Organelles Transport in Health and Disease

3rd - 4th December 2024
in Grenoble, France

4 main topics:

Metabolism | Neuroscience | Cell Biology | Biophysics & structural biology

Plenary Lecture: Volker Haucke (Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin)

Program and registration:

cell-transport.sciencesconf.org

Organizing Comittee: Chiara Scaramuzzino, Marta Prieto, Anthony Procès

Institutional partners

Gold sponsor

Silver sponsor

Bronze sponsor

Organelles transport in health and disease

Tuesday, December 03rd, 2024

08h00-09h00: Registration and welcome coffee break

Morning session: Metabolism

Chairs: Roberto Zoncu and Anja Zeigerer

09h00-09h10: Welcome speech

09h10-09h50: Julien Courchet *(Institut NeuroMyogène, Lyon, France)* – "Fine-tuning of cortical circuit development through a local regulation of mitochondria distribution and function"

09h50-10h30: Anja Zeigerer *(Heidelberg University, Mannheim, Allemagne)* – "Linking membrane transport to metabolic control"

10h30-11h05: Coffee break and poster session

11h00-11h40: Roberto Zoncu *(California University, Berkeley, San Francisco, USA)* - "The Lysosome in Nutrient Sensing and Cellular Growth Control"

11h40-11h55: Myriam Saliba *(Grenoble Institut Neurosciences, Grenoble, France)* – "Creatine Kinase provides energy for the retrograde axonal transport of signaling endosomes"

11h55-12h05: Bio-Techne (sponsor)

12h05-13h20: Lunch and poster session

Afternoon session: Neuroscience

Chairs: Marina Mikhaylova and Max Koppers

13h20-13h30: HuntX Pharma presentation by Laure Jamot, CEO (Gold sponsor)

13h30-14h10: Frédéric Saudou *(Grenoble Institut Neurosciences, Grenoble, France)* – "From Huntington's disease to axonal transport, neurotrophin signaling and energy metabolism"

14h10-14h50: Max Koppers *(Vrije Universiteit Amsterdam, Netherlands)* – "Local role of the endoplasmic reticulum in organizing axonal protein synthesis"

14h50-15h05: Zoë Van Acker (*VIB Center for Brain Disease Research, Leuven, Belgium*) – "Impact of lysosomal PLD3 dysfunction on axonal trafficking and synaptic dynamics in the hippocampal CA3 region"

15h05-15h50: Coffee break and poster session

15h50-16h30: Marina Mikhaylova *(Humboldt-Universität zu Berlin, Germany)* – "Synaptic control of secretory organelle localization in neurons"

16h30-16h45: Lucas Alves Tavares (*Center for Virology and Department of Cell and Molecular Biology, São Paulo, Brazil; Institute Curie, Paris, France*) – "The Contribution of AP-1γ2 to the Early-to-Late Endosome Maturation and Extracellular Vesicles Release"

16h45-17h00: Break and group photo

17h00-18h00: Plenary - Volker Haucke *Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Germany)* – "How the presynapse forms and functions"

19h30-23h00: Gala dinner at *Ciel Rooftop* (Bastille, Grenoble)

Wednesday, December 04th, 2024

8h30-9h00: Welcome coffee

Morning session: Cellular biology
Chairs: Frederik Verweij and Sara Sigismund

09h00-09h10: Proteintech (sponsor)
09h10-09h50: Ludger Johannes *(Institut Curie, Paris, France)* – "GlycoSwitch: a new signalling circuit to control endocytosis"
09h50-10h30: Sara Sigismund *(IEO, Milan, Italy)* – "Regulation of growth factor signalling via membrane contact sites"
10h30-10h45: Gabriele Zaffagnini (*Center for Genomic Regulation, Barcelona, Spain*) – "How endolysosomal super-organelles manage protein aggregates in the acentrosomal mouse oocyte"
10h45-11h20: Coffee break and poster session
11h20-12h00: Frederik Verweij *(Utrecht University, Netherlands)* – "Developing molecular toolboxes to study the loading and secretion of Extracellular Vesicles"
12h00-12h15: Sonia Ruggiero (*Insitute Curie, Paris, France*) – "Investigating the role of the protein arginine methyltransferase PRMT4/CARM1 in the regulation of vesicle trafficking in triple-negative breast cancer"

12h15-13h20: Lunch and poster session

Afternoon session: Biophysics and structural biology
Chairs: Aurélien Roux and Christophe Lamaze

13h20-13h30: 3i Intelligent Imaging (sponsor)
13h30-14h10: Bruno Antonny *(Institut de Pharmacologie Moléculaire et Cellulaire, Nice, France)* – "Changes in lipid arrangement upon surface tension or membrane curvature as cellular information for protein targeting"
14h10-14h50: Aurélien Roux *(Université de Genève, Switzerland)* – "Archaea Asgard reveals the conserved principles of ESCRT-III membrane remodeling"
14h50-15h05: Nicolas Fuggetta (*CEMIPAI, Montpellier, France*) – "Perilipin 4, a novel amyloid-forming protein implicated in skeletal muscle degeneration"
15h05-15h50: Coffee break and poster session
15h50-16h30: Christophe Lamaze *(Institut Curie, Paris, France)* - "Remote control of signaling by caveolae mechanics: A new paradigm in mechanotransduction"
16h30-16h45: Emma Pasquier (*École Normale Supérieure, Paris, France*) – "Bioengineered condensates to interact and manipulate membrane-bound organelles in cells"

16h45-17h00: Closing speech and awards ceremony

17h00-20h00: Happy hour with charcuterie and regional cheeses (*Fromagerie Les Alpages*) and wine tasting by *Maison Delmas* (Languedoc)

Plenary lecture

Tuesday, December 3, 2024

Volker Haucke

Leibniz-FMP

Berlin, Germany

Lipid switches in cell physiology: From nutrient signals to disease

Volker Haucke*[1]

[1]Department of Molecular Pharmacology Cell Biology, Leibniz Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany – Germany

Abstract

Phosphoinositides (PIs) form a minor class of phospholipids with crucial functions in cell physiology, ranging from cell signalling and motility to a role as signposts of compartmental membrane identity and dynamics. In my talk I will discuss the localization, regulation, and molecular mechanism of action of PI kinases and phosphatases and their roles in endocytosis and within the endolysosomal system. Moreover, I will cover our recent advances in the analysis of the metabolic pathways that regulate the cellular synthesis and turnover of distinct PI phosphates at endosomes or lysosomes in response to altering nutrient conditions, discuss the mechanisms by which these lipids regulate endolysosomal membrane dynamics and metabolism, and provide examples of how dysregulation of these pathways may cause human disease.

*Speaker

INVITED SPEAKERS

From intracellular transport to metabolic activity: pleiotropic functions of mitochondria in axonal development

Julien Courchet*[1]

[1]Institut NeuroMyoGène, Inserm, CNRS, Université Claude Bernard Lyon 1, Lyon, France – Institut NeuroMyoGene (INMG-PGNM) – France

Abstract

The correct patterning of long-range axonal connections relies on the coordinated activation of cell-intrinsic signaling pathways in response to environmental cues such as trophic factors, guidance molecules and synaptic activity. The kinase LKB1 has been linked to several aspects of axon development through the sequential activation of intracellular signaling pathways involving AMPK and related kinases. We previously identified that LKB1 regulates axon outgrowth and terminal branching through the activation of NUAK1, an AMPK-related kinase whose mutations are associated to ASD and other neurodevelopmental disorders. NUAK1, but not AMPK, regulates axonal mitochondria trafficking and metabolic activity to support axonal branching. We furthermore uncovered that NUAK1 has a novel function in the regulation of neuronal alternative splicing and identified targets linking gene expression and mitochondrial metabolism. I will finally present evidence that the LKB1/NUAK1 axis acts as an integrator of extracellular cues controlling axon branching, providing neural correlates to behavioral alterations found in LKB1- and NUAK1-deficient mice. Overall, our results suggest that LKB1 integrates extracellular signals to adapt axon branching to the local cellular context and suggest that a local regulation of metabolic activity participates in the balance between short and long-range axonal projections.

*Speaker

Coupling endosomal trafficking with liver metabolism

Anja Zeigerer*[1]

[1]Institute for Diabetes and Cancer, Helmholtz Center Munich, Neuherberg, Germany – Germany

Abstract

Over the last decades, obesity and type-2 diabetes have reached epidemic proportions. They are closely associated with other metabolic and cardiovascular diseases, such as fatty liver disease and myocardial infarction, and the current pharmacological treatments are minimal. This presents a heavy burden on societies worldwide and underscores the need to identify processes and pathways that can be used as novel therapeutic targets. Intracellular membrane trafficking has recently emerged as critical for maintaining metabolic homeostasis. When dysregulated, it may lead to metabolic diseases, but also cancer, neurodegeneration, and immune system disorders. Membrane trafficking is responsible for the sorting and distributing of proteins between the plasma membrane and different intracellular compartments. Central to this are endosomes, which internalize and sort signaling receptors, membrane transporters, hormones, and other ligands. Membrane trafficking is thus vital to the liver, a primary metabolic organ. To maintain metabolic homeostasis, hepatocytes must sense external nutritional cues and promptly integrate this information intracellularly. This is especially important for the fasting-feeding transition, during which glucose and lipid metabolism must be tightly regulated. In response to fasting, the liver downregulates glucagon-driven processes; in response to feeding, it upregulates insulin-driven processes. Hepatic endosomes make these fast responses possible by regulating the plasma membrane proteome and downstream signaling within minutes of food intake. Focussing on the emerging connection between liver metabolism and membrane trafficking, regulated by endosomes, I will present our ongoing study of the dynamics of endosomal protein localization during the fasting-feeding transition. Our analysis of the liver organelle proteome reveals that the endosomal system responds to refeeding cues as a broad network by quickly adapting protein internalization and recycling kinetics. I will discuss our current efforts to understand the signaling mechanisms underlying this complex protein redistribution and its potential for developing novel therapeutic strategies for cardiometabolic diseases.

*Speaker

Dissecting and disabling cholesterol-mTORC1 signaling in neurodegeneration.

Roberto Zoncu*[1]

[1]California University, Berkeley, San Francisco, USA – United States

Abstract

The lysosome plays a central role in cellular homeostasis through its participation in autophagy and its physical and functional association with the master growth regulator, mechanistic target of rapamycin complex 1 (mTORC1) kinase. mTORC1 integrates signals from nutrients, hormones and energy to control the balance between cellular growth and repair programs. Nutrients, including cholesterol, drive the localization of the master growth regulator, mechanistic Target of Rapamycin Complex 1 (mTORC1) to the lysosomal membrane, where mTORC1 regulates its downstream programs. Cholesterol sensing by mTORC1 occurs upstream of the heterodimeric Rag guanosine triphosphatases (GTPases) via the lysosomal cholesterol sensor LYCHOS, and is negatively regulated by the cholesterol exporter, Niemann-Pick C1 (NPC1) protein. In NPC1-deleted cells, mTORC1 becomes dysregulated, leading to disruption of autophagy and mitochondrial function. These data implicate aberrant cholesterol-mTORC1 signaling as a candidate pathogenic driver in the neurodegenerative disease, Niemann-Pick type C (NPC), but the molecular mechanisms of mTORC1-dependent cellular dysfunction in NPC remain to be determined. I will present our recent effort to unravel lysosomal cholesterol sensing through a combination of organelle proteomics, bioinformatic analysis and functional assays. Moreover, I will discuss ongoing work leveraging functional genomics to identify new pathways for mTORC1 regulation and cellular quality control that may have implications for neuronal cell homeostasis and NPC pathogenesis.

*Speaker

Energy supply for axonal transport of vesicles by glycolysis is a crucial determinant of neurodegeneration in health and Huntington disease

Frédéric Saudou*[1]

[1]Grenoble Institut Neurosciences – Université Grenoble Alpes, Grenoble Institute des Neuroscience – France

Abstract

Huntington's disease (HD), a late onset neurological disease characterized by accelerated aging and death of neurons, is caused by the abnormal polyglutamine expansion in the N-ter part of huntingtin (HTT), a large protein of 350kDa. Over the past years, we proposed that HTT associates to vesicles and acts a scaffold for the molecular motors and through this function, regulates the efficiency and directionality of vesicular transport in neurons. HTT controls the microtubule-based fast axonal transport (FAT) of neurotrophic factors such as BDNF. Importantly, polyQ expansion in HTT alters this function, leading to a decrease in neurotrophic support and death of striatal neurons. By developing microfluidic approaches allowing to study healthy and defective corticostriatal networks in vitro that are compatible with high-resolution videomicroscopy and the use of biosensors, we found that HTT also scaffolds the whole glycolytic machinery on vesicles to supply constant energy, independently of mitochondria, for the transport of vesicles over long distances in axons. Here we investigated the contribution of the glycolytic machinery to axonal transport in health and HD. Altering the local production of energy via the selective inactivation of Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) on vesicles reduced axonal transport, synapse number and postsynaptic signaling in vitro and induced neurological defects in mice. We next investigated energy production in HD conditions. We found that the vesicular, but not cytosolic GAPDH is altered in HD. Increasing GAPDH stoichiometry on vesicles rescued axonal transport and circuit functioning in HD-brain-on-chips. In vivo, it restored sensorimotor function, synapse density and striatal integrity as well as brain structure and circuit function in HD mice. Together, these results reveal that local modifications of energy supply on vesicles are crucial factors that control transport efficacy and neurodegeneration in vivo.

*Speaker

Local role of the endoplasmic reticulum in organizing axonal protein synthesis

Max Koppers*[1]

[1]Vrije Universiteit Amsterdam, Netherlands – Netherlands

Abstract

Local mRNA translation in axons is critical for the spatial and temporal regulation of the axonal proteome and neuron function. A wide variety of mRNAs are localized and translated in axons, however how protein synthesis is regulated at specific subcellular sites in axons remains unclear. Here, we establish that the axonal endoplasmic reticulum (ER) supports axonal translation in developing neurons. Axonal ER tubule disruption impairs local translation and ribosome distribution. Using nanoscale resolution imaging, we find that ribosomes make frequent contacts with ER tubules in the axon in a translation-dependent manner and are influenced by specific extrinsic cues. We identify P180/RRBP1 as an axonally distributed ribosome receptor that binds specific mRNAs and regulates local translation in an mRNA-dependent manner. Finally, we find that impairment of axonal ER – ribosome interactions cause defects in axon morphology. Our results establish an important role for the axonal ER in dynamically localizing mRNA translation which is important for proper neuron development.

*Speaker

Synaptic control of secretory organelle localisation in neurons

Marina Mikhaylova*[1]

[1]Humboldt University of Berlin, Berlin, Germany – Germany

Abstract

Secretory trafficking is essential for many neuronal processes including development, homeostasis and synaptic plasticity. Membrane proteins and lipids are processed through the ER, the Golgi complex and the endosomal system on their way to the plasma membrane from where they can be recycled back or targeted for subsequent degradation via lysosomes or autophagosomes. In addition to the cell body, most these components are found in dendrites and axons where they could serve local needs of the synapse. Here, I will speak about how neuronal activity can regulate trafficking and localisation of simplified Golgi-related structures called Golgi satellites (GS). Previously, we have shown that GS are present in the dendrites of primary hippocampal neurons. These organelles are distinct from the somatic Golgi complex and are involved in *de novo* glycosylation and local forward trafficking of membrane proteins. Our new data showed that GS are present all along the axon, extending to the distal tips of the growth cone. Similar to dendritic GS, the axonal organelles are labeled by the same GS markers and are capable of mature glycosylation. Live imaging experiments revealed the presence of both mobile and immobile GS in the axon, and that the switch between active transport and stalling of GS was modulated by neuronal firing. We found that GS frequently pause at en passant synapses and remain stationary for longer time periods at active pre-synaptic sites. This behavior is dependent on the actin cytoskeleton and the actin-based motor protein myosin VI. Overall, our study demonstrates that neuronal activity can dynamically regulate the positioning of GS in the axon, shedding light on the intricate mechanisms underlying organelle trafficking in neurons.

*Speaker

GlycoSwitches control glycolipid-lectin driven endocytosis

Ludger Johannes*[1]

[1]Institut Curie, Paris, France – Institut Curie – France

Abstract

It is commonly assumed that the glycan makeup of glycoproteins is final and static once they have reached the cell surface. Here, we challenge this notion by the discovery of two molecular switches that at the plasma membrane induce acute and reversible changes of glycan structure or arrangement in space. The two switches have a common denominator - the unexpectedly dynamic nature of glycans to drive endocytosis and repurposing of membrane glycoproteins. The physiological contexts in which the GlycoSwitches operate will be presented.

*Speaker

Regulation of growth factor signalling via membrane contact sites

Sara Sigismund*[1,2]

[1]University of Milan, Dipartimento di Oncologia ed Emato-oncologia, Milan – Italy
[2]European Institute of Oncology IRCCS, Department of Experimental Oncology, Milan – Italy

Abstract

The integration of distinct internalization routes is crucial to determine the fate of plasma membrane (PM) receptors and the output of their signalling pathways. Contact sites between cellular organelles adds a further layer of regulation by creating microdomains that governs different signalling and metabolic pathways. These regulatory mechanisms are relevant to the epidermal growth factor receptor (EGFR). Our research has revealed that, while clathrin-mediated endocytosis (CME) is mainly involved in EGFR recycling and sustaining signalling, EGFR internalization through non-clathrin endocytosis (NCE) leads primarily to receptor degradation and signal extinction, representing a crucial safety mechanism to protect cells from overstimulation. NCE involves contact sites between the PM, the endoplasmic reticulum and the mitochondria, that work as platforms for the modulation of localized calcium signalling and mitochondrial energetics. Importantly, this mode of regulation extends beyond EGFR to encompass other growth factor receptors, and is anticipated to be relevant in tumours. In my laboratory, we are currently elucidating how the integration of distinct endocytic pathways and inter-organelle crosstalk regulate growth factor receptor signalling and its interplay with cell metabolic functions in physiology and cancer.

*Speaker

What goes in, sometimes comes out: developing molecular toolboxes to study the loading, maturation and fate of Multivesicular Bodies

Frederik Johannes Verweij*[1,2]

[1]Utrecht university, Faculty of Science, Department of Biology, 3584 CH Utrecht, The Netherlands – Netherlands

[2]Centre for Living Technologies, Alliance Eindhoven University of Technology, Wageningen University Research, Utrecht University, University Medical Center Utrecht, The Netherlands – Netherlands

Abstract

Exosomes are lipid bound extra-cellular vesicles that are formed within so called Multi Vesicular Bodies (MVBs) by protein- and lipid cargo driven inward budding of the limiting membrane. These MVBs undergo endo- somal maturation and release exosomes when they MVBs fuse with the Plasma Membrane (PM) instead of being targeted for lysosomal degradation. The exact mechanism by which endosomes are formed, and in particular 1) loaded with cargo, and are 2) either rendered competent to fuse with the PM to release their exosomal cargo or undergo degradation by fusion with lysosomes is still unclear. We develop and apply molecular tools to decipher these processes by live microscopy.

Here we combine a previously developed CD63-based pH-sensitive optical EV reporter with opto- and chemogenetic approaches, and a novel EV-cargo reporter to study EV-cargo loading and MVB maturation and fate. To study this, we combine traditional and advanced microscopy methods, including spinning-disk, lattice-light sheet and dual-color TIRF-microscopy.

We identify exosome secretion as a multi-step process, where MVBs travel to the peri-nuclear area first, before they move towards the cell periphery. This step-wise maturation can render MVBs fusion competent with the PM. During this process, EV-cargo loading with newly synthesized proteins appears to occur via both direct and indirect pathways. Interestingly, MVBs that do not fuse with the PM, also reside in the perinuclear area, where instead, they are marked for degradation. As proposed and strongly suggested by previous results, degradation and secretion appear to be mutually exclusive fates that balance each other out.

*Speaker

Membrane curvature and surface tension as cellular information for protein targeting in the cell

Bruno Antonny*[1]

[1]Institut de Pharmacologie Moléculaire et Cellulaire (IPMC) – CNRS : UMR7275, Université Côte d'Azur (UCA) – CNRS-IPMC 660 Route des lucioles 06560 VALBONNE, France

Abstract

In the last two decades, cellular and biochemical approaches have shown that cells have proteins with exquisite ability to recognise membrane curvature. In general, membrane curvature sensing cooperates with the recognition of other membrane features resulting in specific targeting to defined organelles. Here, I will discuss recent approaches that suggest that cells used deceptively similar protein modules, notably regions with a high content in amphipathic helices, to recognise the surface tension of lipid droplets. Surface tensions appears as a decisive factor for the targeting of different perilipins to lipid droplets and might contribute to the heterogeneity of lipid droplets as seen in vivo.

*Speaker

Archaea Asgard reveals the conserved principles of ESCRT-III membrane remodeling

Aurélien Roux*[1]

[1]Université de Genève – Switzerland

Abstract

ESCRT-III proteins assemble into composite polymers that undergo stepwise changes in composition and structure to deform membranes across the tree of life. Here, using a phylogenetic analysis we demonstrate that the two ESCRT-III proteins present in eukaryote's closest Asgard archaeal relatives are evolutionarily related to the B-type and A-type eukaryotic paralogues that initiate and execute membrane remodelling, respectively. We show that Asgard ESCRT-IIIB assembles into parallel arrays on planar membranes to initiate membrane deformation, from where it recruits ESCRT-IIIA to generate composite polymers. Finally, we show that Asgard ESCRT-IIIA is able to remodel membranes into tubes as a likely prelude to scission. Taken together, these data reveal a set of conserved principles governing ESCRT-III-dependent membrane remodelling that first emerged in a two-component ESCRT-III system in archaea.

*Speaker

Remote Control of Signaling through Caveolae Mechanics: A new paradigm in mechanotransduction

Christophe Lamaze*[1]

[1]INSERM U1143 - CNRS UMR 3666, Institut Curie, Paris, France. – Institut Curie - CNRS UMR3666 - INSERM U1143 – France

Abstract

Caveolae are small invaginated nanodomains of the plasma membrane that have been classically involved in membrane trafficking and signaling. These multifunctional organelles were recently shown to play a key role as mechano-sensors that adapt the cell response to mechanical stress. Here, we investigated the role of caveolae mechanics in the control of signaling pathways. Using state-of-the-art super resolution imaging combined with machine-learning network analysis, we show that in response to mechanical stress, caveolae disassemble into so-called smaller scaffolds made of non-caveolar caveolin 1 (Cav1) - which display increased mobility at the plasma membrane. This promoted the direct interaction of the caveolin-1 scaffolding domain with several signaling molecules, resulting in inhibition of their catalytic activity. These results therefore establish caveolae as mechano-signaling hubs that couple sensing of membrane tension variations to the remote control of intracellular signaling through the release of diffusing Cav1 scaffolds at the plasma membrane.

*Speaker

SELECTED TALKS

Creatine Kinase provides energy for the retrograde axonal transport of signaling endosomes.

Myriam Saliba*[1], Emeline Cuoc[1], Marta Prieto[1], Chiara Scaramuzzino[1], and Frédéric Saudou[1]

[1][GIN] Grenoble Institut des Neurosciences – Univ. Grenoble Alpes, Inserm, U1216, CHU Grenoble Alpes, Grenoble Institut Neurosciences, GIN, F-38000 Grenoble, France – France

Abstract

Signaling endosomes are specific organelles retrogradely transported within the axons and essential for neuronal survival, synaptic plasticity, and circuit maintenance. This dynein-mediated retrograde transport of endosomes from the axon terminals towards the soma requires ATP. We previously reported that vesicles carry on-board their own fueling system composed of the glycolytic machinery. Interestingly, we found that the glycolysis only fuels the long-range retrograde trafficking of TrkB endosomes in proximal axonal area, but not in distal axons. So, what is the energy provider that fuels endosomal trafficking in distal axons? In addition to the glycolytic enzymes, we identified by proteomic approaches the presence of the brain creatine kinase (CKB) on motile vesicles, thus raising the possibility that CKB could function as an additional energy source. Using biochemical approaches, we confirmed the presence of CKB on small vesicles and in particular, its enrichment on signaling endosomes. Downregulating CKB completely blocked the BDNF-induced retrograde axonal transport of TrkB signaling endosomes. Combining the proximity ligation assay with immunostaining and enzymatic approaches, we found that BDNF activation of TrkB receptors at synapses increases the activity of CKB on endosomes in a Ca2+/Calmodulin dependent mechanism, thus providing the energy necessary for the transport of endosomes.

*Speaker

Impact of lysosomal PLD3 dysfunction on axonal trafficking and synaptic dynamics in the hippocampal CA3 region

Zoë Van Acker*[1,2], Assunta Verrengia[1,2], Kristofer Davie[3], Ine Vlaeminck[4], Suresh Poovathingal[3], Keimpe Wierda[4], and Wim Annaert[1,2]

[1]Laboratory for Membrane Trafficking, VIB Center for Brain Disease Research – Belgium
[2]KU Leuven, Department of Neurosciences, Leuven Brain Institute – Belgium
[3]Single Cell Microfluidics Expertise Unit, VIB Center for AI Computational Biology – Belgium
[4]Electrophysiology Expertise Unit, VIB-KU Leuven Center for Brain and Disease Research – Belgium

Abstract

Background: Synaptic dysfunction in the hippocampus, especially in the CA3 region, is implicated in memory deficits associated with Alzheimer's Disease (AD). Phospholipase D3 (PLD3), a lysosomal 5'-3' exonuclease linked to late-onset AD risk, is involved in both lysosomal and mitochondrial pathways, but its effect on synaptic transmission and lysosomal trafficking in the hippocampus is still unclear.
Materials and Methods: In a PLD3 knockout (KO) mouse model, we examined synaptic function in the CA3 region using multi-electrode array electrophysiology, paired-pulse facilitation, and transmission electron microscopy (TEM) to assess synaptic structure and vesicle dynamics. Additionally, we utilized lysosomal tracking assays in microfluidic devices to investigate axonal trafficking, particularly focusing on vesicle speed, movement directionality, and proportion movement. We are investigating underlying molecular pathways, including STING signaling, lysosomal homeostasis and transcriptional changes (transcriptomics) to uncover mechanisms driving synaptic alterations.

Results: PLD3 KO mice show reduced long-term potentiation in the CA3 region, coupled with increased response amplitudes at various stimulation strengths, suggesting altered vesicle release dynamics. TEM analysis reveals smaller synapses, shorter postsynaptic densities, and increased synaptic vesicle heterogeneity. Preliminary data indicates that PLD3 KO neurons exhibit impaired lysosomal trafficking, with reduced movement proportions and slower lysosome movement along axons.

Conclusion: PLD3 dysfunction disrupts lysosomal trafficking and synaptic transmission in the CA3 region. These findings offer new insights into the connection between lysosomal and synaptic functioning.
Ref. 1. Van Acker, Nat Commun. (2023) doi: 10.1038/s41467-023-38501-w.

*Speaker

The Contribution of AP-1γ2 to the Early-to-Late Endosome Maturation and Extracellular Vesicles Release

Lucas Alves Tavares*[1,2], Mireia Gomez Duro[2], Mara Elisama Da Silva-Januario[1], Mathilde Dimarco[2], Roger Luiz Rodrigues[1], Murilo Henrique Anzolini Cassiano[1], Andreia Nogueira De Carvalho[1], Bergam Ptissam[2], Maryse Romao[2], Gisela D'angelo[2], Raposo Graça[2], and Luis Lamberti Pinto Dasilva[1]

[1]Center for Virology Research and Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil – Brazil

[2]Institut Curie, Paris Sciences Letters Research University, CNRS, UMR144, Structure and Membrane Compartments, Paris Cedex 05 75248, France – Institut Curie, PSL Research University, CNRS – France

Abstract

AP-1γ2 belongs to a family of five heterotetrameric complexes, which mediate intracellular protein trafficking. However, the physiological role of AP-1γ2 has been poorly characterized. We recently reported that AP-1γ2 is required for HIV-1 Nef-mediated downregulation of CD4 and MHC-I, as well as the retrograde transport of CI-MPR and ATP7B. In this study, we report that AP-1γ2 also participates in early-to-late endosome maturation. We developed AP-1γ2 KO cells by CRISPR-Cas9 approach and by immunofluorescence observed morphological alteration in both early and late endosomes marked, respectively by Rab5/EEA1 or Rab7/CD63/Lamp-1/LBPA. Indeed, our transmission electron microscopy analysis revealed that AP-1γ2 depletion causes an apparent aberrant formation of MVBs. AP-1γ2 ablation also compromised cargo protein degradation, since Alexa-Fluor-488-EGF uptake assay reveals that internalized EGF remains trapped in the early endosome in AP-1γ2 KO cells. Moreover, overexpressing a GTP-locked form of Rab5 leads to the accumulation of LBPA in the early endosomes in WT cells, but not in γ2 KO cells. Furthermore, nanoparticle tracking, western-blot, and immuno-electron microscopy analysis of Extracellular Vesicles (EVs) secretion revealed that the ablation of AP-1γ2 decreases small EVs (CD63) and increases large EVs (CD9) secretion, with an alteration of EVs markers in the Lipid Rafts. Therefore, the results suggest a crucial role of AP-1γ2 in the maturation of early endosomes to late endosomes, possibly in the formation of ILVs of MVBs, resulting in altered secretion of EVs.

*Speaker

How endolysosomal super-organelles manage protein aggregates in the acentrosomal mouse oocyte

Gabriele Zaffagnini*[1], Shiya Cheng[2], Marion Salzer[3], Barbara Pernaute[3], Juan Manuel Duran Serrano[3], Manuel Irimia[3], Melina Schuh[2,4], and Elvan Boke[3]

[1]Center for Genomic Regulation – C/ Dr. Aiguader, 88 08003 Barcelona, Catalonia, Spain, Spain

[2]Department of Meiosis, Max Planck Institute for Multidisciplinary Sciences – 37077 Göttingen, Germany

[3]Center for Genomic Regulation – Spain

[4]Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Universität Göttingen – 37077 Göttingen, Germany

Abstract

Lysosome positioning is crucial for their activity and function. Juxtanuclear clustering of lysosomes around the centrosome promotes the delivery of endocytic and autophagic cargos, thereby facilitating the clearance of protein aggregates. Here, we present our recent finding (1) that, in the acentrosomal mouse oocyte, lysosomes are clustered in liquid-like super-organelles that we named EndoLysosomal Vesicular Assemblies (ELVAs). ELVAs harbor the major intracellular protein degradation systems, and store ubiquitinated protein aggregates targeted for degradation, including TDP-43.

Largely non-degradative in immature oocytes, ELVAs migrate towards the cell cortex and activate degradation shortly before fertilization, thereby clearing the stored aggregates. Unlike microtubule-dependent endolysosomal transport in somatic cells, ELVAs' migration relies entirely on the actin cytoskeleton, and it is required for the activation of protein degradation in ELVAs. Disturbance of ELVAs activation, as well as forced inheritance of protein aggregates in the embryo, compromise oocyte maturation and preimplantation embryonic development. Thus, ELVAs represent a strategy to safeguard the oocyte proteome from aggregation in the absence of pericentrosomal lysosomal clustering.

References:

1. Zaffagnini, G. *et al.* Mouse oocytes sequester aggregated proteins in degradative super-organelles. *Cell* (2024) doi:10.1016/j.cell.2024.01.031.

*Speaker

Investigating the role of the protein arginine methyltransferase PRMT4/CARM1 in the regulation of vesicle trafficking in triple-negative breast cancer

Sonia Ruggiero*[1], Solene Huard[1], Samyuktha Suresh[1], Vanessa Masson[2], Kevin Moreau[3], Didier Meseure[4], Damarys Loew[2], Nicolas Servant[5], Reini Luco[3], Nicolas Reynoird[6], and Thierry Dubois[1]

[1]Breast Cancer Biology Group – Département de Recherche Translationnelle, Institut Curie, PSL Research University, CNRS, UMR144 – France

[2]Proteomics platform – Institut Curie, PSL Research University – France

[3]Chromatin and RNA splicing team – Institut Curie, PSL Research University, CNRS UMR3348, F-91405 Orsay, France – France

[4]Experimental Pathology platform – Institut Curie, PSL Research University – France

[5]Bioinformatics platform – Bioinformatics, Biostatistics, Epidemiology and Computational Systems Unit, Institut Curie, PSL Research University, INSERM U900, Paris, France – France

[6]Institute for Advanced Biosciences / Institut pour l'Avancée des Biosciences (Grenoble) – Université Alpes Grenoble – France

Abstract

The protein arginine methyltransferase CARM1 (PRMT4) has recently emerged as a promising therapeutic target for triple-negative breast cancer (TNBC). Our laboratory found that CARM1 is overexpressed in TNBC compared to normal breast tissue and is required for TNBC cells survival.
To better understand CARM1 functions in TNBC cells, we characterized its interactome using immunoprecipitation coupled to mass spectrometry. I identified the ERGIC-resident protein TFG as a partner of CARM1. TFG is a well-known mediator of the uncoating of COP-II-positive vesicles travelling from the ER to the ERGIC, and has also been reported to regulate endocytosis in neurons. My results thereby envision a new role for CARM1 in vesicle trafficking.

I uncovered that CARM1 methylates TFG on two C-terminal arginines in TNBC cells. Since arginine methylation can interfere with protein-protein interactions, we wondered which proteins interact specifically with methylated TFG and what could be the downstream effect of this interaction. In collaboration with Nicolas Reynoird (IAB, Grenoble), we unveiled that proteins involved in retrograde transport of AP1-derived vesicles to the Golgi are potential effectors of CARM1-mediated dimethylation of TFG, which are still under validation.

On the other hand, PRMTs are reported to be alternative splicing (AS) regulators. Transcriptomic analyses performed in CARM1-depleted TNBC cells implied that CARM1 can

*Speaker

tune retrograde transport also by regulating the expression of genes involved in this pathway through modulation of their AS. We therefore hypothesize a dual, parallel action of CARM1 in orchestrating endosome-to-Golgi targeting: via methylation of TFG and via post-transcriptional regulation of trafficking genes.

Perilipin 4, a novel amyloid-forming protein implicated in skeletal muscle degeneration

Nicolas Fuggetta* , Charbel Choufani , Bayane Sabbagh , Cyril Moulin , Sébastien Lyonnais[1], Luc Bousset[2], and Alenka Čopič

[1]CEMIPAI – CNRS : UMS3725, Université de Montpellier – 1919 Route de Mende, Montpellier, France
[2]Molecular Imaging Research Center [Fontenay-aux-Roses] – Université Paris-Saclay, Centre National de la Recherche Scientifique, Institut de Biologie François JACOB – France

Abstract

Perilipin 4 (PLIN4) belongs to the family of perilipins – proteins that localize to lipid droplets (LDs) and regulate lipolysis and LD stability. PLIN4 binds to LDs using an amphipathic helix (AH), which folds on the LD surface and can substitute for phospholipids in the LD monolayer, thereby regulating LD surface tension (1, 2). Strikingly, the AH of PLIN4 contains close to 1000 amino acids (aa), arranged in highly homologous tandem repeats of 33 aa. Mutated PLIN4 resulting from a genetic expansion of its repetitive region accumulates inside skeletal muscle cells in patients suffering from a late-onset vacuolar distal myopathy (MRUPAV) (3). We demonstrate that purified PLIN4 AH fragments aggregate in vitro, forming amyloid fibrils, which we have characterized using cryo-EM and atomic force microscopy. We have analyzed the influence of PLIN4 sequence, as it varies between different repeats, on its aggregation propensity. Importantly, we show that the MRUPAV mutation drastically increases the kinetics of fibril formation in vitro and induces aggregation in budding yeast, suggesting a causative link between PLIN4 amyloids and muscle degeneration. We propose that the determining factor is the multiplication of identical repeats in mutant PLIN4. We are now establishing experimental models to assess how the abundance and nature of LDs influence the formation of PLIN4 fibrils, to study the interplay between LDs, PLIN4 status and skeletal muscle function.
(1) Giménez-Andrés et al. Elife 2021, DOI: 10.7554/eLife.61401.

(2) Araujo et al. JCB 2024, DOI : 10.1083/jcb.202403064.
(3) Ruggieri et al. Acta Neuropathol. 2020, DOI: 10.1007/s00401-020-02164-4.

*Speaker

Bioengineered condensates to interact and manipulate membrane-bound organelles in cells

Emma Pasquier*[1]

[1]École normale supérieure - Paris – Ecole Normale Supérieure de Paris - ENS Paris – France

Abstract

The dynamic interactions between the location and timing of biochemical processes within organelles is crucial for determining cell fate and function. Advanced biochemical, genetic, imaging, and "omics" approaches have revealed many important features of the biogenesis and functions of cellular organelles. In particular, recent studies suggest that communication between membrane organelles, through the establishment of membrane contact sites (MCS), could play crucial roles for their functions. However much less is known about putative contact sites between membrane-bound and membrane-less organelles (also known as biomolecular condensates). One key limitation comes from the biochemical complexity of condensates that make them difficult to study and manipulate in a cell context. To further advance in that direction, we are developing a novel methodology allowing the controlled assembly/disassembly in cells of bioengineered membrane-less organelles and that recapitulate some biophysical features of liquid-like condensates. Our engineered condensates are based on protein scaffolds designed to undergo phase separation in cells in a reversible manner. We demonstrated that it is possible to specifically target the assembly of engineered condensates on the surface of lysosomes, making them suitable for addressing fundamental questions about the interplay between condensates and lysosome interactions: How condensate nucleation and growth are modulated when interacting with the lysosomes? Conversely, how lysosome spatiotemporal dynamics is impacted when interacting with condensates? This method is versatile and could, in principle, be applied for studing other cellular organelles.

*Speaker

ABSTRACTS

Creatine Kinase provides energy for the retrograde axonal transport of signaling endosomes.

Myriam Saliba*[1], Emeline Cuoc[1], Marta Prieto[1], Chiara Scaramuzzino[1], and Frédéric Saudou[1]

[1][GIN] Grenoble Institut des Neurosciences – Univ. Grenoble Alpes, Inserm, U1216, CHU Grenoble Alpes, Grenoble Institut Neurosciences, GIN, F-38000 Grenoble, France – France

Abstract

Signaling endosomes are specific organelles retrogradely transported within the axons and essential for neuronal survival, synaptic plasticity, and circuit maintenance. This dynein-mediated retrograde transport of endosomes from the axon terminals towards the soma requires ATP. We previously reported that vesicles carry on-board their own fueling system composed of the glycolytic machinery. Interestingly, we found that the glycolysis only fuels the long-range retrograde trafficking of TrkB endosomes in proximal axonal area, but not in distal axons. So, what is the energy provider that fuels endosomal trafficking in distal axons? In addition to the glycolytic enzymes, we identified by proteomic approaches the presence of the brain creatine kinase (CKB) on motile vesicles, thus raising the possibility that CKB could function as an additional energy source. Using biochemical approaches, we confirmed the presence of CKB on small vesicles and in particular, its enrichment on signaling endosomes. Downregulating CKB completely blocked the BDNF-induced retrograde axonal transport of TrkB signaling endosomes. Combining the proximity ligation assay with immunostaining and enzymatic approaches, we found that BDNF activation of TrkB receptors at synapses increases the activity of CKB on endosomes in a $Ca2+$/Calmodulin dependent mechanism, thus providing the energy necessary for the transport of endosomes.

*Speaker

Control of lipid droplet dynamics in cells using engineered compartments

Chems Amari*[1,2], Damien Simon , Abdou Rachid Thiam , and Zoher Gueroui

[1]Processus d'Activation Sélective par Transfert d'Energie Uni-électronique ou Radiatif (UMR 8640) – Département de Chimie - ENS Paris, Institut de Chimie - CNRS Chimie, Sorbonne Universite, Centre National de la Recherche Scientifique – France

[2]Physiologie cellulaire et végétale – Centre National de la Recherche Scientifique, Institut de Recherche Interdisciplinaire de Grenoble, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Université Grenoble Alpes, Centre National de la Recherche Scientifique : UMR5168, Institut de Recherche Interdisciplinaire de Grenoble : DRF/IRIG/DBSCI, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement : UMR1417 – France

Abstract

Understanding the spatial and temporal regulation of lipid droplets (LDs) is crucial for unraveling their roles in cellular metabolism, stress responses, and cell fate. However, the mechanisms governing LD dynamics and the tools to manipulate them in cells remain limited. Here, we have developed ControLD (Controlled Trapping Of LDs), a novel approach to selectively confine LDs using engineered compartments that act as a dense protein-based meshwork. The trapping of LDs in these compartments contributes to their physical isolation from the rest of the cell, and their release into the cytosol can be triggered within a minute time scale. Finally, we have found that the confinement of LDs affects their dynamics, as LD consumption during metabolic demand is greatly perturbed. We anticipate that ControLD will provide a tool to manipulate and study LD biology more effectively, contributing to uncovering the role of LD dynamics in various cellular processes.

*Speaker

Label-free quantification of organelle trafficking inside a single axon

Baptiste Marthy*[1], Maxim Cazorla[2], and Guillaume Baffou[3]

[1]Institut Fresnel – Institut Fresnel – France
[2]Institut de Neurosciences de la Timone (INT) – Centre National de la Recherche Scientifique : UMR7289, Aix Marseille Université : UMR7289 – France
[3]Institut Fresnel – Aix Marseille Université, Ecole Centrale de Marseille, Centre National de la Recherche Scientifique - CNRS – Faculté des Sciences et Techniques avenue Escadrille Normandie Niemen 13 397 Marseille cedex 20, France

Abstract

The study of organelle trafficking traditionally relies on fluorescent protein tagging methods. These tags enable the visualization of organelle lifecycles, thus refining models of cellular trafficking. However, fluorescence-based techniques face significant limitations. First, fluorescent proteins are susceptible to photobleaching and phototoxicity, which reduces the observation window for dynamic, long-lasting cellular events. Second, only labeled compartments are made visible, challenging the construction of a comprehensive map of cellular processes when most components cannot be imaged simultaneously.

In this work, we show that quantitative phase imaging (QPI) allows to acquire label-free images of organelle trafficking within a single axon in-vitro. Neural network are reconstructed using microfluidics where single axon can be isolated. QPI quantifies the dry mass of biological structures over extended period of times, without perturbation. We demonstrate that QPI can measure distinct cargoes behaviors (such as dry mass evolution, speed and pauses) while providing contextual information about the axon's morphology, its branches, and its connections with other neurons. We are currently correlating QPI with fluorescence imaging, advancing towards computational _in-silico_ labeling.

*Speaker

The axonal transport and maturation of autophagosomes is regulated by the gigaxonin-E3 ligase

Caroline Liénard*[1], Leticia Arias[2], and Pascale Bomont[1]

[1]Institut NeuroMyoGene (INMG)-Physiopathology Genetic of Neuron and Muscle (PGMN) Inserm U1315, UCBL, UMR 5261 – Institut National de la Santé et de la Recherche Médicale - INSERM, Université Claude Bernard-Lyon I - UCBL (FRANCE), CNRS : UMR5261 – France

[2]Institut NeuroMyoGene (INMG)-Physiopathology Genetic of Neuron and Muscle (PGMN) Inserm U1315, UCBL, UMR 5261 – Institut National de la Santé et de la Recherche Médicale - INSERM, Université Claude Bernard-Lyon I - UCBL (FRANCE), CNRS : UMR5261 – France

Abstract

Autophagy is a major cellular degradation pathway, particularly essential in neurons due to their post-mitotic nature and their morphology. In neurons, this process is compartmentalized: the formation of autophagosomes occurs in the distal axon, and the maturation and fusion with lysosomes in the soma. In the laboratory, we study giant axonal neuropathy (GAN) as a pathological model to investigate the spatial regulation of neuronal autophagy. This disease is caused by mutations in the gene encoding for gigaxonin, an E3 ligase controlling the degradation of ATG16L1, a key protein for autophagosome elongation. In GAN neurons, gigaxonin mutation induces an aggregation of ATG16L1, an impairment of LC3 lipidation, leading to a decrease production of autophagosomes. Considering the unexpected localization of ATG16 aggregates in the soma, we use GAN as an interesting biological model to study the compartmentalization of autophagy. Using the tandem probe RFP-GFP-LC3, we evidence different spatial distribution of autophagic vesicles in GAN and WT neurons, in basal condition and after starvation. Using real-time imaging techniques, we demonstrate the involvement of gigaxonin in the transport-maturation coupling in neurons. Indeed, GAN neurons exhibit a general defect of autophagosome transport, in both anterograde and retrograde directions. In addition to sheds light into the pathophysiological mechanisms involved in GAN, this work enlightens our knowledge of the understanding of the autophagy compartmentalization in the neuron.

*Speaker

Deciphering the beneficial activity of protein palmitoylation in restoring axonal transport defects in Huntington's disease

Tapuwanashe Chikoore*[1,2], Aurélie Le Lay[2], Maxime Bonnet[2], Léo Morey[2], Fanny Roth[2], Frédéric Saudou[2], and Laure Jamot[2]

[1]Grenoble Institut des Neuroscience – Grenoble Institute des Neuroscience – France
[2]HuntX pharma – HuntX pharma – France

Abstract

Huntington disease (HD) is a devastating neurodegenerative disease for which there is no neuroprotective treatment. Palmitoylation, a post-translational modification that plays a role in intracellular trafficking is dysregulated in HD. We previously reported that increasing brain palmitoylation using HX127, a selective inhibitor of Acyl Protein Thioesterase 1 (APT1) rescues axonal transport and restores behaviour and neuropathology in HD mice. The objectives of my phD are to identify the specific substrates of APT1 involved in HX127 therapeutic effect and to demonstrate the target engagement of APT1.
Using acyl resin assisted exchange (Acyl-RAC) technique we demonstrated that the palmitoylation of HTT is increased by HX127. Then, by mass spectrometry we compared the palmitoyl-proteome of WT, HD and HD HX127-treated mice and identified 121 differentially palmitoylated protein (DPP) in HD compared to WT mice. HX127 restored the palmitoylation level of about 30% of them . Interestingly, the list is enriched in proteins that are involved in axonal transport (kinesin & dynein), in mitochondria homeostasis and in synapse plasticity. For APT1 target engagement we used an HD cell model (StHdhQ7/Q111) where we showed that the palmitoylation of HTT was significantly reduced compared to control cells (StHdhQ7/7). We will overexpress APT1 to decrease the palmitoylation of HTT and show that our molecule counteracts this effect.
We are currently validating the most relevant substrates using Acyl-RAC assays. A better understanding of HX127 mechanism of action will allow us to define the key substrates that mediate the beneficial effects of HX127 and may identify biomarkers to track disease progression and treatment efficacy in patients.

*Speaker

FROM TARGETTING TO RESTORING ENDOSOMAL SIGNALLING TO CURE HD.

Anna Trevisan*[1], Claire Séris[1], Frédéric Saudou[1], and Chiara Scaramuzzino[1]

[1][GIN] Grenoble Institut des Neurosciences – Univ. Grenoble Alpes, Inserm, U1216, CHU Grenoble Alpes, Grenoble Institut Neurosciences, GIN, F-38000 Grenoble, France – France

Abstract

Neurotrophin signaling is crucial for neuronal survival and brain connectivity. In Huntington's disease (HD), abnormal neurotrophic signaling, particularly involving brain-derived neurotrophic factor (BDNF), contributes to cortico-striatal neurodegeneration. BDNF is a key neurotrophic factor produced by the cortex which regulates brain development, neuronal survival, and plasticity. We recently showed that BDNF binding to its receptor TrkB triggers a molecular cascade involving calcium release, calcineurin (CaN) activation, and huntingtin (HTT) dephosphorylation, which promotes retrograde movement of TrkB signaling endosomes in cortical axons. We showed that CaN and HTT colocalize on the endosomal surface, allowing calcium-dependent regulation of endosomal transport. Importantly, this retrograde transport extends beyond the nucleus to sustain presynaptic dendrites far from the endosomal formation site.

The HTT mutation causing HD disrupts cortico-striatal connectivity; we showed that it alters the interaction HTT-CaN, thus impacting retrograde endosomal transport and therefore neuronal survival. Recent evidence suggests that membrane-bound organelles, such as endosomes, may coordinate mRNA trafficking and local translation, forming a rapid and localized transduction pathway responsive to neurotrophins. We propose that the TrkB-CaN-HTT complex functions as a motile translation platform, delivering specific signals via local translation to maintain network integrity. Using ribopuromycilation (RPM) techniques, we found that synaptic BDNF treatment increases local translation on retrograde TrkB+ endosomes under normal conditions, but not in HD.

We hypothesize that impaired HTT-CaN interaction disrupts this retrograde signaling, leading to dysregulated cortico-striatal communication. However, it remains unclear how TrkB-CaN-HTT signaling endosomes control long-distance translational programs to maintain circuit integrity. We aim to identify which mRNAs are selectively transported and how they are directed to specific subcellular compartments. To this end, we are optimizing RNA and protein extraction protocols to profile subcellular transcriptomic, translatomic, and proteomic changes, potentially revealing new mechanisms to preserve cortico-striatal network integrity and improve intracellular signaling to restore neurotrophic support to distal compartments.

*Speaker

Altered expression of Presenilin2 impacts endolysosomal homeostasis and synapse function in Alzheimer's disease-relevant brain circuits

Wim Annaert*[1], Anika Perdok[1], Zoë Van Acker[1], Céline Vrancx[1], Ragna Sannerud[1], Inge Vorsters[1], Assunta Verrengia[1], Zsusanna Vegh-Callaerts[2], Eline Creemers[1], Sara Gutierrez Fernandez[1], Britt D'hauw[1], Lutgarde Serneels[1], Keimpe Wierda[1], and Lucia Chavez-Gutiérrez[1]

[1]VIB Center for Brain and Disease Research KU Leuven – Belgium
[2]mINT, Faculty of Psychology, KU Leuven – Belgium

Abstract

Rare mutations in the gene encoding presenilin2 (PSEN2), a catalytic subunit of γ-secretase, cause familial Alzheimer's disease (FAD). Mutations in PSEN2 shift production of amyloid β (Aβ) peptides to the longer, more aggregation prone Aβ42. Moreover, given PSEN2/γ-secretase mainly localizes to late endosomes/lysosomes (LE/Lys), it also contributes particularly to the intracellular pool of Aβ, which surges early in disease pathogenesis. However, the underlying molecular and cell biological mechanisms on how mutant PSEN2 leads to neuronal demise remain elusive. Using newly generated APP knock-in (KI) mouse models we now demonstrate that PSEN2 knockout (KO) as well as FAD-associated N141IKI mutant both accelerated AD-related pathologies. Both models showed significant deficits in working memory compared to control mice, that linked to the elevated PSEN2 expression in the hippocampal CA3 region. The mossy fiber circuit of both APPxPSEN2KO and APPxFADPSEN2 mice had smaller pre-synaptic compartments, distinct changes in synaptic vesicle populations and significantly impaired long term potentiation compared to APPKI mice. In primary hippocampal neurons derived from APPKI, APPxPSEN2KO and APPx-FADPSEN2 mice, altered PSEN2 expression resulted in endolysosomal defects, decreased lysosomal exocytosis and lowered surface expression of synaptic proteins; underscoring recycling defects. These defects caused as well an increased autophagic flux, with accumulating autophagosomes en autolysosomes in primary neurons. As PSEN2/γ-secretase is restricted to LE/Lys, we propose PSEN2 impacts endolysosomal homeostasis, affecting synaptic signaling in AD-relevant vulnerable brain circuits; which could explain how mutant PSEN2 accelerates AD pathogenesis.

*Speaker

α-Synuclein-induced autophagy dysfunction in neuronal cells contributes to tunneling nanotube-mediated interaction with microglia

Ranabir Chakraborty*[1] and Chiara Zurzolo[2]

[1]Institut Pasteur – Institut Pasteur de Paris – France
[2]Membrane Traffic and Pathogenesis Unit – Institut Pasteur de Paris – France

Abstract

Tunneling nanotubes (TNTs) represent a major form of intercellular communication, allowing material exchange over long distances between the connected cells, events that are less stochastic than secretory mechanisms and lead to direct and specific communication. Different kinds of materials can shuttle via TNTs, ranging from small ions to large organelles and protein aggregates. Previous reports have demonstrated efficient directional transfer of α-Synuclein (α-Syn) aggregates from neuronal cells to astrocytes and microglia. However, the underlying reason for such directionality remains unclear. Using quantitative fixed- and live-cell microscopy, we observed differential localization of aggregates on lysosomes of neuronal and microglial cells. This resulted in increased permeabilization of lysosomal membranes. Although lysosomal biogenesis increased for both neuronal and microglial cells upon exposure to aggregates, the ability of microglia to target lysosomes for degradation via lysophagy was significantly higher than neuronal cells, both at a basal level and in the presence of aggregates. We found that neuronal lysosomes had impaired degradative ability compared to microglia, with reduced autophagic flux and fewer aggregates being targeted for degradation. Further inhibition of autophagy in neuronal cells led to increased aggregate transfer to microglia and a rise in the number of homotypic TNTs between cells. Thus, we propose a framework of dysfunctional autophagy in neuronal cells as a functional explanation behind directional α-Syn transfer to microglia.

*Speaker

Defective mitochondria and ER transport to distal Purkinje cell processes results in calcium dysregulation finally leading to neurodegeneration and neuroinflammation in the ARSACS mouse model

Andrea Del Bondio*[1,2], Fabiana Longo[1], Daniele De Ritis[1], Erica Spirito[1], Bernard Brais[3], Angela Bachi[4], Angelo Quattrini[1], and Francesca Maltecca[1]

[1]IRCCS San Raffaele Scientific Institute [Milan, Italie] – Italy
[2]Institut NeuroMyoGène - Appui à la recherche – Institut NeuroMyoGene (INMG-PGNM) – France
[3]McGill University – Canada
[4]IFOM, Istituto FIRC di Oncologia Molecolare – Italy

Abstract

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is caused by mutations in *SACS* gene encoding sacsin, a huge protein highly expressed in cerebellar Purkinje cells (PCs). ARSACS patients, as well as mouse models, display early degeneration of PCs, but the underlying mechanisms remain unexplored, with no available treatments.

We demonstrated aberrant calcium (Ca2+) homeostasis and its impact on PC degeneration in ARSACS. Mechanistically, we found pathological elevation in Ca2+-evoked responses in *Sacs–/–* PCs as the result of defective mitochondria and ER trafficking to distal dendrites and strong downregulation of key Ca2+ buffer proteins. Alteration of cytoskeletal linkers, which we identified as specific sacsin interactors, likely account for faulty organellar trafficking in *Sacs–/–* cerebellum. Based on this pathogenetic cascade, we treated *Sacs–/–* mice with Ceftriaxone, a repurposed drug that exerts neuroprotection by limiting neuronal glutamatergic stimulation and, thus, Ca2+ fluxes into PCs.

Ceftriaxone treatment significantly improved motor performances of *Sacs–/–* mice, at both pre- and postsymptomatic stages. We correlated this effect to restored Ca2+ homeostasis, which arrests PC degeneration and attenuates secondary neuroinflammation. These findings disclose key steps in ARSACS pathogenesis and support further optimization of Ceftriaxone in preclinical and clinical settings for the treatment of patients with ARSACS.

*Speaker

Axonal proteostasis: Focus on re-routing of degradative cargo for secretion

Yannes Popp*[1], Alexander Biermeier[1], Kadi Fobie[1], Pathma Muthukottiappan[2], Dominic Winter[2], and Marina Mikhaylova[1]

[1]Humboldt-Universität zu Berlin = Humboldt University of Berlin = Université Humboldt de Berlin – Germany

[2]Rheinische Friedrich-Wilhelms-Universität Bonn – Germany

Abstract

The autophagy-lysosomal system plays a central role in synaptic remodeling. In addition to rather well-investigated functions of lysosomes and autophagosomes in degradation of proteins and other cellular components, new, unconventional roles have emerged. In this project we focus on axonal autophagosome and lysosome exocytosis as a mechanism responsible for a rapid clearance of 'aged' synaptic components as well as a way of intercellular communication. As a model system, we use adult dissociated primary hippocampal mouse neurons cultured in microfluidic devices, in which axons are separated from somas and dendrites so that neurons can be treated and studied in a compartment-specific manner. Proteomic analysis of somato-dendritic and axonal compartments indicated a cell-compartment-specific differential regulation of the secretome under autophagy-inducing conditions. In a next step, it would be interesting to investigate whether there is a link between the re-routing of autophagosomes/lysosomes to exocytosis and synaptic activity to maintain cellular homeostasis and enable synaptic remodelling.

*Speaker

Lack of myotubularin phosphatase activity is the main cause of X-linked Centronuclear Myopathy

Jocelyn Laporte*[1]

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire – université de Strasbourg, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale : U964, Centre National de la Recherche Scientifique : UMR7104, université de Strasbourg : UMR7104, Institut National de la Santé et de la Recherche Médicale : U1258 – France

Abstract

The *MTM1* gene encodes myotubularin (MTM1), a phosphatidylinositol 3-phosphate (PI(3)P) lipid phosphatase. Loss-of-function mutations in *MTM1* cause X-linked myotubular myopathy (XLMTM), a severe congenital myopathy linked to organelles mis-positioning and with no available cure and a poorly understood pathomechanism. The importance of MTM1 enzymatic activity and its PI(3)P substrate in physiology under normal conditions and in XLMTM is unclear. We generated the *Mtm1* KI C375S mice in which the endogenous MTM1 was converted to a phosphatase-dead protein. Mutant mice survived a median of 12 weeks and demonstrated progressively impaired motor skills. Observed muscle atrophy and reduced force production compared to their WT littermates (_˜3.9-fold reduction in absolute maximal force) were responsible for these severe phenotypes. Increased level of PI(3)P was found in the muscle of *Mtm1* KI C375S mice. Muscle histology and molecular characterization revealed XLMTM hallmarks, with alteration of the mTOR and autophagy pathways correlating with muscle atrophy, and abnormal myofiber intracellular organization correlating with decreased muscle force. Overall, this study reveals the importance of MTM1 phosphatase activity and related PI(3)P substrate for postnatal muscle maintenance, and highlights the significance of MTM1 phosphatase activity in the development of X-linked myotubular myopathy.

*Speaker

How endolysosomal super-organelles manage protein aggregates in the acentrosomal mouse oocyte

Gabriele Zaffagnini*[1], Shiya Cheng[2], Marion Salzer[3], Barbara Pernaute[3], Juan Manuel Duran Serrano[3], Manuel Irimia[3], Melina Schuh[2,4], and Elvan Boke[3]

[1]Center for Genomic Regulation – C/ Dr. Aiguader, 88 08003 Barcelona, Catalonia, Spain, Spain
[2]Department of Meiosis, Max Planck Institute for Multidisciplinary Sciences – 37077 Göttingen, Germany
[3]Center for Genomic Regulation – Spain
[4]Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Universität Göttingen – 37077 Göttingen, Germany

Abstract

Lysosome positioning is crucial for their activity and function. Juxtanuclear clustering of lysosomes around the centrosome promotes the delivery of endocytic and autophagic cargos, thereby facilitating the clearance of protein aggregates. Here, we present our recent finding (1) that, in the acentrosomal mouse oocyte, lysosomes are clustered in liquid-like super-organelles that we named EndoLysosomal Vesicular Assemblies (ELVAs). ELVAs harbor the major intracellular protein degradation systems, and store ubiquitinated protein aggregates targeted for degradation, including TDP-43.
Largely non-degradative in immature oocytes, ELVAs migrate towards the cell cortex and activate degradation shortly before fertilization, thereby clearing the stored aggregates. Unlike microtubule-dependent endolysosomal transport in somatic cells, ELVAs' migration relies entirely on the actin cytoskeleton, and it is required for the activation of protein degradation in ELVAs. Disturbance of ELVAs activation, as well as forced inheritance of protein aggregates in the embryo, compromise oocyte maturation and preimplantation embryonic development. Thus, ELVAs represent a strategy to safeguard the oocyte proteome from aggregation in the absence of pericentrosomal lysosomal clustering.

References:

1. Zaffagnini, G. *et al.* Mouse oocytes sequester aggregated proteins in degradative super-organelles. *Cell* (2024) doi:10.1016/j.cell.2024.01.031.

*Speaker

Investigating the role of the protein arginine methyltransferase PRMT4/CARM1 in the regulation of vesicle trafficking in triple-negative breast cancer

Sonia Ruggiero*[1], Solene Huard[1], Samyuktha Suresh[1], Vanessa Masson[2], Kevin Moreau[3], Didier Meseure[4], Damarys Loew[2], Nicolas Servant[5], Reini Luco[3], Nicolas Reynoird[6], and Thierry Dubois[1]

[1]Breast Cancer Biology Group – Département de Recherche Translationnelle, Institut Curie, PSL Research University, CNRS, UMR144 – France

[2]Proteomics platform – Institut Curie, PSL Research University – France

[3]Chromatin and RNA splicing team – Institut Curie, PSL Research University, CNRS UMR3348, F-91405 Orsay, France – France

[4]Experimental Pathology platform – Institut Curie, PSL Research University – France

[5]Bioinformatics platform – Bioinformatics, Biostatistics, Epidemiology and Computational Systems Unit, Institut Curie, PSL Research University, INSERM U900, Paris, France – France

[6]Institute for Advanced Biosciences / Institut pour l'Avancée des Biosciences (Grenoble) – Université Alpes Grenoble – France

Abstract

The protein arginine methyltransferase CARM1 (PRMT4) has recently emerged as a promising therapeutic target for triple-negative breast cancer (TNBC). Our laboratory found that CARM1 is overexpressed in TNBC compared to normal breast tissue and is required for TNBC cells survival.
To better understand CARM1 functions in TNBC cells, we characterized its interactome using immunoprecipitation coupled to mass spectrometry. I identified the ERGIC-resident protein TFG as a partner of CARM1. TFG is a well-known mediator of the uncoating of COP-II-positive vesicles travelling from the ER to the ERGIC, and has also been reported to regulate endocytosis in neurons. My results thereby envision a new role for CARM1 in vesicle trafficking.

I uncovered that CARM1 methylates TFG on two C-terminal arginines in TNBC cells. Since arginine methylation can interfere with protein-protein interactions, we wondered which proteins interact specifically with methylated TFG and what could be the downstream effect of this interaction. In collaboration with Nicolas Reynoird (IAB, Grenoble), we unveiled that proteins involved in retrograde transport of AP1-derived vesicles to the Golgi are potential effectors of CARM1-mediated dimethylation of TFG, which are still under validation.

On the other hand, PRMTs are reported to be alternative splicing (AS) regulators. Transcriptomic analyses performed in CARM1-depleted TNBC cells implied that CARM1 can

*Speaker

tune retrograde transport also by regulating the expression of genes involved in this pathway through modulation of their AS. We therefore hypothesize a dual, parallel action of CARM1 in orchestrating endosome-to-Golgi targeting: via methylation of TFG and via post-transcriptional regulation of trafficking genes.

How do flotillins influence the endolysosomal function to promote cancer cell invasion?

Layal Rajeh*[1], Josefina Casas[2], Cécile Gauthier-Rouviére[1], and Stéphane Bodin[1]

[1]Centre de recherche en Biologie cellulaire de Montpellier – Centre national de la recherche scientifique - CNRS (France) – France

[2]Research Unit on BioActive Molecules (RUBAM), Department of Biological Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC), Spanish Council for Scientific Research (CSIC), Spain – Spain

Abstract

Endolysosomes are cellular organelles responsible for the degradation and secretion of biomolecules. Dysregulation that enhances their secretory function has been shown to increase the invasiveness of breast cancer cells (Machado et al. 2021). In this context, we aim to investigate the molecular mechanisms by which flotillins, proteins overexpressed in invasive breast cancer cells, promote endolysosomal secretion. Flotillins 1 and 2 are membrane-bound proteins known to be overexpressed in highly invasive cancers. In triple-negative breast cancer cells, flotillins localize to 80% of CD63 and LAMP-1 positive endolysosomes. We hypothesize that flotillins alter the lipid composition of endolysosomes, thereby modulating their function. This hypothesis is supported by the ability of flotillins to bind sphingosine, a key component of sphingolipid metabolism. Our recent lipidomics data showed that flotillin overexpression disrupts the sphingosine-1-phosphate (S1P)/ceramide balance, favoring S1P production-a known metabolic signature of tumor progression. Recent studies have shown that S1P facilitates membrane contact sites between endolysosomes and the endoplasmic reticulum (ER), promoting cholesterol transfer from endolysosomes (Palladino et al. 2022). We propose that the accumulation of flotillins in endolysosomes enriches this compartment with sphingosine, thereby enhancing S1P-mediated cholesterol transfer to the ER. This results in endolysosomes with reduced cholesterol content, a condition that affects their functions in multiple ways, including favoring their peripheral positioning and facilitating their fusion with the plasma membrane to promote the secretion of endolysosomal cargos. Understanding the role of flotillins in this process may reveal how their overexpression in invasive cancers contributes to enhanced tumor cell invasion and metastasis.

*Speaker

Adaptation of the secretory routes upon cell differentiation

Justine Guguin[1], Delphine Desigaud[2], Pauline Goddard[1], Vincent Gache[1], Franck Perez[2], and Gaelle Boncompain*[1]

[1]Institut Neuromyogene - PGNM – UCBL CNRS UMR5261 INSERM U1315 – France
[2]Institut Curie, CNRS UMR144 – Institut Curie, CNRS UMR144 – France

Abstract

The molecular architecture of our cells is paramount for their specific function and is partly fashioned from membrane trafficking. Both defects in the intracellular organization and in protein transport contribute to various diseases. The precise delivery of proteins is essential for the proper functioning of signal transduction, tissue integrity or regulation of ion fluxes. It is now clear that diversity in the Golgi-dependent secretory routes does exist and that diverse trafficking mechanisms and machineries are at work. Specific regulators of the trafficking of cargos transported by a given specialized cell type are still unknown.
In this study, we explore the adaptability of the secretory routes to fit specific secretion needs using human induced pluripotent stem cells (iPSc) differentiated into chondrocytes and cardiomyocytes. Chondrocytes abundantly secrete components of the articular cartilage (collagens). Cardiomyocytes secrete small proteins or peptides involved in maintenance of the viability of cardiomyocytes.

The secretory function of the Golgi apparatus in iPSc-based models is monitored using the RUSH (Retention Using Selective Hooks) assay and real-time imaging. Kinetics of transport of RUSH-synchronized EGFP-GPI have been measured in iPSc and iPSc-differentiated cells. Our study aims to get a better understanding of the adaptive capacity of the Golgi apparatus while specific secretion needs are required and to explore specialization of cellular functions upon cell differentiation.

*Speaker

The Contribution of AP-1γ2 to the Early-to-Late Endosome Maturation and Extracellular Vesicles Release

Lucas Alves Tavares*[1,2], Mireia Gomez Duro[2], Mara Elisama Da Silva-Januario[1], Mathilde Dimarco[2], Roger Luiz Rodrigues[1], Murilo Henrique Anzolini Cassiano[1], Andreia Nogueira De Carvalho[1], Bergam Ptissam[2], Maryse Romao[2], Gisela D'angelo[2], Raposo Graça[2], and Luis Lamberti Pinto Dasilva[1]

[1]Center for Virology Research and Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil – Brazil

[2]Institut Curie, Paris Sciences Letters Research University, CNRS, UMR144, Structure and Membrane Compartments, Paris Cedex 05 75248, France – Institut Curie, PSL Research University, CNRS – France

Abstract

AP-1γ2 belongs to a family of five heterotetrameric complexes, which mediate intracellular protein trafficking. However, the physiological role of AP-1γ2 has been poorly characterized. We recently reported that AP-1γ2 is required for HIV-1 Nef-mediated downregulation of CD4 and MHC-I, as well as the retrograde transport of CI-MPR and ATP7B. In this study, we report that AP-1γ2 also participates in early-to-late endosome maturation. We developed AP-1γ2 KO cells by CRISPR-Cas9 approach and by immunofluorescence observed morphological alteration in both early and late endosomes marked, respectively by Rab5/EEA1 or Rab7/CD63/Lamp-1/LBPA. Indeed, our transmission electron microscopy analysis revealed that AP-1γ2 depletion causes an apparent aberrant formation of MVBs. AP-1γ2 ablation also compromised cargo protein degradation, since Alexa-Fluor-488-EGF uptake assay reveals that internalized EGF remains trapped in the early endosome in AP-1γ2 KO cells. Moreover, overexpressing a GTP-locked form of Rab5 leads to the accumulation of LBPA in the early endosomes in WT cells, but not in γ2 KO cells. Furthermore, nanoparticle tracking, western-blot, and immuno-electron microscopy analysis of Extracellular Vesicles (EVs) secretion revealed that the ablation of AP-1γ2 decreases small EVs (CD63) and increases large EVs (CD9) secretion, with an alteration of EVs markers in the Lipid Rafts. Therefore, the results suggest a crucial role of AP-1γ2 in the maturation of early endosomes to late endosomes, possibly in the formation of ILVs of MVBs, resulting in altered secretion of EVs.

*Speaker

Activation of multivesicular bodies (MVBs) dynamics via transcription factor EB (TFEB) facilitates integrin trafficking and migration in aggressive bladder cancer.

Julie Patat*[1], Justine Del Rio[2], Nadia Elkhatib[3], Mehdi Khaled[3], and Kristine Schauer[4]

[1]Dynamique des cellules tumorales – U1279 – France
[2]Dynamique des cellules tumorales – U1279 – France
[3]Dynamique des cellules tumorales – U1279 – France
[4]Dynamique des cellules tumorales – U1279 – France

Abstract

Organelle homeostasis in cancer has recently gained attention, as organelle positioning changes have been shown to correlate with cancer aggressiveness. Pioneer results from our laboratory showed that peripheral lysosomes characteristic of 'high-grade' bladder cancer cells result from activation of the transcription factor EB (EB). TFEB is the master regulator of lysosome biogenesis and autophagy, but its role in cancer progression is not understood. Hyperactivity of TFEB was previously observed in several aggressive cancers, including renal adenocarcinoma and pancreatic cancer, thus bladder cancer provides un interesting model to study TFEB function in organelle homeostasis in cancer.
Depletion of TFEB by siRNA in invasive bladder cancer cell lines resulted in a perinuclear accumulation of multivesicular bodies (MVBs), modified integrins ß5 trafficking and slower cell migration. Investigation of the underlying mechanisms showed that TFEB knock-down reduced the expression of the HGS protein of the ESCRT-0 complex that regulates the invagination of intraluminal vesicles at the multivesicular bodies (MVBs) stage. Similarly, MVB-specific small GTPase Rab27, known to regulate anterograde movement of MVBs, was downregulated upon siTFEB. HGS depletion mimicked siTFEB phenotypes, and HGS upregulation correlated with aggressiveness in bladder cancer cell models and different bladder cancer patient cohorts (e.g., TCGA, MSK, BCAN/HCRN).

Our results highlight the link between lysosome homeostasis and cell migration and identify the TFEB/HGS/Rab27/ß5 cascade as a regulator of increased cell motility. We reveal MVBs as a TFEB-regulated intracellular platform and suggest HGS as a potential therapeutical target for future interventions.

*Speaker

Surface tension-driven sorting of human perilipins on lipid droplets

Joëlle Bigay*[1], Abdoul Akim Bello , Ana Rita Dias Araújo , Céline Franckhauser , Romain Gautier , Nicolas Fuggetta , Alenka Copic , and Bruno Antonny

[1]Institut de pharmacologie moléculaire et cellulaire – Centre national de la recherche scientifique - CNRS (France) – France

Abstract

Perilipins (PLINs), the most abundant proteins on lipid droplets (LDs), display similar domain organization including amphipathic helices (AH). However, the five human PLINs bind different LDs suggesting different modes of interaction. We established a minimal system whereby artificial LDs covered with defined polar lipids were transiently deformed to promote surface tension. Binding of purified PLIN3 was strongly facilitated by tension but was poorly sensitive to phospholipid composition and to the presence of diacylglycerol. Accordingly, LD coverage by PLIN3 increased as phospholipid coverage decreased. In contrast, PLIN1 bound readily to LDs fully covered by phospholipids; PLIN2 showed an intermediate behavior between PLIN1 and PLIN3. In human adipocytes, PLIN3/4 were found in a soluble pool and relocated to LDs upon stimulation of fast triglyceride synthesis, whereas PLIN1 and PLIN2 localized to pre-existing LDs, consistent with the large difference in LD avidity observed in vitro. We conclude that the PLIN repertoire is adapted to handling LDs with different surface properties.

*Speaker

A multi-organelle platform regulates EGFR endocytosis and signalling

Deborah Mesa*[1], Elisa Barbieri , Andrea Raimondi[2,3], Stefano Freddi[1,4], Giorgia Miloro[5], Gorana Jendrisek[6,7], Giusi Caldieri , Micaela Quarto[6,7], Irene Schiano Lomoriello[6,7], Maria Grazia Malabarba[6,7], Arianna Bresci[8], Francesco Manetti[8], Federico Vernuccio[8], Hind Abdo[9], Giorgio Scita[6,9], Letizia Lanzetti[10,11], Dario Polli[8,12], Carlo Tacchetti[2,13], Paolo Pinton[14], Massimo Bonora[14], Pier Paolo Di Fiore[6,7], and Sara Sigismund[6,7]

[1]IEO, European Institute of Oncology IRCCS, Milan, Italy – Italy
[2]Experimental Imaging Centre, IRCCS San Raffaele Hospital Scientific Institute, Milan, Italy – Italy
[3]Università della Svizzera italiana (USI), Faculty of Biomedical Sciences, Institute for Research in Biomedicine, Bellinzona, Switzerland – Switzerland
[4]Department of Oncology and Hematology-Oncology, Università degli Studi di Milano, Milan, Italy – Italy
[5]IEO, European Institute of Oncology IRCCS, Milan, Italy – Italy
[6]Department of Oncology and Hematology-Oncology, Università degli Studi di Milano, Milan, Italy – Italy
[7]IEO, European Institute of Oncology IRCCS, Milan, Italy – Italy
[8]Department of Physics, Politecnico di Milano, Milan, Italy – Italy
[9]IFOM, The AIRC Institute of Molecular Oncology, Milan, Italy – Italy
[10]Department of Oncology, University of Torino Medical School, Candiolo, Turin, Italy – Italy
[11]Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Turin, Italy – Italy
[12]CNR Institute for Photonics and Nanotechnology (CNR-IFN), Milan, Italy – Italy
[13]Vita-Salute San Raffaele University, Milan, Italy – Italy
[14]Department of Medical Sciences, Section of Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy – Italy

Abstract

Endocytosis plays a pivotal role in the regulation of Epidermal Growth Factor Receptor (EGFR) signaling. The EGFR can be internalized via different mechanisms: clathrin-mediated endocytosis (CME), active at all EGF concentrations, which destines the majority of receptors to recycle, and non-clathrin endocytosis (NCE), which is activated only at high EGF and targets receptors to degradation. This endocytic route could operate as an intrinsic cellular system for the control of EGFR levels and signaling, particularly in situations where the EGFR or its ligands are aberrantly upregulated. A number of NCE functional regulators have been discovered by a proteomics investigation of EGFR-loaded NCE vesicles coupled to RNA interference screening, including the ER shaping factor Reticulon 3 (RTN3) and mitochondrial proteins. We found that RTN3 is critical for EGFR internalization via NCE, promoting the formation of contact sites between the ER and PM necessary for the

*Speaker

formation/elongation of NCE PM invaginations. IP3R-mediated Ca2+ release at these sites is needed for the completion of EGFR internalization and vesicle fission, in concert with dynamin. A significant finding from our study is that Ca2+ signaling triggered by EGF at NCE sites is influenced by mitochondrial activity. Concurrently, EGFR activation increases the formation of ER-mitochondria contact sites in proximity to the PM, resulting in localized ATP production by mitochondria. The interplay between active EGFR, Ca2+ signaling, and mitochondrial metabolism is crucial for the fission of NCE tubular invaginations and it regulates the remodeling of cortical actin, influencing cell migration. Therefore, we aim to define the role of mitochondria in EGFR-NCE and particularly if there is a crosstalk between the EGFR and a subset of mitochondria at NCE contact sites, defining if EGFR signaling can directly communicate with mitochondria, thereby impinging on their metabolic response.

Altered expression of Presenilin2 impacts endolysosomal homeostasis and synapse function in Alzheimer's disease-relevant brain circuits

Wim Annaert*[1], Anika Perdok[1], Zoë Van Acker[1], Céline Vrancx[1], Ragna Sannerud[1], Inge Vorsters[1], Assunta Verrengia[1], Zsusanna Vegh-Callaerts[2], Eline Creemers[1], Sara Gutierrez Fernandez[1], Britt D'hauw[1], Lutgarde Serneels[1], Keimpe Wierda[1], and Lucia Chavez-Gutiérrez[1]

[1]VIB Center for Brain and Disease Research KU Leuven – Belgium
[2]mINT, Faculty of Psychology, KU Leuven – Belgium

Abstract

Rare mutations in the gene encoding presenilin2 (PSEN2), a catalytic subunit of γ-secretase, cause familial Alzheimer's disease (FAD). Mutations in PSEN2 shift production of amyloid β (Aβ) peptides to the longer, more aggregation prone Aβ42. Moreover, given PSEN2/γ-secretase mainly localizes to late endosomes/lysosomes (LE/Lys), it also contributes particularly to the intracellular pool of Aβ, which surges early in disease pathogenesis. However, the underlying molecular and cell biological mechanisms on how mutant PSEN2 leads to neuronal demise remain elusive. Using newly generated APP knock-in (KI) mouse models we now demonstrate that PSEN2 knockout (KO) as well as FAD-associated N141IKI mutant both accelerated AD-related pathologies. Both models showed significant deficits in working memory compared to control mice, that linked to the elevated PSEN2 expression in the hippocampal CA3 region. The mossy fiber circuit of both APPxPSEN2KO and APPxFADPSEN2 mice had smaller pre-synaptic compartments, distinct changes in synaptic vesicle populations and significantly impaired long term potentiation compared to APPKI mice. In primary hippocampal neurons derived from APPKI, APPxPSEN2KO and APPxFADPSEN2 mice, altered PSEN2 expression resulted in endolysosomal defects, decreased lysosomal exocytosis and lowered surface expression of synaptic proteins; underscoring recycling defects. These defects caused as well an increased autophagic flux, with accumulating autophagosomes en autolysosomes in primary neurons. As PSEN2/γ-secretase is restricted to LE/Lys, we propose PSEN2 impacts endolysosomal homeostasis, affecting synaptic signaling in AD-relevant vulnerable brain circuits; which could explain how mutant PSEN2 accelerates AD pathogenesis.

*Speaker

γ-Secretase Inhibitors Relocate Presenilin1/γ-Secretase to Late Endosomes

Ragna Sannerud*[1,2], Kjen Bogaert[1,2], Abril Escamilla Ayala1[1,2], Katlijn Vints[3], Marine Bretou[1,2], Benjamin Pavie[3], Lucia Chavez-Gutiérrez[2,4], and Wim Annaert[1,2]

[1]Laboratory for Membrane Trafficking, VIB Center for Brain Disease Research – Belgium
[2]KU Leuven, Department of Neurosciences, Leuven Brain Institute – Belgium
[3]VIB BioImaging Core – Belgium
[4]Laboratory of Proteolytic Mechanisms in Neurodegeneration, VIB Center for Brain Disease – Belgium

Abstract

γ-Secretase is a crucial intramembrane protease involved in various physiological processes and linked to Alzheimer's disease (AD) through the generation of amyloid β (Aβ) peptides from APP fragments. Its activity is driven by the presenilin (PSEN1 or PSEN2) subunit. Mutations in the genes encoding APP, PSEN1, and PSEN2 account for most familial AD cases, highlighting the importance of Aβ production in AD. PSEN1 is located at the cell surface and endosomes, while PSEN2/γ-secretase is confined to late endosomes/lysosomes (LE/Lys). These distinct locations contribute to substrate specificity.
In this study, we explored how γ-secretase inhibitors affect PSEN1 subcellular localisation. Inhibitor treatment resulted in the accumulation of PSEN1/γ-secretase to LE, mimicking PSEN2's location. This effect, observed for both transition and non-transition state inhibitors but not modulators, was dose-dependent and reversible. Wash-out experiments demonstrated that the LE accumulation is reversible and not influenced by proteasomal and lysosomal inhibitors nor by blocking protein synthesis, indicating an event unrelated to the degradation or turnover of γ-secretase.

Mechanistically, we showed that inhibitor treatment did not affect endocytosis of PSEN1/γ-secretase but instead obstructed its recycling from LE, leading to intracellular accumulation herein. Interestingly, familial AD-linked PSEN1 mutations near the inhibitor binding site caused similar re-location, suggesting a shared mechanism.
As inhibitor binding alters PSEN1/γ-secretase's conformational flexibility, we hypothesise that conformational changes induced by inhibitors or mutations block efficient recycling from LE, potentially affecting APP processing and Aβ production. These insights should be considered in future drug design strategies that aim to reduce the production of toxic Aβ peptides.

*Speaker

Influenza A virus infection remodels the endoplasmic reticulum membrane and alters phosphoinositides homeostasis via a Rab11/ATG16L1 mechanism

Carla Alemany*[1], Juliane Da Graca[1], Quentin Giai Gianetto[2], Sylvain Paisant[2], Thibaut Douché[2], Lydia Danglot[3], Mariette Matondo[2], Etienne Morel[1], Jean-Baptiste Brault[2], and Nadia Naffakh[2]

[1]Institut Necker Enfants Malades – INSERM U1151- Institut Necker Enfants Malades – France
[2]Institut Pasteur – Institut Pasteur de Paris – France
[3]IPNP – Institut de Psychiatrie et Neurosciences de Paris – France

Abstract

Influenza A virus replicates its genomic RNAs in the nucleus of infected cells, packaging them into ribonucleoproteins (vRNPs) that are transported to the plasma membrane. The small GTPase RAB11A has been identified as essential for this transport, but the underlying mechanisms remain largely unknown. Here, we demonstrate that influenza A virus induces extensive remodeling of the endoplasmic reticulum (ER), where vRNPs accumulate at specific ER membrane subdomains before being trafficked via RAB11A. Using A549 cells stably expressing TurboID-RAB11A, proximity labeling assays revealed that cellular regulators of phosphoinositide metabolism, including ATG16L1, are significantly enriched near RAB11A in virus-infected cells. Notably, we observed a significant alteration in phosphoinositide balance, with increased phosphatidylinositol-4-phosphate (PI4P) levels at ER membranes and reduced phosphatidylinositol-3-phosphate (PI3P) levels during infection. While depletion of both ATG16L1 and RAB11A disrupted phosphoinositide turnover, it did not affect ER remodeling. These findings suggest that ATG16L1 and RAB11A are involved in the local production and regulation of PI4P, which facilitates vRNP maturation and trafficking from ER subdomains to the plasma membrane, highlighting a potential role for PI4P in virus-induced endomembrane remodeling and viral transport.

*Speaker

α-Synuclein-induced autophagy dysfunction in neuronal cells contributes to tunneling nanotube-mediated interaction with microglia

Ranabir Chakraborty*[1] and Chiara Zurzolo[2]

[1]Institut Pasteur – Institut Pasteur de Paris – France
[2]Membrane Traffic and Pathogenesis Unit – Institut Pasteur de Paris – France

Abstract

Tunneling nanotubes (TNTs) represent a major form of intercellular communication, allowing material exchange over long distances between the connected cells, events that are less stochastic than secretory mechanisms and lead to direct and specific communication. Different kinds of materials can shuttle via TNTs, ranging from small ions to large organelles and protein aggregates. Previous reports have demonstrated efficient directional transfer of α-Synuclein (α-Syn) aggregates from neuronal cells to astrocytes and microglia. However, the underlying reason for such directionality remains unclear. Using quantitative fixed- and live-cell microscopy, we observed differential localization of aggregates on lysosomes of neuronal and microglial cells. This resulted in increased permeabilization of lysosomal membranes. Although lysosomal biogenesis increased for both neuronal and microglial cells upon exposure to aggregates, the ability of microglia to target lysosomes for degradation via lysophagy was significantly higher than neuronal cells, both at a basal level and in the presence of aggregates. We found that neuronal lysosomes had impaired degradative ability compared to microglia, with reduced autophagic flux and fewer aggregates being targeted for degradation. Further inhibition of autophagy in neuronal cells led to increased aggregate transfer to microglia and a rise in the number of homotypic TNTs between cells. Thus, we propose a framework of dysfunctional autophagy in neuronal cells as a functional explanation behind directional α-Syn transfer to microglia.

*Speaker

Tumor protein D54 dynamically induces tripartite biocondensates gathering intracellular nanovesicles and lipid droplets depending on cellular cholesterol levels

Sébastien Leterme*[1], Antoine Reynaud[1], Maud Magdeleine[1], Ana Rita Dias Araújo[1], Sophie Abelanet[1], Christelle Boscagli[2], Sandra Lacas-Gervais[2], and Bruno Antonny[1]

[1]Institut de pharmacologie moléculaire et cellulaire – CNRS UMR7275, Université Côte d'Azur – France

[2]Centre Commun de Microscopie Appliquée – Université Côte d'Azur (UCA) – France

Abstract

Lipid droplets form unique organelles in the cell that are made of a core of neutral lipids surrounded by a monolayer of phospholipids and proteins. Beside representing the main carbon source in the cell for various metabolic pathways, lipid droplets also constitute a major source of lipids for organelle membrane biogenesis and maintenance. We recently observed that the poorly characterized protein Tumor Protein D54 (TPD54) promotes the formation of a striking structure in the cell: a biocondensate in which lipid droplets are entrapped in a dense matrix of very small (30-40 nm) vesicles. Precursor work revealed that these tripartite biocondensates also contain lipid scramblases and lipid transporters, suggesting that they might constitute a huge reservoir of both neutral and bilayer-forming lipids that could be easily remobilized. Here, we aim to unravel the organization, the control of assembly, the cellular dynamics and the possible functions of this biocondensate gathering lipid droplets, nanovesicles and TPD54. Biochemical reconstitution and biophysical approaches demonstrated that TPD54 is indeed able to form dynamic biocondensates *in vitro* through its structural attributes. We then concentrated on the regulation of TPD54 subcellular localization by phosphorylation of a particular residue and by cell cholesterol content by combining pharmacological and microscopy approaches. We further investigated the possible role of TPD54 biocondensates as donors or acceptors in cellular lipid fluxes using fluorescent lipid probes. Altogether, our results constitute the first characterization of a new cellular structure that could be implied in the dynamic storage of both neutral and bilayer-forming lipids.

*Speaker

#25

Impact of lysosomal PLD3 dysfunction on axonal trafficking and synaptic dynamics in the hippocampal CA3 region

Zoë Van Acker*[1,2], Assunta Verrengia[1,2], Kristofer Davie[3], Ine Vlaeminck[4], Suresh Poovathingal[3], Keimpe Wierda[4], and Wim Annaert[1,2]

[1]Laboratory for Membrane Trafficking, VIB Center for Brain Disease Research – Belgium
[2]KU Leuven, Department of Neurosciences, Leuven Brain Institute – Belgium
[3]Single Cell Microfluidics Expertise Unit, VIB Center for AI Computational Biology – Belgium
[4]Electrophysiology Expertise Unit, VIB-KU Leuven Center for Brain and Disease Research – Belgium

Abstract

Background: Synaptic dysfunction in the hippocampus, especially in the CA3 region, is implicated in memory deficits associated with Alzheimer's Disease (AD). Phospholipase D3 (PLD3), a lysosomal 5'-3' exonuclease linked to late-onset AD risk, is involved in both lysosomal and mitochondrial pathways, but its effect on synaptic transmission and lysosomal trafficking in the hippocampus is still unclear.
Materials and Methods: In a PLD3 knockout (KO) mouse model, we examined synaptic function in the CA3 region using multi-electrode array electrophysiology, paired-pulse facilitation, and transmission electron microscopy (TEM) to assess synaptic structure and vesicle dynamics. Additionally, we utilized lysosomal tracking assays in microfluidic devices to investigate axonal trafficking, particularly focusing on vesicle speed, movement directionality, and proportion movement. We are investigating underlying molecular pathways, including STING signaling, lysosomal homeostasis and transcriptional changes (transcriptomics) to uncover mechanisms driving synaptic alterations.

Results: PLD3 KO mice show reduced long-term potentiation in the CA3 region, coupled with increased response amplitudes at various stimulation strengths, suggesting altered vesicle release dynamics. TEM analysis reveals smaller synapses, shorter postsynaptic densities, and increased synaptic vesicle heterogeneity. Preliminary data indicates that PLD3 KO neurons exhibit impaired lysosomal trafficking, with reduced movement proportions and slower lysosome movement along axons.

Conclusion: PLD3 dysfunction disrupts lysosomal trafficking and synaptic transmission in the CA3 region. These findings offer new insights into the connection between lysosomal and synaptic functioning.
Ref. 1. Van Acker, Nat Commun. (2023) doi: 10.1038/s41467-023-38501-w.

*Speaker

Understanding the molecular role of RAB24 by identification of its interactors

Mauricio Ramm*[1], Martin Alexander Lopez[1], Karsten Nalbach[2], Roosa Kotiniitty[1], Christian Behrends[3], and Eeva-Liisa Eskelinen[1]

[1]University of Turku – Finland
[2]Ludwig Maximilian University [Munich] = Ludwig Maximilians Universität München – Germany
[3]Ludwig Maximilian University [Munich] = Ludwig Maximilians Universität München – Feodor-Lynen Strasse 17, 81377 Munich, Germany

Abstract

RAB GTPases regulate intracellular membrane trafficking, including organelle biogenesis, transport, and fusion. Despite its link to hereditary ataxia, non-alcoholic fatty liver disease, and hepatocellular carcinoma, RAB24's molecular function is unclear.
To investigate RAB24's molecular mechanisms, we performed APEX2-RAB24 proximity biotinylation and mass spectrometry, identifying 77 high-confidence interactors for wild type (WT) RAB24. Comparative proteomics of the proximal proteins of WT RAB24 and the ataxia mutant RAB24-Q38P revealed potential interactions of WT RAB24 with tethering proteins. This interaction was then confirmed by co-immunoprecipitation.

30% of the APEX2 hits are involved in ER-to-Golgi transport via COPII vesicles. Immunostaining for SEC31A, a COPII coat component, showed that SEC31A-positive vesicles cluster in the perinuclear area in WT and RAB24-rescued knockout cells but disperse in RAB24-knockout cells, suggesting RAB24's role in Golgi tethering.
We will further explore RAB24's role in tethering and fusion of COPII vesicles with the Golgi and endosomes and autophagosomes with lysosomes through in vitro assays. Fluorescently tagged organelles will be mixed, and fusion/tethering events will be quantified in extracts from RAB24 WT or knockout cells.

*Speaker

Unraveling the impact of Presenilin-2/gamma-secretase interactions at late endosomes/lysosomes on their functioning

Céline Vrancx*[1,2], Ragna Sannerud[1,2], Rosanne Wouters[1,2], Rosanna Poll[1,2], Iván Ramos Calvo[1,2], Anika Perdok[3,4], Francis Impens[5,6,7], and Wim Annaert[1,2]

[1]Laboratory for Membrane Trafficking, VIB Center for Brain Disease Research, Leuven – Belgium
[2]Department of Neurosciences, KU Leuven, Leuven – Belgium
[3]Microglia and Inflammation in Neurological Disorders (MIND) Lab, VIB Center for Molecular Neurology, Antwerp – Belgium
[4]Department of Biomedical Sciences, University of Antwerp, Antwerp – Belgium
[5]VIB-UGhent Center for Medical Biotechnology, Ghent – Belgium
[6]VIB Proteomics Core, Ghent – Belgium
[7]Department of Biomolecular Medicine, Ghent University, Ghent – Belgium

Abstract

Aims: Presenilins (PSEN) 1 and 2 act as the catalytic core of gamma-secretase, playing a central role in Alzheimer's disease (AD) by releasing beta-amyloid peptides from APP C-terminal fragments. We previously demonstrated the broad distribution of PSEN1/gamma-secretase at the cell surface and in endosomes, whereas PSEN2 is restricted to late endosomes/lysosomes (LE/Lys). Since endolysosomal abnormalities are early features of AD pathogenesis, and since many genetic risk variants intersect with endosomal and lysosomal homeostasis, we focused on deciphering the precise role of PSEN2/gamma-secretase in these organelles.
Methods: Starting from PSEN knockout (KO) cells, we generated cell lines stably rescued by either GFP- or APEX2-tagged versions of PSEN2 with the aim to identify specific interactors hinting at a functional role at LE/Lys. This allowed us to combine two orthogonal interactomics methods, *i.e.* affinity purification with the GFP-tag and proximity-dependent biotinylation with the APEX2-tag. Identified hits were prioritized based on their functional relevance to endolysosomal homeostasis and were both confirmed for their interaction with PSEN2 and scrutinized for their functioning upon PSEN2 deficiency.

Results: We found a direct interaction between PSEN2/gamma-secretase and regulators of nutrient sensing and lysosomal motility. Using super-resolution imaging and functional assays, we confirmed that PSEN2 participates in lysosomal positioning in response to nutrient availability, with its deficiency impairing these two intrinsically connected molecular pathways. A PSEN2 catalytically inactive mutant mimicked PSEN2-KO, indicating a dependency on activity. Interestingly, in primary neurons, PSEN2-KO and PSEN2-FAD exerted subtle different effects on axonal lysosomal transport, suggesting AD-specific defects.
Conclusions: Our observations link PSEN2/γ-secretase to endolysosomal functioning, which may provide novel insights for a better understanding on the etiology of endolysosomal dysfunctions, as observed at early, preclinical stages of AD.

*Speaker

Control of lipid droplet dynamics in cells using engineered compartments

Chems Amari*[1,2], Damien Simon , Abdou Rachid Thiam , and Zoher Gueroui

[1]Processus d'Activation Sélective par Transfert d'Energie Uni-électronique ou Radiatif (UMR 8640) – Département de Chimie - ENS Paris, Institut de Chimie - CNRS Chimie, Sorbonne Universite, Centre National de la Recherche Scientifique – France

[2]Physiologie cellulaire et végétale – Centre National de la Recherche Scientifique, Institut de Recherche Interdisciplinaire de Grenoble, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Université Grenoble Alpes, Centre National de la Recherche Scientifique : UMR5168, Institut de Recherche Interdisciplinaire de Grenoble : DRF/IRIG/DBSCI, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement : UMR1417 – France

Abstract

Understanding the spatial and temporal regulation of lipid droplets (LDs) is crucial for unraveling their roles in cellular metabolism, stress responses, and cell fate. However, the mechanisms governing LD dynamics and the tools to manipulate them in cells remain limited. Here, we have developed ControLD (Controlled Trapping Of LDs), a novel approach to selectively confine LDs using engineered compartments that act as a dense protein-based meshwork. The trapping of LDs in these compartments contributes to their physical isolation from the rest of the cell, and their release into the cytosol can be triggered within a minute time scale. Finally, we have found that the confinement of LDs affects their dynamics, as LD consumption during metabolic demand is greatly perturbed. We anticipate that ControLD will provide a tool to manipulate and study LD biology more effectively, contributing to uncovering the role of LD dynamics in various cellular processes.

*Speaker

Manipulating functional pleiotropy of macrophage through dynamic control of SRC family kinases (SFKs)

Cristina Torres Torres*[1], Paul Rivier[1], Lucia Campos[1], Christiane Oddou[1], Fabrice Senger[1], Ingrid Bourrin[1], Anne-Pascale Bouin[1], Alexei Grinchine[1], Mylene Pezet[1], Jordan Allard[1], Chantal Thibert[1], Eva Faurobert[1], Emmanuelle Planus[1], and Olivier Destaing[1]

[1]Institute for Advanced Biosciences / Institut pour l'Avancée des Biosciences (Grenoble) – Institute for Advanced Biosciences (IAB), Inserm U1209, CNRS UMR 5309, Université Grenoble Alpes – France

Abstract

Genetic vision of cell signaling develops the concept of redundancy to sustain the idea that the same group of Intramolecular Signaling Elements (ISEs) appears to perform identical functions and be compensated by other members upon a genetic loss. To revisit the concept of redundancy in cell signaling, we focus on the Src Family Kinase (SFKs) since being one of the largest family of tyrosine kinase implicated in numerous aspects of cell signaling. Indeed, single, and multi-knock-out models rather supported a high level of redundancy between the eight members of the family, reinforced by their high level of homology and identical mechanism of activation. To challenge redundancy, we propose to compare the functions of Src and Hck, the most compensating members of the family in myeloid lineage since being one of the most affected tissues to the Src and Hck loss. To do so, we have developed different optogenetic SFKs to identify their function in a light-controlled and dynamic manner. Despite their apparent redundancy in multiple models, it appears that Src and Hck can be relocalized in distinct subcellular regions in macrophages and have opposite effects on invasive structures called podosomes. We have identified for the first time the role of Hck which is able to relocalize in a specific clathrin structure. We showed that other functions such migration, ECM degradation as well as invasion were differently regulated by Src and Hck. Dynamic activation of SFKs by light allowed us to define the molecular basis of different SFKs activation rather than promoting a synergic functional intrication than a simple redundancy between each member of the SFKs.

*Speaker

Adaptation of Golgi-associated molecular machineries upon cell differentiation

Justine Guguin*[1], Pauline Goddard[1], Delphine Desigaud[2], Franck Perez[2], Vincent Gache[1], and Gaelle Boncompain[1]

[1]Physiopathology and Genetics of the Neuron and Muscle, Institut NeuroMyoGene, CNRS UMR 5261, INSERM U1315, Université Claude Bernard Lyon 1, 69008 Lyon, France – Institut NeuroMyoGene (INMG-PGNM), UCBL CNRS UMR5261 INSERM U1315 – France

[2]Institut Curie, PSL Research University, Sorbonne Université, Centre National de la Recherche Scientifique, UMR 144, 75005 Paris, France – Institut Curie, PSL Research University, Sorbonne Universités, UPMC, CNRS – France

Abstract

Secretion of proteins is an essential mechanism for cell homeostasis, signaling or tissue integrity. Upon cell differentiation, specific secretory requirements are needed to ensure cellular functions. For instance, cardiomyocytes need surface expression of gap junctions and calcium channels for action potential propagation while chondrocytes massively secrete articular collagens. At the center of the secretory pathway, the Golgi complex has to ensure correct and precise processing and targeting of the cargos to their destination compartment. However, the question of how this organelle adapts upon cell differentiation to fit specific secretory needs remains to be elucidated, especially when mutations in ubiquitous Golgi proteins have deleterious impact only on some tissues. To investigate that, we analyze the Golgi proteome of three different cell types, using TurboID-based proximity labeling. First, we generated human induced Pluripotent Stem Cells (hiPSC) stably expressing a Golgi-targeted TurboID fusion construct, without altering their pluripotency nor differentiation capacities. These hiPSC were then differentiated into cardiomyocytes and chondrocytes. Second, we performed pull-down using streptavidin-coupled magnetic beads prior to quantitative analysis by mass spectrometry. Our results show a specific and reproducible harvesting of biotinylated proteins from the Golgi apparatus in hiPSC and in differentiated cells. Altogether, our work will lead to the identification of Golgi-associated molecular machineries in differentiated cell types, paving the way to understanding regulation of specific secretory routes.

*Speaker

#31

Unravelling WASHC4 brain-specific function to uncover its implication in intellectual disability

Luca Murrru[1], Giacamo Casati[2,3], Patrizia D'adamo[2,3], and Fabrizia Claudia Guarnieri*[4]

[1]CNR Institute of Neuroscience, Vedano al Lambro, Italy – Italy
[2]Vita-Salute San Raffaele University, Milan, Italy – Italy
[3]IRCCS San Raffaele Scientific Institute, Milan, Italy – Italy
[4]CNR Institute of Neuroscience, Vedano al Lambro, Italy – Italy

Abstract

Genetic variants in the human *WASHC4* gene are rare and cause Autosomal Recessive Intellectual Disability (ARID) and other neurodevelopmental disorders. The WASHC4 protein, also known as SWIP, is part of the WASH (Wiskott-Aldrich syndrome protein and SCAR homology) protein complex, which plays an important role in actin dynamics and endosomal trafficking in non-neuronal cells. However, the function of WASHC4 in neurons is poorly characterized. A recent study revealed that a missense mutation (p.Pro1019Arg) in WASHC4 perturbs the abundance of several endosomal and post-synaptic proteins in knock-in mouse (SWIPP1019R) brains, and a limited behavioural assessment in mice revealed cognitive and movement impairments.
Our major interest is to understand how mutations affecting WASHC4 function may alter synaptic structure, composition and function through a perturbation of intracellular vesicular trafficking and sorting of synaptic proteins. To do so, we are characterizing the SWIPP1019R knock-in mouse model in terms of molecular, electrophysiological and behavioural phenotypes. Preliminary results obtained with patch-clamp recording of hippocampal dentate gyrus granule cells showed significant alterations in neuronal excitability and spontaneous synaptic activity in knock-in mice as compared to controls.
Our results are expected to provide new insights in the role of WASHC4 in neuronal cell biology, brain development and cognition.

*Speaker

#32

Lack of myotubularin phosphatase activity is the main cause of X-linked Centronuclear Myopathy

Jocelyn Laporte*[1]

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire – université de Strasbourg, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale : U964, Centre National de la Recherche Scientifique : UMR7104, université de Strasbourg : UMR7104, Institut National de la Santé et de la Recherche Médicale : U1258 – France

Abstract

The *MTM1* gene encodes myotubularin (MTM1), a phosphatidylinositol 3-phosphate (PI(3)P) lipid phosphatase. Loss-of-function mutations in *MTM1* cause X-linked myotubular myopathy (XLMTM), a severe congenital myopathy linked to organelles mis-positioning and with no available cure and a poorly understood pathomechanism. The importance of MTM1 enzymatic activity and its PI(3)P substrate in physiology under normal conditions and in XLMTM is unclear. We generated the *Mtm1* KI C375S mice in which the endogenous MTM1 was converted to a phosphatase-dead protein. Mutant mice survived a median of 12 weeks and demonstrated progressively impaired motor skills. Observed muscle atrophy and reduced force production compared to their WT littermates (_˜3.9-fold reduction in absolute maximal force) were responsible for these severe phenotypes. Increased level of PI(3)P was found in the muscle of *Mtm1* KI C375S mice. Muscle histology and molecular characterization revealed XLMTM hallmarks, with alteration of the mTOR and autophagy pathways correlating with muscle atrophy, and abnormal myofiber intracellular organization correlating with decreased muscle force. Overall, this study reveals the importance of MTM1 phosphatase activity and related PI(3)P substrate for postnatal muscle maintenance, and highlights the significance of MTM1 phosphatase activity in the development of X-linked myotubular myopathy.

*Speaker

Bioengineered condensates to interact and manipulate membrane-bound organelles in cells

Emma Pasquier*[1]

[1]École normale supérieure - Paris – Ecole Normale Supérieure de Paris - ENS Paris – France

Abstract

The dynamic interactions between the location and timing of biochemical processes within organelles is crucial for determining cell fate and function. Advanced biochemical, genetic, imaging, and "omics" approaches have revealed many important features of the biogenesis and functions of cellular organelles. In particular, recent studies suggest that communication between membrane organelles, through the establishment of membrane contact sites (MCS), could play crucial roles for their functions. However much less is known about putative contact sites between membrane-bound and membrane-less organelles (also known as biomolecular condensates). One key limitation comes from the biochemical complexity of condensates that make them difficult to study and manipulate in a cell context. To further advance in that direction, we are developing a novel methodology allowing the controlled assembly/disassembly in cells of bioengineered membrane-less organelles and that recapitulate some biophysical features of liquid-like condensates. Our engineered condensates are based on protein scaffolds designed to undergo phase separation in cells in a reversible manner. We demonstrated that it is possible to specifically target the assembly of engineered condensates on the surface of lysosomes, making them suitable for addressing fundamental questions about the interplay between condensates and lysosome interactions: How condensate nucleation and growth are modulated when interacting with the lysosomes? Conversely, how lysosome spatiotemporal dynamics is impacted when interacting with condensates? This method is versatile and could, in principle, be applied for studing other cellular organelles.

*Speaker

Perilipin 4, a novel amyloid-forming protein implicated in skeletal muscle degeneration

Nicolas Fuggetta* , Charbel Choufani , Bayane Sabbagh , Cyril Moulin , Sébastien Lyonnais[1], Luc Bousset[2], and Alenka Čopič

[1]CEMIPAI – CNRS : UMS3725, Université de Montpellier – 1919 Route de Mende, Montpellier, France
[2]Molecular Imaging Research Center [Fontenay-aux-Roses] – Université Paris-Saclay, Centre National de la Recherche Scientifique, Institut de Biologie François JACOB – France

Abstract

Perilipin 4 (PLIN4) belongs to the family of perilipins – proteins that localize to lipid droplets (LDs) and regulate lipolysis and LD stability. PLIN4 binds to LDs using an amphipathic helix (AH), which folds on the LD surface and can substitute for phospholipids in the LD monolayer, thereby regulating LD surface tension (1, 2). Strikingly, the AH of PLIN4 contains close to 1000 amino acids (aa), arranged in highly homologous tandem repeats of 33 aa. Mutated PLIN4 resulting from a genetic expansion of its repetitive region accumulates inside skeletal muscle cells in patients suffering from a late-onset vacuolar distal myopathy (MRUPAV) (3). We demonstrate that purified PLIN4 AH fragments aggregate in vitro, forming amyloid fibrils, which we have characterized using cryo-EM and atomic force microscopy. We have analyzed the influence of PLIN4 sequence, as it varies between different repeats, on its aggregation propensity. Importantly, we show that the MRUPAV mutation drastically increases the kinetics of fibril formation in vitro and induces aggregation in budding yeast, suggesting a causative link between PLIN4 amyloids and muscle degeneration. We propose that the determining factor is the multiplication of identical repeats in mutant PLIN4. We are now establishing experimental models to assess how the abundance and nature of LDs influence the formation of PLIN4 fibrils, to study the interplay between LDs, PLIN4 status and skeletal muscle function.

(1) Giménez-Andrés et al. Elife 2021, DOI: 10.7554/eLife.61401.

(2) Araujo et al. JCB 2024, DOI : 10.1083/jcb.202403064.
(3) Ruggieri et al. Acta Neuropathol. 2020, DOI: 10.1007/s00401-020-02164-4.

*Speaker

Surface tension-driven sorting of human perilipins on lipid droplets

Joëlle Bigay*[1], Abdoul Akim Bello , Ana Rita Dias Araújo , Céline Franckhauser , Romain Gautier , Nicolas Fuggetta , Alenka Copic , and Bruno Antonny

[1]Institut de pharmacologie moléculaire et cellulaire – Centre national de la recherche scientifique - CNRS (France) – France

Abstract

Perilipins (PLINs), the most abundant proteins on lipid droplets (LDs), display similar domain organization including amphipathic helices (AH). However, the five human PLINs bind different LDs suggesting different modes of interaction. We established a minimal system whereby artificial LDs covered with defined polar lipids were transiently deformed to promote surface tension. Binding of purified PLIN3 was strongly facilitated by tension but was poorly sensitive to phospholipid composition and to the presence of diacylglycerol. Accordingly, LD coverage by PLIN3 increased as phospholipid coverage decreased. In contrast, PLIN1 bound readily to LDs fully covered by phospholipids; PLIN2 showed an intermediate behavior between PLIN1 and PLIN3. In human adipocytes, PLIN3/4 were found in a soluble pool and relocated to LDs upon stimulation of fast triglyceride synthesis, whereas PLIN1 and PLIN2 localized to pre-existing LDs, consistent with the large difference in LD avidity observed in vitro. We conclude that the PLIN repertoire is adapted to handling LDs with different surface properties.

*Speaker

Tumor protein D54 dynamically induces tripartite biocondensates gathering intracellular nanovesicles and lipid droplets depending on cellular cholesterol levels

Sébastien Leterme*[1], Antoine Reynaud[1], Maud Magdeleine[1], Ana Rita Dias Araújo[1], Sophie Abelanet[1], Christelle Boscagli[2], Sandra Lacas-Gervais[2], and Bruno Antonny[1]

[1]Institut de pharmacologie moléculaire et cellulaire – CNRS UMR7275, Université Côte d'Azur – France
[2]Centre Commun de Microscopie Appliquée – Université Côte d'Azur (UCA) – France

Abstract

Lipid droplets form unique organelles in the cell that are made of a core of neutral lipids surrounded by a monolayer of phospholipids and proteins. Beside representing the main carbon source in the cell for various metabolic pathways, lipid droplets also constitute a major source of lipids for organelle membrane biogenesis and maintenance. We recently observed that the poorly characterized protein Tumor Protein D54 (TPD54) promotes the formation of a striking structure in the cell: a biocondensate in which lipid droplets are entrapped in a dense matrix of very small (30-40 nm) vesicles. Precursor work revealed that these tripartite biocondensates also contain lipid scramblases and lipid transporters, suggesting that they might constitute a huge reservoir of both neutral and bilayer-forming lipids that could be easily remobilized. Here, we aim to unravel the organization, the control of assembly, the cellular dynamics and the possible functions of this biocondensate gathering lipid droplets, nanovesicles and TPD54. Biochemical reconstitution and biophysical approaches demonstrated that TPD54 is indeed able to form dynamic biocondensates *in vitro* through its structural attributes. We then concentrated on the regulation of TPD54 subcellular localization by phosphorylation of a particular residue and by cell cholesterol content by combining pharmacological and microscopy approaches. We further investigated the possible role of TPD54 biocondensates as donors or acceptors in cellular lipid fluxes using fluorescent lipid probes. Altogether, our results constitute the first characterization of a new cellular structure that could be implied in the dynamic storage of both neutral and bilayer-forming lipids.

*Speaker

Mrs. **ALEMANY Carla**
carla.alemany@inserMr.fr
Institut Necker Enfants Malades
Paris - FRANCE

Mr **ALVES TAVARES Lucas**
lucastavares.biotec@gmail.com
Ribeirão Preto Medical School - University of São Paulo
Ribeirão Preto - BRESIL

Mr. **AMARI Chems**
chems.amari@ens.fr
ENS - Chemistry Department
Paris - FRANCE

Mr. **ANNAERT Wim**
wiMr.annaert@kuleuven.be
VIB& KU Leuven
Leuven - BELGIUM

Mr. **ANTONNY Bruno**
antonny@ipmc.cnrs.fr
Institut de Pharmacologie Moléculaire et Cellulaire
Nice - FRANCE

Mrs. **BERTRAND Anne**
anne.bertrand@inserMr.fr
INSERM
Grenoble - FRANCE

Mr. **BIERMEIERAlexander**
alexander.josef.biermeier.1@hu-berlin.de
Humboldt-Universität zu Berlin
Berlin - GERMANY

Mrs. **BIGAY Joëlle**
bigay@ipmc.cnrs.fr CNRS
Valbonne - FRANCE

Mrs. **BONCOMPAIN Gaelle**
gaelle.boncompain@univ-lyon1.fr
Institut Neuromyogene
Lyon - FRANCE

Mr. **BONNET Maxime**
maxime.bonnet@huntxpharma.com
HuntX Pharma
Grenoble - FRANCE

Mr. **CHAKRABORTY Ranabir**
ranabir.chakraborty@pasteur.fr
Institut Pasteur
Paris - FRANCE

Mrs. **CHIKOORE Tapuwanashe**
Tapuwanashe.Chikoore@univ-grenoble-alpes.fr
Grenoble Institut Des Neurosciences
Grenoble - FRANCE

Mrs. **CORMENIER Johanna**
johanna.cormenier@univ-grenoble-alpes.fr
Grenoble Institut of Neurosciences
Grenoble - FRANCE

MR. **COURCHET Julien**
julien.courchet@univ-lyon1.fr
Institut NeuroMyogène
Lyon - FRANCE

Mrs. **DANGLOT Lydia**
lydia.danglot@inserMr.fr
Inserm 1266
Paris - FRANCE

Mr. **DEL BONDIO Andrea**
andrea.del-bondio@univ-lyon1.fr
Institut NeuroMyoGène
Lyon - FRANCE

Mr. **DESTAING Olivier**
olivier.destaing@univ-grenoble-alpes.fr
Institute for Advanced Biosciences
Grenoble - FRANCE

Mrs. **FOLCI Alessandra**
alessandra.folci@in.cnr.it
CNR-Institute of Neuroscience
Milano – ITALY

Mr. **FUGGETTA Nicolas**
nicolas.fuggetta@crbMr.cnrs.fr
CNRS
Montpellier - FRANCE

Mrs. GORY Sylvie
sylvie.gory-faure@univ-grenoble-alpes.fr
Inserm U1216
Grenoble - FRANCE

Mrs. GUARNIERIFabrizia Claudia
fabrizia.guarnieri@in.cnr.it
CNR
Vedano Al Lambro - ITALY

Mrs. GUGUIN Justine
justine.guguin@univ-lyon1.fr
INMG
Lyon - FRANCE

Mr. HAN Yuhao
yuhao.han@hu-berlin.de
Humboldt University zu berlin
Berlin - GERMANY

Mr. HAUCKE Volker
haucke@fmp-berlin.de
Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)
Berlin - GERMANY

Mrs. JAMOT Laure
laure.jamot@huntxpharma.com
HuntXPharma
Paris - FRANCE

MR. JOHANNES Ludger
ludger.johannes@curie.fr
Institut Curie
Paris - FRANCE

Mr. KOPPERS Max
m.koppers@vu.nl
Vrije Universiteit Amsterdam
Amsterdam - NETHERLANDS

Mr. LAMAZE Christophe
christophe.lamaze@curie.fr
Institut Curie
Paris - FRANCE

Mr. LAPORTE Jocelyn
jocelyn@igbmc.fr
CERBM GIE
Illkirch - FRANCE

Mrs. LE LAY Aurélie
aurelie.lelay@huntxpharma.com
HuntX Pharma
Grenoble - FRANCE

Mr. LETERME Sébastien
leterme@ipmc.cnrs.fr CNRS
Valbonne - FRANCE

Mrs. LIENARD Caroline
caroline.lienard@univ-lyon1.fr
UCBL
Lyon - FRANCE

Mr. MARTHY Baptiste
baptiste.marthy@fresnel.fr CNRS
Institut Fresnel
Marseille - FRANCE

Mrs. MASSIT Cécile
cecile.massit@univ-grenoble-alpes.fr
Grenoble Institut des Neurosciences
Grenoble - FRANCE

Mrs. MIKHAYLOVA Marina
marina.mikhaylova@hu-berlin.de
Humboldt-Universität zu Berlin
Berlin - GERMANY

Mrs. MONTEIRO Laloé
laloe.monteiro@univ-lyon1.fr
INMG - PGNM
Lyon - FRANCE

Mr. MOREY Léo
leo.morey@huntxpharma.com
HuntX Pharma
Grenoble - FRANCE

Mrs. MOUTIN Marie-Jo
moutinm@univ-grenoble-alpes.fr
Grenoble Institut Neurosciences
Univ. Grenoble Alpes Inserm U1216
Grenoble - FRANCE

Mr. NAZAC Paul
paul.nazac@inserMr.fr
INSERM
Paris - FRANCE

Mrs. **NICOLAS Barbara**
barbaranicolas1@univ-grenoble-alpes.fr
Grenoble Institut Neurosciences
Grenoble - FRANCE

Mrs. **PASQUIER Emma**
emma.pasquier@ens.psl.eu
ENS Paris
Paris - FRANCE

Mr. **POPP Yannes**
yannes.popp@googlemail.com
Humboldt-University Berlin
Berlin - GERMANY

Mrs. **PRIETO GARCIA Marta**
marta.prieto-garcia@univ-grenoble-alpes.fr
Grenoble Institut des Neurosciences UGA
Grenoble - FRANCE

Mr. **PROCES Anthony**
anthony.proces@univ-grenoble-alpes.fr
Grenoble Institut Neuroscience UGA
Grenoble - FRANCE

Mrs. **RAJEH Layal**
layal.rajeh@crbMr.cnrs.fr
Montpellier - FRANCE

Mr. **RAMM Mauricio**
mauricio-ramm@gmx.de
University of Turku
Turku - FINLAND

Mrs. **ROTHFanny**
fanny.roth@huntxpharma.com
HuntX Pharma
Grenoble - FRANCE

Mr. **ROUX Aurélien**
aurelien.roux@unige.ch
Université de Genève
Genève - Switzerland

Mrs. **RUGGIERO Sonia**
sonia.ruggiero@curie.fr
Institut Curie
Paris - FRANCE

Mrs. **SALIBA Myriam**
myriaMr.saliba@univ-grenoble-alpes.fr
Grenoble institute of neurosciences
Grenoble - FRANCE

Mrs. **SALVI MESA Deborah**
Deborah.Mesa@ieo.itEuropean Institute of Oncology
Milan - ITALY

Mrs. **SANNERUD Ragna**
ragna.sannerud@kuleuven.be
VIB /KULeuven
Leuven - BELGIUM

Mr. **SAUDOU Frédéric**
frederic.saudou@inserMr.fr
Grenoble Institut de Neurosciences
Grenoble - FRANCE

Mrs. **SCARAMUZZINO Chiara**
chiara.scaramuzzino@univ-grenoble-alpes.fr
Inserm
Grenoble - FRANCE

Mrs. **SCHAUER Kristine**
kristine.schauer@gustaveroussy.fr
Institut Gustave Roussy
Villejuif - FRANCE

Mrs. **SCHER Nathalie**
nathalie.scher@univ-grenoble-alpes.fr
Inserm
Grenoble - FRANCE

Mrs. **SÉRIS Claire**claire.seris@univ-grenoble-alpes.fr
Grenoble Institut des Neurosciences
Grenoble - FRANCE

Mrs. **SIGISMUND Sara**
sara.sigismund@ieo.it
IEO
Milan - ITALY

Mrs. STEPHANIE Miserey
smiserey@curie.fr
Institut Curie CNRS
Paris - FRANCE

Mrs. TORRES TORRES Cristina
cristina.torres-torres@univ-grenoble-alpes.fr
Institute for Advanced Biosciences
Grenoble - FRANCE

Mrs. TREVISAN Anna
anna.trevisan@univ-grenoble-alpes.fr
Grenoble Institut Neurosciences
Grenoble - FRANCE

Mrs. TSVIRKUN Daria
daria.tsvirkun@univ-grenoble-alpes.fr
Lab Interdisciplinaire Physique
St. Martin d'Hères - FRANCE

Mrs. VAN ACKER Zoë
zoe.vanacker@kuleuven.vib.be
VIB-KU Leuven Center for Brain & Disease Research
Leuven - BELGIUM

Mr. VERVWEIJ Frederik
f.j.verweij@uu.nl
Utrecht University
Utrecht - NETHERLANDS

Mrs. VRANCX Céline
celine.vrancx@kuleuven.be VIB-KU Leuven Center for Brain & Disease Research
Leuven - BELGIUM

Mrs. YASMINA Saoudi
yasmina.saoudi@univ-grenoble-alpes.fr
INSERM
Grenoble - FRANCE

Mrs. YJJOU Sara sara.yjjou@univ-grenoble-alpes.fr
Université Grenoble Alpes
Grenoble - FRANCE

MR. ZAFFAGNINI Gabriele
gabriele.zaffagnini@crg.eu Centre for Genomic Regulation CRG
Barcelona - SPAIN

Mrs. ZEIGERER Anja
Anja.Zeigerer@medma.uni-heidelberg.de
Heidelberg University
Mannheim - GERMANY

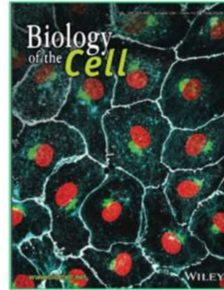
Mr. ZONCU Roberto
rzoncu@berkeley.edu California University
Berkeley - USA

The French Society for Cell Biology

- ✓ > 2000 members and > 350 affiliated labs
- ✓ Promotes cell biology research and education, outreach
- ✓ Travel grants for students and post-docs
- ✓ Annual Young Researcher & PhD thesis Prizes

FREE membership: register www.sbcf.fr

Publish with *Biology of the Cell*



Edited by René-Marc Mège, Paris, France

Broad scope
All aspects of cellular, molecular, structural, and developmental biology, and cell physiology and evolution. Special Issues on a variety of topics

Ease
First decision within 4 working days, no page or color charges

Quality
Published since 1984 by the *Société Française des Microscopies* and *Société de Biologie Cellulaire de France*

Impact
Open access choice – both pay-to-publish (gold) and self-archive (green) options available.

Submit your paper: ReX or www.biolcell.net or rene-marc.mege@ijm.fr

WILEY

journals · meetings · grants

biologists.com

@Co_Biologists

Follow us on WeChat

Development

Journal of Cell Science

Journal of Experimental Biology

Disease Models & Mechanisms

Biology Open