EMBO reports

meeting point

Tales of the autophagy crusaders

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The second EMBO Conference Series meeting on 'Autophagy in Health and Disease' took place in November 2011 in Israel. It brought together researchers from around the globe to cover the biogenesis of the autophagosome, as well as related topics including the regulation of autophagy, selective autophagy and the role of autophagy in disease and cell death.

Finding the source of the autophagosome membrane has become almost a religion to some in the autophagy field, so what better place to discuss this and other fascinating autophagy-related subjects than on a scenic hill above the historic city of Jerusalem. The excellent speaker roster was punctuated by talks from junior faculty, whose outstanding contributions, in particular those from Europe, Israel and Asia, have brought a vibrancy to the field that led to a great atmosphere and highlighted the many fascinating unanswered questions that continue to attract new people to autophagy. The two topics we have chosen to highlight in this meeting point, autophagosome biogenesis and selective autophagy, were a major focus of the workshop.

The origin of the membrane

To the casual scientific bystander, the wealth of recent data on the cellular source of the autophagosome membrane is probably confusing on several levels. First, there are a multitude of names in use—phagophore, isolation membrane, omegasome, phagophore assembly site or PAS—and this is complicated further by data that implicate several different organelles as contributors of membrane to the nascent autophagosome. For simplicity, we use the term phagophore in mammalian cells, and PAS in yeast, to define the first detectable structure in the pathway from which

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the autophagosome membrane grows. The phagophore or PAS is identified as the membrane at which the Atg1–ULK1 complex and the transmembrane protein Atg9 are found after induction of autophagy by acute nutrient deprivation, and where, in mammalian cells, a localized pool of phosphatidylinositol 3-phosphate (PI3P) is produced by the Atg14–Beclin1–Vps34 complex. Regarding the membrane source for autophagosome formation, a general consensus is emerging that the membrane source might be in large part dictated by the induction conditions and influenced by cell and tissue type as summarized in a recent review [1].

To set the stage for the full day session on the biogenesis question, Yoshinori Ohsumi (Tokyo Institute of Technology, Japan) presented technically superb live cell imaging data in yeast. This addressed the dynamics and function of Atg9. Using tagged Atg17, which in yeast is the first Atg protein to relocate to the PAS [2], to ask where and how Atg proteins move during starvation, Ohsumi showed that the mobility of Atg17 does not change in starvation conditions, but that Atg17 concentrates, or remains for a significant time, at the PAS. More intriguing were the data on Atg9, which is the only transmembrane protein among the group of 36 Atg proteins, and resides on vesicles and tubules in a unique compartment known as the Atg9 reservoir [3]. Data from high-speed imaging techniques both *in vivo* and *in vitro* using isolated vesicles revealed the dynamics and properties of Atg9 vesicles in PAS formation and showed that the number of Atg9 vesicles per cell increases after nutrient starvation. The lipid and protein profiles of these isolated 35 nm vesicles are being analysed. Oddly, there is now evidence of asymmetry in the double membrane of autophagosomes, in that Atg9 is only in the outer membrane and PI3P is primarily in the inner membrane. Ohsumi's conclusions suggest that the vesicles fuse with the PAS through a novel mechanism that is unlikely to require SNAREs. Furthermore, he concluded that Atg9 vesicles alone cannot contribute sufficient membrane to grow the PAS during starvation and that therefore additional mechanisms must exist to allow the rapid growth of the PAS. Claudine Kraft (U. Vienna, Austria) discussed the role of

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Atg9 phosphorylation in its multimerization and retrieval from the PAS to peripheral vesicles in yeast.

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Nucleation and growth of the phagophore membrane requires the production of PI3P by the Atg14–Beclin1–Vps34 complex. In yeast, *Caenorhabditis elegans* and mammalian cells, Atg18/WIPI proteins are the Atg effectors of the PI3P produced on the PAS/phagophore. However, in both yeast and *C. elegans*, the correct localization and presumably the function of Atg18/WIPI requires the association of Atg2. Mammalian Atg2 is one of the last mammalian Atg proteins to be studied, in part because there are two genes in humans encoding large proteins (>200 kDa). Noboru Mizushima's group (Tokyo Medical and Dental U., Japan) has studied these orphan Atgs. They presented intriguing data suggesting that these proteins regulate autophagic flux with the striking result that, after depletion of both Atg2A and Atg2B, there is an accumulation of unsealed autophagosomes and abnormal LC3-positive structures in fed cells. Mizushima showed that, surprisingly, Atg2 only partly localizes to the phagophore and a substantial pool is found on lipid droplets. He noted that both the phagophore and lipid droplets are formed from the endoplasmic reticulum (ER). Mizushima's results place Atg2 on phagophore structures where it acts like WIPI2 and WIPI4, with a second role in lipid droplet turnover. Whether these two functions of Atg2 are related remains to be further explored. Mizushima and Hong Zhang (National Institute of Biological Sciences, Beijing, China) also suggested that WIPI4 should join WIPI1 and WIPI2 as a phagophore-localized PI3P autophagy effector, the function of which remains to be elucidated.

Continuing with the theme of the phagophore membrane, Tamotsu Yoshimori (Osaka U., Japan) addressed the recent models of biogenesis of the autophagosome from the ER and/or mitochondria (for a review, see [4]). One way to reconcile these data is to consider the role of the MAM (mitochondria-associated ER membrane) junctions connecting the mitochondria to the ER [5]. By looking at MAM components, Yoshimori showed that MAMs are involved in the formation of the phagophore. Even more revealing were the data on syntaxin 17, an ER-localized SNARE protein required for maintenance of the ER–Golgi intermediate compartment. Yoshimori's data suggest that the formation of ER-derived autophagosomes at MAMs might be catalysed through a syntaxin-17-dependent pathway, with this protein recruiting Atg14L during starvation conditions.

As a follow-up on the role of SNAREs, David Rubinsztein (Cambridge Institute for Medical Research, UK) showed data implicating endosomal SNAREs in the delivery of plasma-membrane-associated Atg16L1 to the early stages of autophagosome biogenesis [6]. The role of SNARE membrane fusion machinery was a talking point in the session. Talks from Zevi Elazar's lab (Weizmann Institute, Israel), published data from Ohsumi's lab, and new data from Fulvio Reggiori (U. Medical Centre Utrecht, The Netherlands) highlighted the pivotal role of LC3 family members in mammals and PI3P phosphatases in yeast in driving expansion and closure of the phagophore, which is clearly SNARE independent. Although SNAREs act in many, if not most, membrane fusion events, their exact role in autophagy requires additional work.

Selective autophagy

Selective autophagy was first recognized by electron microscopy in the 1960s in the maturing erythrocyte, where cytolysosomes (now called autophagosomes) were observed to be engulfing organelles. Selective autophagy has now become a hot topic. One reason for its rediscovery was the identification of the LC3-interacting region (LIR) motif or the corresponding AIM (Atg8-interaction motif; [7]). The LIR motif identified in the ubiquitin-binding protein SQSTM1/p62, mediates binding to LC3 and has become a focus for understanding the mechanism of selectivity in autophagy. Thus, mitophagy, pexophagy, virophagy, xenophagy and aggrephagy were all discussed, and in particular the mechanisms

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underlying selectivity. While the recent progress made has been remarkable (for a review, see [7]), it is apparent that there is yet more complexity and diverse biology underlying selective autophagy, as revealed in a recent genome-wide screen for novel proteins involved in virophagy and mitophagy [8].

The best-characterized selective autophagy pathway in *Saccharomyces cerevisiae* is the cytoplasm-to-vacuole targeting (Cvt) pathway through which cytosolic protein cargos (prApe1, for example) are recognized by cargo receptors (Atg19) and delivered to the vacuole [9]. An emerging theme in selective autophagy is that cargo receptors or adaptors also interact with Atg8 and LC3 through AIM/LIR sequences. This is exemplified by the Cvt pathway receptor, Atg19, which binds to Atg8 on the PAS via an AIM motif. However, as additional Atg proteins with the capacity to target organelles such as mitochondria and peroxisomes are discovered for processes such as mitophagy and pexophagy, the involvement of additional proteins in these selective processes exposes new puzzles.

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While mitophagy in yeast is mediated by the AIM-containing cargo receptor Atg32, the first peroxisome receptor identified in the yeast *Pichia pastoris*, Atg30, does not contain a canonical LIR motif. Suresh Subramani (UC San Diego, USA) and colleagues have now discovered the missing pexophagy link, Atg36, which turns out to be a conserved peroxisomal transmembrane protein. Atg36 and its partner, Atg30, recruit Atg8 to the peroxisomal membrane, thereby delivering the sequestered peroxisome to the vacuole for degradation. However, while Atg30 is degraded in the vacuole during pexophagy, Atg36 is lost by a vacuole-independent mechanism that remains to be elucidated.

Adding to the complexity of organelle degradation, Eli Arama (Weizmann Institute, Israel) presented data regarding fertilization in *Drosophila*: paternal mitochondria are destroyed by autophagy, thus providing an explanation for how the mitochondrial genome is inherited

from the female. Arama's elegant study revealed how mitochondria detach from the axoneme of the sperm, undergo fragmentation and are subsequently engulfed by the phagophore. Selectivity for the fragmented paternal mitochondria was provided by ubiquitination, although the E3 ligase and the target of ubiquitination were not identified. Whether the mitophagy-associated kinase PINK1 and the E3 ligase Parkin are involved in this process is still unclear. Nevertheless, paternal mitochondrial destruction after fertilization is more complex because the mitochondrial cargo must be fragmented before mitophagy can kick in. Further comparison between *Drosophila* and the process described recently in *C. elegans* [10] would further advance our understanding.

> **…proteins with LIR motifs, such as RabGAPs, might be used to coordinate protein transport and the autophagy machinery**

Hong Zhang studies selective autophagy and aggrephagy of cargo known as P granules—germline components that are degraded in somatic cells in *C. elegans*. Zhang's genetic approaches have identified many *epg* genes, some of which are homologues of known Atg genes. His recent screen for the removal of aggregates containing PGL-1 and PGL-3 (proteins in the P granules) and a p62-like protein called SQST-1, which binds to LGG-1 (LC3 in *C. elegans*), revealed a role for WIPI4, a member of the WIPI/Atg18 family not previously studied, and Atg2 (see above). Zhang described a new mutant, *epg-11*, impaired in post-translational modifications of PGL-1 and PGL-3, which regulates their inclusion into aggregates with the receptor SEPA-1. Zhang's results suggest another layer of complexity in the timing and control of selective autophagy during development.

LIR/AIM motifs

The protein p62, and its closely related family member NBR1 (neighbour of BRCA1), are cargo adaptors that bind to cargo and deliver it to autophagosomes through the interaction of the LIR motif on these adaptors with autophagosome-bound LC3 family members. The LIR/AIM sequence featured prominently at the meeting, as much effort has been invested in refining the consensus sequence using bioinformatics. Matthias Peter (ETH Zurich, Switzerland) presented such data from yeast, Trond Lamark (U. Tromsø, Norway) offered results from plants, and Christian Behrends (Frankfurt U., Germany) presented interaction screens in mammalian cells using yeast two-hybrid and proteomic approaches.

Working with Wade Harper (MIT, USA) and Ivan Dikic (Frankfurt U., Germany), Behrends has uncovered a network of RabGAPs (GTPase-activating proteins) that interact with LC3 family members. Surprisingly, this has proven to be 35% of the known RabGAPs. Behrends focused on TBC1D5 and demonstrated that it has a functional LIR motif that binds to LC3 family members, but is also crucial for the interaction with the retromer, a complex of proteins required for retrograde transport from the endosome to the Golgi. LC3 and a retromer subunit, Vps29, compete for binding to the RabGAP. Behrends' data suggests that proteins with LIR motifs, such as RabGAPs, might be used to coordinate protein transport and the autophagy machinery. Coordination and regulation might also be supplied by phosphorylation of the LIR motif, as recently shown for optineurin, and now described by Ivana Novak (U. Split, Croatia) in NIX/BNIP3L and BNIP3, selective receptors for mitochondria. Intriguingly, Novak's data suggest that phosphorylation of the residues surrounding the LIR motif modulates binding to the LC3 family members, in particular LC3 and GATE-16, and supports a model whereby phosphorylation could provide a handover mechanism between LC3 family members.

In conclusion, the breadth of the meeting reflected the enormous diversity of autophagy in essential life and death processes, and showed how the fundamental molecular insights and progress made in the field support an understanding of the complex role that autophagy plays in human health and disease.

ACKNOWLEDGEMENTS

The authors thank Suresh Subramani for help and advice with the article, and all the speakers who agreed to have their work cited here.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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EMBO *reports* (2012) **13,** 175–177; published online 7 February 2012; doi:10.1038/embor.2012.7