EMBO | FEBS
Lecture Course

Membranes, Lipids and Proteins in Organelle Biogenesis

Sunday 26 May - Saturday 1 June, 2024
Spetses, Greece

Anargyrios and Korgialenios School of Spetses

spetses2024.sites.uu.nl

Boehringer Ingelheim
Stiftung

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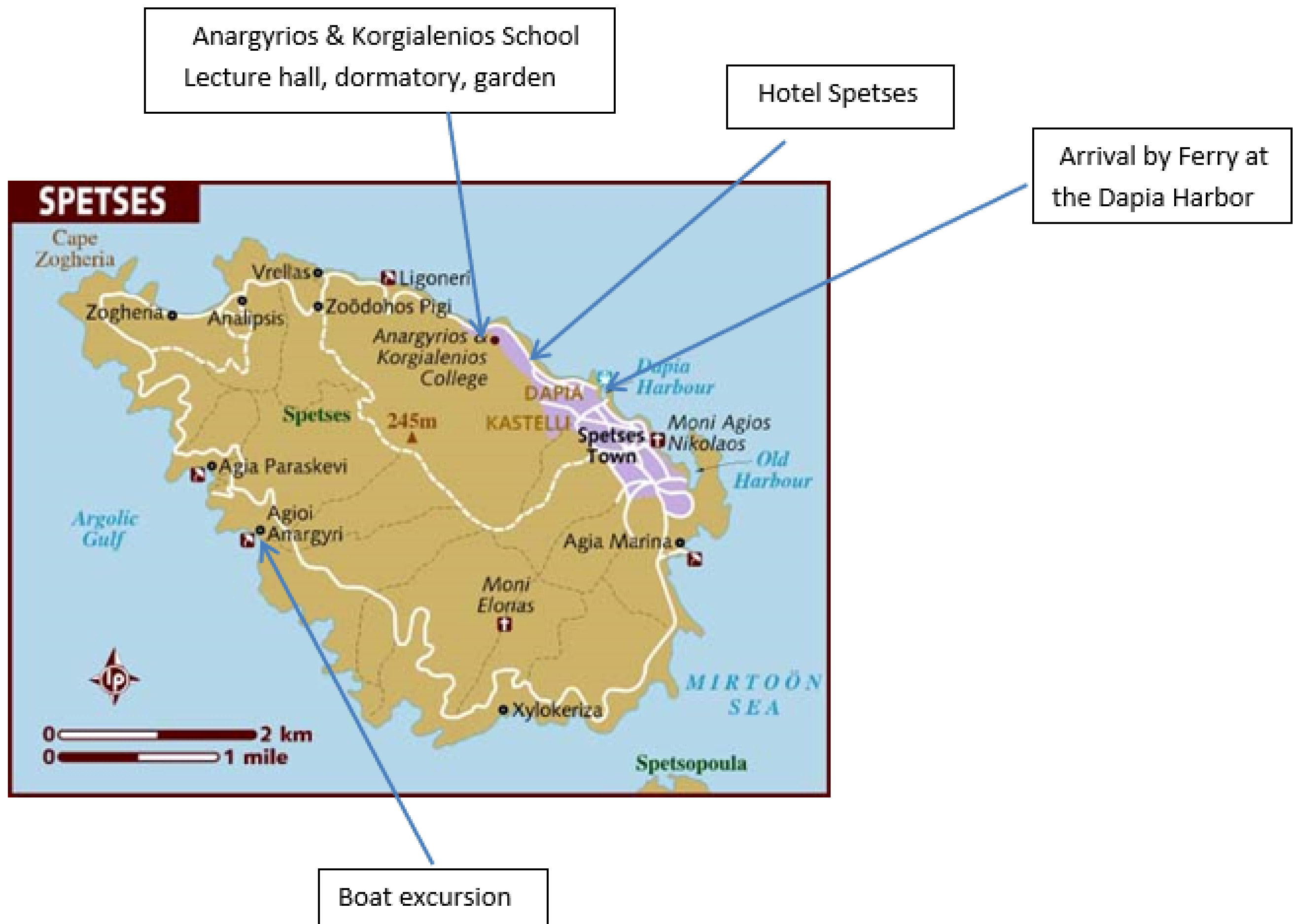
General Information

LOCATION

This lecture course, #19 in a series that started in 1987, will accommodate about 60 graduate students, postdocs and senior scientists during a 6-day summer school in Spetses, a small island of Greece, at the Anargyrios and Korgialenios School of Spetses.

Below you can find a map of the area:



SCHOOL ADDRESS

A.K.S.S. Foundation Schools of Spetses
18050 Spetses, Greece
Tel: +30 22980 72206 or +30 22980 72306
email: info@akss.gr
website: https://akss.gr/main/en/

GETTING TO THE SCHOOL

When you depart the Spetses harbor turn right and follow the road by the sea. After a 15 min walk you pass the Spetses Hotel, which is on your right hand side, and you will see the school a little bit further at the left side of the road. More information can be found on the website: https://spetses2024.sites.uu.nl/

COURSE DATES
Participants should arrive at the latest on Sunday May 26 in the afternoon. The first course lecture starts on Sunday May 26 at 5.30pm. Lectures, poster sessions and discussions will be held from 9am till 12.30pm and from 4pm till 7pm. The course ends in the evening of Friday May 31 and participants should depart on Saturday June 1.

EARLY CHECK-IN
Early arrival is possible on Saturday May 25, only if indicated in the web-form made available in the additional information request that was sent by email. The accommodation for 1 extra night is not included in the registration fee. The costs to stay 1 extra night is 52 euro. If not paid with the registration fee, this has to be paid to us in cash at the venue. Breakfast and lunch are not included that extra first day (on Sunday May 26). There are a lot of possibilities in the neighborhood, even at the Magus Café at the school. So you are free to decide for yourself where to eat.

ON SITE REGISTRATION
All participants must register on site, starting from 5pm on Sunday May 26 at the AKSS school.

ACCOMMODATION
The school at Spetses has multiple buildings. In the main building A; the lectures will be given, the poster sessions will be held, as well as breakfast and lunch are served there.
The rooms are situated in two other buildings: C & D. We mostly have double rooms, a limited number of single rooms and some single rooms with a shared bathroom (shared by 2 rooms) available. We will decide who will get which room. The single rooms are given to participants based on seniority (birth year) and other considerations. We will house participants from the same lab or institute together as much as possible. You will find the room list on Saturday 25 May late in the afternoon at the entrance of the main building A.
A towel for showering is available in the room.

SOCIAL ACTIVITIES
A welcome party for participants will be held at the end of the first lecture day on Sunday May 26. Other social events will be announced during the meeting.
This will include a boat trip (depending on the wheather), which is not included in the registration fee. The boat trip fee of 30 euro can be paid in cash during the course.
At the end of the boat trip on Wednesday May 29, a meal will be served at the school.
On Friday May 31, student sketches will be followed by a Greek BBQ and farewell party.

MEALS
Breakfast and lunch will be served at the school, in building A. In the evening on Monday May 27, Tuesday May 28 and Thursday May 30 tutorial dinners are organized at restaurants in Spetses town where small groups of ~8-10 students will be accompanied by 2 lecturers.

FINANCES
Upon acceptance, a registration fee of €650 will be asked to cover registration, meals during the course, refreshments, as well as accommodation in the school. If your payment has not arrived before the start of the course the amount due will be requested at the time of registration for the course.

Payments (if any) should be made (WITHOUT COSTS FOR THE RECEIVER) to:
Bank: ING Bank N.V. in Amsterdam
Address Bank: Bijlmerdreef 109, 1102 BW Amsterdam
BIC: INGBNL2A
IBAN: NL67INGB0683017136
In the name of E.J. Breukink, Brasemlaan 19, 3984 MN Odijk

Participants are expected to cover their own travel except for those that have obtained a grant that (partially) covered their travel expenses.

PROGRAM
We do not distribute printed versions of the abstracts during the course.

Active participation by the participants in all activities has become a tradition.

All student participants will present their poster during 1 of the 4 poster sessions, according to alphabetical order. The size of your poster should be A0 max, preferably in portrait orientation. Bring your printed poster with you as there is no opportunity to print posters in Spetses.

The organization has selected participants who will be invited to give a talk (10min) based on the abstracts. The selection of graduate student and postdoc participants was based on their poster title and abstract. After each poster session six candidates will also be selected by all participants, by ballot, for a talk. From these six the final three will get a poster prize. So, it is advised to be prepared for a short talk anyway. Please bring your presentation in PowerPoint on a memory USB stick.

ADDITIONAL ARRANGEMENTS
• Please arrange your own travel and/or health insurance. The organizers do not assume any responsibility for this or any other liability.
• Please make personal travel arrangements before you arrive at Spetses.

• Please make sure that you have **the appropriate visa** (especially for non-EU citizens) and a valid **passport**.

ORGANIZING COMMITTEE

Eefjan Breukink, Maya Schuldiner, Ulrich Hartl and Linda Kumeling

ORGANIZATIONAL CONTACT

Linda Kumeling
E-mail: spetses2024@uu.nl
Telephone number in case of urgent matters: **+31 (0)6 42151685**

Program

Sunday May 26th

Afternoon Arrival + settling in rooms

17:00-17:30 Registration

17:30-18:00 Introduction to the course – ***Eefjan Breukink***

18:00-19:00 Talk I: **EMBO Lecture:** Organelle stress responses – ***Agnieszka Chacińska***

19:00 Welcome drinks and snacks
including "speed dating" to get to know each other

Monday May 27th

8:00-9:30 Breakfast

9:30-9:45 Talk II: Introduction to FEBS – ***Mutay Aslan***

9:45-10:30 Talk III: Introduction to lipids – ***Eefjan Breukink***

10:30-11:00 Coffee Break

11:00-11:45 Talk IV: Lipid trafficking / recycling through the lysosomal system – ***Doris Höglinger***

11:45-12:30 Four short talks selected from abstracts: ***Beste Degirmenci, Trong Diep, Sandra Heinen, Selveije Ibraimi***

12:30 Mentoring Lunch & free time to go to the beach

16:00-16:45 Talk V: Lipid Exchange Proteins And How Cells Convert Membranes Into High Definition Signaling Screens – ***Vytas Bankaitis***

16:45-17:30 Talk VI: Membrane protein folding and maturation – ***Ineke Braakman***

17:30-19:00 Poster Session I & coffee (+ vote for best poster in session)

19:00 Dinner

Tuesday May 28th

7:00-8:00 Pre-Breakfast Pilates

8:00-9:00 Breakfast

9:00-9:45 Talk VII: Membrane Contact Sites: Organizers of Cellular Metabolism – ***Hanaa Hariri***

9:45-10:30 Talk VIII: Targeting and translocation to the ER – ***Rebecca Voorhees***

10:30-11:00 Coffee Break

11:00-11:45 Talk IX: Structure, Function and Dynamics of the Nuclear Envelope – ***Ulrike Kutay***

11:45-12:30 Four short talks selected from abstracts: ***Ilia Kalinin, Carla Kirschbaum, Mamta Mamta, Andrea Merino***

12:30 Mentoring Lunch & free time to go to the beach

16:00-16:45 Talk X: Protein Translocation into chloroplasts – ***Hsou-min Li***

16:45-17:30 Talk XI: Mapping the ERAD Highway: An Integrated Picture of All Retrotranslocation Routes – ***Sonya Neal***

17:30-19:00 Poster Session II & coffee (+ vote for best poster in session)

19:00 Dinner

Wednesday May 29th

8:00-9:00 Breakfast

9:00-9:45 Talk XII: Systematic approaches to studying organelles – ***Maya Schuldiner***

9:45-10:30 Talk XIII: Protein targeting to lipid droplets & the importance of inter-organelle communication – ***Bianca Schrul***

10:30-11:00 Coffee Break

11:00-11:45 Talk XIV: Biophysics of Apoptosis – ***Ana García-Saéz***

11:45-12:30 Four short talks selected from abstracts: ***Iris Montes, Leja Perne, Anantha Sen Saji, Anupam Singh***

12:30 Lunch

Afternoon Group boat trip (if weather allows & requires participation fee)

Evening Dinner at the school

Thursday May 30th

7:00-8:00 Pre-Breakfast Yoga/Pilates

8:00-9:00 Breakfast

9:00-9:45 Talk XV: Autophagosomes – ***Florian Wilfling***

9:45-10:30 Talk XVI: Cytosolic delivery by endosomes gone wrong – ***Tom Kirchhausen***

10:30-11:00 Coffee Break

11:00-11:45 Talk XVII: Revisiting COPII in anterograde trafficking – ***Alison Forrester***

11:45-12:30 Four short talks selected from abstracts: ***Shih-En Chou, Anil Sohail, Shota Wada, Evi Zaremba***

12:30 Lunch & free time to go to the beach

16:45-17:30 Talk XVIII: **IUBMB Plenary Lecture:** Distinct types of intramitochondrial protein aggregates protect mitochondria against proteotoxic stress – ***Johannes Herrmann***

17:30-19:00 Poster Session III & coffee (+ vote for best poster in session)

19:00 Dinner

Friday May 31st

8:00-9:00 Breakfast

9:00-9:45 Talk XX: **IUBMB Jubilee Award Lecture***:* Mechanism of protein import into peroxisomes – ***Tom Rapoport***

9:45-10:30 Talk XXI: **Women in Science Lecture** – Sonya Neal

10:30-11:00 Coffee Break

11:00-11:45 Talk XXII: **EMBO YIP lecture**: Intercellular communication and the formation of cell-cell cytoplasmic bridges in plants - ***Emmanuelle Bayer***

11:45-12:30 Four short talks selected from abstracts: ***Nitya Aravindan, Chandini Bhaskar Naidu, Josephine Botsch, Angel Chavez***

12:30 Lunch & free time to go to the beach

16:00-16:45	Mentoring talk: What next? Career advice panel (3-4 scientists from different countries with open questions from students)
16:45-18:15	Poster Session IV & coffee (+ vote for best poster in session)
18:15-18:30	Short break
18:30-18:45	Poster prize awards & talk of best poster (if not already given)
18:45-19:30	Student skits – Farewell words
19:30	Farewell party with Greek BBQ

Saturday June 1st

Departure

Poster sessions

Poster session I : Monday May 27, 17:30-19:00

Domenico	Abete
Petia	Adarska
Stephanie	Agbana
Carla	Alemany
Nitya	Aravindan
Anna	Bartalis
Rana Can	Baygin
Jeremy	Bennett
Chandini	Bhaskar Naidu
Anne-Laure	Boinet
Mayra	Borrero
Josephine	Botsch
Chia-Yun	Chang
Angel	Chavez
Shih-En	Chou

Poster session II : Tuesday May 28, 17:30-19:00

Beste Senem	Degirmenci
Trong Vien Duy	Diep
Francesca	Forno
Anamika	Gaur
Savana	Green
Emanuela	Grillo
Sandra	Heinen
Yu-Ling	Hsu
Selvije	Ibraimi
Akash Kumar	Jha
Ilia	Kalinin
Paulina	Kettel
Ashley	Kidwell
Carla	Kirschbaum

Poster session III : Thursday May 30, 17:30-19:00

Katharina	Kott
Hajnalka	Laczkó-Dobos
H. Mathilda	Lennartz
Joanna	Lewandowska
Paulina	López Carrasco
Sara	Lotfipour Nasudivar
Mamta	Mamta
Klaudia	Maruszczak
Mark	Mcdermott
Andrea	Merino
Kubilay	Mese
Iris	Montes
Johannes	Morstein
Agradeep	Mukherjee
Leja	Perne

Poster session IV : Friday May 31, 16:45-18:15

Thu	Pham
Stefanie	Pritzl
Cristian	Rocha Roa
Anantha Krishnan	Sen Saji
Anupam	Singh
Anil	Sohail
Maria	Soultsioti
Yanwei	Su
Jelmi	Uit De Bos
Rosario	Valenti
Shota	Wada
Linda	Wedemann
Marek	Wilhelm
Gamze Nur	Yapici
Evi	Zaremba

Lecturers: Profiles & Abstracts

Vytas A. Bankaitis

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Vytas A. Bankaitis is the E.L. Wehner-Welch Foundation Chair in Chemistry at Texas A&M University. He earned his PhD degree in Molecular Genetics at the Univ. of North Carolina School of Medicine and was a Helen Hay Whitney Fellow at Cal Tech in the lab of Dr. Scott Emr. After progressing through the faculty ranks at the University of Illinois and the University of Alabama-Birmingham School of Medicine, he was recruited back to the University of North Carolina School of Medicine, where he served as Chair of the Department of Cell & Developmental Biology for 11 years. In September 2012, he was recruited to Texas as the Endowed Wehner-Welch Chair in Chemistry. Throughout, his lab has made seminal contributions to the protein trafficking and lipid signaling fields – particularly in the area of lipid transfer proteins. The lab's present interests focus on how phosphatidylinositol transfer proteins contribute to functional diversification phosphoinositide signaling.

Abstract:

Lipid Exchange Proteins And How Cells Convert Membranes Into High Definition Signaling Screens

An exciting area in contemporary cell biology revolves around the renaissance of an 'old' question – what are the mechanisms by which lipids can be trafficked between organelles by nonvesicular mechanisms? It is from this perspective that lipid transfer proteins now command intense interest and these proteins exist both as components of membrane contact sites, and as soluble proteins. Both will be discussed, but the focus will be on the fascinating biology and biochemistry of the soluble phosphatidylinositol (PtdIns) transfer proteins (PITPs) of fungal and mammalian systems. PtdIns is a metabolic precursor of phosphoinositides (PIPs), and these lipids function as critical intracellular chemical signals in eukaryotes. PIP metabolism is a major mechanism used by eukaryotic cells to convert their membrane surfaces into high-definition signaling screens. While contemporary research efforts focus on enzymes that produce and consume PIPs, key questions regarding how PIP production is regulated and physically organized with respect to downstream effector molecules remain unresolved. It is in those contexts that PtdIns transfer proteins (PITPs) command our interest as PITPs determine the functional channeling of PIP signaling circuits. The biological importance of PITPs is abundantly evident in both uni- and multi-cellular organisms, and PITP deficits are associated with a number of striking phenotypes. The theme that links all of our PITP studies to date is that activities of specific PITPs channel PIP signaling to specific biological outcomes. As example, I will discuss our work on how PITP signaling influences neural stem cell biology and development of the mammalian neocortex and how our findings provide interesting new perspectives regarding developmental brain disabilities and neural tube birth defects.

Relevant Publications:

1. Bankaitis, V. A., J. F. Aitken, A. E. Cleves, and W. Dowhan. 1990. An essential role for a phospholipid transfer protein in yeast Golgi function. Nature 347: 561-562.

2. Cleves, A. E., T. P. McGee, E. A. Whitters, K. Champion, J. R. Aitken, W. Dowhan, M. Goebl, and V. A. Bankaitis. 1991. Mutations in the CDP-choline pathway for phospholipid biosynthesis bypass the requirement for an essential phospholipid transfer protein. Cell 64: 789-800.

3. Kearns, B.G., McGee T.P., Mayinger, P., Gedvilaite, A., Phillips, S.E., Kagiwada, S., and Bankaitis, V.A. 1997. Essential role for diacylglycerol in protein transport from the yeast Golgi complex. Nature 387: 101-105.

4. Schaaf, G., Ortlund, E.A., Tyeryar, K.R., Mousley, C.J., Ile, K.E., Woolls, M.J., Garrett, T.A., Raetz, C.R.H., Redinbo, M.R., and Bankaitis, V.A. 2008. The functional anatomy of phospholipid binding and regulation of phosphoinositide homeostasis by proteins of the Sec14-superfamily. Molecular Cell 29: 191-206.

5. Nile,A.H., Tripathi, A., Yuan, P., Mousley, C.J., Suresh, S., Wallace, I.M., Shah, S.D., Teiotico-Pohlhaus, D., Temple, B., Nislow, C., Giaever, G., Tropsha, A., Davis, R.W., St. Onge, R.P., and Bankaitis, V.A. 2014. PITPs as targets for selectively interfering with phosphoinositide signaling in cells. Nature Chemical Biology 10: 76-84.

6. Wang, Y., Yuan, P., Grabon, A., Tripathi, A., Lee, D., Rodriguez, M., Lönnfors, M.,

Eisenberg-Bord, Wang, Z., Lam, S.M., M., Schuldiner, M., and Bankaitis, V.A. 2020. Non-canonical regulation of phosphatidylserine metabolism by a Sec14-like protein and a lipid kinase. J. Cell Biol. 219: e201907128.

7. Khan, D., Lee, D., Gulten, G., Aggarwal, A., Krieger, I., Wofford, J., Tripathi, A., Patrick, J.W., Eckert, D., Laganowsky, A., Sacchettini, J., Lindahl, P., and Bankaitis, V.A. 2020. An unconventional Sec14-like phosphatidylinositol transfer protein paralog binds heme via a unique coordination mechanism. eLife 9:e57081

8. Xie, Z., Hur, S.K., Zhao, L., Abrams, C., and Bankaitis, V.A. 2017. A Golgi lipid signaling pathway controls apical Golgi distribution and cell polarity during neurogenesis. Developmental Cell 44: 725-744.e4.

9. Xie, Z., and Bankaitis, V.A. 2022. A phosphatidylinositol transfer protein/planar cell polarity axis regulates neocortical morphogenesis by supporting interkinetic nuclear migration. Cell Reports 39: 110869.

10. Chen, X.R., Poudel, L., Hong, Z., Johnen, P., Katti, S., Tripathi, A., Nile, A.H., Green, S., Khan, D., Schaaf, G., Bono, F., Bankaitis, V.A.*, and Igumenova, T.I.* 2023. Mechanisms by which small molecule inhibitors arrest Sec14 lipid transfer activity. J. Biol. Chem. 299: 102861. * -- co-corresponding authors

11. Yeh, Y.-T., Sona, C., Yan, X., Pathak, A., McDermott, M.I., Xie, Z., Liu, L., Arunagiri, A., Wang, Y., Cazenave-Gassiot, A., Ghosh, A., von Meyenn, F., Kumarasamy, S., Najja, S.M.r, Jia, S., Wenk, M.R., Traynor-Kaplan, A., Arvan, P., Barg, S., Bankaitis, V.A., and Poy, M.N. 2023. Restoration of PITPNA in T2D human islets reverses pancreatic beta-cell dysfunction. Nat. Communications 14: 4250 https://doi.org/10.1038/s41467-023-39978-1

Reviews:

1. Bankaitis, V.A., Mousley, C.J., and Schaaf, G. 2010. Sec14-superfamily proteins and the crosstalk between lipid signaling and membrane trafficking. Trends in Biochemical Sciences 35: 150-160.

2. Grabon, A., Bankaitis, V.A., and McDermott, M.I. 2018. The interface between phosphatidylinositol transfer protein function and phosphoinositide signaling in higher eukaryotes. Journal of Lipid Research 60: 242-268.

3. Khan, D., Nile, A.H., Tripathi, A., and Bankaitis, V.A. 2021. Emerging prospects for combating fungal infections by targeting phosphatidylinositol transfer proteins. Intl. J. Mol. Sci. 22: 6754.

Emmanuelle Bayer

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France

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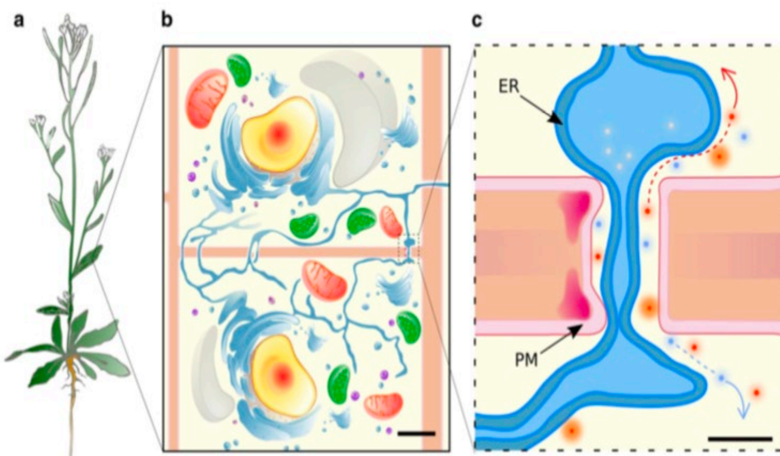
Bayer's lab research focus

Plant cell-cell communication is mediated by intercellular junctions called plasmodesmata, that cross the cell wall and interconnect virtually every single cell within the plant body, allowing for direct exchange of molecules. Plasmodesmata are critical for the cohesive and collective organisation of plant multicellularity and as such essential for plant life. By establishing cytosolic continuity, plasmodesmata allow the dynamic and reciprocal exchange of information between cells. Plasmodesmata are also targets for viruses that exploit them to spread throughout the plant, and they are starting to emerge as 'hubs' for local defence signalling.



Plasmodesmata are atypical membrane contact sites. They consist of a strand of endoplasmic reticulum (ER) running through the pore, tethered extremely tightly (~10 nm) to the plasma membrane (PM). This classifies plasmodesmata as a specialised type of membrane contact site, where both the ER and the PM display extreme, and opposing membrane curvature. Specialisation of both the ER and PM lining plasmodesmata bridge is expected to guarantee unique function. To date however we still don't know how and why the two organelles come together at plasmodesmata cellular junctions. What is the benefit of such membrane contacts for cell-cell communication and plant multicellularity?

Our lab focuses on understanding of the molecular mechanisms underlying plasmodesmata function and biogenesis. We are using multidisciplinary approaches from lipidomics/proteomics, super-high resolution microscopy to access to plasmodesmata 3D architecture, live imaging as well as more classical cell biology and plant genetics.

Abstract:

Intercellular communication and the formation of cell-cell cytoplasmic bridges in plants

Intercellular bridges arising from incomplete cytokinesis act as structural mediators of clonal multicellularity, enabling daughter cells to communicate. Through these membrane-lined bridges, cells exchange signals, nutrients, and organelles to coordinate cell fate and growth. In plants, these communication bridges are called plasmodesmata. Unlike in animals, where only specific cell types form cytokinetic bridges, in plants, every single somatic cell undergoes incomplete division producing daughter cells connected by literally hundreds plasmodesmata. Put simply, plant cells communicate by deliberately halting cytokinesis, a strategy that represents the default state. Plasmodesmata are omnipresent, interconnecting nearly all plant cells, and are indispensable for plant life. During the lecture I will dive into our current understanding of the molecular mechanism responsible for incomplete cytokinesis and plasmodesmata bridge stabilization in plants.

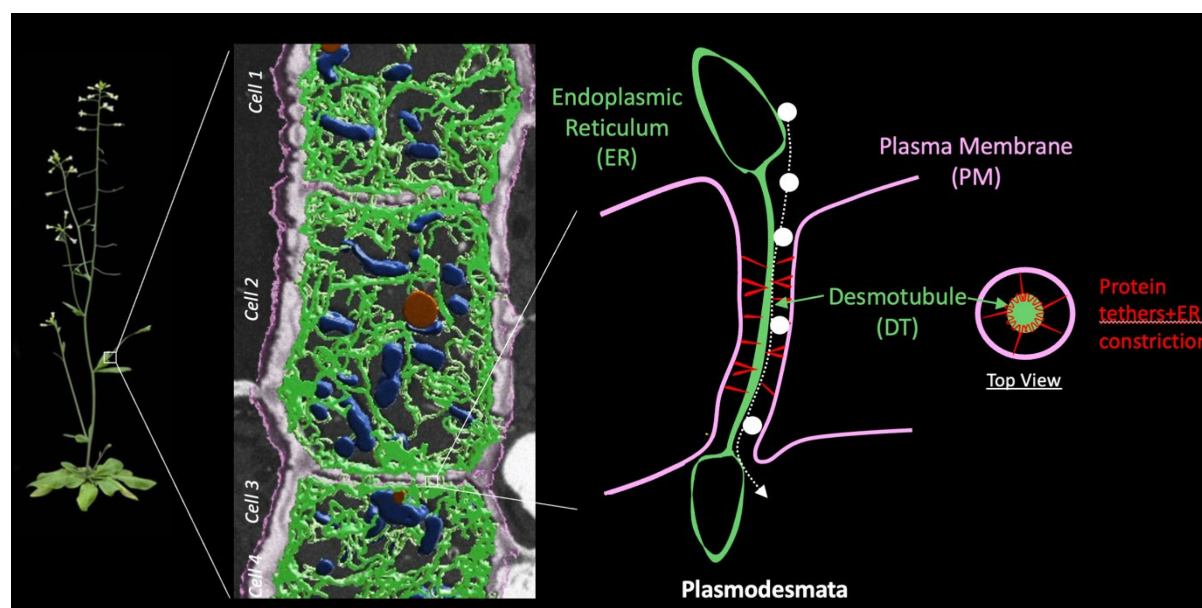


Fig: Within plant tissues, nearly all cells are connected by intercellular ER-PM MCSs called plasmodesmata (middle; ER green, chloroplasts blue, lysosomes brown, cell wall grey). Plasmodesmata have a complex membrane architecture (right) with a hyper-constricted ER-desmotubule (green) tether- and ER shaping proteins (red) in close proximity to the PM (purple). Macromolecules (white) as well as hormones and nutrients are exchanged through plasmodesmata, mediating inter-cellular organelle communication. MCS: membrane contact site

References

1. A. Chaigne, T. Brunet, Incomplete abscission and cytoplasmic bridges in the evolution of eukaryotic multicellularity. *Curr. Biol.* **32**, R385–R397 (2022).
2. Z. P. Li *et al.*, Plant plasmodesmata bridges form through ER-driven incomplete cytokinesis. https://www.biorxiv.org/content/10.1101/2023.12.12.571296v1

Ineke Braakman

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My background is a mixture of pharmacy (undergrad), biochemical pharmacology (PhD, both in Groningen, NL), molecular cell biology, and biochemistry. I now am Professor of Cellular Protein Chemistry at Utrecht University in The Netherlands. The cell biology and biochemistry I have embraced as post-doc in the lab of Ari Helenius, Dept. Cell Biology at Yale University (New Haven CT, USA) Upon retrun to Europe, I started my own lab in the Dept. Biochemistry in the Academic Medical Center in Amsterdam, first as fellow from the Netherlands Academy of Arts and Sciences, later as Associate Professor.

We develop and use radioactive folding assays, cell-free or in intact cells, to establish how proteins fold, how the folding machinery regulates and is regulated by this process. We use any technique that answers the questions, from proteomics, structural biology, high-throughput screens, and live-cell imaging to organellar assays, pull-downs, and assays on purified proteins, exploiting E. coli, S. cerevisiae, mammalian and insect cells.

We have uncovered molecular details of folding pathways of the cystic fibrosis-related CFTR protein, the low-density-lipoprotein receptor (LDLR), and the glycoproteins of influenza virus and HIV-1. This includes the (kinetic & dynamic) relationships between signal-peptide cleavage, disulfide-bond formation, glycosylation, conformational changes and assembly. We have identified novel ER-resident (putative) molecular chaperones and developed cell lines each lacking a chaperone or folding enzyme. Our knowledge on folding pathways we use for mode-of-action studies of chaperones as well as (candidate) modulator drugs, for instance for cystic-fibrosis patients, in collaboration with drug-development industry.

Abstract:

Folding and maturation of membrane proteins

Considering their folding, membrane proteins are not unlike soluble proteins, except for their residence in more than one location, an issue of topology. Extreme examples are multi-spanning membrane proteins that traverse the membrane multiple times, such as ABC-transporters and GPCRs. They form 2 large protein families with important cellular functions and with domains in both cytosol and membrane, plus an ectodomain that may reside in the ER, Golgi, outside the cell, or elsewhere, depending on where the protein travels and functions. Yet, also with a single transmembrane domain, membrane proteins have a topological issue, such as the LDL receptor (type I), influenza virus hemagglutinin (type I) or neuraminidase (type II), each with domains in 3 locations. The complexity this adds is the need for proper translocation, the dependence on chaperones from 3 different compartments, and the increased number of possible modifications. And one more fate-determining level for

proteins in the secretory pathway is that they travel through multiple compartments (ER to Golgi to plasma membrane for instance), with a changing environment in all 3 locations.

You will have heard the basic components explained in Spetses: lipids and membranes, protein translocation and folding. Membrane-protein biosynthesis requires their integration, and you will see that, while a lot of knowledge has been gathered, many basic questions remain.

Recommended reading:

Examples of detailed membrane-protein folding studies:

McCaul N, Quandte M, Bontjer I, van Zadelhoff G, Land A, Crooks ET, Binley JM, Sanders RW, Braakman I. Intramolecular quality control: HIV-1 envelope gp160 signal-peptide cleavage as a functional folding checkpoint. Cell Rep. 2021 Aug 31;36(9):109646. doi: 10.1016/j.celrep.2021.109646.PMID: 34469718

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Reviews:

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Braakman I, Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. Annu Rev Biochem. 2011;80:71-99. doi: 10.1146/annurev-biochem-062209-093836.PMID: 21495850

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Eefjan Breukink received his PhD (in 1994) at Utrecht University. After post-doctoral research at Oxford University with Prof. A. Watts, he returned to Utrecht University, and joined the Department of Biochemistry of membranes of the Utrecht University faculty of Chemistry first as post-doc and since 2003 as faculty member where he became an associated professor in 2013 and a full professor in Microbial Membranes and Antibiotics in 2023. His research focuses on the bacterial cell wall synthesis pathway, determination of the mode of action of antibiotics and on the discovery of novel antibiotics in fungal extracts. He has been involved in a young start-up company QVQ that focuses on development and marketing of lama antibody fragments (VHHs) for imaging and research purposes as CTO and briefly as CEO. He has participated in EU-Networks, and (co-)authored over 100 peer-reviewed publications. In 2014 he became an honorary Professor of the Zhejiang Provincial Key Laboratory of Food Microbiotechnology Research of China. From 2014-2017 he was the Director of Education and Director of the Bachelor Chemistry program. He now heads the Membrane Biochemistry and Biophysics group of the Utrecht University.

Selected publications

1. Shehrazade Jekhmane#, Maik G.N. Derks#, Sourav Maity, Cornelis J. Slingerland, Kamaleddin H. M. E. Tehrani, João Medeiros-Silva, Vicky Charitou, Danique Ammerlaan, Céline Fetz, Naomi A. Consoli, Eilidh J. Matheson, Rachel V. K. Cochrane, Mick van der Weijde, Barend O.W. Elenbaas, Francesca Lavore, Ruud Cox, Joseph H.F.F Lorent, Marc Baldus, Markus Künzler, Moreno Lelli, Stephen Cochrane, Nathaniel I. Martin, Wouter H. Roos, Eefjan Breukink*, Markus Weingarth* (2024) Host defence peptide plectasin targets bacterial cell wall precursor lipid II by a calcium-sensitive supramolecular mechanism, Nat. Microbiol., *in press.*
2. Shukla R, Lavore F, Maity S, Derks MGN, Jones CR, Vermeulen BJA, Melcrová A, Morris MA, Becker LM, Wang X, Kumar R, Medeiros-Silva J, van Beekveld RAM, Bonvin AMJJ, Lorent JH, Lelli M, Nowick JS, MacGillavry HD, Peoples AJ, Spoering AL, Ling LL, Hughes DE, Roos WH, Breukink E, Lewis K, Weingarth M. (2022) Teixobactin kills bacteria by a two-pronged attack on the cell envelope. Nature 608(7922):390-396. doi: 10.1038/s41586-022-05019-y.
3. Medeiros-Silva J, Jekhmane S, Paioni AL, Gawarecka K, Baldus M, Swiezewska E, Breukink E, Weingarth M. (2018) High-resolution NMR studies of antibiotics in cellular membranes. Nat. Comm. 9, 3963. doi: 10.1038/s41467-018-06314-x.
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5. te Welscher YM, van Leeuwen MR, de Kruijff B, Dijksterhuis J, Breukink E. (2012) Polyene antibiotic that inhibits membrane transport proteins. Proc Natl Acad Sci USA 109:11156-11159.
6. Mohammadi, T., van Dam, V., Sijbrandi, R., Vernet, T., Zapun, A., Bouhss, A., Diepeveen-de Bruin, M., Nguyen-Distèche, M., de Kruijff, B. and Breukink, E. (2011) Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. EMBO J. 30:1425-1432.
7. Hasper, H.E., Kramer, N.E., Smith,J.L., Hillman, J.D., Zachariah, C., Kuipers, O.P., de Kruijff, B. and Breukink, E. (2006) Cell wall precursor abduction as a novel antibiotic mechanism. Science, 313, 1636-1637.
8. Breukink E. and de Kruijff, B. (2006) Lipid II as a target for antibiotics. Nature reviews on Drug Discovery 5, 321-332.

Abstract:

Introduction into membrane lipids

At some point during the evolution of life, a single event of a fusion between an archaeon and a protobacterium set the stage for the development of more complex cells containing internal membrane structures and developing vesicular transport. They became the eukaryotic cells. They started to synthesize sphingolipids and sterols. The physical differences between these and the glycerophospholipids must have enabled the cells to segregate lipids in the membrane plane. Localizing this event to the Golgi then allowed them to create membranes of different lipid composition, notably a thin, flexible ER membrane, consisting of glycerolipids, and a sturdy plasma membrane containing at least 50% sphingolipids and sterols.
Why are there so much different lipids? What is their role in important cellular processes? Even relatively simple bacteria have a complex lipid composition that is continuously controlled to be able to react quickly to changing environments. In this lecture the basics of lipids will be discussed, where they are and what they do.

Background reading

1) Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. Nat Rev Mol Cell Biol. 2018 May;19(5):281-296. doi: 10.1038/nrm.2017.138. Epub 2018 Feb 7. Erratum in: Nat Rev Mol Cell Biol. 2019 Nov;20(11):715. PMID: 29410529.
2) Hannich JT, Umebayashi K, Riezman H. Distribution and functions of sterols and sphingolipids. Cold Spring Harb Perspect Biol. 2011 May 1;3(5):a004762. doi: 10.1101/cshperspect.a004762. PMID: 21454248; PMCID: PMC3101845.
3) Lorent JH, Levental KR, Ganesan L, Rivera-Longsworth G, Sezgin E, Doktorova M, Lyman E, Levental I. Plasma membranes are asymmetric in lipid unsaturation, packing and protein shape. Nat Chem Biol. 2020 Jun;16(6):644-652. doi: 10.1038/s41589-020-0529-6. Epub 2020 May 4. Erratum in: Nat Chem Biol. 2020 May 15;: PMID: 32367017; PMCID: PMC7246138.
4) van Meer G, de Kroon AI. Lipid map of the mammalian cell. J Cell Sci. 2011 Jan 1;124(Pt 1):5-8. doi: 10.1242/jcs.071233. PMID: 21172818.

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Agnieszka Chacińska – professor and group leader, director of IMol, the institute of Polish Academy of Sciences. Graduated from Biology Faculty at the University of Warsaw; received a doctoral degree in biochemistry at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences with Prof. Magdalena Boguta, and a habilitation degree from the same institute. Completed post-doctoral training with Prof. Nikolaus Pfanner, followed by a junior group leader position at the University of Freiburg in Germany. Since 2009 the head of Laboratory of Mitochondrial Biogenesis established in the International Institute of Molecular and Cell Biology and currently operating in IMol PAS.

Interested in biochemistry and molecular aspects of cells. This includes biogenesis, transport and degradation of mitochondrial proteins and their failure resulting in pathologies. Her major contribution has been the identification of new mechanisms of protein import into mitochondria and a cross-talk of defective mitochondrial protein import and other mitochondrial deficiencies with cellular protein homeostasis.

Recipient of multiple awards and prestigious grants, including ReMedy project worth nearly 8 mln EUR, financed by the Foundation for Polish Science as part of the International Research Agendas Programme, Welcome Grant from the Foundation for Polish Science, and Maestro grant from the National Science Centre. Elected member of European Molecular Biology Organization, Academia Europaea and German National Academy of Sciences Leopoldina. Corresponding member of Polish Academy of Sciences. Awarded the title of Professor by the President of the Republic of Poland.

Abstract:

Organelle stress responses

Mitochondria are multifunctional organelles, primarily involved in the fundamental biological process of respiration, but also many other biochemical reactions. Their dysfunction causes, or is observed in, many pathologies. The efficient functioning of mitochondria depends on the proper transport, sorting and assembly of mitochondrial proteins that originate either from nuclear or mitochondrial genomes. The nuclear-encoded proteins make up for the large majority of proteins involved in the formation of mitochondria including the respiratory chain complexes. These proteins are transient residents in the cytosol. Stress frequently leads to mitochondrial defects that, in turn, negatively impact mitochondrial protein uptake by the organelles. The cells activate several stress response mechanisms which are aimed at an increase in specific chaperone activities, improving the degradation capacity of the ubiquitin-proteasome system and modulation of cytosolic protein synthesis. This interplay between

defective mitochondria and cytosolic protein homeostasis mechanisms plays an important role in the molecular basis and consequences of mitochondrial diseases, as well as age-related degeneration.

Selected publications recommended for reading:

1. Kim, M., Serwa, R., Samluk, L., Stępkowski, T., Suppanz, I., Kodron, A.,Warscheid, B., **Chacinska, A.** (2023). Immunoproteasome-specific subunit PSMB9 induction is required to regulate cellular proteostasis upon mitochondrial dysfunction, Nat Communications 14(1), 4092. DOI:10.1038/s41467-023-39642-8

2. Nowicka, U., Chroscicki, P., Stroobants, K., Sladowska, M., Turek, M., Uszczynska-Ratajczak, B., Kundra, R., Goral, T., Perni, M., Dobson, C.M., Vendruscolo, M., **Chacinska, A.** (2021). Cytosolic aggregation of mitochondrial proteins disrupts cellular homeostasis by stimulating other proteins aggregation. Elife. 2021 Jul 20;10:e65484. DOI: 10.7554/eLife.65484

3. Sladowska, M., Turek, M., Kim, M-J., Drabikowski, K., Mussulini B. H., Mohanraj, K., Serwa, R. A., Topf, U., **Chacinska, A.** (2021). Proteasome activity contributes to pro-survival response upon mild mitochondrial stress in Caenorhabditis elegans. PloS Biology, 19(7):e3001302. DOI: 10.1371/journal.pbio.3001302

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5. Gold, V.A.M., Chroscicki, P., Bragoszewski, P., **Chacinska, A.** (2017). Visualization of cytosolic ribosomes on the surface of mitochondria by electron cryo-tomography. EMBO Reports (10):1786-1800, DOI 10.15252/embr.201744261

6. Wrobel, L., Topf, U., Bragoszewski, P., Wiese, S., Sztolsztener, M.E., Oeljeklaus, S., Varabyova, A., Lirski, M., Chroscicki, P., Mroczek, S., Januszewicz, E., Dziembowski, A., Koblowska, M., Warscheid, B., **Chacinska, A.** (2015). Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. Nature, 524(7566):485-8. DOI: 10.1038/nature14951

7. Bragoszewski, P., Wasilewski, M., Sakowska, P., Gornicka, A., Böttinger, L., Qiu, J., Wiedemann, N., **Chacinska, A.** (2015). Retro-translocation of mitochondrial intermembrane space proteins. Proc Natl Acad Sci U S A. 112(25):7713-8. DOI: 10.1073/pnas.1504615112

8. **Chacinska, A.**, Koehler, C.M., Milenkovic, D., Lithgow, T., Pfanner, N. (2009). Importing mitochondrial proteins: machineries and mechanisms. Cell 21;138(4):628-44. DOI: 10.1016/j.cell.2009.08.005

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Alison Forrester studied Pharmacology at Newcastle University (UK), graduating in 2007, where she remained to study her PhD in Toxicology and Dermatological Sciences, to identify a biomarker for toxic activation of the transcription factor, the Aryl hydrocarbon Receptor. She graduated in 2012, and to pursue the study of autophagy in disease, moved to the Telethon Institute of Genetics and Medicine (TIGEM) in Naples (Italy) to work under the guidance of Carmine Settembre. Here, Alison worked on the role of autophagy in procollagen trafficking1 and ER-phagy, discovering the ER-phagy complex Calnexin-FAM134B2 responsible for maintaining procollagen trafficking and identifying for the first time a role for autophagy in quality control. To pursue the themes of protein trafficking and advanced microscopy, Alison then moved for a second post-doctoral position to the Insitut Curie in Paris (France), under the guidance of Ludger Johannes. Here she worked on Clathrin independent endocytosis using Lattice light-sheet microscopy3, as well as on the discovery of the mechanism of the first specific inhibitor of endoplasmic reticulum (ER) exit sites (ERES), Retro-24. Alison is now an independent group leader at the University of Namur in Belgium where she works on the pharmacological modulation of ERES, studying the fundamental cell biology of the mechanisms of inhibition and the effects on organelle and cellular homeostasis, as well as working towards developing the ERES as a therapeutic target.

1 Cinque L.*, Forrester A.* et al. Nature. 2015 Dec ; 10;528(7581):272-5. doi: 10.1038/nature16063.

2 Forrester A. et al. EMBO J. 2019 Jan 15;38(2):e99847. doi: 10.15252/embj.201899847.

3 Macdonald E., et al. bioRxiv 2023 doi.org/10.1101/2023.09.12.557183.

4 Forrester A. et al. Nat Chem Biol. 2020 Mar;16(3):327-336. doi: 10.1038/s41589-020-0474-4.

Abstract:

Revisiting COPII in anterograde trafficking

Trafficking of proteins between organelles serves to allow access to enzymes that perform post-translational modifications, that regulate the protein structure and function, and delivers them to their required cellular or extracellular destination. Therefore, trafficking from one organelle to another is tightly regulated at a number of levels: 1) Selection of properly folded and modified proteins for trafficking is a strict quality control step, 2) the amount and rate of protein trafficking, 3) the destination of protein delivery is determined by the transport route, its machinery, and the protein target sequence.

COPI and COPII coated carriers are responsible for anterograde and retrograde trafficking between the endoplasmic reticulum (ER) and the subsequent compartments such as the ER-Golgi Intermediate Compartment (ERGIC) and Golgi apparatus. Although COPI-coated vesicles have been well defined,

COPII coated carriers are still the subject of hot debate. COPII carriers were originally defined as vesicles, however with the advancement of new technologies, this dogma of sole production of COPII *vesicles* is now being challenged. New microscopy techniques suggest that the original hypotheses may need to be revisited, and indicate that instead of ERES producing uniquely classic COPII coated vesicles, other forms of carriers, such as tubules and tunnels, may also originate from there.

COPII organisation is a highly dynamic event, involving the recruitment of a number of proteins to the cytosolic side of the ER. The roles of most COPII coat proteins are relatively well understood, but we are still missing the whole picture for a number of ERES component proteins. Recently, the first specific inhibitors of ERES have been described, which provides an opportunity to dissect the role of these lesser-studied ERES proteins. One of these inhibitors is Retro-2 which targets Sec16A, allowing us to study the role of Sec16A in ERES function and a potential role in cargo selection. It also allows us to study the effects of acute ERES inhibition (the effects of Retro-2 are seen on trafficking after only 30 minutes) on cellular homeostasis and the cellular processes interlinked with the early secretory pathway.

This lecture will discuss COPII in anterograde trafficking, exploring the original understanding of COPII vesicles and how new technologies challenge this. It will also discuss the novel approaches that are now being used to understand the detailed function of ERES component proteins that have so far evaded robust study, and the role of the early secretory pathway in cellular homeostasis.

Recommended reading:

Original COPII hypotheses:
https://www.nobelprize.org/prizes/medicine/2013/summary/

WE Balch et al. Reconstruction of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine. Cell 39:405-16 (1984) doi: 10.1016/0092-8674(84)90019-9.

CA Kaiser & R Schekman. Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. Cell 61(4):723-33 (1990) doi: 10.1016/0092-8674(90)90483-u.

T Söllner et al. SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318-324 (1993) doi.org/10.1038/362318a0

C Gurkan et al. The COPII cage: unifying principles of vesicle coat assembly. Nature Reviews Molecular Cell Biology 7, 727-738 (2006) doi.org/10.1038/nrm2025 (Review)

Revisited COPII hypotheses:
AV Weigel et al. ER-to-Golgi protein delivery through an interwoven, tubular network extending from ER. Cell 184(9):2412-2429e16 (2021)
doi: 10.1016/j.cell.2021.03.035.

O Shomron et al. COPII collar defines the boundary between ER and ER exit site and does not coat cargo containers. J. Cell Biol 220:6 (2021) doi.org/10.1083/jcb.201907224

J McCaughey et al. ER-to-Golgi trafficking of procollagen in the absence of large carriers. J Cell Biol 218(3):929-948 (2019) doi: 10.1083/jcb.201806035

I Raote & V Malhotra. Tunnels for protein export from the endoplasmic reticulum. Annu Rev Biochem 90:605-630 (2021) doi: 10.1146/annurev-biochem-080120-022017 (Review)

M Liu et al. Tango1 spatially organizes ER exit sites to control ER export. J. Cell Biol. 216(4):1035-1049 (2017) doi.org/10.1083/jcb.201611088

AJ Santos et al. TANGO1 recruits ERGIC membranes to the endoplasmic reticulum for procollagen export. Elife 14;4:e10982 (2015) doi: 10.7554/eLife.10982.

D Zeuschner et al. Immuno-electron tomography of ER exit sites reveals the existence of free COPII-coated transport carriers. Nat Cell Biol. 8(4):377-83 (2006) doi: 10.1038/ncb1371

COPII modulation:
A Forrester et al. Functional dissection of the retrograde Shiga toxin trafficking inhibitor Retro-2. Nat. Chem Biol. 16(3):327-336 (2020) doi: 10.1038/s41589-020-0474-4.

CM Robinson, A Duggan, A Forrester. ER exit in physiology and disease. Front. Mol. Biosci. 11:1352970 (2024) doi: 10.3389/fmolb.2024.1352970 (Review)

N Gomez-Navarro et al. Selective inhibition of protein secretion by abrogating receptor-coat interactions during ER export. Proc Natl Acad Sci U S A 119(31):e2202080119 (2022) doi: 10.1073/pnas.2202080119

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Ana J. García Sáez is a professor at the Institute for Genetics, University of Cologne, Germany where she has been a faculty member since 2019. Since October 2023 she has concurrently assumed the position of the Director of the Max Planck Institute (MPI) of Biophysics in Frankfurt am Main, Germany. Her primary areas of research include cell death, biophysics, molecular and cellular biology, biochemistry and advanced microscopy. Her projects have been centered around membrane organization and dynamics, apoptosis regulation, Bcl-2 proteins, and single molecule techniques. Prof. Dr. Garcia Saez's scientific achievements have been recognized by her selection as an EMBO Young Investigator and a Henriette Herz-Scout of the Alexander von Humboldt foundation, and by two prestigious ERC (European Research Council) Grants.

Abstract:

Biophysics of Apoptosis

Mitochondrial permeabilization is a key step in the regulation of apoptosis, which is controlled by the proteins of the BCL-2. We aim to define the composition, dynamics and structure of the apoptotic pore. To this, we have dissected the stoichiometry and interaction preferences of BCL-2 proteins complexes by exploiting the single molecule techniques. We built a 3D model for the structure in the membrane of the proapoptotic protein BAX, which mediates apoptotic pore formation, that reveals a key conformational change critical for activation. We have shown that BAX exists as a mixture of oligomeric species, and we also discovered that active BAX clusters into a broad distribution of distinct architectures, including full rings, linear and arc-shaped oligomers with both rings and arcs being able to perforate the membrane. These features are shared with other family members involved in apoptotic pore formation, yet the comparison of their dynamics of assembly revealed key differences that affect the apoptotic pore growth and downstream cell death and inflammatory signaling. Altogether, our data shed new light on the supramolecular organization of pore forming BCL-2 proteins during apoptosis and support a novel molecular mechanism in which they fully or partially delineates pores to permeabilize the mitochondrial outer membrane.

3 recent publications:

1. Cosentino K, Hertlein V, Jenner A, Dellmann T, Gojkovic M, Peña-Blanco A, Dadsena S, Wajngarten N, Danial JSH, Thevathasan JV, Mund M, Ries J & García-Sáez AJ. (2022) The interplay between BAX and BAK tunes apoptotic pore growth to control mitochondrial DNA-mediated inflammation. Mol Cell, 82(5):933-949.

2. Jenner A, Peña-Blanco A, Salvador-Gallego R, Ugarte-Uribe B, Zollo C, Ganief T, Bierlmeier J, Mund M, Lee JE, Ries J, Schwarzer D, Macek B & García-Sáez AJ. (2022) DRP1 interacts directly with BAX to induce its activation and apoptosis. EMBO J. 41(8):e108587.

3. Flores-Romero H, Hohorst L, John M, Albert MC, King LE, Beckmann L, Szabo T, Hertlein V, Luo X, Villunger A, Frenzel LP, Kashkar H & García-Sáez AJ. (2022) BCL-2-family protein tBID can act as a BAX-like effector of apoptosis. EMBO J. 41(2):e108690.

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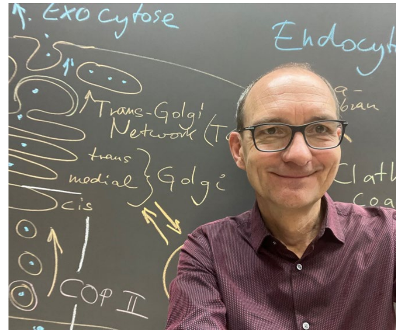


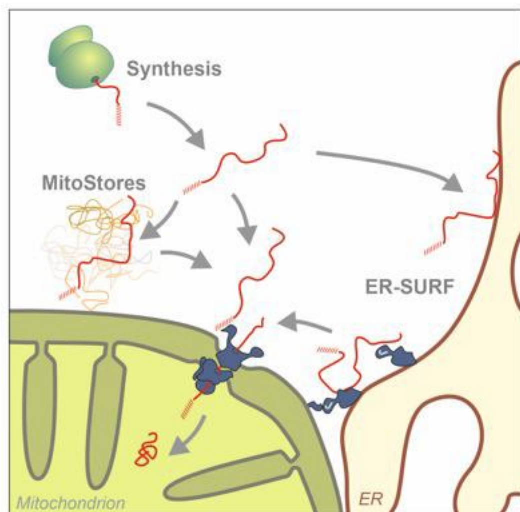
Mitochondria are genetic mosaics as their proteins origin from two distinct genetic systems. A small number of highly hydrophobic membrane proteins are encoded on the mitochondrial genome and synthesized on mitochondrial ribosomes. However, the large majority of all mitochondrial proteins are synthesized on cytosolic ribosomes and imported as precursors into mitochondria. Johannes Herrmann (Hannes) studies the mechanisms of mitochondrial protein biogenesis.
Hannes initially studied Biology and Biochemistry in Bayreuth and Tübingen before he joined the laboratory of Walter Neupert at the LMU in Munich for his PhD, which at that time was a hotspot for mitochondrial research. Subsequently, he worked as a Postdoc with Randy Schekman at the University of California in Berkeley, before he started in 1999 as a group leader in Munich. Since 2006, Hannes is a professor for Cell Biology at the University of Kaiserslautern in Germany.
The students in Hannes' lab mainly work on three different aspects. First, they study the processes by which mitochondrial ribosomes mediate the cotranslational insertion of proteins into the inner membrane of mitochondria. This insertion process is catalyzed by the Oxa1 insertase which is tethered to the mitoribosome by a C-terminal ribosome-binding domain. Second, Hannes' group elucidated the oxidative protein folding in the intermembrane space (IMS) of mitochondria. Most IMS proteins lack mitochondrial presequences but are targeted into the IMS by use of patterns of cysteine residues. These cysteines are oxidized in the IMS by the mitochondrial disulfide relay, which consists of the sulfhydryl oxidase Erv1 and the oxidoreductase Mia40, both being essential for mitochondrial biogenesis. Finally, over the last years, Hannes' lab particularly focused on the early reactions of mitochondrial protein biogenesis, thus at the steps by which proteins are targeted through the cytosol. He will provide an overview about this third topic in his presentation in Spetses.

Abstract

Distinct types of intramitochondrial protein aggregates protect mitochondria against proteotoxic stress

Mitochondria import hundreds of precursor proteins from the cytosol. How precursors are targeted from cytosolic ribosomes to the surface of mitochondria is poorly understood. The situation is particularly difficult for proteins with hydrophobic transmembrane domains which are often aggregation-prone and can be easily confused with ER-destined membrane proteins [1, 2]. Cells employ different strategies to reduce the toxicity of cytosolic precursors. On the one hand, they use the surface of the ER to absorb mitochondrial precursors thereby preventing their aggregation [3]. We recently discovered a targeting route which transports precursors from the ER to mitochondria using ER-mitochondria contact sites. Thereby, the ER mitochondria encounter structure (ERMES) and Tom70 serve as two functionally redundant transfer sites that facilitate the transit of mitochondrial proteins from the ER to the TOM complex [4, 5]. On the other hand, the cytosol induces the formation of transient aggregates which accommodate precursor proteins. These

MitoStores sequester precursor proteins during stress conditions but release them in an Hsp104-dependent manner once stress conditions subsided [6]. This regulated sequestration of precursors is an efficient mechanism to reduce the toxicity of non-imported precursor proteins [7]. The sequestration of mitochondrial proteins is not restricted to the cytosol but is also found in the mitochondrial matrix where Hsp78 serves as disaggregase that operates in an Hsp104-equivalent manner [8]. Thus, eukaryote cells employ several redundant mechanisms in order to store mitochondrial precursor proteins on their way into mitochondria.



Publications (from us and others)

1. Weidberg, H. & Amon, A. (2018) MitoCPR-A surveillance pathway that protects mitochondria in response to protein import stress, *Science.* **360**, eaan4146.

2. Wrobel, L., Topf, U., Bragoszewski, P., Wiese, S., Sztolsztener, M. E., Oeljeklaus, S., Varabyova, A., Lirski, M., Chroscicki, P., Mroczek, S., Januszewicz, E., Dziembowski, A., Koblowska, M., Warscheid, B. & Chacinska, A. (2015) Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol, *Nature.* **524**, 485-8.

3. Hansen, K. G., Aviram, N., Laborenz, J., Bibi, C., Meyer, M., Spang, A., Schuldiner, M. & Herrmann, J. M. (2018) An ER surface retrieval pathway safeguards the import of mitochondrial membrane proteins in yeast, *Science.* **361**, 1118-1122.

4. Koch, C., Räschle, M., Prescianotto-Baschong, C., Spang, A. & Herrmann, J. M. (2023) The ER-SURF pathway uses ER-mitochondria contact sites for protein targeting to mitochondria, *bioRxiv*, 2023.08.10.552816.

5. Backes, S., Bykov, Y. S., Flohr, T., Raschle, M., Zhou, J., Lenhard, S., Kramer, L., Muhlhaus, T., Bibi, C., Jann, C., Smith, J. D., Steinmetz, L. M., Rapaport, D., Storchova, Z., Schuldiner, M., Boos, F. & Herrmann, J. M. (2021) The chaperone-binding activity of the mitochondrial surface receptor Tom70 protects the cytosol against mitoprotein-induced stress, *Cell Rep.* **35**, 108936.

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Dr. Hanaa Hariri was born in Lebanon. She graduated with a BSc in Biology from the Lebanese University in 2007. She completed her MSc in Cell Biology at the American University in Beirut in 2009. She moved to the United States to complete her PhD in Molecular Biophysics at Florida State University where she studied vesicle trafficking using cryogenic electron tomography. Hanaa then moved to UT Southwestern Medical Center to work on her postdoctoral research in the laboratory of Dr. Mike Henne at the Cell Biology department. She joined the faculty of Wayne State University where she has been an assistant professor of Biological Sciences since January 2021.

Hariri serves as a reviewing editor in Scientific Reports and is a reviewer for Nature Communication, Journal of Cell Science, Journal of Lipid Research, and other journals. She is a member of the American Society for Cell Biology and the American Society of Biochemistry and Molecular Biology. She also serves as a panel reviewer for several funding agencies in the US and Europe including the National Institute of Health and the German Research Foundation. In 2023, her lab received an NIH-R35, a competitive five-year research award to study the mechanisms and functions of membrane contact sites. Hariri teaches undergraduate Introduction to Cell Biology and graduate courses in scientific communication and microscopy techniques.

Abstract:

Membrane Contact Sites: Organizers of Cellular Metabolism

Breakthrough studies over the past decade have radically challenged our classical views on the internal organization of eukaryotic cells. These studies revealed close physical connections between membranes of all cellular organelles and yielded exciting findings including the identification of inter-organelle tethering proteins, and their roles in cellular homeostasis. A growing number of human diseases such as cancer, neurodegeneration, and cardiovascular disease have been associated with defects in inter-organelle contacts. My lecture will introduce key discoveries in the field of inter-organelle contact sites and discuss their potential impact on human health. I will also discuss the current and emerging approaches for studying inter-organelle contact sites and their roles in sub-cellular organization of metabolic pathways.

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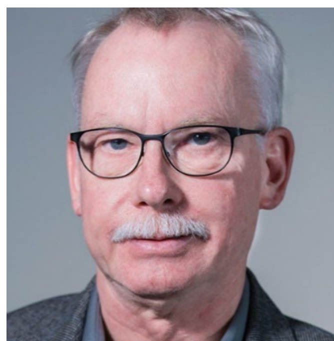

Ulrich Hartl studied Medicine at Heidelberg University. After receiving his doctoral degree in Biochemistry in 1985, he moved to the laboratory of Walter Neupert in Munich, where he worked on protein import into mitochondria, first as a post-doctoral fellow and from 1987 to 1991 as a group leader. In 1988, Ulrich began to work on molecular chaperones and demonstrated, together with Arthur Horwich, the basic role of chaperones in assisting protein folding. The period in Walter Neupert's department was interrupted by a stay in William Wickner's laboratory at UCLA (1989/1990), where Ulrich worked on the mechanism of bacterial protein export. After returning to Munich, he received his Habilitation in Biochemistry and soon after accepted an offer from Sloan-Kettering Cancer Center in New York to join the newly-founded department of James Rothman as an Associate Member. Since then he has been collaborating closely with Manajit Hayer-Hartl. Between 1991 and 1997 they worked mainly on protein folding in the bacterial and eukaryotic cytosol. They reconstituted the pathway of chaperone-assisted folding in which the Hsp70 and the GroEL chaperone systems cooperate and discovered that GroEL and its co-factor GroES provide a cage for individual protein molecules to fold unimpaired by aggregation. In 1993 Ulrich was promoted to Member with tenure, and in 1994 became an Investigator of the Howard Hughes Medical Institute. In 1997, he returned to Munich to head the Department of Cellular Biochemistry at the Max Planck Institute of Biochemistry. At MPIB Ulrich continues to investigate the mechanisms of cellular protein folding using a range of methods from cell biology, biochemistry and structural biology. In addition, he initiated research into neurodegenerative diseases caused by protein misfolding and aggregation.

Abstract:

Molecular Chaperones – Cellular Machineries of Protein Folding

Mammalian cells contain in excess of 10.000 different proteins, most of which must fold upon synthesis into well-defined three-dimensional structures in order to acquire biological activity. While the native, three-dimensional conformation of a protein is specified by the information contained in its amino acid sequence, the folding process is intrinsically error-prone. To deal with this problem, protein folding in living cells is mediated by several types of molecular chaperones that function mainly by preventing undesirable side reactions that would (if allowed) cause the accumulation of misfolded and aggregated states of a newly-made protein. Such side reactions, including aggregation, are commonly observed during the refolding of purified, denatured proteins in a test tube. The relative rate of these processes can be reduced, for a test-tube folding, by lowering the protein concentration and the temperature. However, a solution of this kind is not available inside cells, where the problem of protein aggregation is made particularly severe by the high thermodynamic activities of

folding intermediates, which have to fold while avoiding aggregation in a milieu of very high (> 300 mg/ml) total protein concentration. The latter circumstance, and the attendant effect of macromolecular crowding facilitate aggregation more than monomolecular folding. Thus, chaperone function is central to maintaining protein homeostasis and avoiding the accumulation of potentially toxic protein aggregates that can cause disease (including neurodegenerative pathologies such as Alzheimer's and Parkinson's disease). Many chaperones are known as stress or heat shock proteins (Hsp's), as their expression is induced under conditions of conformational cell stress where the tendency of both newly-synthesized and preexistent proteins to misfold is enhanced.

In my lecture I will discuss the general principles of proteins folding, focusing on the structure and function of two main classes of molecular chaperones, the Hsp70s (and their cofactors) and the cylindrical chaperonins, as well as their functional cooperation in cellular folding pathways.

The Hsp70s (~70 kDa proteins) have a central position in the chaperone network. They have ATPase and peptide binding activities in their N-terminal and C-terminal domains, respectively. They recognize short peptide sequences enriched in hydrophobic amino acid residues that are presented by unfolded or non-native polypeptides such as nascent chains on ribosomes. Peptide binding and release occurs through an ATP-dependent reaction cycle that is regulated by protein cofactors (Hsp40s or J-proteins and nucleotide exchange factors). The main role of the Hsp70 system in the folding of newly-synthesized polypeptides is to prevent (or reverse) misfolding and aggregation until either the fully synthesized chain or a domain thereof is capable of productive folding. In addition, Hsp70s participate in a number of cellular pathways, including protein translocation across membranes and protein degradation via the proteasome. Their role in these reactions is to maintain proteins in an unfolded, non-aggregated state.

In contrast to the Hsp70s, the chaperonins are large cylindrical complexes that form a cage-like structure in which single protein molecules are transiently enclosed for folding to proceed unimpaired by aggregation. These folding machines occur in all three domains of life. They can receive client proteins from Hsp70. The chaperonins are sub-divided into two distantly related groups with overall similar architecture: group I chaperonins are found in bacteria (GroEL), mitochondria (Hsp60) and chloroplasts (cpn60), and group II chaperonins in archaea (thermosome) and the eukaryotic cytosol (TRiC/CCT). E. coli GroEL, the best studied chaperonin, is composed of two stacked heptameric rings of ~60 kDa subunits that enclose a central cavity. The apical domains of the subunits expose hydrophobic amino acid residues towards the ring cavity for the binding of partially folded polypeptides exposing complementary hydrophobic surfaces. Folding initiates when the bound polypeptide is displaced into the central cavity upon binding of the co-factor GroES, a heptameric ring of ~10 kDa subunits that covers the ends of the GroEL cylinder. In the folding-active state the wall of the GroEL cavity is hydrophilic and the folding chamber is large enough for proteins up to ~60 kDa. Recent observations indicate that the chaperonin cage enhances the compaction of encapsulated proteins by an effect of steric confinement, thereby accelerating the overall folding reaction for some proteins.

Recommended reading:

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After teacher's training in Singapore, Manajit Hayer-Hartl graduated with BSc. Honors and a PhD in Chemistry (1984) from the University of Stirling (Scotland). She did post-doctoral training at several Institutions around the world and in 1991 joined Sloan Kettering Institute (New York) as a Research Fellow. In 1997 she moved to Max Planck Institute of Biochemistry (Munich, Germany), where she heads the research group "Chaperonin-assisted protein folding".

Abstract:

Into the Heart of Photosynthesis: Unraveling Rubisco's Cellular Machineries

Photosynthesis is a fundamental process in biology as it converts solar energy into chemical energy and thus, directly or indirectly, fuels nearly all life on earth. The chemical energy generated during the light reaction of photosynthesis is used to fix atmospheric CO2 and produce reduced carbon compounds in the Calvin-Benson-Bassham (CBB) cycle, the second step of the process. The key enzyme responsible for carbon fixation in all photosynthetic organisms is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). In spite of its central importance in photosynthesis, Rubisco is an inefficient enzyme, catalyzing two opposing reactions: photosynthetic carbon fixation (CO2 as the substrate) and photorespiration (O2 as the substrate). Photorespiration results in a decreased efficiency of carbon fixation. Additional complexity comes from the fact that the multistep catalytic reaction is prone to occasional errors, producing so-called 'misfire' products which can remain bound to inhibit catalysis.

Since Rubisco has important implications in improving crop yield and in controlling greenhouse gas induced climate change, understanding its biogenesis and metabolic maintenance, as well as the evolution of micro-compartments for functional efficiency is important in directed evolution of Rubisco to generate unique variants that have a greater specificity for carbon dioxide and a more efficient catalytic rate.

In my lecture I will first introduce the role of Rubisco in oxygenic photosynthesis, discuss its shortcomings as an enzyme and describe the structure of the most abundant form of Rubisco, form 1. Then I will describe our key findings on two aspects of Rubisco's chaperone requirement:

1) The extensive chaperone requirement for folding and assembly
2) The evolution of different mechanisms of the AAA+ chaperone, Rubisco activase, to repair inhibited Rubisco.

To end the lecture, I will describe our more recent findings on the formation of a proteinaceous compartment in cyanobacteria called carboxysome, which is critical for the functional efficiency of Rubisco.

Recommended Reading:

Reviews:

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4. Flecken, M., Wang, H., Popilka, L., Hartl, F.U., Bracher, A. and Hayer-Hartl, M. (2020). Dual role of a Rubisco activase in metabolic repair and carboxysome organization. Cell 183(2), 457-473. (doi: 10.1016/j.cell.2020.09.010).

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Other:

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I am a junior group leader at the Heidelberg university biochemistry center focusing on cross-organelle transport of sphingolipids. I trained as a chemist, but soon figured out that my interests lie in studying the complexities of cell biology. Therefore, I did my PhD at the European Molecular Biology Laboratory in the group of Carsten Schultz, where I learned how to make and use chemically-modified sphingolipid probes to study their impact on cellular processes. After finishing the PhD in 2015, I moved to the group of Prof. Fran Platt at the University of Oxford to study the contribution of organelle contact sites on sterol homeostasis mediated by the Niemann-Pick disease type C1 (NPC1) protein. In 2017, I was recruited to the Biochemistry Center of Heidelberg University where our group designed and applied lysosomally-targeted lipid probes to study transport of sphingosine and found that sterol transporters such as NPC1 but also StAR-related lipid transfer domain containing protein 3 (STARD3) act as sphingosine transporters at lysosome-ER contact sites.

Abstract

Lipid trafficking/recycling through the lysosomal system

Lysosomes are key players not only in degradation and recycling of nutrients, but also in orchestrating a variety of cellular programs depending on the nutrient status of the cell. Not surprisingly, lysosomal dysfunction therefore manifests in large variety of human pathologies, surprisingly many of which show lipid accumulation phenotypes. In this lecture, we will review lipid classes that rely on lysosomes for their turnover, recent advances in identifying lipid metabolizing enzymes, transporters and sensors as well as their impact on physiology. Particular focus will be given to current methods of studying lipids directly and our own attempts in following sphingosine trafficking from lysosomes to the ER. We use multifunctional lipid probes for detection of lipid-protein interaction, lipid visualization, metabolic lipid tracing as well as in vitro reconstitution of lipid transport and have identified unexpected commonalities in sterol and sphingolipid trafficking processes.

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Altuzar J, Notbohm J, Stein F, Haberkant P, Hempelmann P, Heybrock S, Worsch J, Saftig P, Höglinger D (2023) Lysosome-targeted multifunctional lipid probes reveal the sterol tranporter NPC1 as a sphingosine interactor. Proc Natl Acad Sci U S A 120(11):e22138886120

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I received my Ph.D. in Biophysics from the Instituto Venezolano de Investigaciones Cientificas and my post-doctoral training at Harvard where I am now Professor of Cell Biology and Pediatrics, and Senior Investigator at Boston Children's Hospital. My passions include science, nice people, windsurfing and dancing.

Seeing is believing; visual observations are crucial in scientific research, serving both as a primary method of investigation and as a means of engaging the public with science, helping them appreciate its significance.

Our research leverages on the use of emerging technologies – evolving from the early days of molecular cloning to contemporary high-resolution structural visualization and live-cell imaging. Initially we employed x-ray crystallography, cryo-electron microscopy (cryo-EM), and single-molecule biophysics to create a "molecular movie" of clathrin-mediated endocytosis, and in this way related these molecular events to functional properties of the surfaces of living cells. Over three decades, our studies helped define the structure, interactions, and assembly-disassembly mechanisms of clathrin and many of its associated proteins. We achieved the first X-ray structure determination at atomic resolution of large portions of clathrin and AP-1, key components of the clathrin coat. We used cryo-EM to obtain the first visualization of a complete clathrin coat at 8 Å resolution revealing the basic structure of the triskelion leg and established the way triskelions pack when they form the clathrin coat. Further cryo-EM studies showed how auxilin and Hsc70 facilitate ATP-dependent uncoating by binding to the clathrin coat, and we explored the interactions of ß-arrestins and adaptors with clathrin, as well as their links to non-canonical Wnt signaling.

In vivo molecular imaging is a central aim of modern microscopy. Our lab uses Lattice Light Sheet Microscopy (LLSM) and its variant enhanced with Adaptive Optics (AO-LLSM) to bridge molecular and cellular understanding in various biological contexts. These technologies generate vast datasets that challenge us to develop accurate quantitative models of cellular dynamics and underlying biological processes.

We integrate this type of dynamic cellular studies with artificial intelligence and deep learning to fuse high-resolution molecular snapshots with live-cell activities. This approach enables us to create new 'molecular movies' to help elucidate processes such as membrane traffic, organelle biogenesis, ER remodeling and nuclear pore formation during cell division, generation of intraluminal vesicles in endosomes, cell-cell recognition, and the dynamics of virus-host interactions during infection.

My presentation will focus on cytosolic delivery by endosomes gone wrong exemplified by our recent discovery of unique perforations in the endolysosomal membranes of neurons. These perforations facilitate a pathway for toxic a-synuclein aggregation by allowing cytosolic a-synuclein to access internalized a-synuclein pre-formed fibrils. Absent in non-neuronal cells, this neuron-specific mechanism highlights a distinct pathway in synucleinopathies, including Parkinson's disease.

Representative papers:

Molecular architecture of clathrin coats, mechanism of assembly and uncoating

1. Fotin, A. et al. Molecular model for a complete clathrin lattice from electron cryomicroscopy. Nature 432, 573–579 (2004).
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3. Böcking, T., Aguet, F., Harrison, S. C. & Kirchhausen, T. Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. Nat Struct Mol Biol. 18, 295–301 (2011). PMCID:
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6. Kural, C. et al. Dynamics of intracellular clathrin/AP1- and clathrin/AP3-containing carriers. Cell Rep 2, 1111–1119 (2012). PMC4472015.
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11. He, K. *et al.* Dynamics of Auxilin 1 and GAK in clathrin-mediated traffic. J Cell Biol (2020) 219 (3): e201908142. PMCID: PMC7054993.

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12. Lu, L., Ladinsky, M. S. & Kirchhausen, T. Formation of the postmitotic nuclear envelope from extended ER cisternae precede nuclear pore assembly. J Cell Biol 194, 425–440 (2011).
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15. Adell, M. A. Y. et al. Recruitment dynamics of ESCRT-III and Vps4 to endosomes and implications for reverse membrane budding. Elife 6, e31652 (2017). PMC5665648.
16. Sanyal, A. *et al.* Constitutive Endolysosomal Perforation in Neurons allows Induction of alpha-Synuclein Aggregation by Internalized Pre-Formed Fibrils. (2024) doi:10.1101/2023.12.30.573738.

Virus-cell host interactions:

17. Kang, Y.-L. et al. Inhibition of PIKfyve kinase prevents infection by Zaire ebolavirus and SARS-CoV-2. PNAS 15, 202007837 (2020). PMC7263545.
18. Luo, S. et al. An Antibody from Single Human VH-rearranging Mouse Neutralizes All SARS-CoV-2 Variants Through BA.5 by Inhibiting Membrane Fusion. Sci Immunol eadd5446 (2022) doi:10.1126/sciimmunol.add5446. PMCID: PMC9407951.
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20. Liu, T.-L. et al. Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. Science 360, eaaq1392 (2018). PMC6040645.
21. Hoffman, D. P. et al. Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. Science 367, eaaz5357 (2020). PMC7339343.
22. Gao, R. et al. Cortical column and whole-brain imaging with molecular contrast and nanoscale resolution. Science 363, eaau8302 (2019). PMC6481610.
23. Gallusser, B. *et al.* Deep neural network automated segmentation of cellular structures in volume electron microscopy. *J Cell Biol* 222, e202208005 (2022). PMCID: PMC9728137.
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Abstract

Cytosolic delivery by endosomes gone wrong

This talk will highlight the exchange of soluble proteins observed between the lumen of endo-lysosomes and the cytosol. I will exemplify how this unexpected delivery process is mediated by endosomes gone wrong in neurons with our recent discovery of unique nanoscale perforations in the limiting membrane of a subset (<5%) of neuronal endolysosomes. In contrast, the limiting membranes of endosomes and lysosomes in non-neuronal cells are all completely intact.

We have used several complementary approaches to gather evidence for constitutive endolysosomal perforations in neurons, both in brain and human iPSC-derived neurons (iNs) induced in the laboratory. We have visualized damaged structures directly by volumetric FIB-SEM and indirectly by single time point spinning disc or 3D live lattice light sheet fluorescence microscopy using probes for endolysosomal pH (the lumen of endolysosomes will approach neutrality in the case of a perforated limiting membrane).

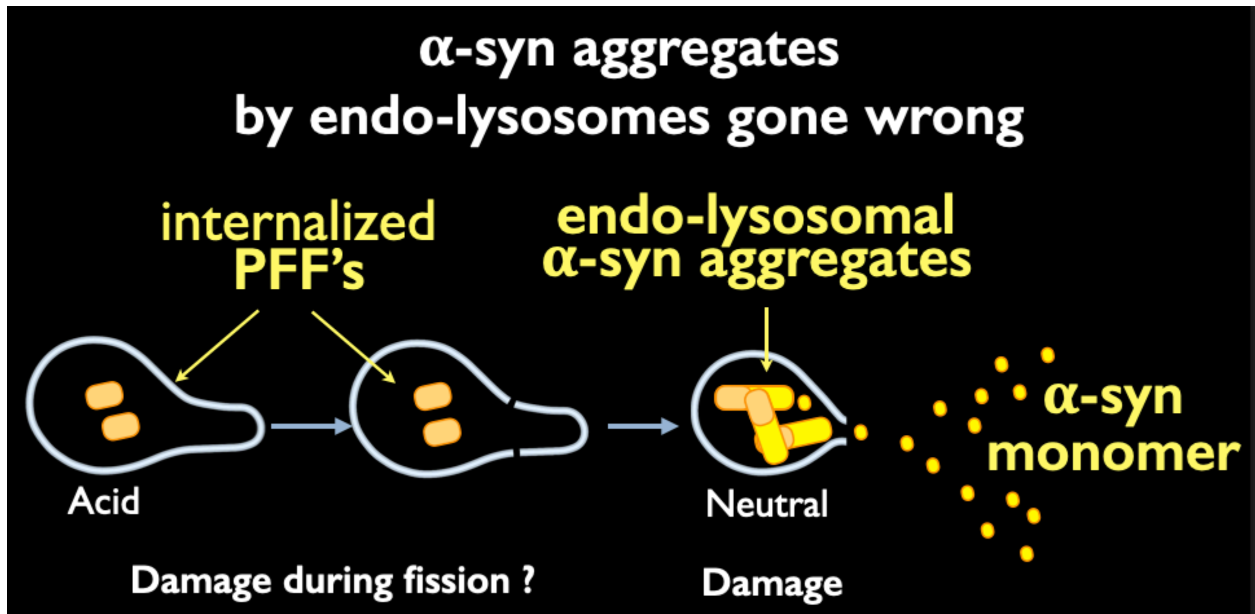
We have also detected damage by probing the recruitment of cytosolic, soluble mCherry-galectin-3, eGFP-galectin-3 or mCherry-galectin-8 to damaged endo-lysosomes. We found galectin fluorescent spots in iNs but very rarely in non-neuronal cells such as iPSCs and SVG-A cells.

Why do neurons have a subset of what appear to be constitutively leaky endolysosomes? One possibility is that their luminal content is viscous and while escaping to the cytosol it behaves as a local physical barrier to prevent the reseal of the damaged membrane. A second possibility is simply inefficiency in the membrane damage repair mechanism, which could also provide a functional signal to accelerate autophagic recycling in these cells, which cannot dilute toxic content by lack of cell division.

Although delineating the complexities of this phenomenon remain to be determined, we suggest that the constitutively perforated endolysosomal membranes detected in neurons could facilitate cytosolic access of endocytosed neurotoxic aggregates, including -- in addition to ɑ-synuclein -- Huntingtin, Aβ, and tau.

Indeed, we found that these perforations facilitate a pathway for toxic a-synuclein aggregation by allowing cytosolic a-synuclein to access internalized a-synuclein pre-formed fibrils (PFFs). Absent in non-neuronal cells, this neuron-specific mechanism highlights a distinct pathway in synucleinopathies, including Parkinson's disease.

I will end the talk by showing that inhibition of the endosomal PIKfyve phosphoinositide kinase by the small molecule Apilimod or Vacuolin-1 prevented PFF-induced endolysosomal ɑ-syn aggregation, even though the PFFs had reached these subcellular compartments. By preventing endolysosomal damage, these compounds also prevented PFF induced toxicity in iNs. Hence, preventing toxicity by inhibiting PIKfyve kinase suggests a potential avenue for therapeutic intervention.



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Ulrike Kutay studied Biochemistry in Berlin. Following her PhD on the integration of tail-anchored proteins into the ER membrane (Rapoport lab; Max-Delbrück Center (Berlin); Harvard Medical School (Boston), from 1992-1996), Ulrike investigated the mechanisms of nucleocytoplasmic transport of proteins and RNAs (Görlich lab; University of Heidelberg, 1996-1999). In 1999, Ulrike became Assistant Professor at the ETH in Zurich, where her lab initially studied various nuclear transport pathways and the biogenesis of nuclear pore complexes. Ulrike was promoted to tenure in 2006. Since then, science in the Kutay lab has mainly been focused on the structure, function and dynamics of the nuclear envelope and ribosome synthesis in mammalian cells. Work of her team helped to unravel the mechanisms governing nuclear envelope breakdown for open mitosis, membrane protein targeting to inner nuclear membrane, the biogenesis and organization of NE-spanning LINC complexes as well as the mechanisms driving the assembly of ribosomal subunits. Current work of the Kutay lab is focused on nuclear organization and proteostasis, exploiting a wide repertoire of approaches, ranging from biochemical reconstitution of cellular processes, over multi-omics approached, high-end microscopy to imaging-based screening methods.

Representative Publications:

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Abstract

Structure, Function and Dynamics of the Nuclear Envelope

The nuclear envelope (NE) is a functionally specialized domain of the endoplasmic reticulum. The architecture of the NE is ideally adapted to its function as a compartment boundary and protective shell of the genome. Its importance for organismal homeostasis is reflected by a suite of genetic disorders termed '*nuclear envelopathies'* that are caused by mutations in genes encoding for NE proteins, including severe diseases such as muscular dystrophies or premature aging.

The NE consists of two juxtaposed lipid bilayers, the inner and outer nuclear membranes (INM, ONM). To enable nucleo-cytoplasmic communication, INM and ONM are connected at numerous places forming membrane pores that contain nuclear pore complexes (NPCs). NPCs constitute the major sites for nucleo-cytoplasmic exchange and define the barrier and permeability properties of the nuclear boundary. Linker of nucleo- and cytoskeleton (LINC) complexes serve as another element of nucleo-cytoplasmic communication. They form bridges across the NE and connect the nucleus to the cytoskeleton, serving as force-coupling devices for nuclear migration and anchorage, meiotic chromosome movements, and centrosome positioning.

While the NE appears as a rather static boundary in interphase cells, it can be rapidly broken at the onset of open mitosis to allow for formation of the mitotic spindle. NE breakdown (NEBD) is a major event during the drastic intracellular reorganization that prepares cells for division. In as little as 10 minutes, all elements of the NE are dispersed. The disassembly of NPCs is a key step initiating NEBD. It leads to mixing of nuclear and cytoplasmic components and sets the stage for spindle assembly. Further, interactions of membrane proteins to the condensing chromatin and the lamina are broken, enabling retraction of NE membrane components into the ER. Disassembly of the nucleus directly exploits the activity of protein kinases involved in mitotic entry and is supported by microtubule-dependent NE restructuring. Defects in NEBD can lead to chromosome segregation errors, binucleation and genome instability.

ONM and INM possess a strikingly distinct protein composition, generating an asymmetry in the NE that reflects the functional specialization of both membranes. Proteins of the INM play important roles in regulating chromatin organization and gene expression. They are tightly associated with chromatin and the nuclear lamina - an intermediate filament network associated with the nuclear face of the NE. Transcriptionally repressed heterochromatin is organized in so-called lamina-associated domains (LADs) underneath the NE. Importantly, heterochromatin tethering to the nuclear lamina has been suggested to play decisive roles in genome stability and regulation.

In my lecture, I will first give an overview of nuclear envelope organization, function, and biogenesis, to then describe how it is remodeled for open mitosis, and finally present some snapshots of our recent work on heterochromatin tethering to the nuclear envelope by inner nuclear membrane proteins.

Recommended Reading:

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Dr. Hsou-min Li was born in Taichung, Taiwan. She graduated with a BSc from the Botany Department of National Taiwan University, was awarded a PhD in Cell and Molecular Biology from the University of Wisconsin-Madison, and underwent postdoctoral training at the Salk Institute in California. She returned to Taiwan in 1995 to join the Institute of Molecular Biology of Academia Sinica, where she is currently a Distinguished Research Fellow and Deputy Director. Her research focuses on investigating the mechanism of protein transport into chloroplasts and the functions of galactolipids in chloroplast membranes.

Abstract

Protein Translocation into chloroplasts

Chloroplasts evolved from a cyanobacterial ancestor engulfed by a eukaryotic host. During the endosymbiotic evolutionary process, genes have been transferred from the chloroplast genome to the host nuclear genome. New proteins have been added to the chloroplast to facilitate its biogenesis as an organelle strictly dependent on the host cell. In Arabidopsis, approximately 3000 nucleus-encoded proteins are predicted to be targeted to chloroplasts, whereas the Arabidopsis chloroplast genome contains only 88 protein-coding genes. Most of the nucleus-encoded chloroplast proteins are synthesized in the cytosol as precursors with an N-terminal targeting signal called the transit peptide, which functions as an “entry ticket” into chloroplasts. Machinery on the chloroplast envelope, the TOC and TIC translocon, recognizes this transit peptide and translocates the precursor proteins across or into the double-membrane envelope. Another unique and fascinating feature of chloroplasts is that they develop into different functional types of differing colors depending on the tissues in which they reside.
In my lecture, I will discuss our current understanding of how proteins are transported into chloroplasts, including the evolution, composition and structure of the translocon. I will also discuss the multiple ways the transport process could be regulated.

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Dr. Sonya Neal received her Ph.D. from U.C. Los Angeles in 2013 in the laboratory of Dr. Carla Koehler where she studied the redox-regulated mitochondrial import pathway using *in vitro* reconstitution approaches. As a graduate student, she was the recipient of the Cota Robles Fellowship and Ruth L. Kirschstein NRSA Predoctoral Fellowship. She then carried out her postdoctoral studies at U.C. San Diego in the laboratory of Dr. Randolph Hampton where she studied the mechanistic actions of ER-associated degradation (ERAD) pathways and more specifically, how ER-resident misfolded membrane substrates are removed (retrotranslocated) from the Endoplasmic Reticulum (ER) and degraded by the cytosolic proteasome. As a postdoc, she received the Burroughs Wellcome Postdoctoral Diversity Enrichment Award and Ruth L. Kirschstein NRSA Postdoctoral Fellowship. Dr. Neal joined the faculty in 2018 in the Department of Cell and Developmental Biology at University of California San Diego where her lab studies the role of the rhomboid superfamily in mediating ERAD retrotranslocation and lipid metabolism. She is the recipient of the NIH MIRA, NSF CAREER, Pew, Chan Zuckerberg Initiative in Science Diversity Leadership Award, and HHMI Freeman Hrabowski Scholar Award. She is also a champion for diversity; she co-founded the Biology Undergraduate & Master's Mentorship (BUMMP) program, serving over 1,300 URM and first-generation students to date. Moreover, she teaches a new DEI graduate course, "Social Issues in Biology." For these efforts, she received the campus-wide Inclusive Excellence and the Faculty Mentorship awards along with the American Society for Cell Biology Junior Award for Excellence in Research.

Abstract:

Mapping the ERAD Highway: An Integrated Picture of All Retrotranslocation Routes

ER Protein Quality Control
The Endoplasmic Reticulum (ER) contains an elaborate protein quality control network that guarantees high-fidelity protein synthesis and folding. In addition, terminally misfolded proteins that do not reach their native conformation are eventually eliminated to minimize proteotoxicity. These terminally misfolded proteins are retained in the ER and targeted for degradation by the proteasome through ER-associated degradation (ERAD). ERAD is the principal degradation pathway in the ER and well conserved from yeast to mammals. ERAD starts with recognition of ER-localized substrates, ubiquitination by multi-spanning membrane E3 ligases, and delivery to proteasome for degradation.

ERAD Retrotranslocation
A fascinating aspect of ERAD is the spatial separation of its key components: while the ER houses the E3 ligase and misfolded substrates, the cytosol accommodates other ubiquitination machineries, adaptors, and the proteasome. This spatial dichotomy leads to one of the most interesting, and still perplexing, aspects of ERAD, the requirement for moving ERAD substrates from their initial locations in the ER lumen or membrane, to their final destination in the cytosol where they are degraded. This energy-dependent movement of substrates from the ER to the cytosol, a process known as

retrotranslocation, has been a three-decade endeavor within the field for identifying the channels or routes responsible for mediating substrate removal.

In this lecture, we will thoroughly explore a range of complementary in vivo and in vitro studies that have provided crucial mechanistic insights into retrotranslocation routes, enabling us to move towards an integrated picture of ERAD.

Background reading:

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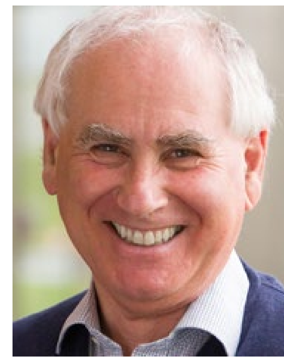

Tom Rapoport earned his Ph.D. from Humboldt University (Berlin) in 1972. He then worked at the Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften der DDR in Berlin, which later became the Max-Delbrück-Institute. In 1995, he joined the faculty of Harvard Medical School as Professor of Cell Biology and group leader. In 1997, Dr. Rapoport was appointed as a Howard Hughes Medical Institute Investigator. Rapoport is a member of the National Academies of the USA and Germany. He has received a number of awards.

Rapoport has worked in several different research areas. He did his graduate work on the mechanism of the enzyme "inorganic pyrophosphatase". He then worked on mathematical modeling of metabolism, developing Metabolic Control Analysis (MCA), a theory that describes the regulation of metabolic networks (together with Reinhart Heinrich). At the same time, his lab began to investigate the mechanism of protein translocation across the endoplasmic reticulum (ER) membrane and the bacterial plasma membrane. Major achievements include the reconstitution of protein translocation with purified components, the identification of the Sec61 complex as the protein-conducting channel, the determination of the first structure of a protein-conducting channel (together with Steve Harrison), and the clarification of the molecular mechanism of different translocation modes. Another area of research concerns ER-associated protein degradation (ERAD), the process by which misfolded ER proteins are retro-translocated into the cytosol and discarded. Major discoveries include the identification of the essential role of the p97 (Cdc48) ATPase, the reconstitution of a basic ERAD process with purified components, the elucidation of the mechanism of the Cdc48 ATPase, and the structure of the retro-translocon. The Rapoport lab also addresses the fundamental problem of how the characteristic shape of an organelle is generated, a field they developed. His lab has identified proteins that form ER tubules, discovered fusion GTPases that connect the tubules into a network, and has reconstituted ER network formation with purified proteins. More recently, his group has worked on the mechanism by which proteins are imported into peroxisomes. His lab showed that proteins are imported together with a soluble receptor by a mechanism that resembles nuclear transport, and that the receptor returns to the cytosol through a separate retro-translocon, formed by a hetero-trimeric ubiquitin ligase complex. Studies from the Rapoport lab have thus identified several different mechanisms by which proteins cross membranes.

Abstract

Mechanism of protein import into peroxisomes

Peroxisomes are ubiquitous organelles whose dysfunction causes fatal human diseases. Most peroxisomal enzymes are imported in a folded state from the cytosol by the receptor PEX5. Recent work demonstrated that PEX5 shuttles cargo completely into the peroxisomal lumen and then returns to the cytosol. The analysis led to the following model for the import cycle. PEX5 enters peroxisomes by a process resembling nuclear transport. A meshwork is formed inside the membrane by a conserved tyrosine/glycine-rich YG domain of PEX13, and resembles the meshwork of

nucleoporin FG domains inside nuclear pores. PEX5 selectively partitions into this phase, using conserved aromatic motifs, and brings bound cargo along. Directionality of import is determined by an interaction of PEX5 with a lumenal PEX14 domain. PEX5 returns to the cytosol through a retro-translocon formed by a ubiquitin ligase complex, consisting of PEX2, 10, and 12. The ligase complex has an open pore, into which the import receptors insert a flexible N-terminal segment from the lumenal side. Following mono-ubiquitination, PEX5 is pulled out of peroxisomes by the PEX1/6 ATPase. During retro-translocation, PEX5 is unfolded, which results in cargo release inside the organelle. After folding and deubiquitination, PEX5 can start a new import cycle.

References

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Towards solving the mystery of peroxisomal matrix protein import.
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Protein import into peroxisomes occurs through a nuclear pore-like phase.
Science 378:eadf3971. doi: 10.1126/science.adf3971. PMID: 36520918

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Prof. Maya Schuldiner was born in Israel. She graduated magna cum laude with a BSc in Biology from the Hebrew University in Jerusalem in 1998. She went on to complete both her MSc and a PhD in genetics, also at the Hebrew University, in 1999 and 2003. Maya conducted postdoctoral research in the Laboratory of Jonathan Weissman at the University of California in San Francisco from 2003 until 2008, when she joined the faculty of the Weizmann Institute of Science, Israel. She has been a tenured associate professor since 2015 at the department of Molecular Genetics at the Weizmann Institute of Science and a Full Professor since 2020.

Schuldiner serves as a reviewing editor in eLife and is a member of the editorial board of Life Science Alliance, Current Opinion in Cell Biology, BBA-Molecular Cell Research, PLoS Biology and Science Open. Schuldiner received a Human Frontiers Science Program Career Development Award in 2008 and became a member of the EMBO Young Investigator Programme in 2011 and of EMBO in 2017. She received three consecutive European Research Council grants (StG in 2010, CoG in 2015 and in 2020). Schuldiner is also the recipient of the FEBS Anniversary and National prizes (2015, 2017), the EMBO Gold Medal award (2017), the Jean Vance prize for breakthroughs in Contact Site research (2018), the Ira Herskowitz Award for leading yeast geneticist (2022), the FEBS Bucher medal (2023). She was elected as a TUM ambassador and as a member of Leopoldina, the German National Academy of Sciences in 2020. Schuldiner currently holds the Dr. Omenn and Martha Darling Professorial Chair in Molecular Genetics and serves as the Chair of the Weizmann Institute of Science "Scientific Council".

Schuldiner's research focuses on uncovering functions for uncharacterized proteins using the bakers yeast as a central eukaryotic model. She does this by using high content screening approaches coupled with dedicated follow-ups and with an interest on processes that occur inside organelles.

Abstract:

Systematic approaches to studying organelles

While targeting and translocation of proteins synthesized in the cytosol to any organelle is complex, mitochondria present the most challenging of destinations. First, import of nuclear-encoded proteins needs to be balanced with production of mitochondrial-encoded ones. Moreover, as mitochondria are divided into distinct sub-domains, their proteins harbor a number of different targeting signals and biophysical properties. I will discuss the process of translocation into the mitochondrial membranes that has been well studied as well as what is known about the cytosolic steps of protein import and also what is not….. We will also touch upon current understanding of the cellular programs that respond to accumulation of mitochondrial precursor proteins in the cytosol.

Recommended publications:

https://pubmed.ncbi.nlm.nih.gov/36164978/
https://pubmed.ncbi.nlm.nih.gov/35714584/

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Bianca Schrul studied Biology at Heidelberg University, Germany. During her Diploma thesis work in the lab of Irmgard Sinning (BZH, Heidelberg University, Germany), she analyzed the membrane interaction of the bacterial signal recognition particle (SRP)-receptor FtsY and its relation to the FtsY GTPase activity. In 2007, she joined the lab of Bernhard Dobberstein (ZMBH, Heidelberg University, Germany) as a PhD student, and functionally characterized the human intramembrane-cleaving protease signal peptide peptidase (SPP), which cleaves a subset of signal peptides after they have been released from their parent pre-proteins during endoplasmic reticulum (ER) translocation. In 2010, she joined Blanche Schwappach´s lab (Göttingen University and Max-Planck Institute for Biophysical Chemistry, Germany) for a short first post-doctoral training during which she obtained in-depth training in various electron-microscopy techniques. Her main focus was on the spatio-temporal localization of GET-pathway proteins, which mediate the post-translational insertion of tail-anchored membrane proteins into the ER. During her postdoc time in Ron Kopito´s lab at Stanford University (USA) from 2012 to 2017, she became interested in the biogenesis and function of lipid droplets (LDs) and discovered a novel ER targeting pathway for LD-destined membrane proteins. This laid the foundation for her own research lab, which she established in 2017 as a Junior-Professor at Saarland University, Germany. In 2023, she became Professor for Medical Biochemistry at Saarland University. In her lab, she employs multi-disciplinary approaches to address three major questions: 1. What are the molecular mechanisms underlying protein targeting and insertion into the limiting membrane of LDs? 2. How is LD biogenesis from the ER membrane regulated? 3. How do LDs communicate with other lipid metabolizing organelles to adapt to metabolic changes?

Abstract:

Protein targeting to lipid droplets & the importance of inter-organelle communication

Cells constantly have to adapt to environmental changes such as nutrient availability and, therefore, have to orchestrate multiple metabolic pathways, which are catalyzed in distinct organelles. Lipid droplets (LDs) are ubiquitous cytoplasmic organelles with a key role in cellular physiology as they dynamically balance storage and consumption of the majority of metabolic energy in the form of neutral lipids. Their fundamental importance in lipid metabolism is reflected by their implication in numerous pathologies including hallmark metabolic diseases of modern times, such as diabetes, obesity, atherosclerosis and neurodegenerative disorders. LDs, however, remain poorly understood organelles, as the molecular mechanisms of their biogenesis, function and turnover are incomplete. This knowledge gap stems from the enduring dogma of LDs being inert fat storage compartments; a viewpoint that was only recently challenged by the development of fluorescent dyes that allow visualization of LD dynamics and by the discovery that key metabolic enzymes are located on LDs.

LDs originate from the endoplasmic reticulum (ER) where neutral lipids are synthesized and, upon lipolysis, supply fatty acids for beta-oxidation in mitochondria and peroxisomes and are, therefore, central hubs in lipid metabolism.

LDs create a unique physicochemical environment in the cell as their hydrophobic neutral lipid core is segregated from the aqueous cytosol by a phospholipid monolayer. In contrast, all other known organelles are surrounded by phospholipid bilayers that separate two aqueous milieus. The dynamic metabolic function of LDs relies on specific proteins that integrate into the limiting monolayer membrane in a unique monotopic hairpin-type topology. In contrast to the well-characterized bilayer- spanning transmembrane domains, the biophysical parameters governing the membrane insertion of monotopic hairpin proteins are unknown. Interestingly, many hairpin proteins dynamically partition between the ER bilayer and the LD monolayer membrane. It has been proposed that the unique monotopic topology of hairpin proteins is necessary and sufficient for ER-to-LD partitioning, potentially by their lateral diffusion during LD emergence at the ER; yet, the collective processes enabling hairpin proteins to reside in these distinct membrane environments as well as the partitioning between them remain unknown.

In this lecture, I will discuss how we discovered a novel biogenesis pathway for a subset of membrane proteins that partition from the ER to LDs. Surprisingly, these proteins are first inserted into discrete subdomains of the ER by a machinery hitherto exclusively implicated in peroxisome biogenesis. This novel concept of inter-organelle communication raises the question why two distinct organelles use shared protein biogenesis machinery. Since LDs and peroxisomes cooperate in lipid metabolism - LDs provide fatty acids, which are metabolized in peroxisomes and peroxisomes synthesize ether lipids, which are stored in LDs - their mutual biogenesis regulation likely contributes to the adaptation to metabolic changes. In addition, I will present recent data from our lab that indicate that hairpin proteins can adopt distinct conformations in ER bilayer and LD monolayer membranes and that they need to undergo unexpected major structural rearrangements during ER to LD partitioning. We will discuss how this may provide mechanistic explanations for regulated protein recruitment to LDs to control lipid metabolism.

Recommended Reading:

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Rebecca performed her graduate work in Venki Ramakrishnan's lab at the Medical Research Council Laboratory of Molecular Biology (LMB), where she studied the molecular mechanism of protein synthesis using X-ray crystallography. For her postdoctoral studies, Rebecca joined Manu Hegde's lab, just down the hall in the LMB's Cell Biology division. In Manu's lab Rebecca used single particle cryoelectron microscopy (cryo-EM) to study how secreted and integral membrane proteins are specifically targeted to the endoplasmic reticulum. Rebecca moved to Caltech in 2017, where she joined the Division of Biology and Biological Engineering. Her lab studies the molecular basis for protein biogenesis and quality control using a variety of techniques spanning from genetics to structural biology.

Abstract:

Targeting and translocation to the ER

Secreted and integral membrane proteins compose ~30% of the eukaryotic proteome, and are essential for a range of cellular functions including intracellular trafficking, cell signaling, and the transport of molecules across the lipid bilayer. Defects in membrane protein maturation underlie numerous protein misfolding diseases, and more than half of all therapeutic drugs bind a membrane protein target. The critical roles of these proteins, as well as the consequences of their failed maturation, underscore the importance of understanding the molecular details of membrane protein biogenesis. The definitive steps for biogenesis of all secreted proteins, and the majority of integral membrane proteins, occur in the endoplasmic reticulum (ER). In this lecture we will focus on the molecular mechanisms that underlie the two major routes used by nascent proteins to reach the ER membrane: the co- and post-translational pathways for protein targeting and translocation.

Co-translational protein targeting
The first identified and most extensively studied is the co-translational pathway, which is responsible for the targeting and translocation of many secreted and integral membrane proteins into the ER. Targeting begins with the identification of a hydrophobic element as it emerges from a translating ribosome. This element, either an N-terminal signal sequence or a transmembrane domain (TM), is recognized by the signal recognition particle (SRP). Upon recognition, the ribosome-nascent chain complex is delivered to the ER through an interaction with the SRP receptor and subsequently transferred to the universally conserved Sec61translocon in a GTP hydrolysis-dependent step. The heterotrimeric Sec61 complex (composedof α, β, and γ subunits) forms the translocation channel and provides access to both the ER lumen through an aqueous pore and the hydrophobic bilayer via a lateral gate.
High-resolution crystal structures of the archaeal and bacterial homologs of the Sec61 complex have provided a putative mechanistic model for lateral release of TMs into the lipid bilayer. A key feature

of the Sec61 pore is a short helix on the luminal side that acts as a plug. Cryo-EM structures of the mammalian Sec61 have suggested that the initial signal sequence interaction with Sec61 encourages plug dislodging and primes lateral gate opening by displacing helices 2 and 7 of the α subunit. Once the channel is in an open state, it can house the nascent chain in its predominately hydrophilic channel while providing access to the lipid bilayer for hydrophobic TM stretches. Structural evidence suggests that both SRP and Sec61 use a qualitatively similar mechanism for selectively recognizing substrates to the ER membrane, ensuring that nascent proteins are accurately sorted into the correct cellular compartment.

Post-translational protein targeting

Despite the widespread role of the co-translational targeting pathway, many membrane proteins require alternative strategies for reaching the ER. One important class of such proteins are tail-anchored (TA) proteins, which have a single TM immediately preceding their stop codon and must therefore be targeted and inserted post-translationally. Despite only accounting for roughly 3-5% of the eukaryotic proteome, TA proteins are essential for a variety of cellular processes including protein biogenesis and quality control, apoptosis, signal transduction, lipid biosynthesis, and vesicular trafficking. TA proteins are inserted into the ER primarily by two pathways: GET, which relies on the heterooligomeric Get1/2 complex for insertion, and the EMC, which is a multifunctional insertase that caters to a subset of TA proteins. Additional alternative pathways may also contribute to TA biogenesis under particular cellular conditions. Decades of elegant biochemical and genetic experiments have defined the molecular players required for these parallel pathways, but until recently the molecular details of the insertion step have remained elusive.

A series of cryo-EM structures of the EMC and Get1/2 have revealed unparalleled mechanistic insight into how TA proteins are inserted into the bilayer. We will discuss how these structures inform the current models for how the EMC and GET pathways cooperate to ensure the selective and efficient targeting and insertion of TA proteins into the ER.

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Florian Wilfling received his Bachelor's and Master's degree in biochemistry from the Technical University of Munich. His subsequent doctoral work at the LMU Munich and the Yale School of Medicine, supervised by Tobias Walther, was focused on the translocation mechanisms of ER-resident transmembrane proteins to lipid droplets and their impact on lipid droplet growth. His postdoctoral research at the Max Planck Institute of Biochemistry in Planegg, Germany, with Stefan Jentsch and Wolfgang Baumeister focused on selective autophagy pathways and the role of intrinsic receptors for the targeted degradation of complex macromolecular machines. As a project leader in the group of Wolfgang Baumeister and Brenda Schulman, Florian Wilfling then proceeded to use cryo-electron tomography for the study of autophagosome biogenesis in situ. In 2021, Florian Wilfling established his own research group at the Max Planck Institute of Biophysics in Frankfurt am Main, Germany. His team is investigating how cargo properties influence autophagosome biogenesis and how this process occurs at the structural level on the cargo surface.

Figure 1: The tomogram, derived from correlated cryo-electron tomography, depicts a cargo-loaded autophagosome within the cellular environment.

Abstract:

Autophagosomes

The capacity to degrade cellular components, is a fundamental aspect of eukaryotic cell homeostasis and survival, both under physiological and stressed conditions. During macroautophagy (hereafter referred to as autophagy), autophagosomes are synthesized de novo to engulf and transport cytosolic material for lysosomal degradation. The targeted material can be highly diverse in size and nature, ranging from proteins to organelles to invading pathogens such as bacteria. The significance of autophagy in cellular homeostasis is exemplified by its involvement in a multitude of diseases, including infections, neurodegenerative disorders, and cancer. Consequently, the inhibition or promotion of autophagy has garnered increasing interest in the clinical setting. In this lecture, I will discuss the fundamental principles and stages of autophagy. I will elucidate the required protein

machinery and illustrate how in situ cryo-electron tomography can assist us in comprehending both autophagosome formation and cargo enrichment.

Recommended reading:

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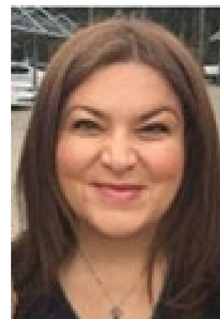

Mutay Aslan MD., PhD. completed her medical residency training at Akdeniz University Faculty of Medicine, in Turkey. She received her Ph.D. in Biochemistry and Molecular Genetics from the University of Alabama at Birmingham, in the United States. She is currently working as General Director of the Clinical Lab. at Akdeniz University Hospital and is also a lecturer at Akdeniz University Faculty of Medicine. Prof. Aslan currently serves as a member and was on the Management Committee of EU-COST. Dr. Aslan has been an independent expert/evaluator for the European Commission in selection of research proposals and has also been elected to the Advanced Courses Committee of FEBS for a four-year term. Aslan's research focuses on lipoproteins, fatty acids and sphingolipids in Diabetes and Cancer.
(https://avesis.akdeniz.edu.tr/mutayaslan)

Introduction to FEBS
The Federation of European Biochemical Societies

I shall introduce to all participants the main activities of FEBS, The Federation of European Biochemical Societies. FEBS is a registered charitable organization advancing research in the molecular life sciences across Europe and beyond. It was founded in 1964, is self-funded and is made of 39 constituent Societies (i.e., national biochemistry and molecular biology societies) that account for >35,000 members. Through its various programmes FEBS warrants innovative and extensive support for research training, education, interaction and communication. For instance, it grants FEBS Fellowships – stipends supporting training and mobility, and a FEBS Excellence Award – funds for early-career group leaders. Of note, FEBS supports every year Lecture Courses (like this FEBS-EMBO Advanced Lecture Course "Lipids, proteins and their interactions in organelle biology"), Practical Courses, Workshops and Special Meetings, in order to stimulate on a regular basis education, training and networking among young European (and non-European) biochemists. Finally, I shall remark that a online forum for the molecular life science community is offered by the FEBS Network, and that FEBS wholly ownes high-quality, peer-reviewed journals whose income funds FEBS programmes. As a general reference, the FEBS official website (www.febs.org) should be visited.

Juanita Perera – FEBS

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I did my Ph.D. at the University of Rochester in New York. After completing my post-doctoral research work at the German Cancer Research Center and at the University of Heidelberg, I joined FEBS Letters as an Editor in 2016.

Talk to a FEBS Press editor

I am attending the FEBS Advanced Course as a FEBS representative. I will be happy to talk to you and help you with any questions you may have about science publishing, preparing your manuscript for submission to a journal (e.g., how to write a good abstract and title), and promoting your published work. If you have questions about a career in science publishing, then we could discuss that too! I look forward to meeting you in Spetses!

William Teale - EMBO

EMBO journal, EMBO Press

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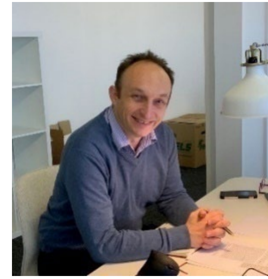

Since October 2021, William Teale has been a Scientific Editor at the EMBO journal, working on the EMBL campus in Heidelberg, Germany. William’s subject areas at the EMBO journal are membranes and trafficking, plant biology, autophagy, and channels and transporters. Before this, William worked as a group leader at Freiburg University, Germany, and was primarily interested in the regulation of polarly localized plasma membrane transporters.

William’s scientific career started with a PhD on gibberellin biosynthesis at Bristol University, UK and continued at Cambridge University with a post-doc looking into the characteristics of high-specificity, low-affinity protein-protein interactions. After this, William moved to Freiburg, Germany, where he contributed to the characterization of auxin transport complexes.

Perspectives from a journal editor

Scientific progress depends on efficient mechanisms to select, quality control and share rigorous, reproducible research. Peer reviewed scientific journals play an important role in this process. I will discuss my impressions of a recent move from bench to EMBO journal editorial team and introduce some initiatives that have been designed to make editorial processes fairer, more informed and more efficient.

Abstracts from participants

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THE TRANSCRIPTIONAL PROGRAM OF GOLGI BIOGENESIS

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The transcriptional regulation of Golgi biogenesis has yet to be fully understood. Considering the fundamental importance of the Golgi complex for intracellular membrane trafficking, this dearth of knowledge represents a serious gap.

To solve this problem, we developed a reliable system to study de novo Golgi biogenesis. We used a cell line expressing HRP-tagged mannosidase II (ManII-HRP), which allowed us to destroy the preexisting Golgi with high precision via HRP-mediated formation of insoluble DAB polymers in the Golgi lumen. Live cell imaging and electron microscopy demonstrated that such loss of the "old" Golgi induced the formation of the "new" fully functional Golgi complex in a substantial subpopulation of cells.

We combined this Golgi inactivation approach with single cell RNA-seq to analyze how the cell transcriptome changes during the process of de novo Golgi biogenesis. In particular, we addressed the following questions:
- Are genes encoding different components of the Golgi activated simultaneously or sequentially?
- How does the activation of certain groups of Golgi genes correlate with structural and functional changes in the rebuilding of the Golgi apparatus?
- Is there any order in the activation of genes encoding components of early (cis) or late (trans) Golgi sub-compartments, which differ from each other in structure, function, and composition?

We found that cells rebuilding the new Golgi transactivate more than 100 Golgi genes that encode different classes of Golgi components including structural proteins, glycosylation enzymes and membrane tethering/trafficking complexes. Upregulation of these genes occurred in a coordinated manner and coincided in time with the recovery of the typical stack architecture and reactivation of transport through the newly-forming Golgi organelle. We also noted that activation of Golgi genes was not compartment-specific as induction of cis-, medial- and trans-Golgi genes occurred simultaneously. Finally, we found that the cells treated with transcription inhibitors failed to rebuild the new Golgi. Thus, our findings suggest that orchestrated transactivation of various Golgi genes supports de novo biogenesis of the Golgi by providing building blocks for morphogenesis of the Golgi stacks and induction of their functional activities.

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Multi-functional ARF1 compartments serve as a hub for short-range cargo transfer to recycling endosomes

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Cellular membrane homeostasis is achieved through a balance of secretory transport of de novo synthesized proteins, endocytic recycling of plasma membrane components and degradation. All these pathways intersect at the level of the trans-Golgi network (TGN) and endosomal compartments. Here, cargo selection and sorting relies on adaptor protein complexes which are recruited to the membranes by the small GTPase ARF1. Combining CRISPR/Cas9 based gene-editing with live-cell stimulated emission depletion (STED) microscopy and correlated light electron microscopy we have previously discovered a tubulo-vesicular ARF1 compartments that facilitates post-Golgi trafficking between the TGN and endosomes. Rather than long-range vesicle shuttling, we observe transient interactions of ARF1 compartments and recycling endosomes (RE) which facilitate sorting of secretory and endocytic recycling cargo. Utilizing a proximity-based mass spectrometry approach (split Turbo-ID) we could identify the interactome at the ARF1/RE interface which includes cargo adaptors, endosomal tethers as well as ARF1 regulators and effectors. Our findings suggest that tubular ARF1 compartments coordinate post-Golgi trafficking through transient interactions with RE, where cargo transfer is mediated by a kiss-and-run mechanism rather than by fusion of transport vesicles.

Reference(s):
Multi-functional ARF1 compartments serve as a hub for short-range cargo transfer to endosomes (currently in revision, preprint on BioRxiv)

ARF GTPases and Their Ubiquitous Role in Intracellular Trafficking Beyond the Golgi (review published in Front Cell Dev Biol.)

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Androgen Mediated Metabolic Perturbation in Endocrine Resistant Breast Cancer

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Introduction: Although breast cancer (BC) is considered a treatable disease, >30% of patients with hormone receptor positive tumours will suffer recurrence post endocrine therapy. One of the fundamental features of cancer cells is their ability to modulate cell metabolism to facilitate survival, stress response, and proliferation. Increasing evidence suggests that these mechanisms play key roles in the development of treatment resistance. Recent reports from our group have shown that an androgenic steroid environment is associated with poor response to aromatase inhibitor (AI) therapy. Of significance, we have previously shown that androgens mediate increased expression of serum-and-glucocorticoid kinase-3 (SGK3), a known substitute for AKT, that is associated with second-line resistance in BC. Here we investigate the role of androgens in modulating cellular metabolism in BC cell lines via SGK3 regulated mechanisms.

Methodology: Using in-house isogenic models of endocrine resistance and a novel SGK3-PROTAC1 degrader, we explore the impact of androgenic steroids and SGK3 inhibition on intracellular lipid accumulation and cell metabolism using a combination of Seahorse Mito Stress assays, flow cytometry, protein analysis and imaging studies.

Results: Cell viability assays showed that androgen-responsive AI-resistant cells were more responsive to SGK3 inhibition than parental endocrine-sensitive cells. SGK3 degradation is associated with the stabilisation of 17β;HSD4, a D-bifunctional enzyme that plays a key role in peroxisomal lipid b-oxidation and androgenic steroid inactivation. Additionally, SGK3 degradation altered intracellular lipid accumulation and distribution under androgenic conditions.

Conclusions: Regulation of 17β;HSD4 highlights the potential role of androgen driven SGK3 in modifying the steroidogenic tumour environment. This altered steroid microenvironment mediates significant changes in cell metabolism, which mirrors disorders of androgen excess in women, such as PCOS, which are linked to lipid toxicity and deranged metabolism. Further studies will explore androgen mediated SGK3 in regulating steroid homeostasis and associated metabolic reprogramming as drivers of endocrine resistance.

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Rab11 endomembranes and phosphoinositides interplay in response to stresses

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Mammalian cells are facing different type of stresses, and to overcome acute changes in homeostasis, they have to adapt immediately. Beside the classical autophagy pathway, cells adapt by dedicated organelles and endomembranes mobilization and crosstalk. In this work, I investigated the molecular mechanisms by which mammalian cells utilize their endosomal arsenal to cope with homeostasis alteration, by comparing nutritional, mechanical and infectious stresses. Importantly, both nutritional and mechanical stresses are often at the benefit of the host (recycling to survive nutritional stress and cell differentiation upon shear stress for mechanical stress). On the contrary, infectious stress adaptations are at the benefit of the pathogen. All situations could lead to different activations of the same machinery. I particularly focused on endosomal subpopulation positive for the small GTPase Rab11, in putative association with dynamic levels of endosomal phosphoinositides PI3P and PI4P. I was able to show that Rab11 positive structures display increased membrane deformations upon starvation and mechanical stress, such situations being associated with autophagy induction. I then investigated further what mechanisms were involved in Rab11 membranes mobilization in these situations.

I show that following stress induction, there is a change in PI3P/PI4P phosphoinositides homeostasis at the vicinity of Rab11 membranes. Depleting Rab11 impairs the PI3P/PI4P switch. The depletion of ATG16L1, an autophagy-related protein able to bind both Rab11 and phosphoinositides and involved in different stress responses, also impairs the PI3P/PI4P signature associated with stress response. Our data suggest that one of the mechanisms leading to Rab11-positive membranes mobilization is a switch of phosphoinositides, controlled by ATG16L1 and other phosphoinositides enzymatic regulators, which takes places on, or at the vicinity of Rab11-positive membranes.

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Proteins at the crossroads: Unravelling key factors that regulate the dual localization of Fis1 to mitochondrial and peroxisomal membranes

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Eukaryotic cells have evolved complex machineries that direct cytosolically synthesized proteins to their specific intracellular location. Some proteins localize to more than one organelle, like the tail-anchored protein Fis1 which is dually localized to mitochondria and peroxisomes. We aimed to identify novel factors and mechanisms that regulate this dual localization. To that goal, we used an automated mating approach to introduce fluorescently labelled tail-anchored proteins to a yeast deletion library. Using high throughput screening, the subcellular localization of mCherry-Fis1 was visualized on the background of mutants in all yeast genes. Employing fluorescence microscopy and subcellular fractionation, we found that the deletion of TOM71 and the uncharacterized gene YNL144C (re-named as MPF1 in this study) led to a greater localization of Fis1 to peroxisomes as compared to the control cells. Surprisingly, Tom71 overexpression (OE) resulted in greater Fis1 distribution to mitochondria compared to Tom70 (OE), suggesting a unique function for Tom71. We further proceeded to characterize Ynl144c (Mpf1) and found it to be loosely associated with the mitochondrial outer membrane (MOM). Mpf1-3HA(PH*), a Pleckstrin homology domain (PH domain) mutant of Mpf1 did not affect its localization to the MOM and displayed elevated steady-state (SS) levels as well as a partial enhancement in its stability compared to its native counterpart. Furthermore, deleting both Tom70 and Tom71 drastically reduced SS levels of Mpf1 suggesting a potential involvement of Tom70/71 in the biogenesis of Mpf1. Additionally, the deletion of MPF1 in yeast is slightly beneficial on FA containing media. Altogether, our findings suggest the potential involvement of Mpf1 and Tom71 in regulating the dual distribution of Fis1 to mitochondria and peroxisomes.

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Investigation of the effect of Parkinson's disease associated mutations in RHOT1 on mitochondrial-ER contact sites, calcium homeostasis and lipid transfer.

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3) Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, Rostock, Germany.
4) Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, Rostock, Germany.

The pathogenesis of Parkinson's disease (PD) is highly complex, including dysfunction of multiple organelles such as mitochondria, lysosomes, ER and peroxisomes. In addition, cellular processes such as calcium homeostasis, proteostasis, autophagy and, lipid homeostasis are altered. To understand all these processes involving different organelles, research has focused recently, on the study of organelle contact sites, particularly on mitochondria-endoplasmatic reticulum contact sites (MERCS). Some studies suggested an involvement of peroxisomes in PD as well, however, the role of peroxisomes and lipid biosynthesis in neurodegenerative diseases has hardly been studied so far. In this context, MIRO1 is a protein of particular interest because it is not only involved in regulation of mitochondrial transport, and mitochondria and peroxisome dynamics, but also in the regulation of MERCS and, as a calcium sensor, serves as a crucial modulator of mitochondrial calcium homeostasis. Notably, organelle-organelle contact sites have been shown to be essential for lipid transfer between organelles. Recently, we have described heterozygous mutations in the RHOT1 gene, encoding MIRO1, in four PD patients. However, a comprehensive understanding of how PD-related mutations in MIRO1 affect MERCS, mitochondria, peroxisomes and thus lipids is currently lacking. To better understand the role of MIRO1 and its putative contribution to PD pathogenesis, we used iPSC-derived neurons carrying CRISPR/Cas9-generated mutations in the RHOT1 gene which either were reported in PD patients or which interfere with MIRO1's interaction with PINK1/Parkin, two well-known proteins involved in the PD pathophysiology. Effects on mitochondria, peroxisomes, ER, lipid biosynthesis and on neuronal function will be investigated. For this purpose, high resolution live cell imaging, Western blot analysis and immunostainings are used. Here we show that mutations in MIRO1 caused significant differences in

calcium handling and alterations in the number of contact sites and their interaction with MIRO1. In particular, thapsigargin-induced calcium stress revealed impaired mitochondrial calcium buffering capacity in Miro1-mutant neurons. Furthermore, Miro1-mutant neurons showed increased calcium levels in the mitochondria compared to control neurons. Thus, alterations in calcium handling might cause defects of neuronal function.

In addition, preliminary results examining lipid droplet formation and lipid trafficking at MERCS showed differences between control and mutant neurons. As a more complex picture emerges, our study on MIRO1 may help to investigate the missing link of organelle interaction impairment, resulting in changes of calcium and lipid homeostasis, in the pathology of different forms of PD.

Funding: Nachwuchsforderung 2021 der Deutschen Gesellschaft für Parkinson und Bewegungsstorungen e.V.; LipiSYN: Untersuchung des Zusammenhangs zwischen Störungen der Membranlipiddynamik und der α;-Synuclein-Proteostase.

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The profiling of CLIC4-lipid interactions in a cell cycle-dependent manner

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CLIC4 is a member of the well-conserved chloride intracellular channel (CLIC) protein family and is structurally related to glutathione-S-transferases. It exists in both soluble and membrane-integrated forms. Our previous study revealed that CLIC4 is important for successful cell division. It enriches on the mitotic cell surface and accumulates at the cleavage furrow during cytokinesis. However, when its Cys35 residue is mutated, which is structurally critical as its equivalent in GST-omega 1 is located at the enzymatically active cleft, the cell division-specific translocation of CLIC4 to the cell surface is abolished1. In this study, we aim to systematically compare the lipid interactomes of wild-type CLIC4 and its mutant counterpart (C35A) in a cell cycle-dependent manner to identify wild-type CLIC4-specific lipid interactors in mitosis and cytokinesis. Given that the interaction partners of CLIC4 such as ezrin and anillin1, tether to the plasma membrane via PI(4,5)P2, we extended our study to map the specific interactions of CLIC4 with phosphatidylinositols. Collectively, this study will give insight into the docking of CLIC4 to the plasma membrane during cell division.

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Mechanism for PEX7 Mediated PTS2 Protein Transport into Peroxisomes

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Peroxisomes are ubiquitous organelles existing in all eukaryotic cells. They contain important functions such as oxidizing fatty acids and producing hydrogen peroxide for redox homeostasis. Proteins that are imported into the peroxisomal matrix are synthesized in the cytosol and transported post-translationally in a unique manner. Unlike protein import into the endoplasmic reticulum or mitochondria, peroxisomal proteins can be imported in folded and even oligomerized states. Imported proteins contain a peroxisomal targeting signal (PTS) on either the C-terminus (PTS1) which mediates interactions with the soluble receptor PEX5 or N-terminus (PTS2) which mediates interactions with both the PEX5 receptor and the PEX7 adaptor. Most peroxisomal matrix proteins contain the PTS1 motif, but mutations that affect PTS2 import can nonetheless cause diseases such as rhizomelic chondrodysplasia punctata. The translocation of PEX5 into peroxisomes with PTS1 cargo has recently been characterized, but it is unknown how PEX7 is recycled back into the cytosol after importing PTS2 cargo. Whereas PEX5 is recycled through a retro-translocon consisting of a hetero-trimeric ubiquitin ligase complex, PEX7 lacks the features required for this pathway, such as an unstructured N-terminus and a cysteine that can be mono-ubiquitinated. Our preliminary results show that PEX7 utilizes a novel mechanism to return to the cytosol.

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Mechanotransduction at the Golgi apparatus - Implications of lipid reorganization in Golgi membrane tension regulation

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Cells can sense and respond to external forces and mechanotransduction events appear to be critical for most cellular functions. While mechanotransduction has been extensively studied at the plasma membrane and at the nucleus, the impact of forces on other organelles is still poorly known. Our project focuses on the study of mechanotransduction at the Golgi apparatus (GA), a central organelle regulating intracellular transport pathways. We aim to answer the following questions: 1) Can external and internal forces propagate to the GA and impact its membrane tension? 2) Is the tension of the GA regulated by actin dynamics and/or the composition of lipids in Golgi membranes and the Golgi matrix? 3) Do post-Golgi trafficking and polarized secretion depend on the tension of the Golgi membrane?

Our findings suggest that the GA is mechanosensitive and can modulate its membrane tension in response to

external or internal constraints mostly by it's lipid reorganization. Cytoskeletal protein disruption/modifications effectively modulate Golgi membrane tension. Furthermore, we have shown that external constraints such as substrate stiffness has a strong impact on the function of the GA as it affects the trafficking kinetics especially the post-Golgi traffikcing of some selected cargoes. Our results should provide new fundamental insights in the role played by mechanical tension in force transduction at the level of the Golgi membranes.

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Characterization of the dynamic behaviour of clathrin and its adaptors along the endocytic timeline in yeast

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Clathrin-mediated endocytosis (CME) is a fundamental process in eukaryotic cells, that happens through the coordinated assembly of over 50 different proteins at the endocytic site. Clathrin and adaptor proteins form a coat around the plasma-membrane-derived vesicle. This coat provides both force necessary for membrane deformation as well as the regulation of cargo recruitment and vesicle formation.

Clathrin itself has been extensively studied over the years and its structural properties are now well characterized. Yet, there are still many unanswered questions regarding its role in endocytic site maturation: its scaffolding nature places it at the centre of the complex interaction network ensuring proper vesicle formation, and its specific assembly properties seem to hint at a regulatory function.

We aimed to characterize clathrin's assembly properties over time at the molecular scale. Using a combination of simultaneous dual-colour Total Internal Reflexion Fluorescence (TIRF) microscopy and Fluorescence Recovery After Photobleaching (FRAP) experiments in S. cerevisiae, we followed real-time clathrin recruitment at single endocytic sites. We related its dynamic behaviour to its various interactors along its lifetime, and showed that clathrin dynamics are distinct from those of its adaptor Yap1801. We demonstrated that the early phase of endocytosis is characterized by a clathrin exchange regulated by the uncoating machinery Hsp70/Auxilin. The clathrin coat is then stabilized in concomitance with the recruitment of late coat protein Sla1 to the endocytic site.

Our results highlight that clathrin assembly during CME is an active and temporally regulated process, that is important for proper endocytic site maturation.

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New interaction partners of human TOM complex

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Mitochondria are vital organelles involved in a number of crucial processes such as energy production, calcium homeostasis, and apoptosis regulation. To fulfil these functions, human mitochondria consist of around 1500 proteins of which only 1% are synthesized inside the organelle. The other 99% of proteins are synthesized in the cytosol and imported into mitochondria, primarily by the Translocase of the Outer Mitochondrial Membrane (TOM) complex, situated in the outer mitochondrial membrane. The protein import process is crucial for the functioning of mitochondria and, consequently, for the survival of the cell. Hence, this process is thoroughly regulated by mitochondrial quality control mechanisms. Unlike its well-studied homologue in yeast, the regulatory processes around protein import via TOM have not been deeply explored in human cells. Our research explores the interactome of the human TOM complex to elucidate quality control mechanisms that regulate human mitochondrial protein import. To study the interactome of TOM complex, we purified the TOM complex from WT HEK293T cells by immunoaffinity purification via TOMM22-FLAG. The purified TOM complex was analysed by mass spectrometry proteomics and novel interactions observed in the MS analysis were further studied by western blot SDS-PAGE. The MS analysis confirmed the main components of the human TOM complex (TOMM22, TOMM40, TOMM20, TOMM5, TOMM6, TOMM7 and TOMM40L) as the most enriched proteins. Interestingly, we found human homologs of yeast proteins involved in mitochondrial quality control such as FAF2 (homolog of Ubx2) and PTRH2 (homolog of Pth2). Further, the MS analysis of the human TOM complex interactome also revealed candidate interactors without yeast homologs, the involvement of which in quality control processes of protein import into mitochondria is the subject of current investigation.

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Structural and functional insights into the ER-resident membrane E3 ubiquitin ligase MARCH6/Doa10

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Transmembrane E3 ligases play crucial roles in protein and organelle quality control, and metabolism. Here, we present insights into Doa10/MARCH6 structure and function by cryo-EM and AlphaFold analysis, and a structure-based mutagenesis campaign. The majority of Doa10/MARCH6 adopts a unique circular structure within the membrane. This channel is established by a lipid-binding scaffold and a flexible helical bundle. The ubiquitylation active site is positioned over the channel by positioning the catalytic RING domain through connections with the scaffold and the flexible helix bundle. By assaying 95 MARCH6 variants for effects on stability of the well-characterized substrate SQLE, which regulates cholesterol levels, we reveal crucial roles of the formed channel and RING domain consistent with AlphaFold-models of substrate-engaged and ubiquitylation complexes. SQLE degradation further depends on connections between the channel and RING domain, and lipid binding sites, revealing how interconnected Doa10/MARCH6 elements could orchestrate metabolic signals, substrate binding, and E3 ligase activity.

Reference(s):

Doa10/MARCH6 architecture interconnects E3 ligase activity with lipid-binding transmembrane channel to regulate SQLE

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Chloroplast import motor subunits FtsHi1 and FtsHi2 are located on opposite sides of the inner envelope membrane

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Protein import into chloroplasts is powered by ATP hydrolysis in the stroma. Establishing the identity and functional mechanism of the stromal ATPase motor that drives import is critical for understanding chloroplast biogenesis. Recently, a complex consisting of Ycf2, FtsHi1, FtsHi2, FtsHi4, FtsHi5, FtsH12, and malate dehydrogenase was shown to be important for chloroplast protein import, and it has been proposed to act as the motor driving protein translocation across the chloroplast envelope into the stroma. To gain further mechanistic understanding of how the motor functions, we performed membrane association and topology analyses on two of its subunits, FtsHi1 and FtsHi2. We isolated cDNA clones encoding FtsHi1 and FtsHi2 preproteins to perform in vitro import experiments in order to determine the exact size of each mature protein. We also generated antibodies against the C-termini of the proteins, i.e., where their ATPase domains reside. Protease treatments and alkaline and high-salt extractions of chloroplasts with imported and endogenous proteins revealed that FtsHi1 is an integral membrane protein with its C-terminal portion located in the intermembrane space of the envelope, not the stroma, whereas FtsHi2 is a soluble protein in the stroma. We further complemented an FtsHi1-knockout mutant with a C-terminally tagged FtsHi1 and obtained identical results for topological analyses. Our data indicate that the model of a single membrane-anchored pulling motor at the stromal side of the inner membrane needs to be revised and suggest that the Ycf2-FtsHi complex may have additional functions.

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Identification of lipid-protein interactions at Plasmodesmata

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Plasmodesmata (PD) serve as intercellular channels that traverse plant cell walls facilitating the communication of adjacent cells. PD environment is constituted by the cell wall, an extension of the endoplasmic reticulum known as desmotubule, and a continuous plasma membrane (PM) between the neighbor cells. Previous studies have reported that PD-PM exhibits a specific protein composition and lipids resembling PM nanodomains, enriched with saturated phospholipids, sphingolipids, and sterols (Grison et al, 2015).

While the significance of lipids in modulating PD protein targeting, function, and ultrastructure has been well documented, the specific lipid-protein interactions governing these processes remain largely unexplored (Grison et al. 2015, Zhang, et al. 2022). To contribute to a global understanding of PD biogenesis and function, our research project centers on the identification and characterization of protein-lipid interactions in PD. To achieve this, we employ Arabidopsis mutants deficient in various lipid classes such as phospholipids, sphingolipids, and sterols to characterize PD properties, conduct transportation assays, and employ proteomics and lipidomics techniques.

Our primary objective is to elucidate how alterations in PD density and permeability correlate with changes in the PD proteome induced by perturbations in lipid composition. By shedding light on these intricate lipid-protein interactions, we strive to advance our understanding of PD dynamics and contribute to broader insights into plant cellular communication and physiology.

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2. Sphingolipids at Plasmodesmata: Structural Components and Functional Modulators

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Investigation of glycosomal protein import machinery for novel anti-leishmanial drug targets

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Vector-borne protozoan parasites like Trypanosoma brucei, Trypanosoma cruzi and Leishmania cause life threatening neglected tropical diseases (NTDs). Especially, leishmaniasis is caused by more than 20 species of Leishmania parasites and annually around one million new cases occur. Visceral leishmaniasis, also known as kala-azar, is lethal without treatment in 95% cases. Cutaneous and mucocutaneous leishmaniasis cause skin lesion and destruction of mucous membrane in nose, mouth and throat and leave irreversible scars on patients' faces and affect their life quality. Glycosomes, which are essential for the parasite survival, are peroxisome-related organelles and maintained by peroxin (PEX) protein through recognition and transportation of proteins into glycosomes. PEX3, a glycosomal membrane protein, has low sequence similarity to human PEX3 and hence it is a good drug target. Previous studies have shown that blocking the interaction between the import receptor PEX19 and the corresponding docking factor PEX3 kill Trypanosoma brucei by disrupting glycosomal biogenesis [1,2]. In this study, we first identified the PEX3 in Leishmania by sequence alignment and localization. Following, the interaction between PEX3 and PEX19 in Leishmania was validated using in vitro pull-down assays and yeast two-hybrid analysis. Next, we established and optimized an AlphaScreen based assay using recombinant L. donovani PEX3 and PEX19 proteins. By screening compounds from an FDA-approved drug repurposing library, candidates that disrupt the LdPEX3-PEX19 interaction in vitro were identified and their anti-leishmanial activities were validated in an in vitro cell-based assay. Further, we are investigating the therapeutic efficacy in animal models of leishmaniasis.

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The Dynamics of CLIC4 in Membrane Blebs

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CLIC1 and CLIC4 are members of the chloride intracellular channel protein family that have several roles in cell signaling, cell adhesion, migration, and protein trafficking. Our previous study showed that CLIC1 and CLIC4 are required for successful cytokinesis in mammalian cells. The absence of both proteins leads to abnormal blebbing at poles during cytokinesis due to the detachment of the cortical actomyosin network from the plasma membrane. Additionally, the localization of both CLIC1 and CLIC4 to the polar membrane blebs during cytokinesis reveals their importance in bleb regulation1. In this study, we aim to gain a better understanding of the spatiotemporal dynamics of CLIC4 during membrane blebbing using lattice light sheet microscopy (LLSM), which which offers a high 3D temporal resolution of living cells with low phototoxicity. By co-imaging CLIC4 with markers of different bleb stages, such as ezrin, actin, and non-muscle myosin II, we aim to determine the hierarchical position of CLIC4 in the cortex assembly at membrane blebs. Our objective is to uncover the role of CLIC4 during the membrane bleb lifecycle and identify its spatio-temporal dynamics relative to other cortical proteins. This study will contribute to a better understanding of the molecular mechanisms underlying membrane blebbing dynamics and the role of CLIC4 in this cellular process.

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A metabolically controlled contact site between lipid droplets and vacuoles

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Lipid droplets (LDs) are ubiquitous organelles specialized for lipid storage. They maintain a continuous supply of lipids during starvation and protect cells from lipotoxicity. The Lipid Droplet Organization (LDO) proteins, Ldo16 and Ldo45, have been identified as multifunctional proteins that affect several aspects of the LD life cycle. They are linked to the LD biogenesis machinery seipin and affect protein targeting to LD surface, spatial LD distribution and autophagy. Despite these clear LDO-related phenotypes, molecular roles of these proteins were unclear. In this study, we find that Ldo16 and Ldo45 form a complex with the vacuolar surface protein Vac8 and act as molecular tethers for the vacuole-lipid droplet contact site (vCLIP). The phosphatidylinositol transfer protein Pdr16 is recruited to vCLIP by Ldo45. Both the abundance and the protein composition of vCLIP respond strongly to metabolic cues. vCLIP-mediated LD-vacuole tethering is prerequisite for efficient autophagic LD engulfment into the vacuole during prolonged nutrient deprivation. In summary, our studies shed light on the molecular machinery and functional role of a previously poorly described contact site, vCLIP.

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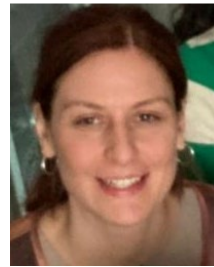

THE TRANSCRIPTIONAL PROGRAM OF GOLGI BIOGENESIS

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The transcriptional regulation of Golgi biogenesis has yet to be fully understood. Considering the fundamental importance of the Golgi complex for intracellular membrane trafficking, this dearth of knowledge represents a serious gap.

To solve this problem, we developed a reliable system to study de novo Golgi biogenesis. We used a cell line expressing HRP-tagged mannosidase II (ManII-HRP), which allowed us to destroy the preexisting Golgi with high precision via HRP-mediated formation of insoluble DAB polymers in the Golgi lumen. Live cell imaging and electron microscopy demonstrated that such loss of the "old" Golgi induced the formation of the "new" fully functional Golgi complex in a substantial subpopulation of cells.

We combined this Golgi inactivation approach with single cell RNA-seq to analyze how the cell transcriptome changes during the process of de novo Golgi biogenesis. In particular, we addressed the following questions:

- Are genes encoding different components of the Golgi activated simultaneously or sequentially?

- How does the activation of certain groups of Golgi genes correlate with structural and functional changes in the rebuilding of the Golgi apparatus?

- Is there any order in the activation of genes encoding components of early (cis) or late (trans) Golgi sub-compartments, which differ from each other in structure, function, and composition?

We found that cells rebuilding the new Golgi transactivate more than 100 Golgi genes that encode different classes of Golgi components including structural proteins, glycosylation enzymes and membrane tethering/trafficking complexes. Upregulation of these genes occurred in a coordinated manner and coincided in time with the recovery of the typical stack architecture and reactivation of transport through the newly-forming Golgi organelle. We also noted that activation of Golgi genes was not compartment-specific as induction of cis-, medial- and trans-Golgi genes occurred simultaneously. Finally, we found that the cells treated with transcription inhibitors failed to rebuild the new Golgi. Thus, our findings suggest that orchestrated transactivation of various Golgi genes supports de novo biogenesis of the Golgi by providing building blocks for morphogenesis of the Golgi stacks and induction of their functional activities.

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Exploring the role of the arginine side chain in the aggregation prone mutant of phospholamban

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N/A Heart contractility is determined by the influx of calcium ions inside the cardiomyocyte. Phospholamban (PLN) and SERCA 2A Ca2+ pump help in regulating this calcium influx. PLN is a 6 kDa, type II membrane protein present in the sarcoendoplasmic reticulum (SER) of cardiomyocytes. During a beta-adrenergic response (e.g. adrenaline rush), PLN is phosphorylated by PKA and CAMK2D. Phosphorylated PLN dissociates from SERCA. This leads to an increased affinity of SERCA towards intracellular calcium ions which culminates in enhanced relaxation rates and contractility of the heart. PLN exhibits itself in an equilibrium between a monomer and pentamer, where the unphosphorylated monomer inhibits SERCA. A deletion of an arginine at the 14th position of PLN (PLN R14del) is associated with dilated cardiomyopathy (DCM).[1] [2]

Immunohistopathological evaluations of hearts of patients with PLN R14del show PLN aggregates that are colocalized with autophagic (p62) and microtubule binding proteins (LC3). It is hypothesized that the cell's protein quality control mechanism is unable to clear out these aggresomes. Misfolding and mislocalization of PLN R14del may contribute to such aggregation.[3]

Our aim is to understand the differences in stability of PLN wild type (PLN WT) and PLN R14del to potentially discover an underlying disease mechanism. We therefore intend to study changes in secondary structure characteristics by CD between the WT and mutant. Through nuclear magnetic resonance (NMR), we observe that only PLN R14del exhibits chemical shift perturbations of amino acid resonances around the deletion site in the presence of guanidium ions. This is interesting as arginine 14 in PLN WT is preceded by the protonated guanidino group in arginine 13. Intriguingly, it has been argued that guanidinium ion can form like-charge ion pairs stabilized by van der Waals forces. Interestingly, the structure of PLN WT bound to 14-3-3 (PDB: 6Y40) reveals an intermolecular arginine-arginine pi-stacking interaction, suggesting that arginine-arginine pairing could be a key mechanism underlying the protein-protein interaction of PLN.

We hypothesize that the absence of R14 in PLN R14del increases its propensity for binding to available guanidinium groups in its surroundings, potentially enhancing its 'stickiness'. Currently, we are investigating the binding of guanidinium ions to a short PLN peptide (3-17) from both wild-type (WT) and R14del variants using NMR and circular dichroism (CD) studies. These data may provide insights into the underlying mechanism of misfolding in PLN R14del and its potential contribution to the formation of aggresomes

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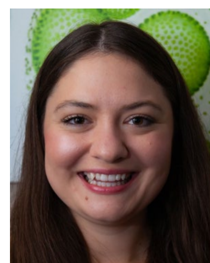

Understanding the Structural Basis of Sec14 Mediated Lipid Exchange

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Phosphoinositide signaling is a critical factor of intracellular signal transduction in all eukaryotic cells. Even subtle perturbations in phosphoinositide metabolism can lead to notable phenotypic changes in unicellular organisms and devastating neurodegenerative and neurodevelopmental diseases in multicellular organisms – including humans. Phosphatidylinositol (PtdIns) transfer proteins (PITPs) serve as central regulators of phosphoinositide signaling from yeast to mammals. These proteins execute an unusual energy-independent lipid exchange cycle that is coupled to the activation of PtdIns 4-OH kinase to produce the essential lipid PtdIns-4-phosphate. Current evidence suggests that the PITP driven lipid exchange cycle is the engine that drives presentation of PtdIns as a superior substrate to the intrinsically inefficient lipid kinase such that sufficient PtdIns-4-phosphate is produced to overcome the action of signaling PtdIns-4-phosphate antagonists. Consequently, understanding the PITP lipid exchange cycle is critical to understanding how phosphoinositide signaling is regulated in cells. Sec14 represents the major yeast PITP and thus serves as our model of choice for elucidating the mechanism of this PITP's lipid exchange cycle. Using a combination of techniques including 19F NMR spectroscopy, X-ray crystallography, molecular dynamics simulations, and biological assays we study the structural basis of membrane association and subsequent lipid transfer by Sec14. The results of our research provide insight into the membrane binding interface of Sec14 and probe the local conformational dynamics that occur during lipid exchange activity.

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Exploring the role of lipid droplets: insights into brain aging and neurodegeneration

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Lipid droplets (LDs) affect the functionality and survival of neurons in several brain disease conditions. This project aims to elucidate the role of these organelles on two human in vitro models of neurodegenerative diseases (NDs): the neuroblastoma SH-SY5Y cell line subjected to 6-OHDA for Parkinson's disease, and primary human fibroblasts obtained from Alzheimer's disease (AD) patients.

In SH-SY5Y, robust and trustworthy experimental methods for detecting and measuring LDs were established. Confocal laser scanning microscopy was integrated with image analysis, facilitating an automated and unbiased assessment of LDs under basal conditions and in reaction to LDs manipulation and/or mitochondrial stress. Additionally, mitochondrial structure was assessed across all experimental settings.

SH-SY5Y cells treated with 6-OHDA behave similarly to untreated cells regarding LDs content and size. Nevertheless, upon exposure to OA, the 6-OHDA-treated cells exhibited elevated LD quantities in contrast to the controls. This rise was effectively prevented by pharmacological inhibition of LD synthesis.

There was notable variability in LDs abundance among human fibroblasts, yet no statistically significant variance in LD content was detected in two out of three pairs of samples from AD patients and healthy individuals. AD fibroblasts exhibited heightened mitochondrial interconnectivity compared to their respective controls.

Although the significance of lipid metabolism in NDs is recognized, the precise alterations in lipid content and metabolism, alongside the involvement of lipids in mitochondrial stress and neuronal function, remain unclear in both normal and pathological conditions.

This study offers essential tools and initial insights into comprehending the involvement of LDs and their potential impact on mitochondria within pertinent in vitro models of PD and AD.

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Mitochondrial protein import is linked to lipid droplet homeostasis

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The outer membrane of mitochondria contains a few dozen integral membrane proteins that are all produced as precursor proteins on cytosolic ribosomes. In baker´s yeast Saccharomyces cerevisiae, five proteins contain a transmembrane beta-barrel structure, while other proteins are inserted into the membrane via a single or multiple alpha-helical membrane spans. All outer membrane proteins are produced as precursors on cytosolic ribosomes and are imported via protein translocases. The sorting and assembly machinery (SAM complex) integrates proteins with a transmembrane beta-barrel. The mitochondrial import (MIM) machinery constitutes the major insertase for most of the proteins that contain alpha-helical membrane anchor. Here we report that Ayr1 is a novel interaction partner of the mitochondrial import complex (MIM complex). Ayr1 is a short-chain dehydrogenase that was found in the ER, lipid droplets and the mitochondrial outer membrane. Ayr1 has been shown to function as triacylglycerol lipase in lipid droplets and plays a role in the biosynthesis of phosphatidic acids. We found that Ayr1 interacts with a specific MIM fraction that is not involved in protein import. It promotes the association of lipid droplets with mitochondria and thereby modulates the homeostasis of lipid droplets. In conclusion, the MIM-Ayr1 interaction links mitochondrial protein import to lipid droplet homeostasis.

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Identification of cytosolic factors for targeting precursor proteins to chloroplasts

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Specific sorting factors that recognize organelle-targeting signal on proteins enable translocation from the cytosol to target organelles. Sorting factors responsible for targeting proteins to the nucleus, endoplasmic reticulum, and peroxisome have been identified and studied. However, the sorting factor for chloroplasts remains elusive. Here, affinity purification approaches are used to identify chloroplast sorting factor candidates. Chloroplast transit peptide-GFP fusions are in vitro translated in high-yield wheat germ extract translation system and immunoprecipitated. In addition, chloroplast transit peptide-GFP fusions accumulated in an Arabidopsis mutant defective in chloroplast protein import were also immunoprecipitated. Proteins specifically coimmunoprecipitated with the transit peptides were identified by mass spectrometry. We report initial characterizations of the identified sorting factor candidates.

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Investigating the Mechanisms of Lipid Transport at ER-Vacuole Contact Sites in Yeast

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The distinct function and architecture of organelles are dependent on the wide repertoire of lipids that constitute their membranes. As most of the lipid synthesis machinery is confined to the endoplasmic reticulum (ER), interorganelle lipid transport becomes an essential process for organelle biogenesis and expansion. Membrane contact sites (MCSs) - 10-30 nm zones of close proximity between organelles – host lipid transport proteins (LTPs) and facilitate lipid exchange between organelles.

Previous studies have shown that, during nutrient stress, there is a massive expansion of ER-vacuole contact sites, with a concomitant recruitment of Vps13, a highly conserved LTP. These observations suggest an important role of ER-vacuole lipid transport during nutrient starvation. In this work, we aim to characterize the role of Vps13 in ER-vacuole lipid exchange and uncover the physiological consequences of blocking Vps13 activity at these contact sites.

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Exploring the Interplay: Mechanistic insight into the interaction of Amylin with Lipid droplets

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Experimental findings indicate that the main culprits triggering the pathological conditions associated with Type-II Diabetes Mellitus (T2DM) are intermediate protein aggregates of Amylin. These aggregates, known as amyloidogenic oligomers, exhibit a propensity to interact with cell membranes, leading to reciprocal structural disturbances. Various membrane surfaces promote the conversion of amyloid-forming proteins into toxic aggregates, which subsequently compromise cell membranes' structural integrity.

The pronounced hydrophobic characteristics inherent in oligomeric species create a heightened susceptibility to interactions with monolayer membranes, notably lipid droplets functioning as pivotal energy-storage components within cells. The potential consequences of membrane disruption extend beyond mere structural alterations, as it can significantly influence cellular homeostasis and disrupt the delicate balance of energy within the cell. Consequently, gaining mechanistic insights into these interactions becomes imperative to design therapeutic interventions that are not only effective but also precisely targeted to mitigate the complexities associated with Type-II Diabetes Mellitus (T2DM).

Despite advancements in our understanding, a critical gap persists in elucidating the intricate relationship between the structural configurations adopted by oligomers and the subsequent impairment of toxicity observed in diverse membrane systems. To address this gap, we employed a comprehensive array of biophysical techniques, including Circular Dichroism (CD), Thioflavin T (ThT) kinetics, Dynamic Light Scattering (DLS), Differential Scanning Calorimetry (DSC), and Nuclear Magnetic Resonance (NMR)-like approaches, along with microscopic methods such as Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM). Through these methodologies, we characterized the impact of toxic conformations on membrane permeabilization and elucidated the factors that enhance the aggregation of Amylin.

Our results not only contribute to a deeper understanding of the mechanisms underlying amylin-induced T2DM but also pave the way for the rational design of drugs aimed at mitigating the deleterious effects associated with Amylin aggregation. This research provides valuable insights into potential therapeutic strategies for addressing the molecular basis of T2DM.

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2. Myricetin encapsulated chitosan nanoformulation for management of type 2 diabetes: Preparation, optimization, characterization and in vivo activity

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Membrane protein sequence features direct post-translational insertion

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An essential step of membrane protein folding is the insertion of transmembrane helices (TMs) into the membrane. Multispanning membrane proteins typically insert into the membrane during translation via the Sec translocon. However, in many cases, translation terminates before the C-terminal transmembrane domain (cTM) can access the Sec translocon, necessitating an alternative post-translational insertion process. The principles governing this post-translational insertion mechanism are still not well comprehended.

Through computational analysis of membrane protein sequences across various organisms, we discovered that these sequences tend to favour smaller lengths and decreased hydrophilicity for the exocytosolic portion of the C-terminal tail. We experimentally validated our findings in E. coli, utilising a chemical labelling-based topology assay and site-specific mutagenesis on two proteins. We discovered that the insertion process could be significantly delayed by mildly increasing the hydrophilicity of the C-tail.

Changes in the C-tail sequence of human proteins may disrupt its proper insertion, leading to misfolding and genetic diseases. We found an enrichment of non-stop mutations in proteins with a short exocytosolic C-tail and confirmed experimentally that these C-tail extensions often result in the misinsertion and retention of proteins in the ER.

Further, we explored the bacterial machinery required for post-translational insertion using a knock-down system and single-transmembrane chimeric proteins. This strategy enabled us to identify YidC and SecDF as potential candidates for the machinery responsible for this insertion process. We confirmed our findings by demonstrating the physical interaction between our model proteins and YidC and SecDF.

These discoveries suggest that C-tail sequences have evolved to optimise the productive interaction with insertion machinery factors to ensure efficient post-translational cTM insertion and membrane protein folding.

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Proteotoxic stress induced clustering of the UPR sensor IRE1α; is driven by disordered regions within its lumenal domain

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Endoplasmic reticulum (ER) homeostasis is maintained by a conserved set of signaling cascades called the "unfolded protein response (UPR)". Physiological and pathological conditions that lead to the accumulation of misfolded proteins in the ER (proteotoxic stress) or perturbations of the ER lipid membrane composition (lipid bilayer stress) activate the UPR, which initiates a transcriptional program to restore ER homeostasis. The most conserved branch of the UPR is driven by the type I transmembrane kinase/RNase IRE1. During proteotoxic ER stress, IRE1 forms clusters on the ER membrane, yet the mechanistic base of IRE1 cluster formation remained largely unknown. Here we show that IRE1's lumenal domain (LD) undergoes phase separation in solution and formes clusters when reconstituted on supported lipid bilayers. A mutagenesis screen revealed specific residues within disordered regions of the LD facilitating multivalent transient interactions in vitro and in cells. Our data highlight the importance of multivalent weak interactions of lumenal disordered regions for IRE1 activity and shed light on IRE1 cluster formation. Using lipidomic profiling, we are currently determining how the ER lipid composition changes during proteotoxic ER stress to uncover the contribution of the lipid membrane environment on IRE1 activation.

Reference(s):
Stress-induced clustering of the UPR sensor IRE1 is driven by disordered regions within its ER lumenal domain (Preprint: https://www.biorxiv.org/content/10.1101/2023.03.30.534746v1)

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Chemical Interference with Phosphatidylinositol Transfer Protein Function in Pathogenic Candida glabrata: Prospects For Next-Generation Anti-Fungal Drugs

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Multidrug-resistant fungal “superbugs” impervious to all drugs approved for clinical use have emerged as a public health crisis. The alarming rise of these organisms has created a dire need for lead compounds directed against new fungal targets. Address of this need is the ultimate goal of my research. My research is based on our ability to interfere with the inositol lipid signaling pathways required for viability and virulence of major pathogenic Candida species. Our approach is based on small molecule inhibitors (SMIs) that target Sec14 phosphatidylinositol transfer proteins (PITPs) and inhibit fungal phosphoinositide signaling with exquisite selectivity. A unique aspect of targeting Sec14 is the SMIs do not target the highly conserved lipid kinases that drive phosphoinositide signaling pathways. Because a C. glabrata reference strain is sensitive to the Sec14-directed SMI that has been tested, I am using the rising superbug Candida glabrata as model to determine the biochemical basis for how SMIs of five chemotypes inhibit the Sec14 of C. glabrata. Genome sequence data from 22 clinical isolates of C. glabrata plus the reference strain report a nearly complete conservation of Sec14 among them. These data: (i) reinforce the idea that first-generation SMIs against fungal SEC14 are attractive anti-fungal lead compounds and (ii) identify C. glabrata as a valid model for assessing the potencies and efficacies of these chemically diverse SMIs (and derivatives) in relevant clinical contexts.

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Getting ready for intermembrane transfer: loading of phospholipids onto the lipid transporter MlaC

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The outer membrane of gram-negative bacteria is highly asymmetric, with its outer leaflet containing lipopolysaccharides and inner leaflet composed of phospholipids. This asymmetry must be actively maintained, as phospholipids spontaneously flip into the outer leaflet and render it more permeable to harmful substances including antibiotics. The maintenance of lipid asymmetry (Mla) system removes misplaced phospholipids from the outer leaflet of the outer membrane and transfers them back to the inner membrane. It is composed of the outer membrane lipoprotein MlaA, the periplasmic lipid transporter MlaC, and the inner membrane ABC transporter MlaFEDB. While ATP-driven phospholipid transfer at the inner membrane is comparably well-understood, little is known about the loading of MlaC with phospholipids at the outer membrane. Here we observe MlaA-mediated phospholipid transfer to E. coli MlaC and investigate protein-lipid interactions in detail using native mass spectrometry (MS). We demonstrate that loading of apo MlaC is significantly increased by MlaA, which concurrently recruits phospholipids and MlaC to facilitate lipid transfer. We show that the efficiency of lipid loading is high for endogenous E. coli lipids and low for exogenous lipids, both with respect to the lipid head group and acyl chains. As lipid binding to MlaA is relatively non-selective, we suggest that the observed binding specificity arises from MlaC, which only accepts lipids of suitable geometry. Furthermore, we demonstrate that lipids bound to MlaC in vivo are highly enriched in phosphatidylglycerols despite their low abundance in the outer membrane. Overall, our results provide detailed insight into the surprisingly specific lipid binding properties of the phospholipid transporter MlaC and the role of MlaA in the loading process.

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Disruption of cellular sphingomyelin gradients by nonsense and missense SGMS2 variants linked to skeletal dysplasia

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Sphingomyelin (SM) is a core component of mammalian cell membranes and preferential binding partner of cholesterol. A widely held view is that bulk production of SM in the trans-Golgi provides a sink for cholesterol synthesized in the ER to create a SM/sterol gradient along the secretory pathway. This gradient marks a fundamental transition in physical membrane properties that segregates early from late secretory organelles, allowing an adaptation from biogenic to barrier functions. We recently identified mutations in sphingomyelin synthase SMS2 that cause a rare form a skeletal dysplasia. Strikingly, all pathogenic SMS2 variants retain enzymatic activity but mislocalize to the cis-Golgi and/or ER. Combining organellar lipidomics with the use of lipid biosensors, we show that cells harbouring pathogenic SMS2 variants accumulate SM in the ER and display a disrupted transbilayer SM asymmetry, presumably owing to a constitutive SM scrambling across the ER bilayer. These aberrant SM distributions are accompanied by imbalances in cholesterol organization, glycerophospholipid profiles and lipid order along the secretory pathway. In parallel, we uncovered Ca2+-activated SM scrambling and turnover as a novel mechanism to drive an outward budding of cellular bilayers. Besides interfering with a timely release of bone critical proteins by undermining the biogenic function of the ER, we envision that pathogenic SMS2 variants may affect the formation of matrix vesicles by osteogenic cells, a process critical for bone mineralization. Our ongoing efforts focus on challenging these models.

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PtdIns4P is required for the autophagosomal recruitment of STX17 (syntaxin 17) to promote lysosomal fusion

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The autophagosomal SNARE STX17 (syntaxin 17) promotes lysosomal fusion and degradation, but its autophagosomal recruitment is incompletely understood. Notably, PtdIns4P is generated on autophagosomes and promotes fusion through an unknown mechanism. Here we show that soluble recombinant STX17 is spontaneously recruited to negatively charged liposomes and adding PtdIns4P to liposomes containing neutral lipids is sufficient for its recruitment. Consistently, STX17 colocalizes with PtdIns4P-positive autophagosomes in cells, and specific inhibition of PtdIns4P synthesis on autophagosomes prevents its loading. Molecular dynamics simulations indicate that C-terminal positively charged amino acids establish contact with membrane bilayers containing negatively charged PtdIns4P. Accordingly, Ala substitution of Lys and Arg residues in the C terminus of STX17 abolishes membrane binding and impairs its autophagosomal recruitment. Finally, only wild type but not Ala substituted STX17 expression rescues the autophagosome-lysosome fusion defect of STX17 loss-of-function cells. We thus identify a key step of autophagosome maturation that promotes lysosomal fusion.

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Visualizing Suborganellar Lipid Localizations using a novel Lipid-CLEM approach

Visualizing Lipid Sorting in Endosomes using a novel Lipid Correlative Light and Electron Microscopy Approach

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The membranes of mammalian cells feature a complex composition of over 1000 chemically distinct lipid species. Such differential lipid distributions provide specific membrane shapes and functionalities that are essential for organelle identities and hence cellular function. A major challenge to understand the function of individual lipids in cellular organelles is the lack of methods to study lipid behaviour in a cellular context. Importantly, lipids are extremely sensitive towards structural changes and thus cannot faithfully be visualized by direct tagging with fluorophores. Utilizing bifunctional (crosslinkable and clickable) lipid probes can help mitigating this problem. Moreover, to visualize lipids in their corresponding membrane context at sub-organelle scales, the resolution of electron microscopy is required. To address these challenging requirements, we developed a novel workflow in collaboration with the Avinoam lab that allows us to image lipid species using correlative light and electron microscopy (Lipid-CLEM). This approach was designed to preserve the near native behaviour of the lipid species of interest by introducing the fluorescent label post sample processing. Using Lipid-CLEM, we were able to demonstrate suborganellar lipid segregation from endocytosed protein cargo in endosomes. Following, we aim to unravel whether lipids are sorted differentially in endosomes in dependence of structural differences such as the head groups, fatty acid saturation and chain length. We believe this approach will be a valuable tool for the membrane community to study specific lipid species at sites of functional membrane domains.

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Mitochondrial potassium ion channels present in Guinea Pig cardiomyocytes and modulation of their activity

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Potassium ion channels are very abundant and diverse group of proteins. So far, they have been identified in many cellular organelles: nucleus, plasma membrane, ER and mitochondria.
Mitochondrial potassium (mitoK) channels present in the inner mitochondrial membrane (IMM) are mainly responsible for transporting potassium ions into mitochondrial matrix. They are, however, not limited to this function. These proteins, through mild depolarization of IMM, prevent the overload of mitochondria with calcium ions, averting mitochondrial damage and even cell death. It has been shown that the mitoK channel activation has a cytoprotective effect, reducing the negative outcomes of ischemia-reperfusion injury (I/R). There are several ways to regulate the activity of mitoK channels. The most popular is pharmacological one. Potassium channels' modulators are also commonly used to confirm specific channel type. In our laboratory we examine a new approach - photobiomodulation. Within the cell, next to chloroplasts, mitochondria are the main light absorbers. In guinea pig myocardium we have observed channels with features of both ATP-sensitive (mitoK ATP) and large conductance Ca 2+ -activated (mitoBK Ca) channels. Our team confirmed the presence of mitoBK Ca at the molecular and protein level by PCR and Western-blot analyses, respectively. In spite of untypically low conductance (130 pS), in patch-clamp recordings we noticed that channel was voltage-sensitive, responsive to Ca 2+ ions and was blocked by specific mitoBK Ca inhibitor, paxilline. Furthermore, we have detected that the mitoBK Ca channel activity decreased in response to oxidizing agent K 3 [Fe(CN) 6]. It was remarkably brought back by illumination with infrared (IR) light. The applied wavelength, 820 nm, is the maximum absorbance of the oxidised cytochrome c oxidase Cu A centre. Although, further expertise is needed to understand how IR radiation affects the mitochondrial function and proteins present in IMM, the interaction between mitoBK Ca channel and electron transport chain complexes appears to be part of the protective mechanism of IR photobiomodulation.
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mitoBKCa is functionally expressed in murine and human breast cancer cells and promotes metabolic reprogramming

Redox regulation of mitochondrial potassium channels activity (under review)

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Characterization of MLKL clusters in the plasma membrane during necroptosis

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Necroptosis is a form of regulated cell death characterized by perturbation of the plasma membrane and the release of intracellular contents that can induce and propagate inflammatory responses. It is a regulated process in which the activation of receptor-interacting protein kinases(RIPKs) leads to the phosphorylation and activation of mixed lineage kinase domain-like protein (MLKL) leads to oligomerization of MLKL, which serves as the executor of necroptosis; however, the molecular mechanisms by which MLKL interacts with the plasmatic membrane and drives necroptosis are not well understood.

We will use super-resolution microscopy of endogenous MLKL to unravel the protein structures formed during necroptosis. To this end, we will generate mouse fibroblasts and human macrophages, where MLKL is endogenously tagged with a HALO-tag and mGFP using CRISPR/Cas9 technology. Once the cell lines are established, total internal reflection fluorescence microscopy (TIRF), and stochastic optical reconstruction microscopy (STORM) will be used to characterize the dynamics and stoichiometry of MLKL in the plasma membrane during necroptosis. Furthermore, we will compare the plasma membrane of healthy and necroptotic cells using Cryo-EM to uncover the plasma membrane reorganization that takes place during this form of cell death.

Finally, we will correlate the single-particle stoichiometry with super-resolution DNA-PAINT to determine the oligomeric state of MLKL in correlation with the nanoscale structural organization of the individual MLKL particles and the ultrastructure and alterations of the plasma membrane associated with the same given particle.

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Oxidation in Phospholipid Model Membranes Induces Lateral Disorganization

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The integrity of the plasma membrane is essential for cellular homeostasis and function, serving as a barrier that regulates the exchange of molecules and signals between the cell and its environment. Recently, ferroptosis has emerged as a distinctive mode of programmed cell death characterized by the accumulation of oxidized phospholipids, particularly within the plasma membrane and evoking plasma membrane damage. Therefore, understanding the interplay between oxidized phospholipids and alterations in biophysical membrane properties is imperative for unravelling the underlying mechanisms of ferroptosis. Given the crucial role of oxidized lipid species in plasma membrane breakdown during ferroptosis execution, our study aims to investigate the integrity and organization of lipid-lipid interaction in minimalistic model membranes. Induced lipid oxidation by a Fenton-like reaction show alterations in domain morphology within supported lipid bilayers exhibiting liquid-ordered (Lo)/liquid-disordered (Ld) phase coexistence. Additionally, atomic force microscopy (AFM) force measurements reveal a significant reduction in the force necessary to puncture the membrane when it is in the oxidized state, suggesting a decrease in lipid packing and acyl chain ordering. Another noteworthy finding is the observed impact on permeability in lipid vesicles and fluidity in supported lipid bilayers. Our findings suggest a decreased stability of membranes in presence of oxidized lipid species and a lateral reorganization in fluid phase coexistence in model membranes. The relation of local membrane alterations and ferroptosis execution remain an open question and will be addressed in future work.

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Linking the liquid and structural parts of endocytic machinery

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Clathrin-mediated endocytosis (CME) is spatially and temporally regulated by a choreographed interaction of over 50 proteins though the precise mechanisms of this intricate network remains poorly understood. In budding yeast, the early phase of CME is variable in duration (1-3 min). During this time the early proteins cluster at the plasma membrane and define the endocytic site. Upon the recruitment of the late coat proteins and the actin regulators the endocytic pathway becomes quite regular (30- 35 sec).

Recently, one of the early arriving yeast endocytic protein i.e. Ede1 has been shown to form liquid condensates upon over-expression. These condensation properties of Ede1 are proposed to catalyse the initiation of endocytosis. From the over-expression screen of multiple endocytic proteins, we observed that Pan1, a late coat protein, generates a liquid phase in vivo. These condensates can specifically recruit other endocytic proteins. Strikingly, for more than a four decades, many components of the endocytic machinery have been known to be highly structured e.g. Clathrin.

This project aims to understand the interface between the structural and the liquid components of endocytosis. I propose that a set of clathrin adaptor proteins act as as the linker. I observed that the deletion of this link leads to a very complex phenotype where endocytic machinery behaves in highly abnormal manner and the temporal organization of early and late phase is lost.

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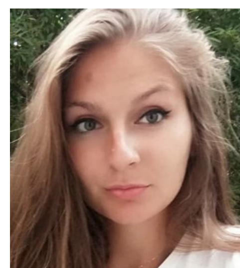

OCIAD1 controls the levels of TIMM17A-containing TIM23 translocase in human cells

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The biogenesis of mitochondria relies on efficient import of the proteins from the cytosol to the organelle. The mitochondrial precursor proteins that are destined to the mitochondrial matrix and, to some extent, inner membrane, utilize the Translocase of the Inner Membrane (TIM23) for their translocation. While the TIM23 complex in yeast was extensively studied over the past decades, we know very little about the human counterpart. The translocating core of the TIM23 complex in yeast constitutes of Tim17 and Tim23; and Tim17 was recently found to play a primary role in protein translocation. Human cells express two orthologs of yeast Tim17, i.e. TIMM17A and TIMM17B. Upon various stressors, TIMM17A was found to be fastly degraded by YME1L to decrease mitochondrial import and rewire mitochondrial metabolism in order to adapt to environmental conditions, whereas the levels of TIMM17B remained unchanged. Such a specific regulation of the two translocase variants denotes the presence of intrinsic regulatory factors, however, these have not been found yet. Here, we show that both TIM23 complex versions are stabilized by the prohibitin complex. Depleting prohibitins resulted in significant loss of both translocase pools. Moreover, we report an unexpected role of ovarian cancer immuno-reactive antigen domain containing 1 (OCIAD1) in controlling the levels of the TIMM17A-containing TIM23 complexes. OCIAD1 depletion lead to reduction of the TIMM17A version of the translocase, while the TIMM17B version was unchanged. In summary, we postulate that OCIAD1 and prohibitins constitute a novel regulatory axis of the human TIM23 complex.

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Understanding the Role of Mammalian PITPNC1 in Melanoma

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PITPNC1, a member of under investigated mammalian phosphatidylinositol transfer proteins (PITPs) is overexpressed in several metastatic tumors including breast, colon, pancreatic and melanoma. Prognosis is frequently inversely related to PITPNC1 expression levels, and in melanoma patients, higher PITPNC1 expression is associated with reduced survivability. The tumor suppressor micro-RNA miR-126 also identifies PITPNC1 as a crucial target, of disease relevance. However, to date, studies addressing a cancer role for PITPNC1 have been inadequate and have failed to address the consequences of complete PITPNC1 knockout or an immunocompetent environment thus, addressing these issues has been our primary focus. Our research demonstrates that PITPNC1 knockout mice are born alive and do not display overt phenotypes, potentiating its importance as a possible drug target. To understand the role of PITPNC1 in the context of melanoma, we employed a syngeneic murine melanoma model in which B16 melanoma cells were introduced to immunocompetent mice by subcutaneous injection. Our data indicate PITPNC1 expression levels correlate with tumor aggression in murine melanoma and stable PITPNC1 overexpression in a low metastatic B16F0 cell line dramatically enhances tumor aggression and metastasis. Furthermore, tumor proliferation was drastically reduced when highly aggressive B16F10 cells were implanted in a PITPNC1 knockout host background or when PITPNC1 lacking cells were injected into mice. In a complete null-null condition, tumor growth was unsupported indicating for the first time that PITPNC1 plays critical roles by both the cells and the host's contribution to tumor development. Further, we report that genetic ablation of PITPNC1 in highly aggressive melanoma cells results in mitochondrial bioenergetic dysfunction and distorted morphology. Preliminary lipidomic screening suggests compromised fatty acid oxidation which potentially underlies mitochondrial dysregulation in the PITPNC1 null condition. These findings represent a major advance in the understanding of PITPNC1 biology and its role in melanoma cancer.

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Mechanism for Vipp1 spiral formation and ring biogenesis on membrane repair.

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Vesicle-inducing protein in plastids 1 (Vipp1) is a ubiquitous plastid component found in cyanobacteria, algae, and plants. It plays essential roles in thylakoid membrane biogenesis and repair. Recently, Vipp1 has been identified as a homolog of ESCRT-III (endosomal sorting complex required for transport-III), a protein family known for constructing dynamic filaments that remodel membranes. However, the process by which these planar filaments transition into 3D membrane budding structures remains unclear. In this study, we combined Fast-Atomic Force Microscopy (F-AFM) and Cryo-EM to elucidate the biogenesis pathway of Vipp1.

Vipp1 accumulated at the edge of supported lipid bilayer (SLB) patches, indicating a sensing capability for highly curved or perturbed membranes. Here, it grew as dynamic planar filaments that curled counterclockwise to form spirals. These spirals then curled inwards until the filaments reached a curvature limit, resulting in filament merging and the formation of closed rings.

Given the structural conservation between Vipp1 and ESCRT-III, our results may represent the general geometric changes required for certain ESCRT-III filaments to switch between two-dimensional and three-dimensional forms.

Reference(s):
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The AAA ATPase Vps4 in ESCRT-III-mediated membrane remodeling

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The membrane remodeling done by Endosomal Sorting Complexes Required for Transport (ESCRT) is one of the key steps toward membrane fission and fusion which are utilized in many cellular events. This is done most likely via the polymerization of ESCRT-III proteins into helices or spirals at points of membrane budding. However, the role of a crucial component of the ESCRT machinery is poorly understood: Vps4, an AAA ATPase that reorganizes membrane-bound, polymerized ESCRT-III subunits. A recent hypothesis is that Vps4 mediates a dynamic subunit turnover by orchestrated removal and addition of individual subunits, which in turn alters the geometry and physical property of ESCRT-III filaments to adjust the membrane expected to undergo fission dynamically. For the yeast ESCRT-III system, the dynamic subunit turnover follows a certain order, but the determining factors for this order orchestrated by Vps4 are not fully understood. We aim to elucidate the function of Vsp4 and its interplay with ESCRT-III subunits in the context of dynamic subunit turnover and membrane fission events that occur during vesicle budding and late cytokinesis. It has been suggested that the interaction between the Vps4-MIT domain and ESCRT-III MIM domain could be one of the determining factors for which ESCRT-III subunit will be removed from the ESCRT-III complex during the dynamic subunit turnover.

We want to describe this role of Vps4 in more detail using in vitro reconstitutions. For this, we will purify recombinant wild-type Vps4 with a TEV-cleavable GST-tag from E.coli through affinity purification. We optimized the purification protocol for Vps4 to achieve high purity while maintaining its enzymatic activity. To assess the ATPase activity of Vps4, we established Malachite Green Phosphate Assay and measured the ATPase activity in the presence of different ESCRT-III subunits that are already being used in the lab. We find that certain ESCRT-III subunits increase Vps4 activity, whereas others do not.

To correlate Vps4 activation with ultrastructural ESCRT-III reorganization, we aim to generate chimeric ESCRT-III subunits (i.e. Snf7 with the MIM domain of Vps2) and assess their effect on Vps4 ATPase activity. We plan to test this effect also in the presence of LUVs (large unilamellar vesicles) and flat lipid surfaces. In parallel, we will use cryo-EM to visualize the structural changes in the ESCRT-III complex formed by chimeric subunits on membrane surfaces, whereas with cryo-ET we will investigate the structural changes on the membrane during the ESCRT-III filament formation and elongation. Later on, we also aim to conduct in vivo experiments with yeast cells to test our hypothesis.

The goal of our project is to increase the knowledge of how ESCRT-III and Vps4 achieve membrane remodeling and budding. Furthermore, by better understanding this mechanism, Vps4 may be investigated further to study cellular events such as cytokinesis in mitosis where membrane remodeling is crucial. Our findings can pave the way for further research on ESCRT-III machinery in humans.

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The p97-UBXD8 Complex Maintains Peroxisome Abundance by Suppressing Pexophagy

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Peroxisomes are ubiquitous organelles that are integrated into essential metabolic functions of eukaryotic cells. Their importance is further underscored by the occurrence of peroxisome biogenesis disorders; serious early childhood pathologies that are often fatal and characterized by altered lipid metabolism. The evolutionarily conserved AAA-ATPase p97 and its membrane embedded adaptor UBXD8 are essential for maintaining protein homeostasis at the ER via ER-associated degradation. In ERAD, the p97-UBXD8 complex recognizes ubiquitin-tagged misfolded proteins in the ER, which are retro-translocated to the cytosol, and delivered to proteasome for degradation. In quantitative proteomic studies comparing the proteomes of wildtype and UBXD8 null cells, we find that loss of UBXD8 perturbs the abundance of numerous peroxisomal proteins. Furthermore, from lipidomics analysis we identified an increase in VLCFAs and a decrease in cholesterol in UBXD8 knockout compared to wildtype cells. Interestingly, it is observed PBD patients accumulate VLCFAs and have consistently reduced cholesterol plasma levels. We have identified a significant decrease in peroxisome number and an increase in peroxisome size in UBXD8 KO cells relative to WT cells. We were further able to rescue this aberrant peroxisome phenotype by complementing UBXD8 KO cells with wildtype UBXD8. Although the mechanism by which UBXD8 regulates ERAD is well understood, its role in peroxisome function is completely unknown. Here I have identified a new role for the p97-UBXD8 complex controlling peroxisome abundance and function by suppressing their degradation through autophagy.

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Direct Targeting of Protein-Membrane Interactions

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Protein-membrane interactions (PMIs) are ubiquitous in cellular signaling. Early transduction modules often rely on transient and dynamic interactions with the inner plasma membrane leaflet to populate signaling hotspots. Methods to target and modulate these interactions could yield many attractive tool compounds and drug candidates. Here, we demonstrate that the conjugation of a medium chain lipid tail to the covalent K-Ras(G12C) binder MRTX849 at a solvent exposed site enables direct modulation of PMIs. The conjugated lipid tail extends to the inner leaflet of the plasma membrane and restricts the lateral mobility of K-Ras(G12C) in live cells. In addition to changing its diffusion behavior, this PMI modulation also affects the relative orientation and conformation of K-Ras(G12C) on the membrane as shown by NMR studies and MD simulations. Finally, we demonstrate that our compounds disrupt K-Ras nanoclustering, which is an essential process for K-Ras signaling. The described strategy could be broadly applicable to the modulation of protein-membrane interactions.

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Direct Modulators of K-Ras–Membrane Interactions

Optical Control of Membrane Fluidity Modulates Protein Secretion

Optical control of sphingosine-1-phosphate formation and function

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Using Artificial Cargo to Pre-deform Liposomes and Understand the Recruitment of ESCRT-III Machinery

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Important cellular events like cytokinesis, vesicular trafficking, damage repair, and many others require the membranes to be severed, fused, or deformed at designated sites. The structurally conserved ESCRT-IIIs are predominantly involved in membrane deformation away from the cytoplasm or excluding cytoplasm, as in the formation of intraluminal vesicles inside multi-vesicular bodies (MVBs). Generally, in yeast, membrane remodeling begins with the ESCRT cascade comprised of the ESCRT – 0, I, and II. The protein Vps20 acts as a nucleator and initiates the formation of membrane-binding ESCRT-III polymer chains, with progressive recruitment of different subunits in the order of Snf7, Vps24, Vps2, Did2, and Ist1. The Vps4 ATPase dynamically remodels the polymeric ESCRT-III filaments by removing subunits, allowing for the insertion of different subunits into the filament, ultimately leading to the scission of the constricted membrane. In addition to their physiological functions, the ESCRT-III is also recruited by certain viruses like HIV-1 and Ebola to bud off virions in a pathophysiological context. This study aims to elucidate the role of ESCRT-III/Vps4 machinery in vesicle formation and fission mimicking viral budding that I plan to reconstitute in vitro.
I use I3-01 artificial self-assembling protein nanocages developed initially by Hsia et al. 2016 from KPDG aldolase to reconstitute the initial stages of membrane deformation. These synthetic 22 kDA monomers can spontaneously polymerize to form a 60-mer shaped as 26 nm wide dodecahedrons (McCarthy et, al. 2022) and act as an inert form of scaffolding cargo. Every complete self-assembled nanocage has hexahistidine tags for purification but can double as membrane-binding motifs to target non-physiological nickel-presenting lipids (DOGS-Ni-NTA). Hexa and deca histidine tag-attached nanocages showed high binding affinity towards 10% NiNTA-DGS containing liposomes after a short incubation period. Though the binding was strong enough to cause tethering of adjacent liposomes, no significant number of nanocage-enclosed invaginations could be achieved. I used membrane-interacting protein domains like the C2 domain to target membranes independent of Nickel lipids. To increase the propensity of creating more invaginations and decrease the reliance on non-physiological lipids, I changed the membrane binding motif to a calcium-independent C2 domain from the Bovine Lactadherin protein, which binds strongly to PS lipids. C2 domains generally rely on hydrophobic interactions, which are also required to self-assemble the nanocages. We hypothesized that some steric hindrance from the C2 domain stopped the formation of complete 60-mer nanocages. To express and link the nanocages separately, I adopted the FKBP-FRB system, which efficiently creates heterodimers in the presence of Rapamycin. The nanocage subunit and the FKBP domain successfully formed complete nanocages, verified by negative stain TEM imaging. Preliminary data shows a successful purification of FRB-LactC2, but more binding studies are needed to confirm dimer formation. Once optimized, the synthetic nanocages with FKBP domains can act as a simple yet effective tool to attach several other membrane-binding domains to our synthetic cargo. Once I achieve the formation of omega-shaped invaginations in sufficient numbers, I can analyze the shape of the membrane neck curvatures. I intend to add Snf7, the first ESCRT-III protein recruited in every remodeling event, and investigate whether it preferentially localizes to the invaginated necks. Gradually, the remaining components of the ESCRT-III machinery will be incorporated to examine the release of the omega-nanocages from inside the liposomes, ultimately yielding a full reconstitution of the essential ESCRT-III dynamics.

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Lipid droplets affect ferroptosis sensitivity through polyunsaturated fatty acid sequestration and release

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Lipid droplets (LDs) are neutral lipid storage organelles that participate in diverse aspects of lipid metabolism and cellular functions. The role of LDs in regulating the availability of fatty acids for incorporation into membranes and its impact on ferroptosis sensitivity is still unclear. Our findings demonstrate that LDs modulate ferroptosis sensitivity in a context- and cell-type-dependent manner. We found that breast cancer cells with compromised ferroptosis surveillance mechanisms undergo lipidome remodelling resulting in enrichment of LDs with polyunsaturated fatty acids (PUFAs) and protection from ferroptosis. This activity was dependent on diacylglycerol acyltransferase 1 (DGAT1)-driven triglyceride synthesis, which lowered membrane phospholipid PUFA content and reduced membrane lipid peroxidation. To find out whether the release of fatty acids from LDs modulated ferroptosis sensitivity, we specifically targeted the two main mechanisms of LD breakdown, lipolysis and lipophagy. Blocking triglyceride lipolysis by depletion of adipose triglyceride lipase (ATGL) led to LD accumulation, which lowered PUFA-phospholipid content and supressed lipid peroxidation and ferroptosis. This protection occurred both under nutrient replete and starvation conditions when LD breakdown is more active. ATGL overexpression, or genetic depletion of its endogenous inhibitor, promoted ferroptotic cell death. These experiments suggest that ATGL-mediated liberation of PUFAs from LD-stored triglycerides enhances ferroptosis sensitivity through changes in membrane composition. We observed a similar, but context-dependent role for lipophagy—a selective type of autophagy that delivers LDs to lysosomes for degradation. The impairment of bulk (macro)autophagy in nutrient replete conditions by depletion of Unc-51 like autophagy activating kinase 1 (ULK1) led to LD accumulation and protection from ferroptosis, suggesting that basal levels of autophagy/lipophagy contribute to ferroptosis in nutrient abundance. On the other hand, blocking lysosomal lipid degradation, which occurs in the late stages of lipophagy, through silencing of lysosomal acid lipase (LAL), did not alter ferroptosis sensitivity in nutrient replete conditions, suggesting that basal lipophagy is not involved in ferroptosis regulation. However, depletion of LAL during serum starvation, when lipophagy is highly active, reduced ferroptosis sensitivity. Our findings demonstrate that LDs display a context-dependent role in ferroptosis modulation, which depends on the cellular state, nutrient availability and the balance between LD biogenesis and breakdown mechanisms, which control the trafficking of PUFAs between membranes and LDs. Our findings suggest that targeting LD metabolism is a valid approach for controlling ferroptosis in cancer and beyond.

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Adaptive responses provoked by chronic ER-stress in neuronal cells – linking ER quality control, metabolic Warburg effect, and lysosomal dysfunction

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Alzheimer's disease (AD), the most common form of dementia, has been studied intensively in the last decades; however, pathogenetic mechanisms remain ambiguous and the exact causes of this disease are still unknown. ER stress and the unfolded protein response (UPR) are commonly observed in AD due to protein deposits including extracellular amyloid β; plaques and intracellular neurofibrillary tangles. Interestingly, several brain regions are able to resist disease-associated pathological changes and neurodegenerative processes. To imitate challenges occurring in pathophysiology, we generated neuronal cell lines (HT22) that are resistant to chronic ER stress as induced by disrupting Ca2+ homeostasis via thapsigargin (TgR) or inhibiting glycosylation using tunicamycin (TmR), respectively. Only TgR cells showed an increased LC3-II flux indicative of a generally enhanced autophagic activity under basal conditions compared with HT22 wild type (WT) cells, whereas both resistant cell lines upregulated ER-phagy, a selective form of autophagy, to maintain ER homeostasis and ER organelle quality control. The expression of one of the known ER-phagy receptors, the long isoform of Reticulon-3, was increased and translocated to lysosomes in both TgR and TmR cells. Excitingly, in ER stress-resistant cells, we observed a strongly increased number of autophagic vesicles and also "giant" lysosomes (diameter > 1500 nm) with decreased overall degradative lysosomal activity. Surprisingly, the "giant" lysosomes were packed with misfolded proteins but also free cholesterol. A full proteome analysis revealed alterations in protein expression linked to numerous cellular pathways, which underlined the overall significant impact of chronic ER stress and the adaptive response of the resistant cells to it. Intriguingly, ER stress-resistant HT22 cells relied on aerobic glycolysis (metabolic shift, 'Warburg effect'), in line with an impaired complex I of oxidative phosphorylation. In addition, chronic ER stress activated the UPR differentially, triggering IRE1α; and ATF6 signaling pathways. Taken together, this work unveils the efficient adaptability of neuronal cells towards chronic ER stress to maintain cell survival and, moreover, reveals a mechanistic link between chronic ER stress, Warburg effect, and lysosomal dysfunction as observed in AD pathogenesis.

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Optical Control of Membranes Enabled by Photolipids

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Cell membranes are composed of a diverse array of lipids and other components, essential for a range of cellular functions and activities. These components' dynamic composition undergoes constant reorganization, influencing key biophysical properties such as fluidity, permeability, and domain formation. Achieving precise control over these membrane properties is challenging using conventional methods, which involve drastic and often irreversible changes of environmental conditions like ion concentration, pH, and temperature.

In this context, we present a method to modulate membrane properties through optical control, utilizing photoswitchable lipids, referred to as photolipids. These molecules contain an azobenzene group in their hydrocarbon tails, which allows for cis/trans isomerization upon exposure to specific wavelengths of light. We demonstrate that this mechanism allows one to control membrane characteristics such as diffusivity, domain formation, permeability, and fusion in model bilayer systems and even protein transport in cellular membranes. Importantly, these changes can be achieved with high temporal precision, offering a novel approach to the study and manipulation of membrane properties and cellular function. Overall, this approach offers a reversible and non-invasive method to study and manipulate artificial and cellular membranes, highlighting its potential for future research and therapeutic strategies.

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Lipid scrambling is a general feature of protein insertases

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Glycerophospholipids are synthesized primarily in the cytosolic leaflet of the endoplasmic reticulum (ER) membrane and must be equilibrated between bilayer leaflets to allow the ER and membranes derived from it to grow. Lipid equilibration is facilitated by integral membrane proteins called "scramblases". These proteins feature a hydrophilic groove allowing the polar heads of lipids to traverse the hydrophobic membrane interior, similar to a credit-card moving through a reader. Nevertheless, despite their fundamental role in membrane expansion and dynamics, the identity of most scramblases has remained elusive. Combining biochemical reconstitution and molecular dynamics simulations, we show that lipid scrambling is a general feature of protein insertases, integral membrane proteins which insert polypeptide chains into membranes of the ER and organelles disconnected from vesicle trafficking. Our data indicate that lipid scrambling occurs in the same hydrophilic channel through which protein insertion takes place, and that scrambling is abolished in the presence of nascent polypeptide chains. We propose that protein insertases could have a so-far overlooked role in membrane dynamics as scramblases.

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The role of polyamines in metabolic elasticity

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Polyamines are an evolutionary conserved class of metabolites and involved in many cellular processes. Our lab has previously shown that dietary supplementation of the polyamine spermidine extends the health and lifespan of various species, including Drosophila. In flies, spermidine supplementation impinges on phospholipid profiles and triglyceride levels (PMID: 25010732), indicating that spermidine (or polyamines in general) might regulate lipid metabolism. Polyamine pathway mutants also exhibit increased weight and elevated triglyceride storage (PMID: 31870547). Recent research indicates that in obese organisms, the "metabolic elasticity," which is the ability to adjust metabolic processes in response to changing nutrient availability, is compromised. (PMID: 37625407)

Here, we aim to investigate how polyamines contribute to metabolic elasticity. To achieve this, we implemented genetic and dietary interventions within a Drosophila model to manipulate the polyamine pathway. These flies with altered polyamine pathway were then examined for the metabolic response under changing nutrient conditions. This study will provide new insight into the interaction between polyamines and metabolic reprogramming in response to differing nutrient availability.

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Mechanochemical modulation of forces in membrane tethering and fusion

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Organelle membranes must be able to fuse to transport molecules inside the cell. However, short vesicle dwell times on target membranes and a high-energy barrier for membrane fusion pose a challenge. The cell solves these challenges by specific binding of tethering proteins to increase dwell time and generating forces with the molecular membrane-fusion machinery to lower the energy barrier. Despite the identification of necessary and sufficient components for membrane tethering and fusion, the orchestration of the process and the mechanics of force generation remain elusive. Thus, cooperativity, such as cooperative kinetics, autocatalysis and synchronization of the molecular complexes consisting of small GTPases, regulators and effectors, must be investigated not only for interactions but also for their ability to generate forces that drive tethering and fusion.

Here, we present our approach from a single molecule study to the collection of molecules in understanding the tethering to the fusion of organelle membranes. I will present our current finding of identifying a two-component molecular motor comprising of long coiled-coil tethering protein EEA1 and small GTPase Rab5 that generates forces to bring vesicles closer to target membranes. These long tethers are in a brush-like formation on early endosomes, and our results show that this brush-like formation enhances the vesicle dynamics on the membrane and thus has implications in vesicle sorting. Finally, to understand the complete molecular machinery driving the tethering and fusion of a native organelle membrane, I will present our strategy to use macrophages' ability to phagocytose particles and then purify the phagosomes containing beads. The optical force spectroscopy performed on the phagosome-coated beads will allow us to measure the forces, distances, and duration of tethering, docking, and fusion between native membranes. Ultimately, this study provides fundamental insights into the underlying mechanisms of mechanochemical transduction of forces driving membrane tethering and fusion.

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1. Two-component molecular motor driven by a GTPase cycle.

2. An endosomal tether undergoes an entropic collapse to bring vesicles together

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Structural studies for inhibition of microsomal triglyceride transfer protein

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Microsomal triglyceride transfer protein (MTP) plays a significant role in the transfer and metabolism of lipids. These lipids, including triglycerides, cholesteryl esters, and phospholipids, are transported by MTP to apo-lipoprotein B. It has been reported that mutants or deletants occurring in MTP can result in abetalipoproteinemia disease, a rare autosomal recessive disorder, leading to abnormal absorption of lipids and vitamins by intestines and liver causing malnutrition. Inhibition of MTP can restrict the secretion of VLDL in the liver and chylomicrons in intestines. The approved drugs by food and drug administration which can be used in controlling malfunctioning of MTP include lomitapide (Juxtapid™) for human.

We solved the first crystal structure of MTP in complex with its inhibitor lomitapide at 2.9 Å resolution. The lomitapide molecule replaces the PEG molecule which was earlier reported in the published MTP-PDI structure (PDB code: 6I7S) and fills the lipid binding cavity of MTP. The crystal structure of MTP-bound to inhibitor shows the interaction of lomitapide molecule with MTP residues (V664F, V778L, F813A, S815L and F819A). A significant conformational change for MTP residue F813 was observed upon the binding of the inhibitor to MTP. Lomitapide is composed of multiple aromatic groups which may repel the aromatic group of F813 and cause conformational changes. Furthermore, a disruption of alpha-helical region into a disorder loop near the enter/exit site of ligands was also observed in the lomitapide bound structure.

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The early autophagosome machinery is involved in the formation of coronavirus replication organelles

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Coronaviruses like SARS-CoV-2 are enveloped, single-stranded, positive-sense RNA viruses. In order to support their replication, they hijack a range of host cell metabolic pathways and resources. For example, they induce the extensive remodelling of ER membranes to generate viral replication organelles (ROs) as a platform for efficient viral RNA synthesis. Although the ultrastructure of coronavirus ROs has been studied in detail, there is limited information on how they are formed and the host factors required for their generation. However, some recent publications have shown that host factors of the autophagosome formation machinery, such as Vps34, TMEM41b, and VMP1, might play a role in the biogenesis of viral ROs. Due to the overall significance of host membranes and lipids in viral replication, our studies initially focused on the pharmacological inhibition of sterol regulatory element binding proteins (SREBPs), which are important transcription factors that regulate the expression of enzymes participating in lipid metabolism. To this end, we employed the small-molecule SREBP inhibitor AM580. Treatment of coronavirus-infected cells with this compound led to a 200-fold reduction of viral RNA replication, while electron microscopy revealed that formation of viral ROs was prevented. Interestingly, while our experiments showed that SARS-CoV-2 and MERS-CoV replication induced an increase in the transcription of TMEM41b, VMP1, Vps34 and Atg2A genes, treatment of infected cells with AM580 prevented their transcription. Furthermore, pharmacological inhibition of SREBPs changed the lipidomic profile of coronavirus-infected cells, which could be a result of the decreased presence or activity of some of these host factors as they play various roles in the intracellular distribution of lipids across membranes. Knockdown studies of TMEM41b and VMP1 led to similar observations and further experiments are under way to understand whether host factors of the early autophagosome machinery participate directly as structural components of the viral ROs or indirectly by promoting a lipid distribution that facilitates RO formation. We also aim to disentangle potential connections between SREBPs and the early autophagosome formation machinery.

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The centronuclear myopathy protein and phosphoinositide phosphatase MTMR14 regulates lysosomal repair

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Myotubularin-related protein 14 (MTMR14) is one of the catalytic active proteins of the myotubularin family, which dephosphorylates PtdIns(3)P and PtdIns(3,5)P2. MTMR14 is an evolutionary conserved protein and has been shown to participate in various biological processes, including lysosome regulation, and autophagy. Mutations in MTMR14 lead to the human genetic disease centronuclear myopathy. However, its intramolecular regulation and membrane recruitment mechanisms are still unclear. In the present study, we attempted to explore the recruitment of MTMR14 to the membrane as well as the underlying molecular mechanisms. We found that lysosomal damage is a potent trigger of MTMR14 recruitment and that MTMR14 is required for efficient lysosomal repair. Specifically, a C-terminal alpha helix is necessary and sufficient to recruit MTMR14 and this process is dependent on cytosolic Ca2+ and sphingomyelin exposure to the cytoplasm. Following recruitment to damaged lysosomes, MTMR14 becomes ubiquitinated and degraded. In line with MTMR14 recruitment and degradation, we found that biphasic metabolism of PtdIns(3)P, the substrate of MTMR14, plays a pivotal role in lysosomal repair and that MTMR14 is required for full lysosome recovery after damage.

Reference(s):
A phosphoinositide signalling pathway mediates rapid lysosomal repair

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Failure in dissociating contacts between Climp63 and microtubules in mitosis causes chromosome mis-segregation and micronucleation

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The endoplasmic reticulum (ER) is a complex membrane system that is continuously remodeled. Its characteristic shape is generated by ER-resident proteins that directly affect membrane curvature and fusion, while interactions with the microtubule (MT) network are critical for global ER positioning. During mitosis, the ER undergoes drastic rearrangements, in which MT-ER interactions are broken to ensure the proper segregation of chromosomes. Mechanisms that regulate ER-MT tethers throughout mitosis are poorly understood. The ER-shaping protein Climp63 is known to bind MTs in a phosphorylation-dependent manner. In this study, we observed that expression of a phosphodeficient Climp63 mutant results in severe mitotic defects, caused by persistent ER-MT interactions. At mitotic exit, cells fail to properly enclose all chromosomes into a single nucleus, leading to excessive micronucleation. Interestingly, all micronuclei contain all tested nuclear envelope components, suggesting that NE reformation is not affected, as is the case for lagging chromosomes. Taken together, our study shows that aberrant Climp63 activity and expression severely affects mitosis, consistent with the poor prognosis associated with Climp63 overexpressing cancers.

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A proteome-wide yeast degron collection for the dynamic study of protein function

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The use of genome-wide collections (libraries) has revolutionized the way yeast studies are carried out. Specifically, libraries that involve a cellular perturbation, as the deletion collection, have facilitated key biological discoveries. However, short-term rewiring and long-term accumulation of suppressor mutations often obscure the functional consequences of genetic perturbation. To overcome these issues, we created a new proteome-wide collection that creates "on demand" protein depletion. In our collection each protein is tagged with a Green Fluorescent Protein (GFP) and an inducible degron, enabling rapid protein depletion that can be quantified systematically using the GFP. We characterized the degradation response of all strains and demonstrated its utility by revisiting seminal yeast screens, including those identifying genes essential for cell cycle progression and mitochondria distribution and morphology. Indeed, in addition to recapitulating known phenotypes, we also uncovered new proteins involved in these central processes. Hence, our tool promises to expand our understanding of cellular biology by facilitating access to phenotypes that are central to cellular physiology and therefore rapidly equilibrated.

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Activation mechanism of endoplasmic reticulum stress sensor ATF6ɑ through redox control

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The endoplasmic reticulum (ER) serves as a site for folding secretory and membrane proteins. However, the various environmental changes or genetic mutations can lead to accumulation of misfolded proteins within the ER, causing ER stress. Prolonged ER stress can result in cell death and is linked to diseases, such as Alzheimer's disease, diabetes, and heart disease. Eukaryotes have a mechanism called the unfolded protein response (UPR) as a strategy to protect cells from ER stress. Activation of the UPR leads to suppression of protein synthesis, upregulation of molecular chaperones, and initiation of ER-associated degradation (ERAD) to preserve ER proteostasis.

Activating transcription factor 6 alpha (ATF6ɑ;), a member of the UPR and ER stress sensor protein, typically forms disulfide bonds within the ER lumen and interacts with the molecular chaperone BiP/GRP78. During ER stress, ATF6ɑ; dissociates from BiP, its disulfide bonds are reduced, and it is then transported from the ER to the Golgi apparatus. In the Golgi apparatus, the N-terminal domain of ATF6ɑ; (ATF6ɑ;-N) undergoes sequential cleavage by site-1 protease (S1P) and site-2 protease (S2P). Subsequently, ATF6ɑ;-N is transported into the nucleus, where it binds to the ER stress response element (ERSE) on gene promoters, thereby inducing the expression of molecular chaperones and oxidases. However, the regulatory mechanisms underlying these steps are not largely understood.

ATF6ɑ; contains two cysteine residues on the ER lumen, which are known to form intermolecular disulfide bonds, forming a homodimer (H. Koba et al., Cell Struct Funct, 2020). However, the functions of these homodimers remain largely unexplored. Our research demonstrated that the ATF6ɑ; homodimer is crucial for its efficient transport from the ER to the Golgi apparatus. Moreover, we discovered that oxidoreductase ERdj5 plays a role in the ATF6ɑ; homodimer formation. In this presentation, we discuss a novel activation mechanism of ATF6ɑ; through redox regulation.

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Tracking the secrets of diacylglycerol transport and metabolism in the cell

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Diacylglycerols are important second messenger lipids involved in many cellular processes, leading to diverse cellular responses ranging from proliferation to apoptosis. Cells produce a plethora of diacylglycerol species varying in fatty acid chain lengths, saturation, or linkage to the glycerol backbone. However, the role of these individual lipid species in signaling pathways is largely understudied.

A tool to investigate individual lipid species involved in cellular signaling in pulse-chase experiments are chemically functionalized lipids. Here, we use trifunctional lipids, which allow for the investigation of individual lipid species in a temporally and spatially defined manner. The probes further enable the visualization of the lipids and omics-analyses. Using trifunctional diacylglycerols, we would like to elucidate species-specific, time-resolved lipid localization and metabolic state as well as identify transporting and metabolizing proteins involved in the post-signaling processes to obtain insights into individual species in cellular signaling pathways.

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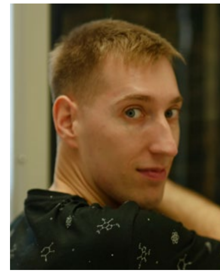

Metabolic and mRNA expression changes in adipose tissue during exercise in older women

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During aging body composition changes with a decrease in muscle mass and an increase in fat mass. At the same time, lipid deposition in visceral and ectopic fat depots predominates, which is associated with an increased risk of developing insulin resistance and other metabolic disorders. Adipose tissue dysfunction, which includes reduced adipogenesis, impaired metabolic flexibility and altered secretory activity, also contributes to these pathologies. Regular physical activity is undeniably part of a healthy lifestyle for people of all ages. In the elderly, its beneficial effects on muscle function, cardiovascular fitness and metabolic health have been described.

In our study, the effect of exercise training on subcutaneous adipose tissue metabolism was analyzed in group of long-term trained (Trained, n=26, age 69±4 years, VO2max 26,4±5,0 ml/kg/min, BMI 24,7±2,5 kg/m2) and untrained (Sedentery, n=54, age 71±5 years, VO2max 16,7±2,6 ml/kg/min, BMI 25,6±2,4 kg/m2) older women. SAT mRNA gene expression and lipidomic profile was determined and anthropometric and insulin resistance parameters of both groups were monitored.

The results showed reduced glucose uptake (GLUT4) and several lipid metabolic genes (ACLY, SCD, ELOVL6, DGAT2, ACOX1) in trained women when compared to sedentary. In contrast, gene expression of lipolytic genes (CGI58 and ADRB1) was higher in trained. At the lipidomic level, the total amount of free FAs does not change, however, the group of very long-chain (>20 C) FAs was significantly higher in „trained" women. Considering the phospholipid profile almost all phospholipid groups (PCs, PUFA PCs, PEs, ePEs, PUFA PEs and ePEs, PGs, PIs, PSs) were significantly reduced in trained women, while ePCs, PUFA ePCs and ePEs remain unchanged. CGI58 gene expression correlated negatively with adiposity and ACOX1 gene expression correlated positively with adiposity and insulin resistance.

These findings suggest adaptation in lipid metabolism by long-term exercise training, which probably leads to changes in membrane composition. The decrease in gene expression of ACOX1 and increased levels of VLC-FAs suggest also reduced VLC-FAs utilization by peroxisomal β;-oxidation of VLC-FAs may be related to insulin resistance.

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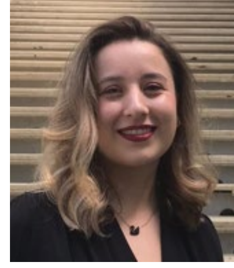

Investigating the Regulatory Role of Palmitoylation During Cell Divisions

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Protein palmitoylation is a reversible lipid modification that plays a crucial role in regulating the localization and function of membrane-associated proteins. Palmitoylation can occur on cysteine residues near the N- or C terminus of proteins, leading to increased hydrophobicity and association with the lipid bilayer. This modification has been shown to affect protein-protein interactions, membrane trafficking, and signaling pathways. Recent studies have also implicated palmitoylation in membrane shaping and remodeling, including membrane curvature generation, membrane fusion, and endocytosis. The current understanding of how protein palmitoylation contributes to membrane shaping and remodeling, and how this modification may be targeted for therapeutic interventions in diseases where membrane dynamics are disrupted.

Reference(s):

Cell cycle-dependent palmitoylation of protocadherin 7 by ZDHHC5 promotes successful cytokinesis

Combining pulsed SILAC labeling and click-chemistry for quantitative secretome analysis

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Study of the vacuolar transport of lysine in the yeast Saccharomyces cerevisiae.

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The vacuole of the yeast Saccharomyces cerevisiae serves as a storage compartment for a large array of metabolites that cells can utilize to survive nutrient shortages1. Particularly, more than 90% of the cellular pool of cationic amino acids is sequestered in the vacuole2. But how they are transported across the vacuolar membrane and in which conditions they are stored or mobilized remains poorly understood.

We study the vacuolar transport of lysine (lys) and the physiological functions of this transport. Our data suggest that Vsb1, a vacuolar membrane protein belonging to the SulP/SLC26A family3, is the vacuolar lys importer. Indeed, when we delete VSB1, we observe a reduction in the vacuolar storage of lys. Moreover, when lys is added in the culture media, it induces an endocytosis more pronounced of Lyp1, the cytoplasmic lys transporter, in a vsb1Δ; mutant, suggesting that in this strain, lys fails to be transported into the vacuole and accumulates in the cytosol. All together, these observations suggest that Vsb1 is implicated in the vacuolar storage of lys.

In contrast, we have indications that Ypq1, another vacuolar membrane protein belonging to the PQ-loop family and an orthologous to PQLC2, a cationic amino acid transporter found at the lysosomal membrane of higher eukaryotes, is responsible for vacuolar lys export. Indeed, during lys starvation, a ypq1 mutant does not mobilize its vacuolar lys reserves, which, if not compensated by biosynthesis, affects cell growth. These observations suggest that Ypq1 is a vacuolar lys exporter.

During nitrogen starvation, a condition where amino acid reserves are normally mobilized, Ypq1 does not contribute to vacuolar lys export. Under this condition, intracellular lys surprisingly accumulates while the levels of all other amino acids decrease. Our data suggest that lys accumulation is due to a massive arrival of lys through autophagy and its sequestration by the vacuolar polyphosphate pool. This sequestration is so drastic that it results in a lack of lys in the cytoplasm which must be compensated by an increase in biosynthesis.

Additional studies are underway to understand the regulation of the Ypq1 activity and the role of lys accumulation in the vacuole under nitrogen starvation.

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