ABCD Meeting

Membrane Trafficking and Organelle Biogenesis

Bertinoro, 3-4 April 2009

Programme and Abstracts

Friday, 3 April

13:00-14:00 Lunch

14:30-15:50 Session I » Endoplasmic reticulum: biogenesis and homeostasis

14:30-14:50 *Jessica Maiuolo (Catanzaro)* A cell model to investigate the mechanism of endoplasmic reticulum expansion in response to increased expression of ER membrane proteins

14:50-15:10 *Roberto Sitia (Milan)* Physiology of ER-to-Golgi protein transport

15:10-15:30 *Giuseppina Amodio (Fisciano)* Endoplasmic reticulum stress reduces the export from the ER of the VSV G glycoprotein and alters the architecture of post-ER compartments

15:30-15:50 *Elisa Mazzoli (Siena)* YIP1 isoforms in skeletal muscle

16:00-16:30 Coffee break

16:30-17:30 Guest lecture

Volker Haucke (Berlin)
Regulation of vesicle-mediated membrane traffic at synapses and beyond

17:30-19:10 Session II » Intracellular traffic in neurons

17:30-17:50 *Gaia Berto (Turin)* Down Critical Region-encoded protein TTC3 inhibits neuronal differentiation in hippocampal neurons

17:50-18:10 *Eugenio Fornasiero (Milan)* Membrane trafficking in neuronal development

18:10-18:30 *Aram Megighian (Padua)* The role of the *Drosophila melanogaster* SNAP-25 C-terminal region in neurotransmitter release

18:30-18:50 *Claudia Verderio (Milan)* Mechanism of microparticle release from glial cells. Role of glia-derived microparticles in the control of neuronal activity

18:50-19:10 *Elisabetta Menna (Milan)* Eps8 regulates axonal filopodia in hippocampal neurons in response to BDNF

19:30-20:00 General discussion and election of Vice-Chairman for next year

20:00-21:00 Dinner

Saturday, 4 April

8:30-9:10 Session III » Mitochondrial biogenesis

8:30-8:50 *Simona Reina (Catania)* Swapping of VDAC domains to investigate structure-function relationships

8:50-9:10 *Teresa Rinaldi (Rome)* Dissection of the carboxyl-terminal domain of the proteasomal subunit Rpn11 in maintenance of mitochondrial structure and function

9:20-11:00 Session IV » From outside to inside the cells:

receptor trafficking

9:20-9:40 *Erika Donà (Milan)* G-protein dependent trafficking of the oxytocin receptor

9:40-10:00 *Monica Fabbri (Milan)* β-arrestins are required for the induction and strengthening of shear stress-resistant adhesion during leukocyte extravasation

10:00-10:20 *Katia Cortese (Genoa)* High resolution study of stimulated ErbB2 endocytosis

endocytosis of virulence factors

10:20-10:40 *Lucia Brandi (Padua)* Anthrax toxins: following their way inside host cells

10:40-11:00 *Zulema Antonia Percario (Rome)* HIV-1 Nef protein is able to enter human primary macrophages activating signal transduction events through a mechanism IRAK1 degradation – independent

11:10-11:30 Coffee break

11:30-13:10 Session V » Intracellular traffic and organelle biogenesis in diseases

11:10-11:30 *Antonella De Jaco (Rome)* Trafficking of Neuroligin3 and disease-associated mutations

11:30-11:50 *Maria Vittoria Barone (Naples)* Gliadin peptide P31-43 enhances IL15 activity by interfering with its intracellular trafficking

11:50-12:10 *Ilaria Palmisano (Milan)* OA1, an intracellular G protein-coupled receptor, regulates both melanosome biogenesis and transport in pigment cells

12:10-12:30 *Daniela Sarnataro (Naples)* Analysis of intracellular localization and interaction between Prion Protein (PrP^C) and its homolog Doppel

12:30-12:50 *Elisa Fasana (Milan)* The early steps in the biogenesis of VAP-B and its mutant form associated with amyotrophic lateral sclerosis

12:50-13:10 *Riccardo Ronzoni (Milan)* Protein folding, protein degradation and quality control in a model of protein misfolding disease

13:30 Lunch and departure

Abstracts

Alternative splicing of TI-VAMP/VAMP7 and other human longins

Marcella Vacca[1,2], Valeria Rossi[1], Andrea Carpi[3], Floriana Della Ragione[2], Lara Albania[1], Ornella Rossetto[3], Maurizio D'Esposito[2], Francesco Filippini[1]
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Alternative splicing (AS) is a common mechanism by which a genome flexibility is acquired to better manage complex biological phenomena. Among the multitude of them, the control of intracellular vesicle trafficking is an ideal target to weight the role of AS in shaping genomes to make cells. Post-transcriptional regulation can concern both v(R)- and t(Q)-SNAREs, which play a pivotal role in the control of subcellular trafficking. Here we provide the first report on AS of human longins, which represent the most widespread v(R)-SNAREs, being essential in eukaryotes and prototyped by TI-VAMP (Tetanus neurotoxin insensitive VAMP)/VAMP7, Sec22b and Ykt6. We demonstrate that the AS of SYBL1, encoding TI-VAMP/VAMP7 results in skipping of whole exons and/or exon fragments. Therefore, isoforms may show in-frame as well as frameshift coding sequence modification, which in turn modulates domain combination and architecture of isoforms (missing whole domains or domain fragments and/or showing variant or extra domains). This post-transcriptional control is conserved among longins, as it concerns also Ykt6 and Sec22 genes. Since TI-VAMP and its longin domain are known to be involved in the regulation of membrane fusion and neurite outgrowth, and longin Ykt6 is highly expressed in neurons, we started monitoring levels of longin isoforms and their variation both in different cell lines and along neuronal differentiation of a neuroblastoma cell line.

Endoplasmic reticulum stress reduces the export from the ER of the VSV G glycoprotein and alters the architecture of post-ER compartments

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Newly synthesized proteins gain their native conformation within the Endoplasmic Reticulum (ER), where Quality Control machinery operates to ensure that only correctly folded molecules leave the compartment in transport vesicles. Several stress conditions induce protein misfolding within the ER. To counteract this event eukaryotic cells evolved a specific pathway, termed Unfolded Protein Response (UPR), which triggers the transcription of chaperones and membrane trafficking factors, attenuates protein synthesis and favours the Endoplasmic Reticulum Associated Degradation of misfolded polypeptides. Since little has been reported on how the UPR relates to post-ER compartments, we analysed the effect of ER stress on the intracellular localization of the cargo receptor protein ERGIC-53 and of the golgin GM130, dynamic markers of the ER Golgi Intermediate Compartment and of the cis-Golgi, respectively. We report that exposure to ER stress severely alters the morphology of the ERGIC and the Golgi complex suggesting that ER stress might interfere with the molecular events required for the biogenesis of post-ER compartments. Moreover, in response to ER stress induced by either TG or NO the localisation pattern of both proteins became more dispersed throughout the cytoplasm and segregates in different membrane structures. In addition, experiments of BFA wash-out showed that in presence of Tapsigargin, ERGIC-53 and GM130 were unable to recover their original intracellular pattern suggesting that ER stress alters the vesicular transport between the ER and the Golgi complex and/or interferes with the process of reconstruction of either the ERGIC or the cis-Golgi. We also studied the effect of the ER stress on the intracellular trafficking from the ER to the Golgi complex by analyzing the rate of transport of the ts045 conditional mutant of the VSV G protein. Confocal microscopy showed that in transfected cells upon shifting to 32°C the amount of green fluorescent ts045G protein transported to the Golgi complex drastically declined. Meanwhile, the greater part of the reporter protein was still retained within the ER suggesting that ER stress reduced the export of the reporter protein. This impairment is most likely due to the reduced COPII coat assembly and/or stability at the ER membranes as revealed by the reduction of the number of exit sites showed by immunofluorescence of the SEC31 marker.

Gliadin peptide P31-43 enhances IL15 activity by interfering with its intracellular trafficking

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Background: We previously observed that A-gliadin peptide P31-43 induces proliferative effects similar to Epidermal growth factor (EGF) both in cultured cell lines and enterocytes from celiac disease (CD) patients. The effect is mediated by delayed EGF degradation and prolonged EGF receptor (EGFR) activation in endocytic vesicles due to P31-43 mediated interference with endocytic maturation.
Aims: To test P31-43 effects on IL15 induction at level of transcription, translation, intracellular trafficking and its role in P31-43 induced proliferation.
Methods: Semi-quantitative and real time PCR investigated P31-43 effects on IL15 mRNA levels. Protein levels and distribution was analyzed by Facs, Elisa and immunofluorescence. Stat5 and IL15 receptor alfa (IL15Ra) activation has been examined by WB. BrdU (Bromodeoxiuridine) analyzed proliferation.
Results: In Caco2 cells P31-43 slightly increases IL15 mRNA levels. IL 15 protein was found increased only on the cell surface together with markers of recycling vesicles, as transferrin receptor and Lamp2, implying a P31-43-mediated interference with IL15 vesicular trafficking. On the cell surface IL15 is linked to the receptor, its increase is not dependent on new protein synthesis and functions as a growth factor for CTLL 2 cells. Stat5 and the IL15Ra are activated after P31-43 treatment. Anti-IL15 blocking antibodies can prevent P31-43 induced increase of proliferation in Caco2 cells and in enterocytes of biopsies from CD patients.
Conclusion: P31-43 induces enhanced presentation of IL15 in trans to the neighboring cells, interfering with its vesicular trafficking. Justacrine signaling of the IL15/IL15-receptor-alfa contributes both to cell proliferation and activation of innate immunity.

Down Critical Region-encoded protein TTC3 inhibits neuronal differentiation in hippocampal neurons

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Neuronal connectivity, which is the basis of brain function, arises from the sprouting and elongation of axon and dendrites at the right time and in the right direction. This first step of neuronal differentiation, also known as neuritogenesis, requires a complex series of intracellular events, leading to a drastic reorganization of the cytoskeleton. In parallel, it requires a polarized delivery of plasma membrane from the Golgi apparatus to the growing neurites. Rho small GTPases are master regulators of these processes and act through a complex network of effector molecules. Citron proteins, known effectors of RhoA, may regulate neuronal differentiation by modulating the dynamics of actin and Golgi apparatus (Di Cunto et al., 2003; Camera et al., 2003). We have previously found that TTC3, one of the Down Critical Region (DCR) genes on the Chromosome 21, physically interacts with Citron proteins and functionally cooperates with RhoA and Citron to inhibit the PC12 cells differentiation (Berto et al. , 2007).
In this work, we investigated the role of TTC3 in rat hippocampal neurons. Even in these cells, TTC3 is a negative regulator of neuritogenesis. Its overexpression leads to a strong inhibition of neurite extension; conversely, TTC3 knockdown stimulates axonal growth. To evaluate the role of the actin cytoskeleton in the TTC3 overexpression phenotype we analyzed how it is modulated by drugs that affect actin polymerization. Interestingly, the actin polymerization inhibitor cytochalasin D is capable to revert the phenotype of TTC3 overexpression. Since Citron proteins and actin filaments are involved in maintaining the structural organization of the Golgi in young neurons (Camera et al., 2003) we asked whether the function of TTC3 is also related to this organelle. Interestingly, immunofluorescence microscopy revealed that endogenous TTC3 has a polarized distribution and is partially colocalyzed with specific Golgi markers. More importantly, the overexpression of TTC3 induces Golgi fragmentation, suggesting that its differentiation-inhibiting activity could be also the result of altered Golgi function.
The results suggest that the TTC3 overexpression modify actin polymerization and could change Golgi architecture leading to neuritogenesis arrest.

Anthrax toxins: following their way inside host cells

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A central role in *Bacillus anthracis* infection is played by two A-B toxins: edema and lethal toxin. They are composed of two different catalytic subunits, edema factor (EF) and lethal factor (LF) which associate to a single binding subunit, protective antigen (PA). PA binds to cell surface receptors, oligomerizes and the binding of LF and EF to PA oligomer is followed by their endocytosis. Thus, EF and LF exploit the host cell endocytic pathway to reach their intracellular targets. Upon exposure to the acidic milieu of the endosomal lumen, PA heptamer undergoes a conformational change that leads to its membrane insertion with formation of a pore that mediates the translocation of LF/EF across the endosomal membrane into the cytoplasm.
However, it is not clear at which stage of the endocytic pathway membrane insertion occurs.
EF and LF are believed to share the same internalization pathway. However, recent studies suggest a different localization after translocation into the cytosoplasm. LF is released into the cytoplasm while EF appears to associate to the cytosolic side of late endosomes around the nucleus.
The aims of this study are to clarify the trafficking of the toxins into the host cell and to investigate the possible implication of EF/LF structure in their different cytoplasmatic localization.
Recombinant EF-EGFP, EF-mCherry and LF-EGFP were successfully expressed in *E. coli*.
To validate the use of chimeric toxins, electrophysiology studies on the translocation of the recombinant subunits across PA heptamer inserted in planar phospholipid bilayers were performed. They revealed that the chimeric proteins preserve the ability to translocate across the PA channel.
Studies on cultured cells intoxicated with EF/LF-EGFP showed that both the toxins reach late endosomes. Moreover, our results confirm that after translocation into the cytoplasm they exhibit different localizations.
To investigate this issue, chimeric EF/LF with swapped PA binding domain and fused to EGFP, were expressed and purified. Here, we will report our preliminary observations on the behaviour of chimeric swapped factors.

Unassisted insertion of tail-anchored proteins into lipid bilayers is modulated by cytosolic proteins

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Tail anchored (TA) proteins are membrane proteins that contain an N-terminal domain exposed to the cytosol and a single transmembrane segment near the C-terminus followed by few or no polar residues. TA proteins with a mildly hydrophobic transmembrane domain, such as cytochrome b5 (b5), are able to insert post-translationally into pure lipid vesicles without assistance from membrane proteins.
Here, we investigated if any cytosolic protein is needed to maintain b5 in a competent state for transmembrane integration. Using b5 constructs translated in vitro or produced in bacteria we demonstrate that cytosolic proteins are neither necessary nor facilitatory for the unassisted translocation of b5. Furthermore, we demonstrate that no cytosolic protein is involved in the translocation of a C-terminal domain of 85 residues appended to the transmembrane domain of b5. Nevertheless, b5 does bind cytosolic proteins, and in their presence but not in their absence, its insertion into liposomes is inhibited by the thiol oxidant diamide and the alkylating agent N-ethylmaleimide. The effect of diamide is observed also in living cells. Thus, the specific in vivo targeting of b5 may be achieved by interaction with redox sensitive targeting factors that hinder its nonspecific insertion into any permissive bilayer.

High resolution study of stimulated ErbB2 endocytosis

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Overexpression of the orphan ErbB2 receptor has a frequent occurrence in human breast cancer and correlates with poor prognosis. Targeting of ErbB2 with HSP90 chaperone inhibitors such as Geldanamycins has proven to be a promising therapeutical approach dowregulating ErbB2 toward the lysosomal compartment. However, ErbB2 early endocytic events have remained elusive. Several endocytic routes do not involve clathrin-coated pits, however alternative non-clathrin pathways lack specific markers. Using complemetary high resolution imaging approaches, we have examined the early stages of internalization and trafficking of ErbB2. We have demonstrated that Geldanamycin induced ErbB2 internalization from the plasma membrane via a major tubular clathrin independent endocytic pathway that shares some features with the CLICs/GEECs pathway in SKBr3 breast cancer cells. To visualize ErbB2 primary carriers at the ultrastructural level, we used Herceptin-HRP to target surface ErbB2 and performed time course endocytic assays. Dynasore, a novel inhibitor of dynamin, dramatically impaired Herceptin uptake, suggesting a role for dynamin in ErbB2 trafficking. To provide further insights in the 3D architecture of ErbB2 carriers we performed 3D tomography analysis. Our results showed that ErbB2-positive and CLICs carriers shared common features with endosomes, such as tubular/stacked domain and a globular domain containing intralumenal vesicles, providing new insights in the structure-function relationship. The early stages of ERBb2 endocytosis were also examined quantitatively in plasma membrane lawns. Geldanamycin induced transient changes in clustering patterns, suggesting a lack of correlation between clustering and endocytosis. However, biochemical analysis showed that a rapid, transient ErbB2/ErbB1 heterodimerization and activation of downstream ERK1/2 signalling pathway occurred at very early stages of Geldanamycin treatment, suggesting that spatial distribution is related to ErbB2 function. We next examined the protein machinery involved in ErbB2 trafficking. RhoGTPases are key regulators of cytoskeleton dynamics and influences many cellular processes including cell migration and endocytic trafficking. We have studied the role of the RhoGAP GRAF1 protein, a BAR sensing/generating curvature protein marker for early GEECs, in ErbB2 trafficking. We found that ErbB2 was internalized into GRAF1 and Rab8 positive tubules before reaching transferrin-positive early endosomes, suggesting a role for GRAF1 in ErbB2 endocytosis/trafficking. Surprisingly, Geldanamycin dramatically modified GRAF1 tubules and induced the formation of both filopodia and stress fibers, suggesting an inhibitory role for HSP90 in GRAF1 and Rho/cdc42 function.

Trafficking of Neuroligin3 and disease-associated mutations

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Proteins of the α/β-hydrolase family including Neuroligins (NLGNs), Acetylcholinesterase (AChE) and Thyroglobulin (Tg) are characterized by a domain with a critical structural role for heterophilic adhesion, hydrolase catalysis and protein secretion. It has recently been proposed that the α/β-hydrolase fold domain might be crucial for folding and exit from the ER of newly synthesized proteins. Mutations in the α/β-hydrolase fold domain are found in the NLGNs in association with autism, in the cholinesterase affecting the metabolism of specific drugs and in thyroglobulin associated to hypothyroidism. Sequence polymorphisms in *NLGN3* and *NLGN4* genes have been reported in autistic patients, indicating NLGNs as candidate target genes in brain disorders. We have characterized the autism-related R451C mutation and showed that when the substitution is inserted in NLGN3, AChE and BChE the mutant proteins result mostly retained in the Endoplasmic Reticulum. We have further investigated folding, biosynthetic protein processing and trafficking of NLGN3 *wild type* in order to study alterations caused by the introduction of mutations in the α/β-hydrolase fold domain. To determine the severity and nature of the export defect, R451C mutation was compared to G221R in NLGN3, homologous to Tg G2320R responsible for congenital hypothyroidism in *rdw/rdw* rats. Our findings show that R451C and G221R mutations affects folding and slow down maturation of NLGN3, but R451C only partially reduces export because it is still able to incorporate into synapses while G221R completely abolishes NLGN3 export, both in a non-neuronal model system and in primary hippocampal neurons. We have identified several proteins belonging to distinct ER chaperones families, which differently associate with NLGN3 *wild type* or mutant proteins, suggesting that the ERQC system is blocking the proteins to exit the ER. Understanding the mechanisms by which these proteins are retained within the cell should provide important insights into common events leading to diseases linked to mutations in proteins belonging to the α/β-hydrolase family. (Supported by NIH R37-GM18360 and U0-1 ES10337 to PT).

G-protein dependent trafficking of the oxytocin receptor

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The oxytocin receptor (OTR) is a promisquous G-protein coupled receptor that couples to both Gαq and Gαi and whose stimulation leads to the activation of different intracelluar signaling pathways. Following oxytocin (OT) treatment, OTR internalizes via clathrin coated vesicles and recycles to the plasma membrane via Rab4/Rab5 vesicles (Conti et al, Am J. Physiol 2009).
OT –derived peptides that activate selectively either the Gαi or Gαq pathways were developed and characterized in our laboratory (Reversi et al 2005 J Biol Chem). In order to assay the capability of these analogs to promote receptor internalization we developed a fluorescent selective OTR/Gaq agonist (dLVT-Alexa568) and a selective OTR/Gai agonist (atosiban-Alexa594) which showed no differences from the non fluorescent peptide concerning their binding and coupling properties. We then performed in vivo imaging experiments using HEK293T cells stably expressing the human OTR fused with EGFP at its C-terminus (OTR-EGFP) to follow the intracellular fate of the receptor and the ligands.
After stimulation of the cells with dLVT-Alexa568, the OTR/Gaq agonist, we observed internalization of both the receptor and the fluorescent peptide. The two molecules were initially colocalized in vesicles, but this colocalization was lost after two hours when the receptor and the ligand were present in different vesicles, suggesting that they followed different trafficking pathways. On the contrary, the selective OTR/Gai agonist (atosiban-Alexa594) did not lead to any change in receptor localization at the plasma membrane even after one hour of stimulation.
Since OTRs are expressed in the CNS in neuronal and glial cells, we used a neuronal cell line (the mouse neuroblastoma Neuro2A cells) transiently transfected with human OTR to further investigate receptor trafficking. Both the OTR-EGPF and OTR tagged at its N-terminus with an HA epitope were localized at the plasma membrane as expected, but, interestingly , no internalization was observed after agonist stimulation in in vivo imaging experiments. Only when Gαq was co-transfected with the OTR, internalization after agonist stimulation was observed in many OTR-EGFP expressing cells. These data, taken together, suggest that Gαq activation plays an important role in OTR internalization.

β-arrestins are required for the induction and strengthening of shear stress-resistant adhesion during leukocyte extravasation

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Leukocyte extravasation involves interdependent signaling pathways underlying the complex dynamics of firm adhesion and diapedesis. While signal transduction by agonist-bound chemokine receptors plays a central role in the above responses, it is unclear how it contributes to the sustained and concurrent nature of such responses, given the rapid kinetics of chemokine-induced trimeric G protein coupling and homologous desensitization. Ubiquitous β-arrestins may contribute to the spatial and temporal dynamics of chemokine-induced adhesion and motility by catalyzing a sustained, spatially confined activation of signaling directly downstream of agonist-bound chemokine receptors. By combining in vivo approaches in β-arrestin knockout mice with in vitro studies in engineered cellular models we show that β-arrestins are required for the onset and maintenance of shear stress-resistant, integrin-mediated adhesion. This requirement is downstream of and complementary to Gi coupling and involves the functional upregulation of the small GTPase Rap1. While rapid chemokine-induced adhesion requires limiting concentrations of β-arrestins but is not isoform specific, adhesion strengthening under flow is selectively dependent on β-arrestin 2, which synergizes with phospholipase C (PLC) in promoting Rap1 activation. Thus, receptor-induced Gi and β-arrestins act sequentially and in spatially distinct compartments to promote optimal chemokine-induced integrin-dependent adhesion during leukocyte extravasation.

Melanosome biogenesis: role of OA1 signaling pathway

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The OA1 protein is a pigment cell specific membrane glycoprotein that belongs to the GPCR (Gprotein Coupled Receptors) superfamily. Unlike all other known GPCRs, it localizes exclusively in intracellular compartments, i.e. endolysosomal and melanosomal membranes, and it is not expressed on plasmamembrane. Absence or misfunctions of OA1 bring to Ocular Albinism type 1, an X-linked disease involved in the visual system. The phenotypic characteristic of this disease is the presence of giant melanosomes (macromelanosomes) in retinal pigmented epithelium (RPE) and skin. These observations suggest that OA1 could play a role in melanosome biogenesis. In spite of this knowledge, the physiological function of OA1 is still unclear. Our results show that OA1 regulates MITF (MIcrophthalmia-associated Transcription Factor), a transcription factor involved all along the life of pigmented cells. MITF is located in the center of multiple signaling pathways, controlling the differentiation, morphology, proliferation, and survival of the melanocyte lineage (melanoblasts and melanocytes). Furthermore, changes in Mitf activity are implicated in melanoma progression. Mitf plays a major role in melanocytes differentiation, by inducing the key enzymes of melanogenesis. We demonstrate that OA1 regulates MITF at transcriptional level in two distinct cell systems: the rate of production of MITF is sustained by the presence of OA1. In cells depleted for OA1, the mRNA and protein levels of MITF show a notable decrease, leading to impaired transcription of a key melanogenesis enzyme (GP100). We further demonstrate that OA1 is involved in the cAMP-signaling pathway, sustaining the MITF transcription under differentiative conditions.

The early steps in the biogenesis of VAP-B and its mutant form associated with amyotrophic lateral sclerosis

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VAP-B is an ER resident protein that functions as adaptor for lipid-exchange proteins. A mutant form, VAP-B P56S, causes a familial form of amyotrophic lateral sclerosis. It has been previously reported that VAP-B P56S forms aggregates, which have been implicated as the cause of motor neuron toxicity. Here, to understand the basis of the pathogenicity of mutant VAP-B, we have focused on the early steps of the biogenesis of mutant and wild-type VAP-B.
VAP-B belongs to the heterogeneous group of so-called Tail anchored (TA) proteins, which, because of their topology (transmembrane (TMD) domain at the C-terminus), do not rely on the classical SRP/Sec61 system of membrane insertion into the ER, and use instead an incompletely characterized post-translational mechanism. Recent studies indicate that moderately hydrophobic TMDs can insert spontaneously into protein-free lipid bilayers, while more hydrophobic TMDs require ER protein(s). Using in vitro translation-translocation systems, we demonstrate that: (i) VAP-B translocates post-translationally with low efficiency into microsomes but not into pure lipid liposomes, as predicted from the hydrophobicity of its TMD; (ii) the low efficiency of VAP-B translocation depends on its TMD, but is not improved by the deletion of its two dimerization sequences; (iii) VAP-B P56S inserts into membranes in the same way as the wild-type protein.
After expression of VAP-B in cultured cells, we demonstrate that: (i) Except at very early time points after inducing its expression (up to 2 hours after microinjection of the cDNA), VAP-B P56S is exclusively contained in aggregates, in contrast to the wild-type protein, which localizes to the ER; (ii) the aggregates colocalize to different extents with different ER markers. Transmission EM analysis of mutant VAP-B expressing cells shows a unique ER morphology, consisting most commonly of pairs of ER cisternae united by an electron-dense layer of cytosol approximately 30 nm thick; these apparent cisternal pairs are most likely due to indentations within single cisternae. Finally FRAP and FLIP analysis show that a GFP-tail anchored reporter freely diffuses between the aggregates and the surrounding ER and that the VAP-B P56S aggregates are continuous with the ER entire compartment. We are presently investigating the relationship to P56S-VAP-B induced structures to other organelles.

Membrane trafficking in neuronal development

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The establishment and maintenance of an organized architecture are essential processes for proper neuronal activity and ultimately for the functioning of the whole brain. The plasmalemma is the neuron's largest organelle undergoing continuous reconfiguration during the first steps of neuronal development. For this reason, spatially-regulated plasma membrane traffic together with polarized exocytosis and endocytosis are tightly regulated, fundamental processes for the development of neurons. In the present work we address the identity and regulation of the membrane paths during neuronal growth and axonal remodelling of young hippocampal neurons in vitro. At early developmental stages the membrane is delivered, retrieved and rearranged in a constitutive manner. At later stages, during synaptogenesis, a program of maturation leads to a change in plasma membrane dynamics corresponding to the emergence of depolarization-induced synaptic vesicle exo-endocytosis. We propose that the control of bulk membrane retrieval accounts for the efficient remodelling of the axonal plasma membrane and growth cone during axonal outgrowth and that its down regulation during and after synapse formation has a role in the preservation of synaptic vesicle specificity.

Essential role of Citron-K in the late stage of mammalian cell division

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Cytokinesis is the final step of mitosis, at the end of which the two daughter cells phisycally separate through a poorly understood process called abscission. Studies conducted in the last years have increased our knowledge of this process, showing that its players and mechanisms are evolutionary conserved and that rearrangements of the actin cytoskeleton and intracellular membranes play a pivotal role in these events. CIT-K is a protein kinase that plays important roles in cytokinesis in several systems. Anillin is a conserved protein, involved in the organization of the acto-myosin ring during cytokinesis. The phenotypes of Drosophila S2 cells depleted of CIT-K and Anillin are very similar, suggesting that CIT-K and Anillin could operate in the same pathway. In this study, we present several data for a functional interaction of these two proteins in mammalian cells. CIT-K and Anillin colocalize during cytokinesis in HeLa cells. By performing reciprocal coimmunoprecipitation studies, we have shown that Anillin and CIT-K strongly interact during the end stages of mitosis. By overexpressing tagged mutants in HeLa cells, we have mapped the interaction domains to a small region of the Anillin N-terminus and to a large portion of the CIT-K C-terminal domain. Finally, we have found that overexpression of CIT-K affects Anillin localization depending on the activation state of CIT-K. Since Anillin is heavily phosphorylated in mitosis, these results make Anillin one of the most likely potential substrates of CIT-K. Moreover, we have shown that human CIT-K is required for the completion of cytokinesis and that its downregulation affected Anillin localization at the midbody during late stages of cytokinesis, but not its localization in the cleavage furrow during earlier stages, suggesting that Anillin could be involved not only in cleavage furrow formation and ingression but also in abscission. Small Rho GTPases are key players in different stages of cytokinesis, CIT-K specifically binds to and is activated by active RhoA and Anillin directly interacts with active RhoA. We found that CIT-K is not required for RhoA localization in the cleavage furrow, but for its localization at the midbody.Furthermore, knockdown of CIT-K abolishes the localization at the midbody of VAMP8, a v-SNARE required for the final step in cell cleavage. Altogether, these results indicate that CIT-K is strongly associated with the cytoskeletric components of the midbody, but could also play a pivotal role in the modulation of membrane trafficking complexes involved in abscission.

Regulation of vesicle-mediated membrane traffic at synapses and beyond

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To maintain neurotransmitter (NT) release during phases of prolonged synaptic activity, synaptic vesicles (SVs) have to undergo rapid recycling within the presynaptic terminal. Following NT release at the active zone triggered by activity-dependent Ca^{2+} influx, SVs are retrieved by compensatory, mostly clathrin-dependent endocytosis at the periactive zone before they rejoin the SV cluster (SVC). Here we show that the scaffolding protein intersectin 1 (ITSN1) plays a prominent role at early steps of SV endocytosis by directly binding to the endocytic clathrin adaptor AP2. Disruption of ITSN1-AP2 complex formation by microinjecting an ITSN1-derived peptide into reticulospinal axons of the lamprey causes the accumulation of early endocytic intermediates, suggesting that ITSN1 regulates the regulates the endocytic limb of the SV cycle. Biochemical and x-ray crystallographic analysis identifies two peptides within the SH3A-B linker region of ITSN1 that accomodate complementary binding pockets within the sandwich subdomains of the AP2 α- and β2 ears. Consistent with this overexpression of the AP2-binding region inhibits endocytosis in cerebellar granule neurons. These data suggest that an ITSN1-AP2 complex is required during early steps of clathrin-coated pit assembly during SV cycling.
Furthermore, we present evidence that ITSN1 via its SH3A domain also forms a complex with synapsin I, a protein implicated in SV clustering and regulation of NT release. Truncation and deletion mutagenesis identified an evolutionary conserved proline-rich motif within the D domain of rat synapsin I sufficient for its association with intersectin. Similar motifs are found within synapsins in lamprey (*Lampetra fluviatilis*) and in *Drosophila melanogaster*. Microinjection of the lamprey synapsin 1 D domain-derived intersectin 1-binding peptide but not of an inactive mutant into the giant reticulospinal axon of the lamprey leads to a near complete loss of the reserve pool of synaptic vesicles, even in the absence of an electrical stimulus. A selective depletion of the reserve pool of synaptic vesicles was also observed following microinjection of antibodies directed against the SH3A domain of intersectin or of the SH3A domain itself. Consistent with these phenotypes synapsin is mislocalized in synaptic boutons derived from intersectin/ DAP160 mutant flies. These data suggest that synapsin-intersectin complex formation is required for the proper organization of synaptic vesicle clusters in the presynaptic terminal. In agreement with this model, synapsin and endocytic SH3 domain-containing proteins including intersectin 1 undergo parallel translocation between the vesicle cluster and the periactive zone during stimulation-induced exo-endocytic vesicle cycling.
In summary, we propose a novel function of the dual adaptor/scaffold protein ITSN1 to link compensatory endocytosis with SV clustering at release sites, therefore implicating it in the regulation of synaptic efficacy.

A cell model to investigate the mechanism of endoplasmic reticulum expansion in response to increased expression of ER membrane proteins

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The endoplasmic reticulum (ER) is a dynamic organelle capable of adjusting its surface area, molecular composition and architecture in response altered cellular conditions. The best characterized signaling pathway involved in ER plasticity, known as the Unfolded Protein Response (UPR), senses protein overload in the ER lumen and transduces this signal to the cytosol and nucleus, with resulting increases in lumenal chaperones and ER associated degradation (ERAD), and attenuation of protein synthesis to decrease load on the ER and restore homeostasis. In addition to overload in the lumen, the ER also senses an increase in membrane proteins, and responds by increasing its surface area. However, the mechanisms underlying this response are poorly understood. To address this question, we have generated a HeLa Tet-off cell line inducibly expressing a GFP fusion protein (GFP-b5-tail) anchored to the ER membrane. GFP-b5-tail consists of a cytosolically located EGFP fused at its C-terminus to the membrane anchor of cytochrome b5 (1). Ultrastructural analysis demonstrated that induction of GFP-b5-tail expression caused an increase in surface area of the ER, which maintained its normal branching tubular/cisternal structure (1). To confirm this observation with a biochemical assay, we compared the incorporation of tritiated choline into phosphatidylcholine cells induced or not induced to express GFP-b5 tail. Already at one day after removal of doxicycline from the medium, we observed a significant increase in 3H-choline incorporation. This increase was maximum at 2-3 days after induction (~5 fold) and decreased somewhat thereafter. To investigate whether the UPR is involved in the observed ER membrane expansion, we (i) analyzed by western blotting the expression of ER membrane proteins known to increase in the UPR, and (ii) assayed for XBP1 mRNA splicing by highly sensitive real time RT-PCR. Upon induction, we observed no increase in UPR-responsive ER lumenal chaperones, but, did detect a marked increase in the translocon subunits Sec61 alpha and beta. In agreement with the lack of increase in lumenal chaperones, RT-PCR failed to reveal any increase in spliced XBP-1 mRNA. In order to establish the relation between increase of phospholipid byosynthesis and the overproduction of ER membrane proteins we are presently investigating the other arms of the UPR and other signal transduction pathways.

References

(1) Sprocati T., Ronchi P., Raimondi, A., Francolini, M. & Borgese, N. Dynamic and reversibile restructuring of the endoplasmic reticulum induced by PDMP in cultured cells. J. Cell Sci 119, 3249-3260 (2006).

YIP1 isoforms in skeletal muscle

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The internal membrane system of skeletal muscle cells, including the endo/sarcoplasmic reticulum, the T-tubules and the Golgi apparatus, presents a very precise spatial organization with respect to the myofibrils. Within the endo/sarcoplasmic reticulum, the transitional endoplasmic reticulum (ER) is localized to the Z lines and at the perinuclear region, the "canonical ER" is mainly located in I bands, whereas the Golgi appears localized in spots along the fibers and around the nuclei.
In the effort to identify genes involved in the development and/or maintenance of this highly regular membrane organization, we isolated a cDNA encoding a protein homologous the yeast Yip1p, named Yip1B. Yip1p was identified in budding yeast as an essential protein that interacts with Ypt1p and Ypt31p. Yeast Yip1p is located on Golgi membranes and its depletion causes massive accumulation of ER membranes and aberrations in protein secretion and glycosylation. Yif1p was also identified as an essential protein, which forms a tight complex with Yip1p. The mammalian hortolog of Yip1p includes two members, Yip1A and Yip1B, which share about 60% of identity. Similarly, the mammalian ortholog of Yif1p includes Yif1A and Yif1B.
To quantify the relative expressions of these proteins in skeletal muscle, we performed Real-Time PCR in different tissues and during skeletal muscle development. Results indicated the Yip1B isoform is expressed only in striated muscle. During skeletal muscle development, Yip1A resulted more abundant in the early developmental phase (E14 and E16), while the expression level of Yip1B reached the maximum in the adult.
To unveil the function of Yip1B we produced polyclonal antibodies. By Western blot, the antiserum was able to recognize the endogenous protein in striated muscle and differentiated C2C12 cells, but not in undifferentiated C2C12 and other tissues, as expected. Moreover, the protein resulted enriched in the skeletal muscle microsomal fraction, confirming its association to the membrane, and more abundant in the adult tissue. Immunofluorescence experiments on skeletal muscle revealed that the Yip1B protein is localized around the nuclei and in spots along the fiber, partially overlaying with the cis-Golgi marker GM130.

The role of the *Drosophila melanogaster* SNAP-25 C-terminal region in neurotransmitter release

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SNARE proteins are the main molecular constituents of the synaptic vesicle fusion machinery. The three SNARE proteins (the v-SNARE synaptobrevin, the t-SNARE syntaxin-1 and SNAP-25) associate by forming a four helix bundle which involves one cytoplasmic domain for each v- and t-SNARE, and two SNAP-25 cytoplasmic domains. The assembly of the SNARE proteins leads to the formation of a heterotrimeric trans SNARE complex. The formation of the SNARE complex (zippering) plays a key role in the fusion process, by releasing the energy for lipid bilayer fusion. Molecular studies have shown that botulinum (BoNT) and tetanus toxins inactivate SNARE complex formation by cleaving the cytoplasmic helices of the assembling proteins, ultimately blocking the zippering process, and leading to the blockage of neurotransmitter release. Thus, the SNARE complex is viewed as the "minimal fusion machinery".
SNARE complexes assemble around the fusion pore forming a supercomplex. Supercomplexes originate from the interactions of transmembrane domains of v and t-SNAREs and palmitoylated SNAP-25. Studies using mutated SNARE proteins, or SNAREs cleaved with different BoNTs, suggest that the number of SNARE complexes placed around the fusion pore in a rosette-like pattern should be between 3 and 10-15.
Cleavage of SNAP-25 by BoNT A occurs within a nine-aminoacid (aa) C-terminal region of SNAP-25. This truncated form of SNAP-25 is still able to bind to syntaxin and synaptobrevin to form a stable SNARE complex, but it has a strong inhibitory effect on neuroexocitosis, probably by preventing the formation of the SNARE supercomplex. In order to better understand the role of SNAP-25 in super-complex formation, we employed the *Drosophila melanogaster* third instar larva neuromuscular junction (NMJ) as a model system.
In mammals, the assembly of SNARE complexes into a SNARE supercomplex appears to require an electrostatic interaction between the C-terminal aa SYNATXIN D55 and SNAP-25 R189. In Drosophila, the homologous SNAP-25 aa site(s) are either R199 or R206. We generated transgenic lines expressing SNAP-25 in which both aa were substituted with alanine. We expressed the transgenes in a wild type background, under the expectation that the mutated SNAP-25 isoform would produce a dominant-negative effect on the formation of SNARE supercomplexes. We found a significant reduction of both evoked and spontaneous neurotransmitter release with respect to controls, in the absence of overt NMJ morphological defects, providing strong support for a key role of SNAP-25 in supercomplex formation.

Eps8 regulates axonal filopodia in hippocampal neurons in response to BDNF

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The regulation of filopodia plays a crucial role during neuronal development and synaptogenesis. Axonal filopodia, which are known to originate presynaptic specializations, are regulated in response to neurotrophic factors. The structural components of filopodia are actin filaments, whose dynamics and organization are controlled by ensembles of actin binding proteins. How neurotrophic factors regulate these latter proteins remains, however, poorly defined.
Here, using a combination of mouse genetic, biochemical and cell biological assays, we show that genetic removal of Eps8, an actin-binding and regulatory protein enriched in the growth cones and developing processes of neurons, significantly augments the number and density of VASP-dependent axonal filopodia. The reintroduction of Eps8 WT, but not an Eps8 capping-defective mutant into primary hippocampal neurons restored axonal filopdia to wild type levels. We further show that the actin barbed end capping activity of Eps8 is inhibited by BDNF treatment through MAPK-dependent phosphorylation of Eps8 residues S624 and T628. Additionally, an Eps8 mutant, impaired in the MAPK target sites (S624A/T628A), displays increased association to actin-rich structures, is resistant to BDNF-mediated release from microfilaments, and inhibits BDNF-induced filopodia. The opposite is observed for a phosphomimetic Eps8 (S624E/T628E) mutant.
Thus, collectively, our data identify Eps8 as a critical capping protein in the regulation of axonal filopodia and delineate a molecular pathway by which BDNF, through MAPK-dependent phosphorylation of Eps8, stimulates axonal filopodia formation, a process with crucial impacts on neuronal development and synapse formation.

OA1, an intracellular G protein-coupled receptor, regulates both melanosome biogenesis and transport in pigment cells

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Ocular albinism type 1 is an X-linked inherited disorder characterized by severe visual defects and by the presence of giant melanosomes (macromelanosomes) in skin melanocytes and retinal pigment epithelium (RPE), suggesting a defect in melanosome biogenesis. The protein product of the ocular albinism gene, named OA1, is a pigment cell-specific membrane glycoprotein, displaying structural and functional features of G protein-coupled receptors (GPCRs), including the ability to activate heterotrimeric G proteins. However, in contrast to canonical GPCRs, OA1 is not localized to the plasma membrane, but is targeted to intracellular organelles, namely melanosomes and lysosomes, by specific sorting determinants. We recently performed a detailed analysis of *Oa1*-KO mouse melanocytes and RPE, and found that OA1 loss-of-function determines not only abnormal biogenesis, but also abnormal transport of melanosomes, which are rare in the perinuclear area and accumulate toward the cell periphery. Organelle tracking analysis revealed that *Oa1*-KO melanosomes display a severe reduction in microtubule-based motility only in the presence of an intact actin cytoskeleton, which, in physiological conditions, is known to compete with microtubules and promote the peripheral versus the perinuclear distribution of the organelles. In addition, we found that OA1 interacts with tubulin and polymerized microtubules by coimmunoprecipitation and cosedimentation assays. Together, these data enlighten a novel function of OA1 in melanosome motility, and in particular in the switch between microfilament and microtubule-based transport, and imply that the cytoskeleton might represent a downstream effector of this receptor. Furthermore, our results suggest that ocular albinism type 1 might result from a different pathogenetic mechanism than previously thought, based on an organelle-autonomous signaling pathway implicated in the regulation of both membrane traffic and transport. Reference: Palmisano I., Bagnato P., Palmigiano A., Innamorati G., Rotondo G., Altimare D., Venturi C., Sviderskaya E.V., Piccirillo R., Coppola M., Marigo V., Incerti B., Ballabio A., Surace E.M., Tacchetti C., Bennett D.C., Schiaffino M.V. The ocular albinism type 1 (OA1) protein, an intracellular G protein-coupled receptor, regulates melanosome transport in pigment cells. Human Molecular Genetics, 17, 3487-3501, 2008.

HIV-1 Nef protein is able to enter human primary macrophages activating signal transduction events through a mechanism IRAK1 degradation – independent

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The HIV-1 protein Nef is a virulence factor that plays multiple roles during the early and late phase of the viral life cycle inducing also immunoevasion. We reported that recombinant HIV-1-Nef (recNef) enters primary monocyte-derived macrophage (MDM) and triggers NFκB and MAPKs activation that is followed by synthesis and release of several cytokines and chemokines (IL-1β, IL-6, TNFα, MIP-1α, MIP-1β and IFNβ) (Alessandrini et al., JGenVirol 2000; Federico et al., Blood 2001; Olivetta et al., JImmunol 2003; Percario et al., JLeukBiol 2003; Mangino et al., JVirol 2007). How the viral protein Nef is able to switch on those events in MDM is unknown. Now we have used MDM purified from healthy donors, treated with myristoylated recNefSF2-Alexa488, to perform time-courses experiments on living or fixed cells using confocal microscopy. The experimental results confirm that Nef is accumulated inside the cell, but it is localized also on the cell membrane. Biological activity of fluorescent recNef was verified by specific signal transduction activation. FACS analysis on cells treated with myristoylated recNefSF2-Alexa488 at +4°C or 37°C suggests the absence of Nef-specific binding receptor on the cytoplasmic membrane. In addition immunoprecipitation and western blot analysis indicate that IRAK1 degradation, involved in the signalling events triggered by Toll Like Receptor (TLR)-4, does not occur in the Nef-induced signalling.

Swapping of VDAC domains to investigate structure-function relationships

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The voltage-dependent anion channel (VDAC), located in the mitochondrial outer membrane, is the main pathway for metabolite diffusion across this membrane and thus controls cross-talk between mitochondria and the cytosol. In mammals three isoforms exist that probably play different roles in the regulation of mitochondrial functions. In particular VDAC3 is unable to form channels in cellular assays and in reconstitution experiments. Using Saccharomyces cerevisiae mutant depleted of endogenous VDAC1 (Δpor1) we studied the function of human VDAC3. It is well known that human VDAC1 and VDAC2 are able to complement the lack of growth of VDAC-deficient yeast on media containing a non-fermentable carbon source such as glycerol at 37°C. In the same conditions VDAC3 cannot restore the wild type phenotype. This difference is due to a limited amount of sequence dissimilarity (VDAC1 to VDAC3 is 67% identical and 85% conserved).
To identify which region of the protein provides the pore-forming properties, we are building swapping domain mutants with protein domains exchanged among the VDAC isoforms. In this work we present the result of the substitution of the 20 N-terminal amino acids moiety of human VDAC3 with the homologous sequence of human VDAC1. After transfection in Δpor1 cells we have found that this chimeric protein is able to restore the functionality of yeast mitochondria and to double the yeast lifespan at variance from the w.t. human VDAC3. The strain shows also a higher resistance to oxidative stress. This result outlines the importance of the N-terminal moiety of VDACs in the function of the protein and of the whole mitochondria.

Dissection of the carboxyl-terminal domain of the proteasomal subunit Rpn11 in maintenance of mitochondrial structure and function

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In *S.cerevisiae*, during cell cycle the mitochondrion is the first organelle to enter the bud, the absence of the mitochondrial structure in the daughter cell results in lethality, consequently the mitochondrial inheritance is tightly regulated. A complex, called mitochore/tubulation apparatus assures the anchorage of the organelle on the actin cytoskeleton: mutations affecting this complex lead to collapsed giant mitochondria. Up to now, the regulation of mitochondrial morphology and inheritance has not been elucidated: some reports suggest the involvement of the ubiquitin-proteasome pathway in regulating fusion and fission events (which dynamically maintain the wild-type mitochondrial tubular network). We now show that the Rpn11 deubiquitinating enzyme of the proteasomal lid is involved in the regulation of the tubulation process, independently from its proteasomal function because the protein is found in a monomeric form close to the mitochondrial membranes. Our recent results showed that the absence of the mitochore genes in an rpn11-m1 strain (bearing a truncated version of the Rpn11 protein) results in a lethal phenotype. We hypothesize that the mitochore could be part of a cross-talk between mitochondrial inheritance and cell cycle, in which the exit from mitosis is delayed if mitochondria are not correctly present into the bud. We will also discuss the involvement of calcium signalling in mitochondrial inheritance.

Protein folding, protein degradation and quality control in a model of protein misfolding disease

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Proteins destined to the extracellular space are synthesized on ribosomes associated to the membranes of the ER and cotranslationally translocate into this orgenalle, where they attain their native structure (ERAF, ER Folding), before proceeding to downstream compartments. Quality control (QC) mechanisms ensure that only correctly folded proteins are secreted (ERAE, ER export), while non-native proteins are recognized and retained for a second chance of folding or for degradation via cytosolic proteasomes (ERAD, ER Associated Degradation). If degradation is insufficient, aggregation can ensue in the exocytic pathway at various stages of the sequential folding and assembly steps, particularly in the case of mutated proteins or when normal proteins are over-produced. It has been recently shown that autophagy can also degrade some ER aggregates. Disturbances in protein QC play a key role in the pathogenesis of protein misfolding and aggregation disease. Russel Bodies (RBs) are dilated Endoplasmic Reticulum (ER) cisternae containing large amounts of aggregated mutant Ig, which are frequently detected in multiple myelomas cells. As a model of ER storage disease, we have analysed the aggregation of a mutant IgM heavy chain deleted of the first constant domain (μΔCH1). μΔCH1 aggregation is a consequence of an unbalance between protein entry in the ER (synthesis) and its exit (degradation or secretion). Aggregation can occur in the ER or in ERGIC, depending on whether Ig-L chains are secreted or not. We show here that ERAF, ERAD and ERAE all concur to modulate RB formation. Moreover, preliminary results suggest that also autophagy could have a role in RB clearance from the ER. The observation that, by controlling folding, degradation and secretion we can modulate protein aggregation can have important implications for the cure of protein misfolding and ER storage disorders.

Elaborating a methodology for isolating vacuoles in the roots of *Phaseolus vulgaris* L

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Vacuoles, multi-function organelles, have a fundamental role in cell strategies for plant development and as a consequence identifying and characterizing the transport systems of the tonoplast are of fundamental importance for understanding the processes regulating cell homeostasis.
In order to isolate the vacuoles several methodologies were compared, reported in the specific bibliography for cell culture and red beets: bean (Phaseolus vulgaris L.) roots were used grown during three weeks in a Knop nutritive solution.
The isolation of morphologically integer and physiologically active vacuoles was done according to two procedures: mechanical homogenization and enzymatic lysis of the cell wall. The vacuole preparation was purified on a Histodenz gradient (15%, 10%, 2.5%).
The analysis of the resulting fractions at the optical microscope showed a higher concentration of particles compatible with vacuole morphology at the interface between the layers 10% and 2.5%
The vacuole integrity was assessed through the absorption and retention of the neutral red. Purity was estimated by an appraisal of the nitrate-sensitive tonoplast ATPase and through the analysis at the scanning microscope.

Analysis of intracellular localization and interaction between Prion Protein (PrP^C) and its homolog Doppel

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Doppel (Dpl) is a homolog of PrP^C, the cellular prion protein which in the misfolded conformation (PrP^{Sc}) is responsible for Transmissible Spongiform Encephalopathies (TSEs). It has been demonstrated that the ectopic expression of Dpl in the brain of some lines of PrP knock out mice provokes cerebellar ataxia which could be rescued by reintroducing the PrP gene, suggesting an interaction between the two proteins. Since mixed findings have been described, it is not yet clear where and in which conditions PrP^C and Dpl interact in the cells.
In the present study we have analyzed the intracellular localization and the interaction between Dpl and PrP^C in FRT (Fischer Rat Thyroid) cells when expressed separately or together. We found that both proteins localize prevalently on the basolateral surface of FRT cells in both single and double expressing clones. Interestingly, we found that they associate with detergent-resistant membranes (DRMs or lipid rafts) from where they can be co-immunoprecipitated in a cholesterol-dependent fashion. Overall our data support the hypothesis that PrP^C and Dpl interact in rafts and that this interaction depends on the integrity of these membrane microdomains.

Physiology of ER-to-Golgi protein transport

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Using IgM secretion during terminal B cell differentiation as a model system, we analyze the mechanisms that couple efficiency and fidelity in the antibody factory. ERp44 and ERGIC-53 synergize to promote IgM polymerization. ERGIC-53 provides a platform that binds IgM monomers and helps their assembly into planar polymers of the proper size and shape; ERp44 retrieves unassembled IgM species back to the ER. ERGIC-53 binds the C-terminal N-glycan of Ig-μ chains, located 12 residues upstream of the cysteine residue (575) involved in polymer formation and ERp44 binding. ERp44 has a clover-like structure, with 3 trx-like domains (a, b and b') and a C-terminal tail which hides the active site in the a domain. The C-terminal tail covers also a hydrophobic pocket formed between domains b and b'. The movements of the tail regulate the accessibility to the active site and exposure of the C-terminal RDEL. The pH gradient between the ER and the cisGolgi could coordinately regulate substrate binding and release to ERGIC-53 and ERp44, and facilitate the transfer of substrate-ERp44 complexes to KDEL-receptors.

Mechanism of microparticle release from glial cells. Role of glia-derived microparticles in the control of neuronal activity

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Cells communicate and exchange informations by different secretory mechanisms. Among these, extracellular vesicles shed from the plasmamembrane are gaining increasing attention as efficient vehicles for release of signalling molecules. We have recently demonstrated that upon ATP exposure cultured astrocytes shed microparticles which contain and release the pro-inflammatory cytokine IL-1b and we have got insights into the mechanism of their shedding (Bianco et al., 2009, in press). Upon activation of the ATP receptor P2X7, microparticle shedding is associated with rapid activation of acid sphingomyelinase, which translocates to plasma membrane outer leaflet. ATP-induced shedding and IL-1b release are markedly reduced by inhibition of acid sphingomyelinase, and completely blocked in glial cultures from acid sphingomyelinase knock-out mice. We also demostrate that p38 MAPK cascade is relevant for the whole process, since specific kinase inhibitors strongly reduce acid sphingomyelinase activation, microparticle shedding and IL-1b release. Besides representing an unconventional secretory pathway, microparticles released from glial cells demand further characterization as they represent new, still unexplored, organelles potentially mediating intercellular communication in the CNS. Our preliminary results indicate that microparticles shed from reactive glia can alter neuronal responsiveness to glutamate agonists, by increasing network excitability. Globally, our data represent the first demonstration that activation of acid sphingomyelinase is necessary and sufficient for microparticle release from glial cells, and define key molecular effectors of microparticle formation and IL-1b release, thus opening new strategies for treatment of neuro-inflammatory diseases.

Characterization of LptC, an *Escherichia coli* protein involved in lipopolysaccharide biogenesis

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Lipopolysaccharide (LPS) is an essential component of the outer membrane (OM) in most Gram-negative bacteria. The chemical structure of LPS and its biosynthetic pathways have been fully elucidated. By contrast only recently details of the transport and assembly of LPS into the OM have emerged.
The inner membrane (IM) transport protein MsbA is responsible for flipping LPS across the IM. After IM translocation the Lpt complex, composed of seven proteins (LptABCDEFG) is responsible for the transport of LPS to the cell surface. These proteins are located in the IM (LptBCFG) in the periplasm (LptA) and in the OM (LptDE) and are proposed to constitute a complex that spans the IM and OM. As depletion of any of the above mentioned proteins results in similar phenotypes it has been proposed that the Lpt machinery operates as a single device. LptA may function as the periplasmic chaperone in LPS transport as it has been shown to bind LPS *in vitro*.
Our most recent data indicate physical interaction between LptC and LptA, suggesting that the LptC protein may represent a IM docking site for LptA and could also be required for LPS binding in vivo. In an effort to define the molecular role of LptA and LptC in the LPS transport we set up a random PCR mutagenesis screening to identify residues important for the functions of these proteins. Three LptC mutant proteins unable to complement an *lptC* conditional mutant under non permissive conditions were selected so far.
Preliminary experiments indicate that in these mutants the LptA level is lower than that observed in the wild type strain, suggesting a role for LptC in LptA induction/stabilization. Further studies are ongoing to better characterize the mutants and to identify LptC residues involved in LptC-LptA interaction.

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