Laudatio for Dr. Thomas Wollert

Max Planck Research Group Leader,
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Winner of the Eppendorf Award for Young Investigators 2015

The laudatio was held by Prof. Reinhard Jahn (Director at the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) at the prize ceremony at the EMBL Advanced Training Centre in Heidelberg on June 25, 2015.

»The generation of waste and the need for waste disposal and recycling is not only a problem of modern societies. Rather, generation of waste is also a feature of every biological system, not only of whole organisms but of every single cell. Principally, biological systems face the same problem as modern societies – what to do with the garbage? However, nature has solved the task of recycling of irreparably damaged parts much better and efficiently than we have done so in our daily lives.

Cells produce sophisticated molecular machines composed of many complex parts. Occasionally, cells wind up with some that are not properly assembled and do not pass quality control checkpoints. Furthermore, like every man-made device biological machines wear down during use and become damaged or even non-functional, and these components need to be dealt with by the cellular garbage recycling services. Indeed, every eukaryotic cell possesses at least three recycling routes for different types of cellular garbage. The first of these routes directs proteins that do not reach their appropriate final shape or soluble proteins that get

damaged later to a shredder located in the cytoplasm, termed proteasomes. Here, the macromolecules are disassembled into their building blocks. The second pathway deals with membrane proteins that get damaged during use. This pathway begins with the flagging of the damaged molecules by ubiquitin, followed by engulfment of chunks of the plasma membrane containing these damaged proteins, a process termed “endocytosis”. The endocytosed membrane vesicles then fuse with endosomes. Then, in a sophisticated process, these endosomes “endocytose” vesicles, i.e. they engulf part of their own membrane containing these damaged proteins, resulting in small vesicles in their interior, so called “multivesicular bodies”. These multivesicular bodies then fuse with lysosomes, the central recycling stations of every cell that are filled with digestive enzymes. Here, not only the damaged membrane proteins but also the membrane lipids are hydrolyzed, resulting again in the generation of the building blocks that can be re-used. If the damage is more profound, e.g. involving aggregates etc., there is a “last resort” pathway to save the cell from death. This pathway is capable of handling almost any type of macromolecular waste: Here, membrane cisternae are being generated from scratch. These cisternae can wrap themselves around cellular constituents of almost any kind, including mitochondria, cytoplasm, even chunks of the nuclear membrane. The pathway is termed “autophagocytosis” – “self-eating cytosis”. Originally discovered as an emergency pathway during cell starvation, it is now being appreciated as being not only essential for every eukaryotic cell but also for being highly adaptable and regulated at multiple levels. The molecular machinery responsible for this rather involved remodeling pathway is known, at least in its basic makeup, but it is still rather enigmatic how they work at the mechanistic level. The system includes two molecular cascades resembling the ubiquitin pathway, and it involves the formation of unique links between some of the proteins and the membrane cisternae. However, it is still enigmatic how the sequence of protein-protein interactions and modifications are performing the tasks: the directed formation of the preautophagosomal structure, followed by the engulfment of substrate and finally the closure of the double-layered vesicle that then delivers its entire content into the lumen of the lysosome. Mechanistic problems of this kind cannot be solved with genetics (although genetic tools are indispensable), and this is probably why progress has been much slower than during the original discovery of the genes. Rather, addressing such problems requires sophisticated biochemistry, i.e. the *in-vitro* reconstruction of individual steps, and ultimately the entire

sequence, in the test tube. It is exactly this challenge that has been taken up by Thomas Wollert, the winner of the 2015 Eppendorf Young Investigator Award. Before Thomas gives his award lecture, let me briefly introduce him.

Thomas Wollert is a biochemist by training who did his undergraduate studies in Potsdam, then moved to Hannover. As he has told me, there was too much plant science in Potsdam which at that time did not really meet his main interests. Hannover was also attractive for him because of the focus on biophysics and structural biology of the curriculum. In particular, he was attracted to structural biology. For his PhD thesis, he therefore went to the Helmholtz Center for Infection Biology in Brunswig to work on the structure of Listeria proteins. This work resulted in an important discovery. Listeria is a human-specific pathogen that does not infect mice, thus preventing the development of convenient animal models. Thomas and his colleagues analyzed the recognition complex of the listerial invasion protein InlA and its human receptor E-cadherin. Based on the structure, Thomas and colleagues predicted, and then proved experimentally, that substitution of only few amino acids in InlA would increase its affinity, in fact, as it turned out, by four orders of magnitude! With this small modification the affinity was sufficiently high to also infect mice by binding to the formerly incompatible mouse variant of E-cadherin, thus creating a versatile animal model of human listeriosis.

Unknowingly to myself, Thomas has told me that I have had some influence on his future path. Many years ago, he learned about our work on the molecular structure of synaptic vesicles. He became fascinated by membranes and membrane remodeling, and it also became clear to him that he did not want to be limited to structural biology. Then he heard a presentation of Jim Hurley, then at the NIH, which fascinated him. Jim Hurley is a structural biologist who is interested in protein membrane interactions. Thomas talked to him and eventually joined his lab as a postdoctoral fellow.

As you may appreciate, Thomas is someone who very much has his own mind and does not like to follow the beaten path. Quite in character, he did not agree with the project proposed to him by Jim, which was not sufficiently ambitious for him – he was craving for a really big challenge that would be worthy to dig in. His plan was nothing less than reconstituting one of the degradative pathways I have told you

about – the formation of multivesicular bodies that constitutes the decisive step in the disposal and recycling of membrane proteins. The protein machinery was again previously discovered by genetics (with seminal contributions by Scott Emr), and structural biologists were in the process of crystallizing all of the components. Intriguingly, however, the invagination of the vesicles at the endosomal surface, i.e. the central membrane remodeling event of the pathway, was structurally not well understood. Thomas boldly took on the challenge to reconstitute this complicated process from scratch *in vitro* using purified components. Apparently, Jim Hurley's enthusiasm about this plan was moderate – probably he thought this young German scientist still has to arrive in reality, this is completely impossible. So Thomas was pretty much on his own which suited him well. He spent a bit of time in a neighboring lab (that of Jennifer Lippincott-Schwartz) to learn how to handle giant liposomes, and then set out to reconstitute membrane invagination.

Amazingly, Thomas was able to eventually pull it off – and the credit for this seminal work, published eventually in *Nature*, is indeed all his own. Essentially he demonstrated the first reconstitution of a protein-driven invagination and vesicle shedding process from purified components that recapitulates essential steps of the ESCRT pathway.

Instead of building on this success as most postdocs do when starting their own laboratory, Thomas, again rather daring, decided that it is not a good idea to continue with his postdoctoral project. Rather, after moving from Bethesda to Martinsried, he was looking for a new challenge where he could benefit from his experience in reconstituting membrane remodeling processes *in vitro*. He selected the process of autophagocytosis, which I am sure he is going to tell you is even more complicated than the ESCRT pathway. He again was able to succeed in this task – very much impressing everyone in the field including the editors of *Cell*. However, let Thomas tell us by himself what he has done – let's welcome this year's winner of the Eppendorf Young Investigator Award, Thomas Wollert!«