Michael Rape, Professor of Cell and Developmental Biology, University of California at Berkeley and Investigator, Howard Hughes Medical Institute, USA
Titia Sixma, Division of Biochemistry, Netherlands Cancer Institute, Netherlands
Henning Walczak, Professor of Cancer Biology, University College London, UK

This issue of EMBO reports highlights a new Review series on 'Ubiquitylation: mechanism and functions' that started last issue with a discussion of the mechanisms of cullin-RING E3 ligase assembly. This month includes an analysis of the role of ubiquitin in the immune system, and subsequent issues of the journal will feature the roles of ubiquitylation in mitochondrial homeostasis and in stem cells, as well as how RING-between-RING (RBR) E3 ligases function and how recent structural work has contributed to our understanding of the ubiquitylation cascade.

Most of the senior authors of the Reviews included in this series came together at the recent EMBO Conference on 'Ubiquitin and Ubiquitin-like proteins: from structure to function', held in Riva del Garda in November 2013. It was a good opportunity to gather their personal insights into the most important recent developments, future challenges and their impressions on working in the ubiquitin field. We also took the opportunity to include the viewpoint of two of our Editorial Advisory Board members—Ivan Dikic and Michael Rape.

The following multi-interview is an excerpt of their comments. Coming from various fields and at different career stages, this collage of their very personal opinions is a good overview of the current state of all things ubiquitin.

EMBO reports: What would you say have been the most significant contributions to the ubiquitin field in recent years?

Ivan Dikic: The biggest advance came from the realization that ubiquitin is involved in most of biological processes in so many unexpected ways. For example, different biological and physiological processes are regulated by specific ubiquitin signals. There are also numerous examples in which not only defects but also rewiring of ubiquitin networks is involved in human diseases. A significant progress in targeting the ubiquitin system for therapy has been accomplished. One good example is the clinically approved drug Bortezomib that targets the proteasome, but there are also other drugs, such as inhibitors of different conjugation enzymes and blockers of the ubiquitin decoding machinery. Altogether, the biggest advance is the spread of ubiquitin functions across biology and medicine.

Titia Sixma: I think it is the realization that, although the interactions are weak, there is a lot more specificity in the system than we had initially realized, that these modifications are selective.

Henning Walczak: In my view, one of the most important realizations has been that different types of ubiquitin linkages recruit different ubiquitin receptors that lead to completely different outcomes. There is a lot more diversity in the ubiquitin system than we originally thought, when ubiquitin was solely thought to be a tag targeting proteins to the proteasome. It started with the discovery that K63 linkages lead to signaling. Then linear linkages were also shown to play a role in signaling, even though there were some controversies about this when this was first reported. I think it is now obvious that not only linear and K63, but also other linkage types, lead to signaling outputs depending on the context they are in. There is a lot of plasticity in all of the signaling complexes that involve ubiquitin.

Wade Harper: One of the major advances is the integration of mechanisms in structural biology. For a long time, you would know that ubiquitin was connected to a given pathway, or you might know what the molecules involved were, but actually knowing how they work together was not really possible. It took time to develop methods to analyze these often complex reactions. Now we have a pretty good understanding of the fundamental biochemical mechanisms for the key enzymatic processes. However, much still needs to be done to understand the entire molecular pathways for the most complex ubiquitin transfer cascades.

Michael Rape: I wouldn't want to point it down to a single finding, rather to general concepts about specificity in the ubiquitin system: that it extends beyond single proteolytic modifications; that there are many different chains, different deubiquinating enzymes (DUBs). How the degree of complexity is currently recognized, not only in terms of numbers, but also of functional interactions, which have been identified through dynamic and quantitative proteomic

Wade Harper is the Bert and Natalie Vallee Professor of Molecular Pathology in the Department of Cell Biology at Harvard Medical School (Boston, MA, USA). He received his PhD in Chemistry at the Georgia Institute of Technology (Atlanta, GA, USA) prior to post-doctoral studies in biological chemistry at Harvard Medical School. In 1988, he joined the faculty in the Department of Biochemistry at Baylor College of Medicine (Houston TX, USA) before moving to Harvard Medical School in 2003. His work focuses on the analysis of ubiquitin-driven signaling systems and the integration of quantitative proteomics to understand mechanism. [Photo credit: Nathan Harper].

analysis. The combination between *in vivo* cell biology and *in vitro* biochemical reconstitution has always been a strength in the field. Over the last years, many interesting developments have occurred at that interface, and new technologies have pushed the field forward.

Ron Hay: For me, the biggest advances have come from the structural analysis of the components of the conjugation machinery. We now have a pretty good understanding of how ubiquitin is activated at the E1 step, how it is transferred onto the E2 and from E2s onto both HECT E3 ligases and substrates. There is also structural work on large complexes—like the anaphase promoting complex—that is benefiting from the combination of electron microscopy and X-ray crystallography. In all, I think the whole ubiquitin transfer cascade has been illuminated over the last couple of years by really nice structural biology.

Er: What kind of translational/medical applications or developments could come from this field in the mid-term?

WH: We have already seen the beginnings of it, with inhibitors in the cullin system. Big pharmaceutical companies have had less impact in the field than a lot of the smaller ones, in terms of actually identifying targets and molecules. Within the biotech industry, there are several compounds that are making significant progress. There is a major emphasis on trying to target the ubiquitin pathway to treat neurodegenerative diseases. I think the role of aberrant protein turnover in cancer is pretty clear and if you can identify drugs that work in cancer, it will have an important impact on other diseases. In addition, a large amount of protein aggregation occurs in neurodegenerative diseases. The challenge is to develop methods that would reverse some of the effects of aggregated proteins, or otherwise find ways to eliminate aggregates.

MR: Having founded a company myself, I strongly believe that there are a lot of opportunities in this field; the proteasome inhibitors and the thalidomides and related compounds are just the tip of an iceberg. We will have to learn to translate the recent findings regarding the mechanism of ubiquitylation—how you generate specific chains, how you get activity and timing right—into drugs. It is not going to be a simple ATP-binding pocket as for kinases, but rather one will need to find allosteric activators, allosteric inhibitors or binding site modulators. Cancer genomics over the last few years has shown that there are very good targets to be attacked for diseases with no good therapeutics available, so there is a big need. I think ubiquitin can fill that. Such drugs could be used to treat cancer, chronic inflammation and neurodegenerative diseases, for example. You can think about autophagy as a pathway linked to ubiquitin, and modulating this pathway would have a lot of therapeutic benefit. An increasing number of ligases are also linked to developmental processes, so their misregulation causes developmental diseases.

RH: Up to now, we really only had inhibitors against the proteasome or the NEDD8 E1. I think the big translational challenge is to find inhibitors of the specificity step, which is catalyzed by the E3 ligase. We now have assays that are suitable for high throughput screening and there are many screens underway to try to identify such inhibitors. However this may be difficult, because the libraries we have may not contain chemical matter that can inhibit the components of the ubiquitin system. Over the next couple of years this may be possible as better libraries come on stream.

Er: In which direction do you see your lab going in the next few years?

Thomas Langer: My group is interested in mitochondria, mitochondrial quality control, mitochondrial proteases and protein turnover, which is how we entered the ubiquitin field. It became clear that ubiquitin plays a major role, not only in regulating the

Titia Sixma is Head of the Division of Biochemistry at the Netherlands Cancer Institute and Professor by special appointment at the Erasmus Medical Center. She was trained as protein crystallographer, received a PhD from Groningen University and performed post-doctoral research at Yale University. Her research uses structural biology and biochemical approaches to understand ubiquitin signalling pathways with particular emphasis on DNA and chromatin-associated pathways. She is a member of the Royal Netherlands Academy of Arts and Sciences, of the Academia Europaea and of EMBO. [Photo credit: Ben Balster].

Thomas Langer is Professor at the Institute for Genetics, University of Cologne (Germany). He studied biological sciences at the University of Regensburg (Germany) and obtained his PhD at the Ludwig-Maximilians-University Munich (Germany). After working as a visiting scientist at the Memorial Sloan-Kettering Cancer Institute in New York, he became group leader at the Ludwig-Maximilians-University Munich. Since 2000 he holds a position as Professor of Genetics at the University of Cologne, where he serves as coordinator of the collaborative research center 635 of the DFG (German Research Council), as vice-coordinator of the Cluster of Excellence Cologne (CECAD) and as prorector for research and young academics. He is interested in mitochondrial quality control, membrane and phospholipid dynamics. In 2008 he was awarded an ERC advanced career grant. Thomas Langer is an elected member of EMBO, of the Academy of Sciences and Arts North-Rhine Westphalia and of the Leopoldina (German National Academy of Sciences).

mitochondrial proteome at the outer membrane, but also regulating the dynamics of other mitochondrial processes, such as autophagy. We are really interested in identifying how ubiquitylation determines the regulation of mitochondrial dynamics, for example. Many components have been identified, but under what physiological conditions is this regulated? Regarding protein turnover at mitochondria, there is a lot of discussion about ERAD pathways at the mitochondrial outer membrane (MOM), but I think this is by far not fully understood and we would like to explore this. Parallels between ERAD and turnover of MOM proteins do exist but there is a tendency to oversimplification. It is likely to be much more complex, in terms of regulation and membrane dynamics, exchange with other lipids and vesicle, etc. I think ubiquitylation plays a central role in the regulation of these processes.

TS: We are now interested in the specificity of ubiquitylation, and we will study this, both on the DUBs and E3 ligases. I am also interested in how DUBs and E3 ligases collaborate and talk to each other, although we have not been working directly on this so far.

MR: We always tend to let the biology drive the questions that we study, so it is hard to predict. About seven years ago, when I started my lab, I would not have predicted I would work on specific ubiquitin chain types, for example. One big knowledge gap in our field is that there are 600–800 E3 ubiquitin ligases in humans and substrates are known for 10% or less of them. There must be a reason for this and I doubt that it is just technical. I think we did not look in the right model systems; we tend to focus on cancer cells, for example. One big direction my lab has recently taken is to move away from cancer cell lines to untransformed systems: stem cells and differentiation models. We can study ubiquitylation, which is a dynamic modification, in these dynamically changing environments. In so doing, we have picked up many interesting ligases and substrates.

WH: In general, the tools that we routinely use to examine interaction networks or map ubiquitylation sites, for example, can be used in traditional cell lines but applying these approaches to more relevant cells can sometimes present a challenge. For example, studying certain pathways in cancer cell lines is not optimal for understanding the actual mechanism *in vivo*, or connecting it with a disease. One of the things that we are trying to do is develop induced pluripotent stem (iPS) cell systems to study some of the ubiquitin-related pathways at a systems level, using the tools that we developed in cancer cells. However, there is a technical hurdle: you don't get a comparable number of cells and so the ability to detect things is much lower, and moreover, the purity of differentiated iPS cells might limit interpretations based on tools that ask questions about the bulk population. Initially, it may be necessary to scale back and focus on either particular questions or particular pathways, and not just generate one more set of data. We are taking this approach in the context of neurodegenerative diseases.

RH: In the short term, our big challenge is to understand how E3 ligases work, particularly the RING type. Within the last year or so, we managed to understand how the RING activates the ubiquitin ~ E2, but we still don't have an analysis of how substrate is really brought to the ubiquitin ~ E2. We are now trying to obtain the structure of SUMO-targeted ubiquitin ligase RNF4 bound to a poly-SUMO chain of a defined length, in complex with Ubiquitin ~ E2. We hope this will allow us to see how the lysine residue

Ron Hay is Professor of Molecular Biology at the Centre for Gene Regulation and Expression and Honorary Member of the MRC Protein Phosphorylation and Ubiquitination Unit, University of Dundee. He attended Heriot-Watt University, Edinburgh and obtained his PhD from the University of Glasgow. His postdoctoral research was carried out at Harvard Medical School, Boston before joining the MRC Virology Unit, Glasgow as an Independent Investigator. In 1985 he joined the Biochemistry Department of the University of St Andrews and in 2005 took up his present position in Dundee. Ron's work focuses on establishing the roles of Ubiquitin and Small Ubiquitin-like Modifier (SUMO) as signaling components. Ron is a Wellcome Trust Senior Investigator and a fellow of the Royal Society, the Royal Society of Edinburgh, the Academy of Medical Sciences, Academia Europaea and is a member of the EMBO. In 2012, Ron was awarded the Novartis Medal and Prize of the Biochemical Society.

Henning Walczak is Professor of Cancer Biology at University College London (UCL) and Chair of the Centre for Cell Death, Cancer and Inflammation (CCCI) at UCL's Cancer Institute. Following his PhD in 1995 at the German Cancer Research Centre in Heidelberg, Henning Walczak worked as a postdoctoral researcher at Immunex Corporation in Seattle (WA, USA), before returning to the German Cancer Research Centre as group leader in 1998. In 2007 he was appointed Chair of Tumour Immunology at Imperial College London, and joined UCL in 2013 to assume his current position. He has received the Biofuture Prize of the German Ministry for Science and Education (2000), an ERC Advanced Grant (2012) and a Wellcome Trust Senior Investigator Award (2012). Henning's lab works on cell death and inflammation with a special focus on the biology of the different death receptor-ligand and the ubiquitin systems. His research aims at developing new therapies for cancer. [Photo credit: Kerstin Schmid].

that is going to be modified is poised in the active site of the enzyme, which will give us some insight into how the acceptor lysine is selected. I think it is going to be quite a difficult challenge.

HW: I am very interested in deciphering the ubiquitin code at the tumor necrosis factor (TNF) receptor-signaling complex, which is a molecular machine that I find fascinating. In fact that is the short title of my ERC Grant! Linear ubiquitination plays a role in TNF signaling, but we have also found K63, K11, K48, and there may be more. We need to find out the exact sequence of events, where exactly is which linkage type placed and how do the different DUBs de-construct this. The other aspect my lab is interested in is to understand the outcome of this signaling in terms of autoimmunity and cancer. Can we harness what we have learned about linear and other types of ubiquitination at receptor signaling complexes to identify new treatments for autoimmunity and cancer?

ID: We need to understand more details about the spatiotemporal dynamics of the ubiquitin system *in vivo*. Along these lines we have recently developed specific engineered sensors (GFP-tagged versions of ubiquitin binding domains) that can decode specific ubiquitin chains and can be used to detect specific ubiquitin signals on depolarize mitochondria or DNA damage foci in the nucleus. I believe that by using the high-resolution microscopy we can better distinguish local ubiquitin signaling complexes, as seen for example on the surface of cytosolic *Salmonella*. We are also focusing on how ubiquitin can regulate selectivity in autophagy, which is one of the processes where Ub plays an important role. The transfer of ubiquitin knowledge to the autophagy field has given us good recognition. I am always intrigued by the possibilities of molecular medicine, and ubiquitin might lead us to another medically relevant application. At the moment, we are focused on infection and cancer.

Er: The ubiquitin field is growing tremendously and spans virtually all aspects of cell biology. What spirit would you say dominates the field?

TL: I think it is both collegial and competitive. It is collegial because many people from different backgrounds come together, so there is a lot of potential for collaboration without having the problem of too much overlap. I think it is a very attractive field for young people. Of course, it is a competitive field, but a field that is not competitive is maybe also less interesting. We came into this field unexpectedly and felt very welcome. We had no problems in interacting with other groups, possibly because we came from a different angle. From that point of view, it is a positive and open field. In general, I have had no bad experiences, quite the opposite.

HW: As I said, there was some controversy when linear ubiquitination was discovered to be important in TNF-induced signaling. Some people misinterpreted that as a statement against a role for K63 in this process but that was never my take on it. And in the end it did turn out that both linear and non-linear ubiquitylation were important in this process. As it now stands the non-linear component actually doesn't have to be K63, it can also be K11 or another linkage type. In general, I find the ubiquitin field very collegial and at the same time highly competitive. I sense a real spirit of excitement in the field because of the recent discoveries of

Ivan Dikic is Professor and Chairman of Institute of Biochemistry 2 at the Goethe University Medical School and Scientific Director of the Buchmann Institute for Molecular Life Sciences in Frankfurt (Germany). Before joining Goethe University, he was a Group Leader at the Ludwig Institute for Cancer Research in Uppsala (Sweden). He was trained as a medical doctor in Zagreb, Croatia and obtained his PhD in molecular biology from the University of Zagreb under the supervision of Joseph Schlessinger at New York University Medical Center. Ivan's research focuses on the role of ubiquitin in the regulation of autophagy, DNA repair, inflammation, cancer, infection, receptor endocytosis, and proteasomal degradation. He contributed to the identification of linear ubiquitination, a signal that can regulate the NF-kB pathway, making a decision between cell survival and death pathways. [Photo credit: Uwe Dettmar].

Michael Rape is Professor of Cell and Developmental Biology at the University of California at Berkeley and Investigator of the Howard Hughes Medical Institute, USA. He obtained his PhD from the Max-Planck-Institute of Biochemistry in Martinsried (Germany), after which he carried out postdoctoral research at Harvard Medical School. In 2006, he joined the faculty of the Department of Cell and Developmental Biology at the University of California at Berkeley, where he has held various positions ever since. In 2013, he joined the Howard Hughes Medical Institute. He has won numerous accolades, including the NIH Director's New Innovator Award, the Vilcek Award for Creative Promise and the Curci Foundation Award. Michael's research is focused in understanding how proteins are modified with ubiquitin, how processes in the cell are regulated by ubiquitylation, and how to alter ubiquitylation to treat disease. [Photo credit: Daily Californian].

ubiquitin's role in so many different aspects of biology. It is great to be part of that.

WH: When I first started, I was in the cell cycle field, which was pretty contentious for a long time; it is highly competitive. I find that, overall, the ubiquitin field is extremely collegial. People tend to share reagents and ideas. That doesn't mean that it is not competitive, it is very competitive, but overall, I think the field is very positive in its outlook and collaborative spirit.

ID: The ubiquitin field has a tradition of professional and friendly relations that was initiated by the pioneers in the field—like Aaron Ciechanover, Avram Herschko, Alexander Varshavsky, and others—who trained an extremely high number of the people who continued to develop the field to where we are today. I think these standards have prevailed; the field is competitive, but there is a very professional competitive atmosphere. Friendships and competition go side by side and it is a real pleasure to work in this field. The acceptance of new PIs has been tremendous; we actually need more young PIs, more people coming from different disciplines, with different ideas. Because of the nature of the knowledge in the ubiquitin field, there was never a lack of topics to study, but a lack of people, because there is a lot to discover. At the end of the day, we sometimes compete, sometimes publish together, sometimes disagree, but with time things are resolved scientifically. This is the strength of the field.

RH: I think it is a very collegial field and there are a lot of new people coming into it. In most cases, people interact very well. Over the last few years in Europe for instance, we had quite a large consortia funded by the EU and there was a very nice spirit within that consortia. People interacted well, they shared reagents, and in general they were supportive of young people establishing new areas.

Er: Would you choose the ubiquitin field now, if you were to start all over?

TS: For structural biology it has never been easy. I'm not sure if 10 years ago I would have chosen it had I known that, but I found it very interesting. The sort of molecular mechanistic focus of the field was very attractive to me. I think there are still many open questions, especially on the interplay between ubiquitin conjugation and other signaling systems, and so there is a lot to do. However, it is not easy to enter the field from the biochemical side, as it is becoming technically quite sophisticated. You have to learn the technology, but there are courses to help people get to that level of sophistication, and that is useful.

MR: Ubiquitin is a very supportive field, so I think it is a wonderful field to start to work in. I received a lot of support in my career from more established people. The questions that have been answered are very few compared to those we don't know anything about. We still have so much to learn, especially from a biological perspective. It is also one of the few fields where you can really combine cell biology, biochemistry and mechanism. It is established that this is the way it should be, which can be a very fulfilling because you can dip your hands in a lot of different areas. I would certainly choose it again.

RH: Yes, I would. In fact, I have worked in a few different fields over the years, in DNA replication and transcriptional regulation mediated by NF-κB. I really enjoy the ubiquitin field because it touches on many different biological problems, and so there is really a wide interest.

WH: Yes; it touches so many aspects of biology. Every day there is something new to think about and you can spend time going down one road or another road, and still have tons to do.

EMBO reports thanks the participants of this multi-interview for their time and effort.
The interviews were conducted and coordinated by Nonia Pariente.

DOI 10.1002/embr.201338230