© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 2229-2233 doi:10.1242/jcs.206219

MEETING REPORT

Meeting report – Emerging Concepts in Cell Organization

David Teis[1,*] and Wanda Kukulski[2,*]

ABSTRACT

New concepts in cell organization emerged in a medieval castle during a snowy week in January 2017 in the middle of the Austrian Alps. The occasion was the 10th Annaberg EMBO workshop in Goldegg am See; organized by Gabriele Seethaler, Catherine Rabouille and Marino Zerial. There were 95 participants, including many who gave talks and presented posters, enjoying a familial and trusting atmosphere that stimulated lively exchange of (unpublished) results, new ideas and thoughts.

As at previous occasions when this community met in the same spot, the aim of the meeting was to identify the key questions in cell biology and set the ground for future research directions. The inspirational character of the meeting and the enthusiasm of the participants certainly achieved that. The new concepts that emerged will be shaping our understanding of how cells are organized and how this organization impacts cellular functions. Overall, it became clear that there is a discrepancy between the textbook views of the cellular building plan and how the field currently envisions a cell is really built. Furthermore, novel methods allow the study of the complexity of cellular organization, and old methods are being revisited. This report is an attempt to recapitulate and integrate the ideas that emerged at the workshop, and thereby provide inspiration to the larger community. All presentations and, in particular the lively poster sessions, were essential for the success of the meeting. However, owing to space restrictions, we could not cover all short talks or the posters here. We apologize to our colleagues whose presentations could not be discussed here.

Secretion: making proteins and membranes and how to get them out

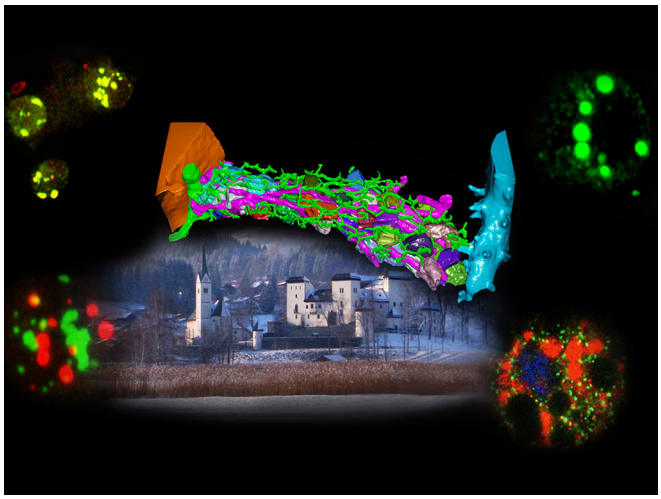
A lot remains to be discovered about intra-organelle communication by 'classical' vesicular trafficking, especially in the secretory pathway. For instance, how do cells ensure that vesicles contain the right cargo? Liz Miller (MRC LMB, Cambridge, UK) explained protein quality control at endoplasmic reticulum (ER) exit sites and presented a cargo-crowding mechanism that helps to exclude ER-resident proteins from being packed into COPII vesicles, thereby preventing their 'spilling over' into the Golgi.

While ER export has been intensely studied, exit sites from the trans-Golgi network (TGN) have remained more elusive. Stéphanie Miserey-Lenkei (Institut Curie, Paris, France) highlighted that RAB6 controls membrane fission of transport vesicles at the TGN through coupling motor proteins of the kinesin and myosin family to the cytoskeleton at specific sites of fission. This coupling ensures the spatial coordination between the fission of RAB6-positive vesicles and their exit along microtubules (Miserey-Lenkei et al., under review).

Another important question is how is specificity in trafficking to and through the Golgi achieved? After all, the Golgi is the major protein distribution center of the cell. Sean Munro (MRC LMB, Cambridge, UK), this year's winner of the famous Grunzelsbacher award (sponsored by the elusive Austrian billionaire and philanthropist Alfons Grunzelsbacher), has asked how long, coiled-coil tethering proteins called golgins are able to capture vesicles arriving at the Golgi. Examining several golgins revealed that short conserved motifs at their N-termini are necessary and sufficient for vesicle capture. Their search for proteins that interact with this motif in golgin-97 has identified at least one candidate protein which mediates endosomal cargo relocation.

Another Golgi tether, GMAP-210, can capture small vesicles trafficked between ER and Golgi. As presented by Bruno Antonny (Université Côte d'Azur, Valbonne, France), this is achieved by an ALPS motive, which recognizes the lipid-packing defects of highly curved membranes, which appear to be particularly prominent in vesicles of the early secretory pathway such as COPI or COPII (Magdeleine et al., 2016).

Do all proteins traffic at the same rate and what controls any differences? Franck Perez (Institut Curie, Paris, France) showed time-resolved proteomics of cargo interaction during Golgi transit using a combination of the RUSH system that his laboratory developed (Boncompain et al., 2012) and ascorbic acid peroxidase (APEX)-mediated protein proximity labeling. This approach revealed that trafficking fellows are transported together as sub-pools of cargos through the Golgi complex. He further showed that



Collage of the 10th EMBO-Annaberg Workshop 2017. The photograph shows Goldegg Castle where the 95 participants gathered in the lecture room to listen to exciting talks and new ideas. This illustration has been kindly provided by Gabriele Seethaler, Marino Zerial and Catherine Rabouille.

[1]Division of Cell Biology, Biocenter, Medical University of Innsbruck, 6020 Innsbruck, Austria. [2]Division of Cell Biology, MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK.

*Authors for correspondence (kukulski@mrc-lmb.cam.ac.uk; david.teis@i-med.ac.at)

Journal of Cell Science (2017) 130, 2229-2233 doi:10.1242/jcs.206219

cargos are subsequently secreted near adhesion sites in non-polarized cells.

Dan Cutler (University College London, UK) discussed how the linkage of Golgi stacks into a ribbon controls the size of the endothelial secretory granules Weibel–Palade bodies (WPBs). Altered Golgi organization reducing the extent of linkage, such as resulting from statin treatment, generates smaller WPBs, that have reduced haemostatic function, thus potentially reducing thrombosis (Ferraro et al., 2016). Changes in Golgi organization, in particular its disassembly in early mitosis, also appear to have a role in controlling mitotic spindle assembly, as shown by Joachim Seemann (UT Southwestern, Dallas, USA) (Guizzunti and Seemann, 2016).

Not only 'classical secretory organelles' but also mitchondria can bud cargo-specific vesicles – so called mitochondria-derived vesicles (MDVs) – that target various destinations, as described by Heidi McBride (McGill University, Montreal, Canada). Amongst other functions, MDVs fuse with ER-derived vesicles to form newly born peroxisomes (Sugiura et al., 2017).

So it seems that some, if not all, organelles may lead 'secret double lives' and have properties that we are only beginning to uncover.

Unconventional secretion

Although secretion largely occurs through the classical secretory pathway (ER to Golgi to plasma membrane), several unconventional secretion pathways are now emerging. Min Goo Lee (Yonsei University, Seoul, Korea) reported that misfolded proteins (including ΔF508-CFTR or H723R-SLC26A4) can be exported from the ER and reach the plasma membrane by recruiting GRASP or DNAJC14 directly to the ER and bypassing the Golgi (Kim et al., 2016; Jung et al., 2016).

A subset of extracellular proteins such as the tumor cell survival factor FGF2 lack a signal peptide and are secreted from cells by direct translocation across the plasma membrane. Walter Nickel (Heidelberg University Biochemistry Center, Germany) presented how his lab successfully reconstituted several steps of the unconventional secretory pathway of FGF2. This process involves (1) sequential molecular interactions with the inner leaflet of the plasma membrane, (2) Tec kinase-dependent tyrosine phosphorylation of FGF2, (3) phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$]-dependent oligomerization and membrane pore formation and (4) extracellular trapping of FGF2 mediated by heparan sulfate proteoglycans on cell surfaces (La Venuta et al., 2016; Rabouille, 2017).

Finally, Bruno Beaumelle (Université de Montpellier, France) explained how unconventional secretion of the HIV-1 transactivator of transcription (Tat) protein could increase the efficiency of HIV infection. Tat lacks a signal sequences and uses direct binding to PI $(4,5)P_2$ to be recruited to the plasma membrane. From there, it translocates directly across the plasma membrane for secretion. Secreted Tat molecules can be taken up by other non-infected cells through endocytic mechanisms and thereby modulate AIDS pathogenesis.

Internalizing, recycling and degrading membranes and proteins

Different endocytic processes internalize cell surface proteins, membranes, extracellular material and pathogens. Gareth Griffiths (University of Oslo, Norway) uses a zebrafish model to test and develop nanoparticles loaded with antibiotics to kill macrophage-resident *Mycobacterium marinum*. These nanoparticles are efficiently engulfed by granuloma-resident marcrophages that are infected with mycobacteria, thereby directly delivering the drug to the infected cell and clearing the infection from the fish (Fenaroli et al., 2014).

Tommy Kirchhausen (Harvard Medical School, Cambridge, USA) spoke about new sensors they developed to report the dynamics of phosphoinositide conversion in clathrin-mediated endocytosis, as well as imaging and quantification of these events by 3D lattice light sheet microscopy (Aguet et al., 2016).

Kay Oliver Schink (The Norwegian Radium Hospital, Oslo, Norway) presented data on a special type of endocytosis, macropinocytosis, and asked how macropinosomes find their way through the dense subcortical actin network before they fuse with the endosomal compartments. From there a substantial portion of endocytosed proteins recycle back to the plasma membrane, and several talks addressed this aspect.

Pete Cullen (University of Bristol, UK) showed that internalized membrane proteins recycle back to the cell surface using different routes; in addition to the retromer complex, he presented a novel recycling complex he termed the retriever complex. This complex appears to function specifically in recycling of α5β1 integrin and other proteins interacting with the extracellular matrix. Along the same line, Judith Klumperman (University of Utrecht, The Netherlands) demonstrated a CORVET-independent role for a new tethering complex for integrin transport, which is formed by Vps3 and Vps8 and presumably acts in a step that follows retriever requirement.

However, not all proteins recycle back to the plasma membrane. Ubiquitylated membrane proteins at endosomes, such as the epidermal growth factor receptor (EGFR), will be sorted along endosomes into the multivesicular body (MVB) pathway for their subsequent lysosomal degradation. The biogenesis of MVBs requires reverse membrane budding through the ESCRT machinery, which also functions at the plasma membrane and at the nuclear envelope. Aurélien Roux (University of Geneva, Switzerland) reconstituted and visualized ESCRT-III assembly and dynamics *in vitro* (Chiaruttini et al., 2015), whereas David Teis (Medical University of Innsbruck, Austria) reported on the dynamic properties of ESCRT-III and Vps4 on endosomes in yeast. Jean Gruenberg (University of Geneva, Switzerland) showed that, in mammalian cells, MVB biogenesis additionally requires F-actin, annexin II and moesin. Jean also presented a new drug that stimulated the efflux of cholesterol from MVBs of NPC1/2 cells.

Not all MVBs fuse with lysosomes. Clotilde Théry (Institut Curie, Paris, France) showed that a subpopulation of MVBs that are marked by Rab27 can fuse with the plasma membrane to release exosomes (Ostrowski et al., 2010). Exosomes and ectosomes (they bud directly from the plasma membrane) are collectively called extracellular vesicles and have attracted a lot of attention because they appear to be involved in intercellular exchange of RNA, proteins and lipids. Clotilde presented the first steps towards a much-needed careful morphological and biochemical characterization of extracellular vesicles of different subcellular origin (Kowal et al., 2016).

Autophagy

Besides the MVB pathway and biosynthetic pathways, autophagy is another cellular pathway that delivers cargo to lysosomes. Growing autophagosomes are large spherical double-membrane structures that trap cytoplasmic contents inside. Once formation of the autophagosome is complete, its outer membrane fuses with lysosomes, and the inner membrane with its cytoplasmic cargo is degraded. Zevi Elazar (Weizmann Institute, Rehovot, Israel) showed that the MVB pathway and autophagy work together to control the degradation of FN14, a member of the tumor necrosis factor (TNF)

Journal of Cell Science (2017) 130, 2229-2233 doi:10.1242/jcs.206219

receptor superfamily. This requires two different members of the LC3 protein family, GABARAPs and GATE16. These LC3/Atg8 proteins are typically conjugated to phosphatidylethanolamine in the membranes of growing autophagosomes and then contribute to their growth and closure. Sharon Tooze (The Francis Crick Institute, London, UK) presented evidence that GABARAP also localizes to the centrosome in a regulated manner and might shuttle from there to growing autophagosomes. The precise function of this localization remains to be explored.

In the Annaberg lecture, Andrea Ballabio (TIGEM, Naples, Italy) highlighted the central importance of lysosomes for cell physiology ("remember: lysosomes are awesome. They act as control center of cell metabolism. They are not just the last step of cellular catabolic pathways! Thus, they should be at the top of the slide and not at the bottom!"). He highlighted the molecular mechanisms that enable signaling from lysosomes and emphasized how these pathways intersect with autophagy for the homeostatic control of metabolic networks.

Organelles display unprecedented levels of interaction and exchange

A major emerging topic is that membrane-bound organelles should not be viewed as separate entities, but instead as highly interactive compartments that constantly undergo molecularly defined, physical membrane contacts to exchange information and material. They appear to be major sites of lipid exchange and metabolic activity, thereby impacting on organelle composition and function. Thus, lipid transfer was given attention by many speakers (see also the section on 'lipids and neurodegeneration' below). Tim Levine (University College London, UK) discussed contact sites between organelles by highlighting the patchwork-type organization of contacts between any pair of organelles (Gatta and Levine, 2017). Tim also presented a bioinformatics approach to search for lipid-transport proteins that contain tubular lipid-binding (TULIP) domains, which revealed their great diversity. This approach has identified a possible TULIP-like domain in VPS13, which is a protein shown by others to be at multiple contact sites and strongly implicated in lipid traffic (Lang et al., 2015; Park et al., 2016).

Bruno Antonny presented data on how oxysterol-binding protein 1 (OSBP) acts as a lipid pump that transfers cholesterol from the ER to the Golgi in a VAP-dependent manner, and massively consumes phosphatidylinositol 4-phosphate (PI4P) to fuel the process. The PI4-kinase PI4K-IIIb, which is localized close to OSBP, allows the transfer reaction to proceed steadily. In contrast, striking regular oscillations are observed when PI4P is synthesized by PI4K-IIα, which is located at distance from OSBP. The VAP proteins are also required to generate yet another contact site, that between the ER and the trans-Golgi as described in detail by Antonella De Matteis (TIGEM, Naples, Italy).

Synaptotagmin-like mitochondrial-lipid binding protein (SMP) domains belong to the TULIP-domain family. Karin Reinisch (Yale University, New Haven, USA) showed that the SMP-containing protein TMEM24 transports phosphatidylinositol in a Ca^{2+}-dependent manner and explained how this regulates slow pulsatile insulin release (Lees et al., 2017). The breadth of the presented work clearly illustrates that direct organelle contacts are a key organizing principle that contributes to lipid diversity.

Furthermore, Cathy Jackson (Institute Jacques Monod, Paris, France) discussed how guanine nucleotide exchange factors (GEFs) for the GTPase Arf1 contribute to lipid droplet homeostasis and to mitochondrial dynamics.

Organelle contacts are, however, not restricted to lipid transfer and contribute to the organization of cells in many exciting ways. Consistent with this idea, Maya Schuldiner (Weizmann Institute of Science, Rehovot, Israel) showed that, in principle, any two different organelles in a yeast cell can contact each other.

Lipids and neurodegeneration

For some time already, lipids have no longer been considered passive components of membranes, but have emerged as key signaling molecules that regulate many cell-organizing processes. Their exchange and metabolism often occurs at contact sites. The central role of lipids is reflected by their involvement in pathological conditions. Gil Di Paolo (Denali Therapeutics, San Francisco, USA and Columbia University, New York, USA) used lipidomics to find that the levels of phosphatidylinositol 3-phosphate [PI(3)P] were specifically reduced in brain tissue from humans with Alzheimer's disease and mouse models thereof (Morel et al., 2013). Reduction of PI(3)P by knocking out or inhibiting Vps34 causes endolysosomal damage associated with galectin-3 immunoreactivity and interferes with lysosomal degradation of C-terminal fragments (CTFs) of the amyloid precursor protein (APP) in neurons. Vps34 inhibition also caused upregulation of sphingolipid-enriched exosomes harboring high levels of APP-CTFs, likely as a mechanism for elimination of toxic proteins or lipids accumulating as a result of endolysosomal/autophagy dysfunction.

Continuing with this theme, Karl Fernandes (University of Montreal, Canada) showed that ependymal cells at the interface between the brain and cerebrospinal fluid (CSF) accumulate neutral lipids in Alzheimer's disease (Hamilton et al., 2015). The aberrant lipid droplet accumulation at the brain-CSF interface triggers the deterioration of the surrounding neural stem cell niche. These results could explain how polymorphisms in ApoE4, the strongest genetic risk factor for late-onset Alzheimer's disease and the main lipid transporting apolipoprotein in the brain, contributes to perturbations of brain fatty acid metabolism and neurodegeneration in the elderly.

Membrane-less compartments

Membrane-bound organelles are characteristic for eukaryotic cells and have been considered the main means of cell organization. Now, membrane-less compartments are recognized to additionally compartmentalize cells. How they are formed and organized is an emerging area of organelle biology, in particular with regard to their interaction with other organelles. Simon Alberti (MPI-CBG, Dresden, Germany) proposed a liquid–liquid phase separation as an organizing principle for this type of compartmentalization. This is particularly relevant for the formation of ribonucleoprotein (RNP) granules, such as stress granules, which normally protect untranslated mRNAs. In amyotrophic lateral sclerosis, liquid stress granules accumulate proteins prone to misfolding and aggregation, for example superoxide dismutase (SOD1), and this leads to an aberrant phase transition of stress granules into a solid-like state (Mateju et al., 2017). Catherine Rabouille (Hubrecht Institute, Utrecht, The Netherlands) showed that nutrient starvation stress triggers – in addition to stress granules – the formation of a novel membrane-less assembly that incorporates ER exit site (ERES) components, including Sec16. Along the same lines, Anne Spang (University of Basel, Switzerland) reported that yeast Pin2 uses its prion-like domain as a TGN retention device by forming stress-dependent oligomers in the TGN, which also contribute to the retention of Flc2. The observations that defining components of membranous organelles can form liquid-droplet compartments

Journal of Cell Science (2017) 130, 2229-2233 doi:10.1242/jcs.206219

upon changes of conditions, predominantly during stress, adds new organizational principles and complexity to inter-organelle exchange and dynamics (Rabouille and Alberti, 2017).

New tools to study the novel complexity and modularity of cellular organization

New tools and technologies are needed to dissect the emerging complexity and the molecular principles that govern cellular and organelle interaction networks.

Marino Zerial's (MPI-CBG, Dresden, Germany) lab has reconstituted endosome fusion with 17 components (Ohya et al., 2009), systematically studying their structure and function. Recently, they focused on endosome tethering using elaborate *in vitro* reconstitution experiments in combination with biophysical approaches. Marino reported that binding of Rab5 to EEA1 induces an entropic collapse of the long coiled-coil tethering protein EEA1 prior to endosome fusion (Murray et al., 2016).

Presenting another *in vitro* approach, Gisje Koenderink (AMOLF, Amsterdam, The Netherlands) showed how reconstituting cytoskeleton–membrane assemblies opens new ways to study and think about the mechanics of their interaction. By using time-lapse imaging, her lab found that activity of actomyosin cortices is controlled by an interplay between myosin pulling forces, cortex-membrane anchoring and actin connectivity (Alvarado et al., 2013). She also showed that anionic lipids cause septin polymerization, which in turn affects membrane organization.

By using giant unilamellar liposomes and purified fluorescent proteins, Dan Fletcher (University of California, Berkeley, USA) outlined how protein size can drive protein segregation at membrane interfaces, in particular at cell–cell contacts (Schmid et al., 2016). A similar principle may contribute to the organization of organelle contact sites. These presentations demonstrated the need to combine reconstitution with biophysical approaches to understand the properties of protein–membrane assemblies.

Furthermore, new imaging approaches and the combination of imaging methods have recently emerged, and now allow unprecedented visualization of subcellular structures. High-speed atomic force microscopy and correlative light and electron microscopy, as well as quantitative live-cell and tissue imaging (e.g. 3D lattice light sheet microscopy) prove to be most useful to identify and image at high resolution transient states of protein complexes and of organelle reshaping.

Richard Scheller (23andMe, USA) explained that it is even possible to use clever questionnaires to phenotype millions of humans and to correlate this phenotypic information with genomic information from the same cohort. The combination of these approaches could provide information on how single nucleotide polymorphisms (SNPs) correlate with social behavior (such as coffee consumption) and diseases, and thus help to identify new drug targets.

Together, these approaches open up novel possibilities for addressing tissue-dependent cellular organization at different scales of resolution, which certainly is one of the next steps in the field.

Are we talking about an identity crisis?

In summary, unexpected levels of organelle dynamics, as well as a significant functional overlap of key components, became apparent during the talks and the vivid poster sessions. Moreover, the prominence of direct lipid-transfer sites is intriguing, given the importance of lipids for organelle function and morphology. These findings raise important questions with regard to organelle identity – how do organelles maintain identity, do they actively prevent constant identity crisis? Are our current definitions of organelles, which are based on prominent lipid and protein markers, still valid? Or should we disband the idea of a strict organelle identity, but rather think of organelles as sets of functional and structural modules with gradual boundaries that function hand-in-hand with organized membrane domains, thus encompassing membrane-less compartments as well? Future research will tell; possibly, a combination of these concepts will apply.

The emerging modularity of cellular organization is also evident at the molecular level. Many of the protein components involved in driving cellular organization are promiscuous with regard to their localization and/or function: besides well-known candidates, such as ESCRTs, clathrin and dynamin, new general molecular organizers emerge. For instance, the WASH complex emerges as a general hub on endosomes. As shown by many labs, the VAP proteins appear to be required for formation of many different membrane contact sites, as well as for the lipid transfer reactions. Similarly, we have already highlighted the emerging ubiquitous role of TULIP domain-containing proteins. So rather than acting in specific, perfectly robust cellular processes, it appears that many protein functions are utilized in different cellular pathways, and, likewise, that many processes involve redundant protein function. In addition, stochasticity, quality control mechanisms and specificity may maintain organizational principles and support the robustness of cellular processes.

After the meeting, it was obvious that exciting discoveries and a lot of work are ahead to advance our understanding of cell organization. Equipped with new tools, plenty of ideas and with increasing collaborative efforts in interdisciplinary approaches, we expect the current and future research to explore unknown and emerging complexity of how cells are built.

Acknowledgements

Work in the group of W.K. is supported by the Medical Research Council (MC_UP_1201/8). Research in the laboratory of D.T. is supported by the Austrian Science Fund (FWF-Y444-B12, P30263, MCBO W1101-B18).

References

Aguet, F., Upadhyayula, S., Gaudin, R., Chou, Y.-Y., Cocucci, E., He, K., Chen, B.-C., Mosaliganti, K., Pasham, M., Skillern, W. et al. (2016). Membrane dynamics of dividing cells imaged by lattice light-sheet microscopy. *Mol. Biol. Cell* **27**, 3418-3435.

Alvarado, J., Sheinman, M., Sharma, A., Mackintosh, F. C. and Koenderink, G. H. (2013). Molecular motors robustly drive active gels to a critically connected state. *Nat. Phys.* **9**, 591-597.

Boncompain, G., Divoux, S., Gareil, N., De Forges, H., Lescure, A., Latreche, L., Mercanti, V., Jollivet, F., Raposo, G. and Perez, F. (2012). Synchronization of secretory protein traffic in populations of cells. *Nat. Methods* **9**, 493-498.

Chiaruttini, N., Redondo-Morata, L., Colom, A., Humbert, F., Lenz, M., Scheuring, S. and Roux, A. (2015). Relaxation of loaded ESCRT-III spiral springs drives membrane deformation. *Cell* **163**, 866-879.

Fenaroli, F., Westmoreland, D., Benjaminsen, J., Kolstad, T., Skjeldal, F. M., Meijer, A. H., Van Der Vaart, M., Ulanova, L., Roos, N., Nyström, B. et al. (2014). Nanoparticles as drug delivery system against tuberculosis in zebrafish embryos: direct visualization and treatment. *ACS Nano* **8**, 7014-7026.

Ferraro, F., Da Silva, M. L., Grimes, W., Lee, H. K., Ketteler, R., Kriston-Vizi, J. and Cutler, D. F. (2016). Weibel-Palade body size modulates the adhesive activity of its von Willebrand Factor cargo in cultured endothelial cells. *Sci. Rep.* **6**, 32473.

Gatta, A. T. and Levine, T. P. (2017). Piecing together the patchwork of contact sites. *Trends Cell Biol.* **27**, 214-229.

Guizzunti, G. and Seemann, J. (2016). Mitotic Golgi disassembly is required for bipolar spindle formation and mitotic progression. *Proc. Natl. Acad. Sci. USA* **113**, E6590-E6599.

Hamilton, L. K., Dufresne, M., Joppé, S. E., Petryszyn, S., Aumont, A., Calon, F., Barnabé-Heider, F., Furtos, A., Parent, M., Chaurand, P. et al. (2015). Aberrant lipid metabolism in the forebrain niche suppresses adult neural stem cell proliferation in an animal model of Alzheimer's disease. *Cell Stem Cell* **17**, 397-411.

Jung, J., Kim, J., Roh, S. H., Jun, I., Sampson, R. D., Gee, H. Y., Choi, J. Y. and Lee, M. G. (2016). The HSP70 co-chaperone DNAJC14 targets misfolded pendrin for unconventional protein secretion. *Nat. Commun.* **7**, 11386.

Kim, J., Noh, S. H., Piao, H., Kim, D. H., Kim, K., Cha, J. S., Chung, W. Y., Cho, H.-S., Kim, J. Y. and Lee, M. G. (2016). Monomerization and ER relocalization of GRASP is a requisite for unconventional secretion of CFTR. *Traffic* **17**, 733-753.

Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J. P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M. and Théry, C. (2016). Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl. Acad. Sci. USA* **113**, E968-E977.

La Venuta, G., Wegehingel, S., Sehr, P., Müller, H.-M., Dimou, E., Steringer, J. P., Grotwinkel, M., Hentze, N., Mayer, M. P., Will, D. W. et al. (2016). Small molecule inhibitors targeting Tec kinase block unconventional secretion of fibroblast growth factor 2. *J. Biol. Chem.* **291**, 17787-17803.

Lang, A. B., John Peter, A. T., Walter, P. and Kornmann, B. (2015). ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. *J. Cell Biol.* **210**, 883-890.

Lees, J. A., Messa, M., Sun, E. W., Wheeler, H., Torta, F., Wenk, M. R., De Camilli, P. and Reinisch, K. M. (2017). Lipid transport by TMEM24 at ER-plasma membrane contacts regulates pulsatile insulin secretion. *Science* **355**, eaah6171.

Magdeleine, M., Gautier, R., Gounon, P., Barelli, H., Vanni, S. and Antonny, B. (2016). A filter at the entrance of the Golgi that selects vesicles according to size and bulk lipid composition. *Elife* **5**, 292.

Mateju, D., Franzmann, T. M., Patel, A., Kopach, A., Boczek, E. E., Maharana, S., Lee, H. O., Carra, S., Hyman, A. A. and Alberti, S. (2017). An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J.* doi:10.15252/embj.201695957. [Epub]

Morel, E., Chamoun, Z., Lasiecka, Z. M., Chan, R. B., Williamson, R. L., Vetanovetz, C., Dall'Armi, C., Simoes, S., Point Du Jour, K. S., McCabe B. D. et al. (2013). Phosphatidylinositol-3-phosphate regulates sorting and processing of amyloid precursor protein through the endosomal system. *Nat. Commun.* **4**, 2250.

Murray, D. H., Jahnel, M., Lauer, J., Avellaneda, M. J., Brouilly, N., Cezanne, A., Morales-Navarrete, H., Perini, E. D., Ferguson, C., Lupas, A. N. et al. (2016). An endosomal tether undergoes an entropic collapse to bring vesicles together. *Nature* **537**, 107-111.

Ohya, T., Miaczynska, M., Coskun, U., Lommer, B., Runge, A., Drechsel, D., Kalaidzidis, Y. and Zerial, M. (2009). Reconstitution of Rab- and SNARE-dependent membrane fusion by synthetic endosomes. *Nature* **459**, 1091-1097.

Ostrowski, M., Carmo, N. B., Krumeich, S., Fanget, I., Raposo, G., Savina, A., Moita, C. F., Schauer, K., Hume, A. N., Freitas, R. P. et al. (2010). Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* **12**, 19-30; sup pp. 1-13.

Park, J.-S., Thorsness, M. K., Policastro, R., Mcgoldrick, L. L., Hollingsworth, N. M., Thorsness, P. E. and Neiman, A. M. (2016). Yeast Vps13 promotes mitochondrial function and is localized at membrane contact sites. *Mol. Biol. Cell* **27**, 2435-2449.

Rabouille, C. (2017). Pathways of unconventional protein secretion. *Trends Cell Biol.* **27**, 230-240.

Rabouille, C. and Alberti, S. (2017). Cell adaptation upon stress: the emerging role of membrane-less compartments. *Curr. Opin. Cell Biol.* **47**, 34-42.

Schmid, E. M., Bakalar, M. H., Choudhuri, K., Weichsel, J., Ann, H. S., Geissler, P. L., Dustin, M. L. and Fletcher, D. A. (2016). Size-dependent protein segregation at membrane interfaces. *Nat. Phys.* **12**, 704-711.

Sugiura, A., Mattie, S., Prudent, J. and McBride, H. M. (2017). Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature* **542**, 251-254.