Associazione di Biologia Cellulare e del Differenziamento

Membrane Trafficking and Organelle Biogenesis

Organisers

Paolo Remondelli (Chair) - University of Salerno

Sara Colombo (vice-Chair) - CNR Institute of Neuroscience

Programme & Abstracts

Pesaro, 4-5 April 2014

http://MTOB2014.azuleon.org

PROGRAMME

Friday, 4 April

12:30 **Registration**

13:00-14:00 **Lunch**

14:30-16:30 **Session 1: Autophagy**

Chair: Roberto Sitia & Carmine Settembre

14:30-14:50 *Laura Cinque (Naples)*

FGF18 regulates bone size through macroautophagy

14:50-15:10 *Katia Cortese (Genoa)*

Heat shock protein 90 inhibition in ErbB2-overexpressing breast cancer cells causes ErbB2 proteolytic fragmentation and lysosomal degradation simultaneously with alterations of endocytic recycling and suppression of autophagy

15:10-15:30 *Riccardo Cristofani (Milan)*

Inhibition of dynein ATPase activity reduces high molecular weight forms of misfolded proteins involved in motoneuron diseases

15:30-15:50 *Maurizio Renna (Cambridge, UK)*

IGF-1R signalling and regulation of autophagosome biogenesis: novel findings against an old paradigm

15:50-16:10 *Thomas Vaccari (Milan)*

Multiple functions of the SNARE protein Snap29 in autophagy, endocytic and exocytic trafficking during epithelial formation in *Drosophila*

16:10-16:30 *Maria Giovanna De Leo (Naples)*

Defining the role of the PtdIns(4,5)P2 5-phosphatase OCRL in the late endosomal compartment and autophagy

16:30-17:00 **Coffee break**

17:00-18:00 **Session 2: Endocytic Pathway**

Chairs: Simona Paladino & Cecilia Bucci

17:00-17:20 *Leopoldo Staiano (Naples)*

Looking for correctors of Lowe Syndrome: a High Content Screening (HCS) approach

17:20-17:40 *Lorena Urbanelli (Perugia)*

MicroRNAs regulate lysosomal system by targeting MPRs and Adaptor Protein complex 1 subunits

17:40-18:00 *Alessia Castagnino (Genoa)*

Role of ErbB1 in ErbB2 membrane redistribution induced by Trastuzumab treatment in SK-BR-3 cells

18:00-19:00 **Plenary Lecture**

Anne Spang (Basel, Switzerland)

Regulation of transport through the endocytic pathway

19:00-19:30 **General discussion and election of next vice-chair**

20:00-21:30 **Dinner**

21:30 **Poster Session**

Saturday, 5 April

8:30-10:30 **Session 3: Endoplasmic Reticulum**

Chairs: Sara Colombo & Stefano Bonatti

8:30-8:50 *Tiziana Anelli (Milan)*

A dynamic study of protein condensation in the secretory pathway

8:50-9:10 *Paola Genevini (Milan)*

ER-derived inclusions generated by ALS-linked mutant VAPB are cleared by cells and do not interfere with proteostasis or protein transport

9:10-9:30 *Sara Sannino (Milan)*

Regulation of protein quality control at the ER-Golgi interface: role of conserved histidines in dictating ERp44 localization and function

9:30-9:50 *Mariano Stornaiuolo (Naples)*

Rescue of folding defects, modulation of receptor activity and anti-tumoral potential of the first organic molecules addressing Frizzled4

9:50-10:10 *Alberto Danieli (Ceriano Laghetto, MB)*

A novel approach for correlative light and electron microscopy

10:10-10:30 *Laura Oliva (Milan)*

Inherent cellular stress and exquisite proteasome sensitivity in amyloidogenic plasma cells

10:30-11:00 **Coffee break**

11:00-12:40 **Session 4: Golgi**

Chairs: Paolo Remondelli & Nica Borgese

11:00-11:20 *Antonino Colanzi (Naples)*

Golgi complex fragmentation in G2/M transition: an organelle-based cell-cycle checkpoint

11:20-11:40 *Mafalda Concilli (Naples)*

Identification and functional study of Wilson disease protein interactors

11:40-12:00 *Carmen Valente (Naples)*

Molecular mechanisms of post-Golgi tubular carrier formation

12:00-12:20 *Simona Paladino (Naples)*

Cholesterol-dependent sorting in the Golgi regulates the supramolecular organization and activity of GPI-anchored proteins at the apical membrane of polarized epithelial cells

12:20-12:40 *Alexandre Mironov (Milan)*

Experimental comparison of prediction power of different models of intra-Golgi transport suggests in favour of the kiss-and-run mechanism

13:00 **Lunch and Departures**

Abstracts

(in alphabetical order of presenting authors)

Poster

Endoplasmic reticulum stress reduces COPII vesicles formation and modifies Sec23a cycling at ERESs

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COPII vesicles bud from the ER at ER Exit Sites (ERESs) to mediate the exit from the Endoplasmic Reticulum (ER) of newly synthesized proteins. Previously, we demonstrated that ER Stress rapidly impairs the anterograde transport to the Golgi complex and the formation of COPII vesicles (Amodio et al., 2009). In a recent work (Amodio et al., 2013) we found that the reduced permanence of Sec23a at the ERES could be the mean through which ER Stress modulates COPII assembling and vesicular trafficking. Sec23a is one of the component of the COPII vesicles coat and its GTPase activating function on Sar1 is one of the key mechanisms of COPII assembly. Interestingly, we found that during ER Stress the association to the ER membrane of Sec23a is reduced. Concomitantly, FRAP and FLIP analysis of Sec23a revealed that ER stress accelerates its recycling kinetics on ER membrane. These results prompted us to analyze the role of post-translational modifications of Sec23a in the regulation of its function during ER Stress. Surprisingly, we found that Sec23a is mono-ubiquitinated in mammalian cells on two different cysteines and that the induction of ER stress reduces the amount of mono-ubiquitinated Sec23a. The biological scope of Sec23a cysteine mono-ubiquitination has yet to be elucidated but recent evidences demonstrating that ubiquitination on cysteines regulates signal transduction and membrane translocation (Grou et al., 2008; Shannon and Weerapana, 2013) open new fields of investigation about Sec23a ubiquitination and modulation of COPII function.

Oral

A dynamic study of protein condensation in the secretory pathway

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Stringent quality control mechanisms at endoplasmic reticulum-Golgi interface maintain the fidelity of the secretory proteome. When synthesis of a molecule destined to the extracellular space exceeds the sum of secretion and degradation, proteins often condense and accumulate in dilated cisternae of the early secretory compartment (ESC) causing cell damage (ER storage disorders). Owing to the central role of ESC trafficking in pathophysiology, dynamic imaging methods are required to follow the fate of cargo proteins and their interactions with resident enzymes and folding assistants. As a model of ER storage disease, here we analyzed the intracellular condensation of a mutant IgM heavy chain μ deleted of its first contant domain. By tagging μΔCH1 with the Halo-tag and exploting the Halo-tag property of binding covalently fluorescent ligands, we were able to discriminate between young and old molecules at the single cell level and dynamically follow in vivo protein biogenesis, degradation and condensation-aggregation.

Poster

Exosomal Hsp60 in human colon cancer

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In previous works (1,2) we showed for the first time that human tumor cells, differently than the non-tumor counterpart, secrete Hsp60 via exosomes, which are considered immunologically active nanovesicles involved in tumor progression. In particular, Hsp60 is present in the plasma membrane of tumor cells, where it is associated to lipid rafts, and it localizes in the exosomal membrane.
In the present work we studied tumor samples obtained from a series of patients affected by colon cancer using a number of techniques including PCR, WB, IHC, IF, TEM and ELISA. Moreover, plasma of patients was obtained before and after surgery. Exosomes were isolated by ultracentrifugation and characterized by TEM, AChE activity and WB. We found that Hsp60 levels are significantly increased in tumor samples of patients affected by cancer, compared to controls. Moreover, Hsp60 levels reduced significantly in exosomes isolated from plasma after surgery. Hence, exosomal Hsp60 can be considered as a new marker for colon cancer diagnosis and follow-up as well as a new player in colon cancer progression.

References

Merendino AM, Bucchieri F, Campanella C, Marcianò V, Ribbene A, David S, Zummo G, Burgio G, Corona DF, Conway de Macario E, Macario AJL, Cappello F. Hsp60 is actively secreted by human tumor cells. PLoS One. 2010 Feb 16;5(2):e9247.
Campanella C, Bucchieri F, Merendino AM, Fucarino A, Burgio G, Corona DF, Barbieri G, David S, Farina F, Zummo G, de Macario EC, Macario AJ, Cappello F. The odyssey of Hsp60 from tumor cells to other destinations includes plasma membrane-associated stages and Golgi and exosomal protein-trafficking modalities. PLoS One. 2012;7(7):e42008.

Oral

Role of ErbB1 in ErbB2 membrane redistribution induced by Trastuzumab treatment in SK-BR-3 cells

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ErbB2 is a member of a family of four closely related receptors (ErbB1-4), involved in several physiological process such as proliferation, differentiation and cell migration. ErbB2 overexpression has been identified in approximately 25-30% of primary breast cancers and correlates with poor prognosis and cancer relapse. For these reasons, ErbB2 represents an attractive target for immunotherapy. Trastuzumab (Tz) is a humanized monoclonal IgG1 against ErbB2 currently used in advanced breast cancers therapy, and it exerts anti-proliferative effects on ErbB2-positive breast tumor cell lines. Despite its therapeutic success, resistance to Tz has been frequently observed. Therefore, the study of the molecular mechanisms involved in Tz action represents an important aim of our research work. Our studies are focused on Tz effects in SK-BR-3 cells, following only the ErbB2 molecules directly bound to the drug. In particular our group has found that Tz short treatment induces ErbB2 phosphorylation, signaling and ErbB1/ErbB2 heterodimerization, promoting the ErbB1/ErbB2/Tz complex endocytosis. As an early response to Tz, we observed formation of Circular Dorsal Ruffles (CDRs).
CDRs are transient actin-based structures that assemble and disappear on the dorsal plasma membrane very quickly. CDRs (waves) are involved in the reorganization of actin cytoskeleton necessary to prepare a static cell for motility and in the internalization of RTKs, such as EGFR and PDGFR, after stimulation by a variety of growth factors. The characterization, by co-IP and IF studies of Tz/ErbB2 complex with specific markers such as N-WASP and Cortactin confirmed the Tz induction of CDRs. In this analysis, through transient silencing of ErbB1, we also observed a loss of CDR formation in absence of ErbB2-ErbB1 heterodimers. We are interested in defining the mechanism of CDRs formation-ErbB1 dependent, and its role as response to Trastuzumab treatment.

Poster

Role of MAPK pathways in the regulation of Golgi fragmentation and entry into mitosis

R.I. Cervigni, R. Bonavita, M.L. Barretta, D. Corda, A. Colanzi
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The Golgi complex is composed of many cisternal stacks that are interconnected by tubules, to form a continuous 'ribbon-like' structure. During mitosis, the Golgi ribbon undergoes extensive fragmentation through a multistage process that promotes its correct partitioning and inheritance by the daughter cells.
When Golgi fragmentation is inhibited the cell cycle is blocked in G2 supporting the existence of a Golgi checkpoint. We have described that the Golgi-dependent G2 arrest is mediated by a failure of centrosome maturation, an event that is essential to achieve activation of the Cdk1/CyclinB complex, the master regulator of mitosis. Indeed, the failure of Golgi fragmentation inhibits the recruitment to and activation at the centrosome of the kinase Aurora-A, that in turn inhibits the activation of Cdk1/CycB at the centrosome. This data contribute to the definition of a previously unidentified point of dialogue between the Golgi apparatus and the centrosome in the regulation of G2/M transition.
Moreover, we have developed novel experimental approaches to induce the block of Golgi fragmentation that integrate a previously developed assay that is based on the microinjection of blockers of Golgi fragmentation, a reliable but demanding approach.
The assays that we have developed are based on the ability of the GRASP65 protein to regulate Golgi fragmentation. As well as being essential for inducing the Golgi checkpoint in a wide cell population, they are also useful for the unravelling of the mechanism through which GRASP65 acts in the Golgi checkpoint.
Together with other Golgi matrix components, GRASP65 contributes to the partitioning of the Golgi apparatus during G2, in particular when it is phosphorylated at serine 277. This residue is phosphorylated along all the cell cycle by different kinases depending on the stimulus. Our work describes the role of the MAPK in these phosphorylations.

Oral & Poster

FGF18 regulates bone size through macroautophagy

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(Macro)autophagy is a metabolic pathway involved in several cellular functions and its levels are finely tuned by multiple cues, including growth factors that regulate different developmental processes. This observation suggests a yet unexplored role for autophagy during development. We investigated the role of autophagy during endochondral ossification, a developmental process whereby long bones form and grow. Bone elongation occurs at the growth plates and chondrocytes are the major players in this process since their rate of proliferation, differentiation and extracellular matrix (ECM) secretion determines the length of the forming bone. By analyzing autophagy levels in growing bones we observed that autophagy is strongly activated in growth plate chondrocytes during post-natal development. We found that autophagy induction regulates post-natal bone size since mice lacking an essential autophagy gene (*ATG7*) in chondrocytes develop normally *in utero* but show reduced bone growth during adulthood. Genetic and cell-based analyses indicate that FGF signaling regulates chondrocyte autophagy during post-natal bone growth by inducing JNK-dependent Bcl-2 dissociation from Beclin1 protein and activation of the VPS34 complex. In conclusion, our data demonstrate that autophagy is a developmentally regulated process with an important role in in the regulation of bone size.

Oral

Golgi complex fragmentation in G2/M transition: an organelle-based cell-cycle checkpoint

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During mitosis, the Golgi complex undergoes a multi-step fragmentation process that is instrumental to its correct partitioning into the daughter cells. To prepare for this segregation, the Golgi ribbon is initially separated into individual stacks during the G2 phase of the cell cycle. Then, at the onset of mitosis, these individual stacks are further disassembled into dispersed fragments. Inhibition of this Golgi fragmentation step results in a block or delay of G2/M transition, depending on the experimental approach. Thus, correct segregation of the Golgi complex appears to be monitored by a 'Golgi mitotic checkpoint'. Using a microinjection-based approach, we recently identified the first target of the Golgi checkpoint, whereby a block of this Golgi fragmentation impairs recruitment of the mitotic kinase Aurora-A to, and its activation at, the centrosomes. Overexpression of Aurora-A can override this cell-cycle block, indicating that Aurora-A is a major effector of the Golgi checkpoint. We have also shown that this block of Aurora-A recruitment to the centrosomes is not mediated by the known mechanisms of regulation of Aurora-A function. We are currently investigating the regulation of Golgi fragmentation during G2 and how this fragmentation is signalled to Aurora-A.

Poster

TRC40-dependent insertion of tail-anchored proteins into proteoliposomes reconstituted from rat liver microsomal detergent extracts

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Many tail-anchored (TA) proteins that are targeted to the endoplasmic reticulum (ER) utilize the TRC40/Get3 ATPase targeting pathway. The sequential interactions of this chaperone have been well characterized in yeast, where the Get1/2 complex functions as ER receptor for Get3, but are less well understood in mammalian cells. Present evidence suggests that a complex composed of the Get1 homologue, WRB, and a newly identified player, CAML, functions as the TRC40 receptor in the mammalian ER. To gain further insight into the mechanism of TRC40-assisted insertion in mammals, we have made use of proteoliposomes reconstituted form rat liver microsomal detergent extracts subjected to immunodepletion. Soluble extract, when reconstituted with phospholipids, was capable of integrating *bona fide* TRC40 substrates. To identify TRC40-interacting proteins in the ER, we took advantage of the abundance of membrane-associated TRC40 in our microsomal fraction. Proteoliposomes reconstituted with an extract from which TRC40 had been depleted by immunoadsorption were inactive towards TRC40-dependent substrates. The loss of activity could not be attributed to depletion of TRC40 itself, as the reticulocyte lysate used for in vitro translation is a rich source of the chaperone; thus, TRC40-interacting microsomal components, required for insertion, were immobilized on the anti-TRC40 resin. To recover these components, we eluted the immunoadsorbed fraction with TRC40 immunogenic peptide, and found that the eluted fraction is capable of restoring activity to the depleted extract. This eluted fraction is both necessary and sufficient since, when reconstituted into pure lipid vesicles, it is able to restore TRC40-dependent TA insertion. Mass spec analysis demonstrated the presence of both WRB and CAML in the eluted fraction. We are presently investigating the role of these proteins, as well as of other identified interactors, in TRC40-dependent TA protein insertion in our reconstituted system.

Identification and functional study of Wilson disease protein interactors

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Wilson's disease (WD) is an inherited autosomal recessive disorder of copper metabolism characterized by liver failure and/or neuronal degeneration. The pathology is a result of mutation in *ATP7B gene*, which encodes the ATP7B copper transporting ATPase. ATP7B protein has a key role in hepatic copper excretion by virtue of its ability to transport Cu across the cellular membrane at the expense of ATP hydrolysis. A unique feature of ATP7B that is integral to this function is its ability to sense and respond to intracellular Cu levels. This response is manifested through its Cu-regulated trafficking from the *trans*-Golgi network to the appropriate cellular membrane domain, where ATP7B can then eliminate excess Cu from the cell. This property of ATP7B is affected by Wilson-disease-causing mutations that, therefore, result in toxic Cu accumulation in hepatocytes, leading to oxidative stress and acute liver failure. Several proteins are probably involved in dealing with the excess copper and oxidative stress. In this work we identified new molecular players that regulate ATP7B transport in health and under pathological conditions and in low and high copper conditions using an immunoprecipitation approach combined with mass spectrometry analysis.
The expression of the molecules obtained from proteomics analysis were suppressed using an RNA interference method, and ATP7B localization and transport in response to elevated Cu was analyzed using confocal microscopy in HeLa and HepG2 cells and Blot analysis.
The experimental approaches proposed in this work should provide a solid basis for the construction of the molecular networks involved in the regulation of ATP7B (and its mutant) transport, through which we will be able to define the roles of these network participants in WD pathogenesis, and to detect suitable candidates for therapeutic approaches.

Oral

Heat shock protein 90 inhibition in ErbB2-overexpressing breast cancer cells causes ErbB2 proteolytic fragmentation and lysosomal degradation simultaneously with alterations of endocytic recycling and suppression of autophagy

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The oncoprotein and receptor tyrosine kinase ErbB2, normally expressed in breast epithelia, is a remarkable therapeutic target because it resists efficient down-regulation in breast cancers. HSP90 inhibitors like Geldanamycin (GA) are efficient drugs that target ErbB2 to degradation, but the overall mechanisms of action are still not completely clear. In this study, we used SKBR3, a human breast cancer cell line overexpressing ErbB2, to dissect the mechanisms of action of GA underlying ErbB2 trafficking and degradation. In particular, we show that GA simultaneously acts at three distinct levels. First, GA potentiates the C-terminus cleavage of ErbB2, and generates a transmembrane fragment of approximately 116-135KDa. Inhibition of lysosomal activity with Bafilomycin A1 stabilizes this fragment, indicating that ErbB2 is preferentially internalized in the cleaved form. Second, we report that early-to-late trafficking of down-regulated ErbB2 and EGFR is functional, while the recycling TfR appears to accumulate in dysfunctional early/recycling/autophagosomal-like compartments. This observation suggests that GA perturbs endocytic recycling and autophagy. Third, immunoelectron microscopy revealed that GA also down-regulates ErbB2 traffic through altered early/late endosomes, which in part show morphological features of autophagy intermediates like amphisomes. The accumulation of these vesicular structures may result from autophagy impairment caused by a decrease of LC3II levels. In conclusion, these results show that HSP90 inhibition induces potent ErbB2 proteolytic fragmentation followed by degradation of the cleaved form in lysosomes. Moreover, ErbB2 fragmentation, alterations in endocytic recycling and inhibition of autophagy are synchronous events.

Inhibition of dynein ATPase activity reduces high molecular weight forms of misfolded proteins involved in motoneuron diseases

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Spinal and bulbar muscular atrophy (SBMA) and Amyotrophic lateral sclerosis (ALS) are two adult onset motoneuron diseases caused by degeneration of specific classes of motoneuron. Different mutant proteins have been found involved in familiar forms of these diseases (e.g.: androgen receptor (AR), SOD1 and TDP43). All these proteins tend to misfold acquiring aberrant conformations, which aggregate. Aggregates may be protective by subtracting misfolded proteins, but they may also impair proteasome and autophagy, two major players of the protein quality control system (PQC). We recently showed that HSPB8 mediated autophagy reduces misfolded proteins aggregation, even when proteasome is inhibited. Dynein, by binding BAG3 and HSPB8, transports misfolded proteins to microtubule organization center (MTOC), where proteins are assembled in aggresome to be degraded by autophagy. Dynein also mediates autophagosome formation, by binding Ambra1-Beclin1-Vps34 complex that enable autophagosome nucleation.
In SBMA cell model we found that dynein is sequestered into aggregates of mutant AR near MTOC. To analyze the role of dynein in SBMA and ALS we used EHNA, an inhibitor of dynein ATPase activity witch impairs dynein-mediated transport. In cell model of SBMA and ALS EHNA induced a drastic reduction of aggregates of the misfolded proteins: ARpolyQ, mutSOD1, and mutTDP43. EHNA, also, increased the solubility of misfolded ARpolyQ. BAG1, witch routes misfolding proteins to proteasome degradation or chaperone-mediated-autophagy, was found increased after EHNA treatment.
Our data are in line with previous observation that showing an increase of survival of the double mutant mice for mutSOD1 and an inactive form of dynein. This suggests that dynein may mediate the process of aggregate formation during the PQC response.

GRANTS: Telethon; Fondazione AriSLA; AFM, France; Fondation Thierry Latran, France; Regione Lombardia; Università degli Studi di Milano.

Oral

A novel approach for correlative light and electron microscopy

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We earlier established that signaling stress from the endoplasmic reticulum (ER) through the unfolded protein response (UPR) entail clustering of two ER stress sensors, IRE1 and PERK, in the ER membrane. To obtain insight at the ultrastructural level of these clusters, we set out to develop a novel approach for correlative light and electron microscopy (CLEM). The approach revolves around the HALO tag, which is a genetically engineered derivative of a hydrolase gene that covalently binds specific substrates. This tag allows visualization indirectly by ligands that carry different fluorescent moieties (Promega). Due to the fact that the HaloTag protein is not endogenous to mammalian cells, high labeling specificity is ensured. For CLEM we explored the usefulness of an eosin-coupled ligand. Eosin HALO-ligand is both fluorescent and able to catalyze photoconvertion of DAB and O_2 into an electron dense polymer that binds OsO_4 and thus allows visualization both in fluorescence light microscopy and electron microscopy. As a proof of principle, we HALO-tagged a mutant of the secretory IgM heavy chain (μΔCH1-Ala_{565}-HALO) that forms large aggregates in the cell, and to our excitement readily gave signal by both microscopic techniques. We next successfully extended the technique to tail-anchored HALO and HALO-RDEL, two artificial constructs that mark the ER. Finally, we obtained preliminary results that suggest we can detect even the ER stress signaling clusters despite their small size and the low expression levels of the proteins involved. We are very excited about our breakthrough in establishing a novel method for CLEM and are convinced our system is amenable to a wide range of questions in biology.

Defining the role of the PtdIns(4,5)P2 5-phosphatase OCRL in the late endosomal compartment and autophagy

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Phosphoinositide lipids play a key role in cellular physiology, participating in a wide range of cellular processes and mutation of phosphoinositide-metabolizing enzymes is responsible for a growing number of diseases in humans. By combining unbiased and hypothesis-driven approaches we found a new role for the PI(4,5)P2 5-phosphatase OCRL in lysosome physiology. Mutations in OCRL are responsible of Lowe Syndrome, which is characterised by congenital cataracts, central hypotonia, and renal proximal tubular dysfunction. Recent advances in our understanding of OCRL function show that OCRL associates with clathrin coated vesicles, early endosomes and the Golgi complex at steady state, but translocates to lysosomes under stress conditions (e.g. serum-starvation).
We show here that OCRL is a key controller of the late endosomal/lysosomal compartments. By controlling the phosphoinositide composition of late endosomes and lysosomes OCRL controls the activity of the lysosomal calcium channel, mucolipin1, which is stimulated by PI35P2 and inhibited by PI45P2. The accumulation of PI(4,5)P2 upon depletion of OCRL inhibits the activity of mucolipin, which is required for autophagosome- and late-endosome-lysosome fusion, and slows down autophagosomal and late endosomal flux.
Importantly, by using selective agonists of mucolipin1, we were able to rescue lysosomal function in cells depleted of OCRL, thus identifying mucolipin1 as a possible drug target for the treatment of Lowe Syndrome.

Oral & Poster

ER-derived inclusions generated by ALS-linked mutant VAPB are cleared by cells and do not interfere with proteostasis or protein transport

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VAP-B is a ubiquitously expressed, ER-resident tail-anchored adaptor protein implicated in interorganellar lipid exchange, generation of membrane contact sites and membrane trafficking. Its mutant form, P56S-VAPB, has been linked to a dominantly inherited form of Amyotrophic Lateral Sclerosis (ALS8). P56S-VAPB generates intracellular inclusions derived from restructured ER domains whose role in ALS pathogenesis has not yet been elucidated. At variance with most pathological inclusion bodies, these inclusions are efficiently degraded by the proteasome with no apparent involvement of autophagy. To investigate whether P56S-VAPB-inclusion formation interferes with general proteostasis (protein degradation mediated by proteasome and/or autophagy) and ER-plasma membrane protein transport, we generated stable HeLa- and NSC34-TetOff cell lines inducibly expressing physiological levels of P56S-VAPB. HeLa cells were transiently transfected with either the ERAD (ER Associated Degradation) substrate CD3Delta, to assess possible proteasome impairment, or VSVG (Vescicular Stomatitis Virus Glycoprotein), to study protein transport. To investigate autophagic flow, cells were treated with autophagy-stimulating drugs. The levels of CD3Delta were comparable in P56S-VAPB-expressing and -nonexpressing cells and no difference in the levels of autophagic markers was detected between the two cellular models upon autophagy stimulation. Thus, neither proteasome-mediated degradation of a classical ERAD substrate nor autophagocytosis appear to be slowed by the presence of P56S-VAPB inclusions. Similarly, protein transport through the secretory pathway is not affected. Our results suggest that clearance of P56S-linked inclusions does not interfere with cell proteostasis or protein transport, thus questioning whether the mechanism by which dominantly inherited mutant VAPB causes ALS is toxic gain of function, negative dominance, or simply haploinsufficiency.

Oral

Experimental comparison of prediction power of different models of intra-Golgi transport suggests in favour of the kiss-and-run mechanism

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Mechanisms of intra-Golgi transport represent a disputable issue. The vesicular, compartment maturation, diffusion and kiss-and-run models of intra-Golgi transport compete with each other for the 'right' to be regarded as the paradigm. Here, we have examined predictions derived from these models using several parameters and assays; namely, the kinetics of exit of cargo and post-Golgi carriers from the Golgi area, and from the Golgi apparatus per se, the volume of the Golgi, and the surface area of Golgi membranes, and the diffusion and concentration of cargo during intra-Golgi transport. Intra-Golgi transport of FP-tagged albumin and AAT included their concentration at the trans side of the GA. Both blockage of formation of inter-cisternal connections and their stabilization inhibited penetration of albumin across the Golgi stacks and its concentration at the trans side of the Golgi. In contrast, not all of these influences affected transport of FP-tagged version VSVG and PC-I. Manipulation with the ionic gradients existing through the Golgi affected concentration of albumin and AAT at the trans side, but not their penetration into the TGN. These results suggest in favour of the kiss-and-run mechanism of intra-Golgi transport. The kiss-and-run model appeared the most encompassing in the explanation of these set of experimental data.

Poster

Multiple functions of the SNARE protein Snap29 in autophagy, endocytic and exocytic trafficking during epithelial formation in *Drosophila*

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How autophagic degradation is linked to endosomal trafficking routes is little known. Here we screened a collection of uncharacterized *Drosophila* mutants affecting membrane transport to identify new genes that also have a role in autophagy. We isolated a loss of function mutant in *matryoshka,* the gene encoding the conserved SNARE protein Snap29 and have characterized its function *in vivo*. Snap29 contains two SNARE domains and a NPF motif at its N-terminus and rescue experiments indicate that both SNARE domains are required for function, whereas the NPF motif is in part dispensable. We find that Snap29 interacts with SNAREs, localizes to multiple trafficking organelles, and is required for protein trafficking and for proper Golgi apparatus morphology. Developing tissue lacking Snap29 displays distinctive epithelial architecture defects and accumulates large amounts of autophagosomes that are unable to fuse with lysosomes to enact degradation of their content. Accumulated autophagosomes are at time nested, and are often secreted, highlighting a major role of Snap29 in autophagy. Mutants for autophagy genes do not display epithelial defects, suggesting that the epithelial alterations of the *matryoshka* mutant are not caused by the impairment of autophagy. In contrast, we find evidence of elevated levels of JAK/STAT ligand, receptor and associated signaling, which might underlie the epithelial defects. In summary, our findings support a role of Snap29 at key steps of membrane trafficking, and predict that signaling defects, rather than impaired autophagy, may contribute to the pathogenesis of CEDNIK, a congenital syndrome due to loss of Snap29.

Poster

Molecular Features of Russell Bodies Deposits

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Secretory proteins that fail to reach the Golgi or dislocate to the cytosol for proteasomal degradation often condense in dilated cisternae of the early secretory compartment (ESC). These are common features of endoplasmic reticulum storage disorders (ERSD). Different proteins, however, follow distinct pathways and sites of condensation. For instance, immunoglobulins (Ig) deposit as extracellular fibrils in systemic amyloidosis or as intracellular inclusions in ESC, called Russell Bodies (RB), in plasma cell dyscrasias, autoimmune diseases and chronic infections. RB biogenesis can be recapitulated in lymphoid and non-lymphoid cells by expressing suitably mutated Ig-μ chains, providing powerful models to investigate the pathophysiology of ERSD and Heavy chain disease. Here we analyse the aggregation propensity, biochemical features and localization of the intra- and extra-cellular Ig deposits in human cells, as well as the role of the cellular context in determining their fate.

Poster

The Rab-interacting lysosomal protein (RILP) regulates vacuolar ATPase acting on the V1G1 subunit

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RILP (Rab-Interacting Lysosomal Protein) is a downstream effector of the Rab7 GTPase. GTP-bound Rab7 recruits RILP on endosomal membranes and, together, they control late endocytic traffic, phagosome and autophagosome maturation and are responsible for signaling receptor degradation. We have identified, using different approaches, the G1 subunit of the V1 domain (V1G1) of the V-ATPase as a RILP interactor. V-ATPases are composed of a peripheral domain (V1), responsible for ATP hydrolysis, and an integral membrane domain (V0) that translocates protons. The V-ATPases are ATP-dependent proton pumps that function to acidify intracellular compartments and, in some cases, transport protons across the plasma membrane of eukaryotic cells. The activity of V-ATPase in different endocytic compartments results in a pH gradient from early endosomes to lysosomes, while the acidification of the extracellular space promotes cells migration. We demonstrated that RILP interacts directly with V1G1 and that RILP regulates V1G1 stability, via a proteasome-dependent mechanism, and its localization on endosomes and lysosomes. Furthermore, we established that V1G1 has a fundamental role in controlling V-ATPase activity. In addition, our data suggest that also Rab7, through RILP, acts on V1G1 protein stability. Thus, Rab7 and RILP control the proton pump activity and thus endosomes to lysosome maturation. Importantly, both RILP and acidification triggered by V-ATPase in early endosomes, are required for the biogenesis of MVBs (multivesicular bodies) in the endocytic pathway, supporting the idea that RILP could coordinate this process. Given the involvement of V-ATPase in several human diseases, the discovery of a new regulatory mechanism coordinated by RILP opens the way to future development of new therapeutical means. Furthermore, the discovery of the functional link between RILP and V-ATPase opens new scenarios on the cellular role of RILP that will have to be further investigated.

Oral

Inherent cellular stress and exquisite proteasome sensitivity in amyloidogenic plasma cells

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Light chain amyloidosis (AL) is a protein misfolding disease caused by a small clone of bone marrow plasma cell (PC) secreting unstable light chains (LC) which deposit systemically as amyloid fibrils, affecting multiple organs and leading to death. Virtually nothing is known about the strategies activated by PCs to cope with the production of aggregation-prone proteins but the recent success of proteasome inhibitors (PIs) in improving patients' survival, raised the thought-provoking possibility that amyloidogenic PCs suffer from proteostatic stress. We thus characterized, for the first time, primary AL cells biochemically, functionally, and morphologically by electron microscopy (EM) and EM cytochemistry, revealing markers of higher cellular stress in AL PCs as compared to multiple myeloma (MM) cells, the paradigmatic proteasome-sensitive cancer cells. In line with higher stress, AL PCs proved significantly more sensitive than primary MM cells to PI-induced apoptosis. Our data reveal that primary AL PCs are intrinsically extremely sensitive to PIs and that suffer from cellular stress which can be exploited to identify novel therapeutic targets to achieve selective elimination of amyloidogenic clones.

Oral

Cholesterol-dependent sorting in the Golgi regulates the supramolecular organization and activity of GPI-anchored proteins at the apical membrane of polarized epithelial cells

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The dramatic changes in the lipid profiles of polarized vs non-polarized cells might impact protein organization and function. Here, by combining classical biochemistry with novel biophysical approaches we studied with high spatial and temporal resolution the organization of GPI-anchored proteins (GPI-APs) at the plasma membrane of polarized epithelial cells. We found that in polarized MDCK cells, following sorting in the Golgi, each GPI-AP reaches the apical surface in homo-clusters. Golgi-derived homo-clusters are required for their subsequent plasma membrane organization into cholesterol-dependent hetero-clusters. By contrast, in non-polarized MDCK cells GPI-APs are delivered to the surface as monomers in an unpolarized manner and are not able to form hetero-clusters. We further demonstrated that this GPI-AP organization is regulated by the content of cholesterol in the Golgi apparatus and is required to maintain the functional state of the protein at the apical membrane. Thus, different from fibroblasts, in polarized epithelial cells a selective cholesterol-dependent sorting mechanism in the Golgi regulates both the organization and the function of GPI-APs at the apical surface.

Oral

IGF-1R signalling and regulation of autophagosome biogenesis: novel findings against an old paradigm

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Inhibition of the insulin/insulin-like growth factor signalling pathway increases lifespan and protects against neurodegeneration in model organisms, and has been considered as a potential therapeutic target. This pathway is upstream of mTORC1, a negative regulator of autophagy. Thus, we expected autophagy to be activated by insulin-like growth factor-1 (IGF-1) inhibition, which could account for many of its beneficial effects. Paradoxically, we found that IGF-1 inhibition attenuates autophagosome biogenesis. The reduced amount of autophagosomes present in IGF-1R depleted cells can be, at least in part, explained by a reduced formation of autophagosomal precursors at the plasma membrane. In particular, IGF-1R depletion inhibits mTORC2, which, in turn, reduces the activity of protein kinase C (PKCα/β). This perturbs the actin cytoskeleton dynamics and decreases the rate of clathrin-dependent endocytosis, which negatively impacts on autophagosome precursor formation. Finally, with important implications for human diseases, we demonstrate that pharmacological inhibition of the IGF-1R signalling cascade reduces autophagy also in zebrafish and mice models. The novel link we describe here has important consequences for the interpretation of genetic experiments in mammalian systems and for evaluating the potential of targeting the IGF-1R receptor or modulating its signalling through the downstream pathway for therapeutic purposes under clinically relevant conditions, such as neurodegenerative diseases, where autophagy stimulation is considered beneficial.

Oral

Regulation of protein quality control at the ER-Golgi interface: role of conserved histidines in dictating ERp44 localization and function

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To guarantee the integrity of the secretory proteome, cargo proteins undergo stringent quality control at the ER-Golgi interface. Amongst the many chaperones and enzymes known to be actors in the "quality control farm", ERp44 is rather unique in that its activity is regulated by the pH gradient existing between the ER and Golgi. The more acidic Golgi pH (about 6.5) induces conformational changes in the ERp44 C-terminal tail that simultaneously expose the substrate-binding site and RDEL motif. So, both ERp44 and its client proteins can be retrieved into the ER, where the higher pH (about 7.1) likely induces complex dissociation. We show that conserved histidines located at the border between the b' domain and the flexible tail are important for regulating ERp44 localization and function. Unlike wild type ERp44, mutants lacking key histidines are O-glycosylated in the Golgi and in part secreted. Interestingly, co-expression of client proteins restores retention of ERp44, suggesting that conserved histidines regulate RDEL exposure in the absence of clients. Surprisingly, not all the clients bind ERp44 at the same compartment level. In fact, depending on their affinity for ERp44 binding site and on the surrounding pH, ERp44-substrate complexes form before or downstream the subregion where O-linked glycosylation takes place. The client-induced retrieval mechanisms we describe here may allow the distinct and sequential localization of ERp44 interactors along the early secretory pathway.

Poster

Rac1 inhibition affects polarity and modulates E-cadherin dynamics

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We are studying the role of Rac1 in the polarization process in the FRT thyroid epithelial cells. To this aim, we analyzed the effects on cell polarization of a specific inhibitor of Rac1, NSC23766, and of a tamoxifen-inducible, dominant-negative form of Rac1, ER-RacN17, which was stably expressed in FRT cells. Directional migration, transepithelial resistance acquisition by confluent monolayers grown on filters, cell aggregation and formation of polarized follicles in suspension cultures were found to be affected by inhibition of Rac1, indicating a critical role of Rac1 in the acquisition of the polarized phenotype. To understand by which molecular mechanisms Rac1 regulates this process, we investigated whether its inhibition affected E-cadherin dynamics and/or function since E-cadherin mediated cell-cell adhesion is the first necessary step of polarization process. We found that Rac1 inhibition determines a progressive loss of E-cadherin from the plasma membrane. Consistently upon Ca-switch assays Rac1 inhibition drastically reduced the amount of E-cadherin that re-localizes at cell-cell contacts from the cytosol where it is translocated following EGTA treatment. Thus, these data suggest that Rac1 regulates the physiological recycling of E-cadherin to the plasma membrane. Accordingly, we found that upon Rac1 inhibition E-cadherin is directed to intracellular degradation as demonstrated by its presence in endo-lysosomal compartments and by detecting its degradation products. In addition, both at the steady-state and upon Ca-switch, we found that the loss of Rac1 activity determines a significant shift of E-cadherin from the TX-100 insoluble pool to the soluble fraction, reflecting a loss of its binding to cytoskeleton. Taken together these data indicate that the crucial role of Rac1 in cell polarization is elicited by modulating the E-cadherin dynamics and membrane organization and, therefore, ensuring functional adherens junctions.

Plenary Lecture

Regulation of transport through the endocytic pathway

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Endocytosis plays an essential role in the cell surface expression of various proteins and in signaling cascades. Sequential transport from early to late endosomes requires the coordinated activities of the small GTPases Rab5 and Rab7. The transition between early and late endosomes is mediated by Rab conversion, a process in which the loss of Rab5 from an endosome occurs concomitant to the acquisition of Rab7. We identified SAND-1/Mon1 as the critical switch for Rab conversion in metazoa. SAND-1 serves a dual role in this process. First, it interrupts the positive feedback loop of RAB-5 activation by displacing RABX-5 from endosomal membranes and, second, it times the recruitment of RAB-7 probably through interaction with the HOPS complex to the same membranes. SAND-1/Mon1 thus acts as a switch by controlling the localization of RAB-5 and RAB-7 GEFs.
CORVET and HOPS are tethering complexes in the endosomal pathway in yeast. They share a common core and differ only in two subunits, which are important for the interaction with Rab GTPases. We show that both complexes also exist in metazoans. One of the core components, the SM protein Vps33 is present in two isoforms VPS-33.1 and VPS-33.2, each of which is specific for one complex. The effect of knockdown of individual HOPS and CORVET components was studied in oocytes, in epithelial cells and in coelomocytes. These analyses revealed that the predominant role of the CORVET complex is to promote homotypic fusion of early endosomes and the fusion of endosomal carriers with early endosomes, while the HOPS complex performs functions in endosome maturation and endolysosome formation The specificity of CORVET and HOPS complex function is at least in part dependent on the individual VPS-33 subunit.

Looking for correctors of Lowe Syndrome: a High Content Screening (HCS) approach

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Lowe Syndrome is a rare X-linked genetic disease (1:500,000 newborn males) caused by mutations in OCRL1, a gene encoding a phosphatidylinositol 4,5-bisphosphate 5-phosphatase. Patients with Lowe Syndrome present congenital cataracts, central hypotonia, mental retardation and renal Fanconi syndrome (loss of salts and low molecular weight proteins from the urine). The OCRL protein associates with the endosomal compartment and the Golgi complex. We have previously shown that OCRL is a key component in the endocytic trafficking of different receptors, including the mannose-6-phosphate receptor (MPR), transferrin receptor and megalin (Vicinanza et al., EMBO J. 2011). In fact, the knock-down of OCRL induces the redistribution of the mannose-6-phosphate receptor (MPR) into peripheral early endosomes due to impaired retrieval of MPR to the Golgi complex and impairs the internalization of ligands of TfR (transferrin) and of megalin (RAP), as these receptors fail to efficiently recycle from the early endosomes to the plasma membrane.

We exploited the endocytic defects induced by OCRL knock-down (KD) to set up a "phenotypic" microscope-based assay suitable for high content screening (HCS) of small molecules to search for correctors of the MPR and RAP phenotypes. We screened small molecules from the LOPAC collection (an annotated library of 1280 Pharmacologically-Active Compounds from Sigma) in a stable OCRL-KD-inducible HeLa cell line and in Human Kidney cells (HK2) transiently interfered with OCRL targeted siRNA. 113 positive hits emerged from the primary screening and have been evaluated in a secondary screen. Positive hits were tested on OCRL-KD HK2-cells in 384-well plates, at 1, 3 and 10μM, and 59 of these were confirmed to be correctors also of the RAP uptake phenotype. We are now analysing some of the 59 drugs, screening them for their ability to revert other OCRL-KD induced phenotypes. These correctors represent potential drugs for the treatment of Lowe Syndrome.

Oral

Rescue of folding defects, modulation of receptor activity and anti-tumoral potential of the first organic molecules addressing Frizzled4

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Molecular screening toward pharmacological folding chaperones leads here to the identification of the first small organic molecules that modulate Frizzled4 (Fz4). This member of the Class F GPCRs is involved in the Wnt signaling and controls cellular stemness, invasiveness of brain tumors and occurance of the Familial Exudative Vitreorethinopaty (FEVR). The molecules here described revert the folding defect induced by the autosomic dominant Fz4 mutation L501fsX533. Moreover they display antagonist activity on Fz4, inducing β-catenin degradation and drastically reducing the growth of a highly invasive Fz4 dependent glyoblastoma cell line. Differently from the Fz4 agonist Wnts, these molecules bypass the need for coreceptor LRP-5/6 to modulate the Wnt signaling. All suggest future therapeutic applications for the treatment of tumors and genetic diseases dependent on alteration of the Fz4/Wnt pathway.

MicroRNAs regulate lysosomal system by targeting MPRs and Adaptor Protein complex 1 subunits

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Lysosomes are single membrane surrounded cytoplasmic organelles present in almost all eukaryotic cells, implicated in degradation and recycling processes. Mutations that cause defect in specific lysosomal components result in more than 50 lysosomal storage disorders, characterized by intracellular deposition of undegraded substrates and protein aggregation, events also found in neurodegenerative diseases. A database on human lysosomal genes and their regulation was realized (http://lysosome.unipg.it). The database integrates miRNA binding predictions from five softwares (TargetScanS, picTar 4-way and 5-way, PITA, miRanda). Even if no specific miRNA coordinating lysosomal genes have been indentified, bioinformatic analysis revealed that genes coding for proteins involved in lysosome biogenesis, vesicle trafficking and autophagy are indeed trageted by a higher number of miRNAs. In particular genes relevant for acid hydrolases sorting to lysosome, such as receptors for mannose 6-phosphate (M6PR, IGF2R) or other receptors (SORT1) and subunits of the Adaptor Protein complex 1 (AP1), have been shown to be regulated by miR-9 and miR-211 by luciferase assays. Moreover, the over-expression of miR-9 in cells impared the processing of cathepsin D (CATD), an aspartic protease that acquire its functional form only after proteolytic cleavage at acid pH in lysosome, suggesting that miR-9 could affect CATD transport to lysosome.

Oral

Multiple functions of the SNARE protein Snap29 in autophagy, endocytic and exocytic trafficking during epithelial formation in *Drosophila*

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How autophagic degradation is linked to endosomal trafficking routes is little known. Here we screened a collection of uncharacterized *Drosophila* mutants affecting membrane transport to identify new genes that also have a role in autophagy. We isolated a loss of function mutant in *matryoshka,* the gene encoding the conserved SNARE protein Snap29 and have characterized its function *in vivo.* Snap29 contains two SNARE domains and a NPF motif at its N-terminus and rescue experiments indicate that both SNARE domains are required for function, whereas the NPF motif is in part dispensable. We find that Snap29 interacts with SNAREs, localizes to multiple trafficking organelles, and is required for protein trafficking and for proper Golgi apparatus morphology. Developing tissue lacking Snap29 displays distinctive epithelial architecture defects and accumulates large amounts of autophagosomes, some of which secreted or nested inside each other, highlighting a major role of Snap29 in autophagy. Mutants for autophagy genes do not display epithelial defects, suggesting that the epithelial alterations of the *matryoshka* mutant are not caused by the impairment of autophagy. In contrast, we find evidence of elevated levels of JAK/STAT ligand, receptor and associated signaling, which might underlie the epithelial defects. In summary, our findings support a role of Snap29 at key steps of membrane trafficking, and predict that signaling defects, rather than impaired autophagy, may contribute to the pathogenesis of CEDNIK, a congenital syndrome due to loss of Snap29.

Molecular mechanisms of post-Golgi tubular carrier formation

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Transport from the Golgi complex to the basolateral plasma membrane is mediated by large tubular pleiomorphic carriers that bud at the trans-Golgi network by *en-bloc* extrusion of specialised tubular domains, and undergo fission during extrusion along microtubules. The molecular mechanism of formation of these carriers is partially understood. We have described a complex made of proteins that are involved both in budding and fission. These include ARF, frequenin and the kinases PKD and PAK as well as PI4KIIIβ bridged by 14-3-3γ with the fission-inducing protein BARS (for brevity, PB complex). Disrupting this complex inhibits the fission of carrier precursors, indicating that the complex couples budding with BARS-mediated fission. We have also previously reported that BARS expresses a slow lysophosphatidic acid acyltransferase activity (LPAAT) *in vitro*; later work showed however that the LPAAT activity associated with BARS is not intrinsic to this protein. In addition, we reported that Golgi membranes possess an active LPAAT which, upon provision of appropriate substrates, produces significant levels of phosphatidic acid (PA), and promotes the fission of Golgi membranes and, that BARS potentiates the PA production and Golgi membrane fission induced by the endogenous LPAAT. We now report that BARS a) binds to, and potently stimulates, an LPAAT isoform (LPAAT4) that localizes at the Golgi as well as on TGN-derived tubular carrier precursors, b) the activity of LPAAT4 is required for the fission of the Golgi carriers and, c) the incorporation of BARS in the PB complex is necessary for the stimulation of LPAAT4 as well as for carrier fission. Thus, BARS activates LPAAT4 to form PA, a bilayer-destabilizing lipid that is essential for the fission event.

Poster

The secretory IgM heavy chain potently activates the unfolded protein response through its CH1 domain

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All proteins destined to travel along the secretory pathway fold and assemble in the endoplasmic reticulum (ER) with assistance of molecular chaperones and folding enzymes. A stringent quality control ensures that only fully folded proteins exit the ER. If the folding capacity of the ER becomes insufficient to handle the protein folding load, the unfolded protein response (UPR) is invoked to restore ER homeostasis. Most studies on UPR signaling involve drugs that perturb productive protein folding in the ER, such as tunicamycin, which abrogates N-glycosylation. We decided to trigger the UPR instead with a proteostatic challenge. To this end, we inducibly expressed the secretory IgM heavy chain (μ_S) in HeLa cells. We found that μ_S activates both the IRE1 and PERK branches of the UPR in a dose and time dependent manner. Interestingly, the μ_S did not signal the UPR anymore when it lacked the CH1 (constant) domain (μ_Δ) or when this domain has become inaccessible because of the pairing with the light chain (λ). Conversely, the CH1 domain was sufficient to activate the UPR when fused to the VL (variable domain of λ). Our data identify the CH1 domain as a potent activator of the UPR, a feature which may be key for full-blown ER expansion during B to plasma cell differentiation.

Poster

Medaka as a model for Spondyloepyphyseal dysplasia tarda

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Spondyloepyphyseal dysplasia tarda, caused by mutation in the Sedlin gene, is a human X-linked genetic disease characterized by disproportionate short stature with short neck and trunk, barrel chest and absence of systemic complications. Distinctive radiological signs are platyspondyly with hump-shaped central and posterior portions, narrow disc spaces, and mild to moderate epiphyseal alterations. Sedlin is part of a big complex named TRAPP (TRAnsport Protein Particle) which is involved in membrane trafficking along the secretory pathway. In chondrocytes, Sedlin mutations result in defects in deposition of extracellular matrix by controling the exit of the procolagen II from the ER.
So far, no SEDT animal model have been generated.
Here we show that morpholino Sedlin Medaka is a useful model system to study mechanism leading SEDT and identify disease-modifying compounds. Sedlin is exclusively expressed in *vertebrae centrum* and Knock-down of Sedlin in Medaka causes short body length, an abnormal bone mineralization with the absence of both neural arches and caudal fin with increased space between vertebrae centrums.
These preliminary data suggest that Sedlin function is conserved among the species. Sedlin morphants recapitulate features of SEDT disease making the Medaka system a suitable model to screen potential drugs and identify molecular mechanisms involved in PCII secretion.

Design and layout

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