Meeting Report

Meeting Report From the 2019 "Organelle Zone" Symposium in Osaka, Japan

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Tim P. Levine[1], Franck Perez[2], Yasunori Saheki[3], and Julia von Blume[4]

Abstract

On May 29, 2019, at the Osaka University Hospital, Japan, the "Organelle Zones" research grant group (see http://organellezone.org/english/) organized a 1 day symposium for its own members and four guest speakers, with about 60 attendees. The research group studies three different ways in which regions within organelles carry out functions distinct from other parts of the organelle. Work at this suborganellar level is increasingly recognized as an important aspect of cell biology. The group's projects are divided into these themes with 9 Principal Investigators and 18 Coinvestigators over 5 years. The symposium followed a similar meeting in 2018 and had four speakers from within the consortium as well as the external speakers. The talks were divided into three sessions, each showcasing one way of subcompartmentalizing organelles into zones.

Keywords

organelle, microscopy, membrane contact site, symposium

Session 1 focused on how communication between organelles creates heterogeneous specialized zones within organelle pairs ("Communication Zones"). Yasushi Tamura (Yamagata University, Japan) spoke about his laboratory's work on endoplasmic reticulum (ER)–mitochondrial communication in both yeast and mammalian cells. He developed a split-GFP-based approach to mark various membrane contact sites in yeast and mammalian cells. In yeast, his laboratory's main focus is the ER–mitochondrial encounter structure (ERMES). He has used protein fusion and superresolution live cell microscopy to study protein dynamics. Through genetic studies he has identified previously unknown regulators of ERMES. The creation of new ERMES foci is linked to mitochondrial dynamics and also seems to correlate with phospholipid synthesis, for example, during the unfolded protein response. Extending the screening approach to human cells has identified regulation of ER–mitochondrial bridging by new components that reside in multiple nonoverlapping sites of contact between ER and mitochondria.

Yasunori Saheki (Nanyang Technological University, Singapore) focused on two aspects of tethering and lipid traffic at specialized zones where the ER and the plasma membrane (PM) directly communicate. The first topic was the extended synaptotagmin family (E-Syt1-3, in yeast: tricalbins Tcb1-3). E-Syts are PI(4,5)P_2-dependent and Ca^{2+}-regulated ER-PM tethers. E-Syts transport glycerolipids via the synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domain, an evolutionarily conserved lipid-harboring module present in various membrane contact sites. They are important for the PM lipidome, not for steady-state phospholipid levels

[1]UCL Institute of Ophthalmology, London, UK
[2]Cell Biology and Cancer, Institut Curie, PSL Research University, CNRS UMR144, Paris, France
[3]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
[4]Department of Cell Biology, Yale School of Medicine, New Haven, CT, USA

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Corresponding Author:
Tim P. Levine, UCL Institute of Ophthalmology, London EC1V 9EL, UK.
Email: tim.levine@ucl.ac.uk

but for rapid adaptation to signaling, where recycling of diacylglycerol following phospholipase C activation requires E-Syts, as can be seen in cells lacking E-Syts. In unpublished studies, his laboratory is working on the three human GramD1a-c proteins in the family of lipid transfer proteins anchored at membrane contact sites (LAMs, also called lipid transfer and contact site, LTC), which have been shown in multiple systems to contribute to sterol transport from the PM to the ER. He showed that the GRAM domain, a pleckstrin homology-like domain named for its first discovery in glucosyltransferases, Rab-like GTPase activators and myotubularins, is a coincidence detector for cholesterol and phosphatidylserine, binding the PM because it is enriched for both ligands. The lipid transfer domain catalyzes intermembrane transfer of cholesterol. In gene-edited human cells lacking GramD1 proteins, PM cholesterol homeostasis is impaired. An acutely regulatable system which the laboratory has created shows that cholesterol is extracted from the PM within minutes of the recruitment of GramD1 proteins to ER-PM contacts.

Tim Levine (University College London, UK) talked about expanding the known range of intracellular communication by identification of more "two phenylalanine in an acidic tract" (FFAT) motifs, which target proteins on other organelles to VAPon the cytoplasmic face of the ER. He described how the originally tight definition of the motif by their discovery in oxysterol-binding protein and other lipid transfer proteins had been widened so far as to be impossible to detect by a simple linear description. He then introduced his unpublished bioinformatics study that aimed to identify all FFAT motifs present in yeast. Using a position weighted matrix, he predicted that approximately 1% of all proteins possess FFAT or FFAT-like motifs. Among these were some that implicate the existence of zones of intracellular communication previously either unknown (ER–eisosome) or largely overlooked (ER–nucleolus).

The second session addressed how the biosynthetic organelles have separate zones for distinct functions ("Response Zones"). Kazutoshi Mori (Kyoto University, Japan) spoke about multiple organelle zones present in the ER, which his group identified using super-resolution microscopy, namely, the folding zone, the sensing zone for the unfolded protein response, and ER-associated degradation (ERAD) zone.

Next, Julia von Blume (Yale University, USA) described her work on cargo sorting in the trans-Golgi network (TGN). Proteins destined for secretion such as Lysozyme-C and Cartilage Oligomeric Protein depend on the TGN-localized membrane-anchored Ca^{2+} ATPase SPCA1 (secretory pathway Ca^{2+}-ATPase isoform 1), and the calcium-binding protein Cab45 to exit the TGN. She showed how Ca^{2+} induces Cab45 to cluster into oligomers, both in vitro and in vivo, where Cab45 and client were shown to bud together in vesicles that then reach the PM. The identification of TGN-derived sphingomyelin-rich vesicles uncovered an exciting link between synthesis of sphingolipids and Cab45-dependent sorting. Purification and proteomics analysis of these vesicles identified Cab45 as one of the most abundant native cargoes of this pathway. Using the retention using selective hooks (RUSH) technique (see later), she showed that the lipid sphingomyelin is required in the TGN to allow cargo to reach the plasma membrane, but not for other destinations, such as the lysosome. She then linked the lipid requirement to previous findings, by showing that SPCA1 accumulates in detergent-resistant membranes, that the enzyme requires sphingomyelin for in vitro reconstitution, and that it can be cross-linked with a derivatized sphingosine. Using an in vivo sensor of Ca^{2+} levels in the TGN, she confirmed the link with calcium levels by showing that the enzyme sphingomyelin synthase (SMS1), which makes sphingomyelin in the TGN, is required for normal levels of Ca^{2+}.

Taroh Kinoshita (Osaka University, Japan) discussed his laboratory's work on regulation of a specific step in synthesis of glycosylphosphatidylinositol (GPI) anchors at two stages in the secretory pathway. Early on, GPI is affected by components of the ERAD pathway. A genome-wide CRISPR screen identified B3GALT4 (Beta-1,3-Galactosyltransferase 4), known before only for its role as monosialotetrahexosylganglioside (GM1) synthase, as the Golgi-localized enzyme that modifies GPI anchors by adding a terminal galactose to its sugar side chain. In addition, there were unexpected hits among ERAD components. The link to ERAD was exemplified by showing that this pathway prevents massive buildup of free GPI anchors (the glycolipid with no attached protein) when the transamidase enzyme that links the GPI to proteins is missing. Thus, ERAD is part of the cell's response that inhibits the GPI pathway when its capacity is excess to requirement. Later in the secretory pathway, GPI synthesis is tightly linked to production of lactosylceramide in the Golgi. This glycosphingolipid, which itself is produced by addition of a terminal galactose, is a critical cofactor for B3GALT4's terminal modification of GPI, as it interacts with both enzyme and substrate to permit glycosylation.

The final session was on technical aspects of imaging and focused on organelle zones that operate in protein transport ("Sorting Zones"). Akihiro Nakano (RIKEN, Japan) talked about his laboratory's work on techniques to visualize such zones. He has developed and improved 4D superresolution confocal live imaging microscopy systems to study the secretory pathway in action. The first iteration, SCLIM1, has at its core a spinning disk microscope and highly sensitive camera for ultrafast single molecule detection. SCLIM1 microscopy of

budding yeast showed that when early in secretion the cis-Golgi cisternal elements capture COPII vesicles, these are not floating free, but likely they are directly handed over from ER. The cis-Golgi approaches ER exit sites in an oscillatory manner, remaining nearby for a few seconds. The laboratory's initial work in yeast has now been generalized to plant and animal cells. The second microscopy setup, SCLIM2, has vastly improved sensitivity, speed, and resolution and was able to detect and track every cisterna and even individual clathrin-coated vesicles in real time. This system allowed his team to show that Golgi cisternae are highly dynamic at the 50-millisecond timescale. He visualized cisternal dynamics in the Golgi ribbon of an animal cell, transient tubular connections between cisternae, and cargo moving through the ribbon by applying the RUSH technique (see next talk). This leads to the proposal that the cisternal maturation model does not explain all aspects of intra-Golgi traffic.

The final speaker was Franck Perez (Institut Curie, France), who started by presenting his RUSH technique. An expressed protein is retained in one organelle, typically the ER by binding a second expressed protein. The interaction is broken by adding biotin, and synchronous wave of forward movement can reveal new details of secretory pathways. Within the Golgi, different secretory cargos show distinct behavior in terms of both speed of transit and sub-Golgi location. After leaving the Golgi, preferential sites of exocytosis can be found. These sites were more accurately defined either by patterning cells in a crossbow shape or by capturing exocytosed GFP-tagged cargo with an antibody coated onto the slide preplating. Both tools suggest adhesion sites as exocytic hot spots, and these results were confirmed using total internal reflection fluorescence (TIRF) microscopy. Using these assays, he started to study some of the mechanisms responsible for such restricted exocytosis. For example, he showed that the Rab6-dependent machinery is involved, together with a subset of microtubules. Even further along the secretory pathway, he has studied the cell-to-cell repulsion system based on ephrin signaling. He found that cells transport GPI-anchored Ephrin A1 to actin-rich filipodial extensions which are endocytosed by EphR-expressing cells. The receiving cell eats part of its neighbor in trans—hence, the process is called "transendocytosis" (also known as trogocytosis). The EphR-EphrinA1 complex remains in a modified endosome for many hours, signaling continually. Thus, the repelled cell takes away with it a part of the cell that repelled it, presumably as a molecular memory of the contact.

All eight talks use the study of single proteins to throw fresh light on how whole organelles have suborganelle specializations in the way they communicate, respond, and sort. By addressing the complexity of membrane cell biology at this level, the Organelle Zones group are making progress toward a more complete multidimensional understanding of how cells achieve the myriad tasks required for life.

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ORCID iD

Tim P. Levine https://orcid.org/0000-0002-7231-0775