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Traffic Report

Meeting Report: Seventh Annaberg EMBO Workshop 'Membrane Traffic in the Secretory Pathway', Goldegg, Austria, 9–14 January 2007

Joëlle Morvan[1,*], Sara Salinas[2,*] and Veronika E. Neubrand[2,*]

[1]Secretory Pathways Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, UK
[2]Molecular Neuropathology Laboratory, Cancer Research UK, London Research Institute, Lincoln's Inn Fields, London WC2A 3PX, UK
**Corresponding authors: Joëlle Morvan, joelle.morvan@cancer.org.uk or Sara Salinas, sara.salinas@cancer.org.uk or Veronika E. Neubrand, veronika.neubrand@cancer.org.uk*

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The seventh Annaberg Conference entitled 'Membrane Traffic in the Secretory Pathway' was, like previous meetings, very successful. The scenic Austrian village of Goldegg and its castle gave the meeting a great atmosphere, although this time we missed the wintry landscape. Nevertheless, many outstanding speakers presented the latest research in membrane dynamics along the secretory and endocytic pathways, and the poster sessions in the evenings were no less exciting. Within the topic of membrane trafficking, the talks and posters ranged from lipids to autophagy to signaling, and from mitosis to mitochondria, which made this conference very diverse and dynamic. The high quality of the talks ensured that all these different topics were effectively conveyed and provoked lively discussions.

We would like to apologize that we can only report on a selection of the presented talks because of limited space, and therefore will not mention all the invited speakers in this report.

Lipids in the Regulation of Membrane Traffic

Lipids play an important role in the regulation of the transport along the secretory and endocytic pathways. In particular, phosphoinositides assist in binding proteins to specific sites at the membrane, establish organelle identity and hence are able to regulate the recruitment of the transport machinery (1).

Phosphatidylinositol (4) phosphate [*PtdIns*(4)P] is the main phosphoinositide present at the Golgi apparatus (Figure 1) and one of the key regulators of membrane transport at the *trans*-Golgi network (TGN). In yeast, Golgi *PtdIns*(4)P is synthesized by the essential *PtdIns*(4)-kinase Pik1. However, effector molecules of this enzyme, which are involved in membrane transport, remain unidentified up to date. **Christiane Walch-Solimena** (Dresden, Germany) and her coworkers performed a screen for *PtdIns*(4)P effector molecules using a *pik1* mutant background (*pik1-101*) with reduced *PtdIns*(4)P levels. This mutant strain showed a severe defect in transport within the secretory pathway and a delay in maturation of the vacuolar enzyme carboxypeptidase Y. Deletion of the Golgi-localized, gamma-ear-containing ADP-ribosylation factor (Arf)-binding protein 2 (GGA2) in this background resulted in an enhancement of the *pik1* mutant growth defect, while overexpression of GGA2 could rescue the *pik1-101* mutant phenotype. GGA2 was therefore a good candidate for such an effector. This protein is a clathrin adaptor protein (AP) involved in the transport of proteins from the TGN to late endosomes. Christiane Walch-Solimena presented data demonstrating that *PtdIns*(4)P, together with the small GTPase Arf1, could recruit GGA2 onto liposomes. Consistent with this result, in the *pik1-101* mutant, there was less green fluorescent protein (GFP)-GGA2 detectable at the TGN, although Arf1 was still bound to the membrane, indicating that *PtdIns*(4)P played an important role in recruiting GGA2 onto membranes. This recruitment is mediated by the Vps27, Hrs, Stam (VHS) domain of GGA2, which contains a stretch of hydrophobic and basic amino acids that is similar to a phosphoinositide-binding motif found in other clathrin-associated proteins. Christiane Walch-Solimena concluded by emphasizing that in yeast GGA2 requires both Arf1 and *PtdIns*(4)P for its recruitment onto the membrane, describing another example for coincidence detection, which is necessary for the correct membrane targeting of the transport machinery.

This 'coincidence' idea, standing for the assembly of multiple proteins or lipids to create highly specific sites, which in turn attract molecules involved in membrane

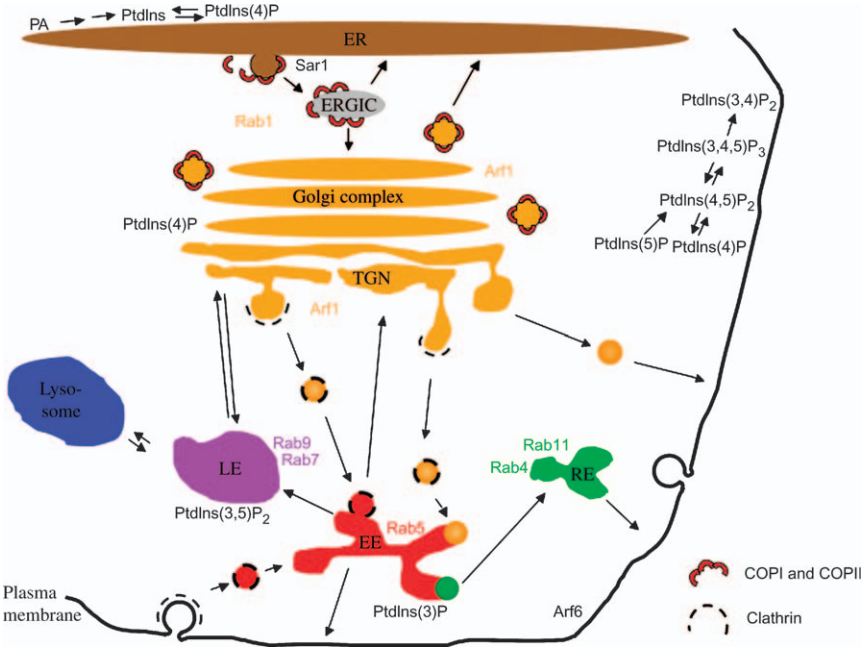

Figure 1: Secretory and endocytic pathways. Each intracellular compartment is characterized by a subset of certain phosphoinositides that can recruit numerous effector proteins. *PtdIns*(4,5)P_2 is mainly found at the plasma membrane, *PtdIns*(3)P is localized on EE and *PtdIns*(3,5)P_2 on LE, whereas the major pool of *PtdIns*(4)P is present at the Golgi complex. Small GTPases, e.g., from the Rab and Arf families, are also specifically localized along the secretory and endocytic routes. They are responsible for the maintenance of organelle identity and cargo progression. Only the best-characterized examples are depicted in this figure. The generation of carriers is mainly mediated by coat proteins (COP), such as COPI and COPII for the transport between ER and Golgi complex, and clathrin involved in post-Golgi trafficking and endocytosis. EE, early endosome; LE, late endosome; RE, recycling endosome; PA, phosphatidic acid.

traffic, has become familiar in recent years. It was mentioned by various speakers throughout this conference, e.g., by **Sean Munro** (Cambridge, UK). His laboratory is interested in the Arf-like GTPases (Arls) and their effectors. Most of these Arls contain, similar to the ARF family, an N-terminal amphipathic helix and myristoyl group by which they can associate with membranes upon GTP binding. Arl4 is one of the 22 known human Arls. Sean Munro and his coworkers showed that it bound directly the pleckstrin homology (PH) domain of ADP ribosylation factor nucleotide-binding site opener (ARNO), the GTP exchange factor for Arf6, which in turn promotes *PtdIns*(4,5)P_2 synthesis at the plasma membrane (2). He presented data showing that Arl4 required the interaction with ARNO for its efficient recruitment to the plasma membrane, and *vice versa*, ARNO needed to interact with Arl4 for its own membrane recruitment (3). By using several targeting signals, the cell ensures that the right proteins are at the correct place, and therefore, the Arls – in addition to the Arfs, Rabs and phosphoinositides – could also act as spatial landmarks.

By the mechanism described above, *PtdIns*(4,5)P_2 can be enriched at the plasma membrane (Figure 1), where it plays a variety of different roles in cells (1). One of its well-characterized functions is the recruitment of the endocytic machinery, such as clathrin adaptors. This is an important step for synaptic vesicle recycling, which involves clathrin-dependent endocytosis. In order to complete the synaptic vesicle cycle, *PtdIns*(4,5)P_2 has to be cleaved into *PtdIns*(4)P by which the endocytosed vesicle also loses its plasma membrane identity. The hydrolysis is performed by the presynaptic phosphoinositide phosphatase synaptojanin 1, with *PtdIns*(4,5)P_2 as its main physiological substrate. **Gilbert Di Paolo** (New York, USA) showed in his presentation that, through its interaction with the Bin/amphiphysin/Rvs (BAR) domain-containing endophilin (4,5), synaptojanin 1 could sense membrane curvature and promote *PtdIns*(4,5)P_2 hydrolysis *in vitro* preferentially on smaller liposomes, which have a diameter comparable with that of endocytic vesicles.

In the second part of his talk, Gilbert Di Paolo pointed out a probable relationship between Down syndrome, one of the most common genetic causes of mental retardation, and synaptojanin 1. The gene encoding synaptojanin 1 is localized on chromosome 21, a very small chromosome containing only 200–300 genes, which is duplicated in Down syndrome. Using a bacterial artificial chromosome (BAC), Di Paolo and his collaborators produced a transgenic mouse with an additional copy of synaptojanin 1. In the brain of these mice as well as in that of a mouse model for partial trisomy 21, synaptojanin 1 was expressed in higher amounts than in control mice and *PtdIns*(4,5)P_2 levels were decreased, in contrast to the synaptojanin 1 knockout

mice, where *PtdIns*(4,5)P_2 levels were elevated resulting in a delay of synaptic vesicle reformation (6,7). Because other knockout mice, which exhibit defects in synaptic vesicle recycling have major learning deficiencies (8), his group is now establishing whether synaptojanin 1 BAC mice also suffer from learning difficulties. In a preliminary data set, they observed that the synaptojanin 1 BAC mice perform poorly in behavioral tests assessing spatial learning, thus implicating synaptojanin 1 overexpression as a possible contributor to the cognitive disability in Trisomy 21 patients, although the origin of Down syndrome is thought to be multigenic.

Sima Lev (Rehovot, Israel) talked about the involvement of lipid transfer proteins (LTPs) in the regulation of membrane traffic. These proteins transport lipids between intracellular organelles in close proximity, and hence are called membrane contact sites. Nir2 is an LTP that contains a phosphoinositol transfer domain, which exchanges phosphatidylinositol or phosphatidylcholine between two lipid bilayers *in vitro*. Her group could show that small interfering RNA (siRNA) depletion of Nir2 resulted in a redistribution of protein Kinase D from the Golgi apparatus to the cytoplasm. Diacylglycerol (DAG) levels were reduced in these Nir2-depleted cells, while at the same time phosphatidylcholine levels were elevated. Nir2 downregulation led to a fragmented Golgi apparatus and inhibited transport by impairing the fission of carriers from the TGN (9). Their data therefore strongly suggest that Nir2 plays a role in regulating the exit of proteins at the Golgi apparatus by modifying the levels of DAG.

The Golgi Apparatus and Its (Dis-)Assembly

The Golgi apparatus is composed of *cis*-, *medial*- and *trans*-Golgi cisternae. Newly synthesized proteins and lipids destined for secretion or other intracellular organelles traverse this organelle, and if necessary they can be posttranslationally modified here. This highly dynamic organelle is maintained by the continuous addition and retrieval of proteins and lipids, and undergoes a complete fragmentation during mitosis. Therefore, tightly controlled and coordinated mechanisms must be in place for its formation and maintenance of its integrity.

To further characterize the maintenance of the Golgi apparatus, small molecules that affect its morphology can be of great help. **Tom Kirchhausen** (Boston, USA) and his laboratory have therefore established a screen looking for such small, cell-permeable molecules that can interfere with the Golgi apparatus assembly and disassembly (10). Among more than 10 000 screened chemicals, they identified a molecule, named Dispergo, which leads to consumption of the Golgi apparatus. Dispergo blocked the transport of the vesicular stomatitis virus glycoprotein (VSV-G), a membrane-bound marker protein for the transport along the secretory pathway, at the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). In addition, using specific markers, they showed that in these dispersed Golgi structures, the *cis*-, *medial*- and *trans*-cisternae were completely separated and did not form Golgi ministacks, typically observed after treatment with the microtubule-depolymerizing drug nocodazole. Because one prerequisite for the selection of those small molecules was that their effect was reversible, a functional Golgi apparatus could completely reassemble after a washout period at 37°C through a mechanism independent of *de novo* protein synthesis from the ER. These experiments support the hypothesis that the Golgi apparatus is an independent organelle, which is capable of self-organization and -reassembly after fragmentation.

Mitosis is a physiological situation in which the Golgi apparatus undergoes fragmentation. One essential protein for this process is the fission-inducing protein CtBP1-S/BARS (BARS) (11), whose malfunction leads to an arrest of the cell cycle at the G2 phase (12). **Daniela Corda** and collaborators (Santa Maria Imbaro, Italy) elucidated the function of BARS in fissioning the tubules, which interconnect the stacks of the Golgi ribbon, before entering mitosis (Figure 2). Cells treated with siRNA against BARS were

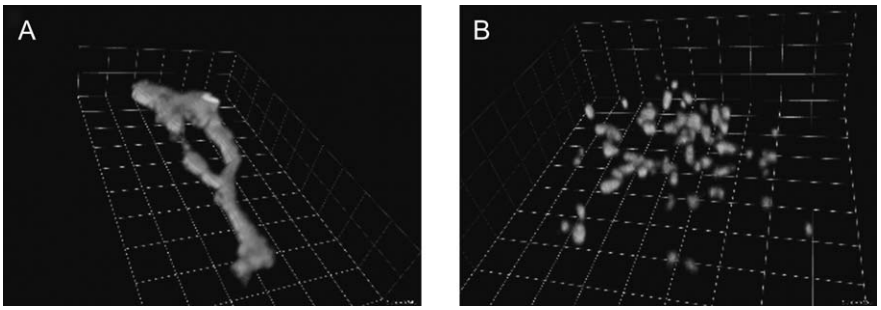

Figure 2: Disassembly of the Golgi apparatus. In mammalian cells, the Golgi apparatus is organized in various stacks during interphase, which are disrupted at the entry of mitosis, representing a 'putative' Golgi apparatus checkpoint (see text for details). A) In this electron microscopy tomograph, a single Golgi ribbon is shown, as it is found during interphase. B) Before entering mitosis, the Golgi ribbons have to be unlinked in order to overcome the Golgi apparatus checkpoint, which was demonstrated by various speakers to be essential for mitotic progression and successful completion of the cell cycle. This figure was kindly provided by Adam Linstedt (Pittsburgh, USA).

not able to divide, a phenotype that could be rescued by microinjection of BARS into those cells. In cells, which did not have an intact Golgi ribbon, as observed in embryonic or GM130-knockout cell lines, BARS was not essential for the transition from the G2 to M phase, indicating that there is an essential checkpoint to monitor the disassembly of the Golgi ribbons for mitotic progression. Using fluorescence recovery after photobleaching, Corda's laboratory showed that in cells with inhibited BARS function, Golgi-resident proteins could still diffuse back to the photobleached area in G2 phase, indicating an intact Golgi ribbon as seen for an interphase Golgi apparatus; in untreated cells, there is no recovery of fluorescence of Golgi-resident proteins at this stage of the cell cycle (13). This implies that BARS is necessary for fission of the Golgi ribbons, which seems to be a prerequisite for cells to complete the G2 phase and to enter mitosis (13).

The signaling pathways involved in this Golgi apparatus disassembly checkpoint have been a focus of controversy for a long time. **Adam Linstedt** (Pittsburgh, USA) studied the role of the mitogen-activated protein (MAP)/ERK kinase (MEK1) and the Golgi matrix proteins, which link the Golgi ribbons laterally, in this 'Golgi checkpoint' in intact cells. Depletion of MEK1 by siRNA and the specific inhibition of MEK1 activity delayed entry of cells into M phase (14). Normal kinetics was restored if the Golgi ribbons were fragmented before M phase, e.g., by brefeldin A treatment or downregulation of the Golgi re-assembly stacking protein (GRASP)65 which is part of the Golgi matrix. Interestingly, another GRASP family member, namely GRASP55, has been previously identified as a MEK1 pathway substrate (15). Like GRASP65, downregulation of GRASP55 led to Golgi complex unlinking. Using phosphomimic or nonphosphorylatable mutants of GRASP55, Adam Linstedt and his group found that GRASP55 phosphorylation by MEK1 was essential for the unlinking of the Golgi ribbons into ministacks at the late G2 phase, representing the putative Golgi checkpoint (Figure 2). Subsequently, at early M phase, cyclin-dependent kinase (CDK)1 is activated to promote further disassembly of the ministacks into vesicular-tubular structures. These structures are then separated into two pools that constitute the new Golgi complexes in the daughter cells after complete cell division.

Coats, Adaptor Proteins and Sorting Motifs

Coat proteins are essential for the generation of carriers between the Golgi apparatus and endosomes/lysosomes, as well as in protein internalization at the plasma membrane. Their associated adaptors are recruited by specific sorting signals in cargo proteins and allow the clustering of these cargos in the nascent vesicle.

Matthew Seaman (Cambridge, UK) discussed results about studies of the motifs and the machinery required for retromer-mediated endosome-to-Golgi apparatus retrieval. Several sorting motifs required for TGN-to-endosome/lysosome transport have been identified. However, until now, no sorting motif for retromer-mediated endosome-to-Golgi retrieval was known. Using a CD8-reporter protein-based approach, and systematic mutagenesis of the cation-independent (CI)-mannose-6-phosphate receptor (MPR) cytoplasmic tail, they identified the novel, conserved sorting motif WLM, essential for endosome-to-Golgi transport of a CD8-CI-MPR reporter protein. This motif was necessary for the interaction with retromer subunits and adaptor protein (AP)1, although AP1 itself was not essential for this endosome-to-Golgi retrieval. A similar motif (FLV) present in the tail of sortilin (another cargo protein retrieved in a retromer-dependent fashion) was found to be necessary for the retrieval of a CD8-sortilin chimera. This motif seems to be a consensus motif recognized by the retromer complex for endosome-to-TGN retrieval of proteins.

Juan Bonifacino (Bethesda, USA) discussed the mechanism of the HIV-1 Nef-dependent downregulation of CD4. Nef messenger RNA (mRNA) is the most abundant mRNA in cells infected by HIV. The most obvious phenotype of these cells is that the virus receptor CD4 is not present at the cell surface, but in an intracellular compartment in a juxta-nuclear region. This phenotype could be explained by an acceleration of endocytosis or a delay in the secretion of CD4. The adaptors that are potentially involved in Nef-induced CD4 downregulation are AP1, AP2 and AP3, as Nef is able to interact with these three AP complexes through a di-leucine motif. To identify which of these adaptors and which other host cell factors are involved in the Nef-driven downregulation of CD4, Bonifacino's group performed an RNA interference (RNAi) screen targeting membrane traffic genes. They used the *Drosophila* S2 cells coexpressing CD4 and Nef. In this system, CD4 was downregulated by Nef in the same way as in mammalian cells and depended also on di-leucine motifs in both CD4 and Nef. This screen identified clathrin and AP2 as key factors involved in CD4 downregulation. The requirement of AP2 was reproduced in the human cell line HeLa. In addition, a three-hybrid screen in yeast also demonstrated that a robust interaction between the di-leucine motif of HIV-Nef and the α and $\sigma 2$ subunits of AP2 were required for the CD4 downregulation. Bonifacino proposed a model in which HIV-1 Nef promotes endocytosis of CD4 by inducing its capture into clathrin-coated pits leading to enhanced CD4 internalization from the cell surface (16).

Endosomal Sorting Machineries

Endosomes are platform organelles where proteins destined for degradation are sorted from proteins that have to be recycled to the cell surface or to the TGN. The different mechanisms involved in these sorting events were illustrated and discussed by several speakers.

Jean Gruenberg (Geneva, Switzerland) presented results about the intra-endosomal membrane trafficking. There is

now evidence that different subpopulations of vesicles coexist in the lumen of late endosomes. While internal vesicles present in the lumen of multivesicular endosomes are known to transport downregulated epidermal growth factor receptor (EGFR) to lysosomes for degradation, some internal vesicles, perhaps corresponding to a different population, can undergo selective back fusion with the limiting endosomal membrane. This latter process would allow the escape of proteins and lipids from lysosomal degradation. This phenomenon was illustrated by following the intracellular trafficking of the VSV. The VSV entered the cells through clathrin-dependent endocytosis, then the virus envelope fused with the endosomal membrane, preferentially with internal membranes. The viral RNA release into the cytosol finally occurred from late endosomes after back fusion of internal vesicles with the outer membrane (17). This back fusion event required the late endosomal lipid lysobisphosphatidic acid (LBPA) and the putative LBPA effector Alix/AIP1. Interestingly, the anthrax toxin lethal factor is delivered to the cytoplasm through a similar pathway, which also depends on LBPA and Alix (18). By contrast, degradation of the EGFR in lysosomes does not depend on LBPA and Alix. The LBPA is a lipid enriched in internal vesicles of multivesicular bodies (MVB) but is very poorly degraded. Jean Gruenberg hypothesized that LPBA-containing vesicles could be the ones that fused back with the endosomal membrane, while the other pool of internal vesicles enriched in *PtdIns*(3)P and cholesterol might be degraded in the lysosome.

The endosomal sorting of growth factor receptors to the internal vesicles of the MVB depends on ubiquitination and ubiquitin-binding factors. Ubiquitination is reversible by the activity of de-ubiquitination enzymes (DUBs). **Sylvie Urbé** (Liverpool, UK) presented studies of the DUB associated molecule of SH3 domain of SATM (AMSH) and ubiquitin specific processing protease Y (UBPY) and their role in the downregulation of the EGFR. Both enzymes were found by their ability to bind the Src homology (SH)3 domain of the endosomal adaptor STAM, and interacted with other components of the endosomal sorting complex required for transport (ESCRT) machinery, responsible for the ubiquitin-dependent sorting of cargo proteins. Urbé showed that AMSH is specific to the Lys^{63}-linked ubiquitin chains *in vitro*. AMSH was able to bind the clathrin heavy chain as well as the Vps24 subunit of the ESCRT III complex, in addition to STAM. These interactions could allow the formation of a bridge between ESCRT0 and ESCRT III complexes (19). Furthermore, AMSH siRNA-mediated knock down enhanced the EGFR degradation rate in HeLa cells (20). This result suggested that AMSH could antagonize the E3-ligase activity of Cbl, which is responsible for the ubiquitination and subsequent downregulation of the EGFR at the cell surface. In contrast to AMSH, UBPY has no preference for Lys^{63}- or Lys^{48}-linked ubiquitin chains *in vitro*. The siRNA-mediated knock down of UBPY resulted in inhibition of EGFR de-ubiquitination and of both EGFR and Met receptor downregulation (21). This was accompanied by the partial loss of Hrs and a severe reduction in the level of STAM. The UBPY seemed to stabilize STAM by removing the Lys^{48}-linked ubiquitin chains and thereby protecting STAM from proteasome-mediated degradation. However, UBPY depletion also had severe effects on MVB morphology in cells, suggesting multiple targets for this enzyme in endosomal sorting. In summary, AMSH and UBPY appear to have opposing roles in growth factor receptor downregulation.

Anjon Audhya (La Jolla, USA) is using *Caenorhabditis elegans* to identify new regulators of membrane transport during development, with a particular focus on members of the ESCRT family. In a similar way to mammalian cells where growth factors and their receptors are downregulated by degradation, *C. elegans* oocytes degrade certain membrane components following fertilization. Using tandem affinity purification of Vps37, the ESCRT-I complex was isolated. Four main proteins were recovered, Tsg101, Vps28, Vps37 and a new member of the complex, conserved in higher eukaryotes, ESCRT-binding protein 1 (EBP1). The recombinant version of EBP1 was able to interact with ESCRT1 *in vitro,* and when overexpressed in HeLa cells, EBP1 colocalized with Tsg101 and perturbed endosomal morphology. Furthermore, long-term EBP1 overexpression resulted in a three-fold increase in binucleated cells. The EBP1 was also shown to accumulate at the mid-body during cell division. Moreover, following cell abscission and separation, the EBP1-labeled mid-body associated with one of the daughter cells and was then degraded. Audhya postulated therefore that ESCRT-I may function during mid-body removal and/or cell abscission.

In a special lecture, The Annaberg lecture, acknowledging his large contribution to the membrane trafficking field, **Scott Emr** (La Jolla, USA) provided an overview of endosomal sorting mechanisms and of proteins involved in this process in yeast. Together with the groups of E. Jones and T. Stevens, the group of Scott Emr identified the vacuole protein sorting (VPS) genes in 1980s. This was followed by the functional description of the Vps proteins. Among the VPS genes, *VPS34* codes for a phosphatidylinositol (PI)3-kinase. This discovery was the starting point for understanding the role of phosphoinositide-modifying enzymes in membrane trafficking and in the recruitment/activation of effector proteins required for vesicle-mediated transport processes. The PI-specific-binding domains were then identified, the Fab1, YOTB, VAC1 and EEA1 (FYVE), phox homology (PX), PH and epsin N-terminal homology (ENTH) domains. Proteins that contain one of these domains are localized to specific membrane compartments, as e.g., *PtdIns*(3)P is enriched in endosomes, while *PtdIns*(4,5)P2 is enriched at the plasma membrane (Figure 1). Unexpectedly, at the plasma membrane, the *PtdIns*(4)-kinase Stt4p and the *PtdIns*(4,5)-kinase Mss4p are localized in dynamic patches that do not overlap. This observation reinforces the idea that lipids in general, and phospholipids in particular, are key regulators of many cellular processes by

defining functional subdomains in organelle membranes. They are also involved in the recruitment of the ESCRT machinery, a set of protein complexes required for receptor downregulation and protein sorting at the multivesicular endosomal compartment. The Vps27/Hse1 complex subunit Vps27p is recruited to the endosomal membrane through its FYVE *PtdIns*(3)P-binding domain. The ESCRT-II also binds to *PtdIns*(3)P through the GRAM-like Ub binding in EAP45 (GLUE) domain in the Vps36 protein. It is still not clear how the different ESCRT complexes drive membrane invagination and the formation of the MVB internal vesicles. It is also not known how the cargo is transferred from the ubiquitin-binding site of Vps27p to the one of ESCRT-I Vps23 subunit. Until now, no ESCRT-I/II supercomplex could be purified from yeast cell extracts. However, a key protein involved in the association of the two complexes has recently been identified as a new subunit of ESCRT-I, Mvb12. This protein could be a direct regulator of the formation of the ESCRT-I/II complex as this supercomplex can be copurified from *mvb12Δ* cells. The fine regulation of the recruitment and function of each of the ESCRT complexes is an essential step for cargo sorting and MVB vesicle formation. These proteins are also required for the budding of HIV virus. It is an exciting time of discovery in this area of cell biology, but many important questions remain to be answered.

Membrane Fusion and Fission

During membrane remodeling, fusion and fission mechanisms need to be particularly well regulated. Classical examples comprise endosome fusion and the regulation of size and number of mitochondria. Few molecular components involved in these processes are known to date. For instance, proteins of the SNARE family are key actors of membrane fusion and have been intensively studied.

Paul Luzio (Cambridge, UK) presented data regarding the mechanisms involved in the delivery of material to lysosomes. Four different models had been proposed: the maturation of late endosomes, the vesicular transport from late endosomes, the fusion of late endosomes with lysosomes to form a hybrid organelle and a 'kiss and run' mechanism. In order to distinguish between these different hypotheses, they used a very simple and elegant approach consisting of loading cells with rhodamine–dextran in order to label lysosomes. Subsequently, they incubated these cells with Oregon green–dextran for a short period of time to label late endosomes. They then followed by time-lapse confocal microscopy the appearance of merged structures, which is because of the transfer of material from late endosomes to lysosomes. They found that this mechanism was due in part to direct fusion, but occurred mainly by 'kiss and run' events. Furthermore, some tubular structures were observed emerging from lysosomes, possibly involved in the reformation of these organelles (22). In order to characterize the molecular events occurring during endosomal fusion, they studied the SNAREs involved in homotypic (late endosome to late endosome) and heterotypic (late endosome to lysosome) fusions in a cell-free assay, using purified late endosomes and lysosomes from rat liver. They found that Syntaxin 7, Vti1b, syntaxin 8 and VAMP8 mediated homotypic fusion, whereas Syntaxin 7, Vti1b, syntaxin 8 and VAMP7 mediated heterotypic fusion.

Mitochondrial homeostasis is obviously crucial for cell survival, and extensive membrane remodeling has been shown to occur in the course of apoptosis. However, the mechanisms and the molecules controlling these events are not well understood. **Luca Scorrano** (Padova, Italy) provided insights into the molecular mechanisms involved in the remodeling of the inner membrane (*cristae*) during apoptosis. His laboratory is interested in particular in the function of optic atrophy-1 (OPA-1). This protein is related to dynamin and is localized in the inner membrane of mitochondria. It has been shown to promote mitochondrial fusion and its overexpression inhibits apoptosis. Furthermore, a delay in cytochrome c release was observed in OPA-1 overexpressing cells, but surprisingly, this effect was independent of OPA-1 fusion activity. Hetero-oligomers of transmembrane and cleaved OPA-1 were observed to be the target of Bid action during apoptosis (23). Scorrano and coworkers then took advantage of the knockout mouse for Parl1, an inner mitochondrial membrane rhomboid protease. These mice showed an increase in apoptosis and died around 8–12 weeks of age. Interestingly, a soluble form of OPA-1 was able to rescue the phenotype of Parl1 −/− mouse embryonic fibroblasts (24). Consequently, the authors proposed that both OPA-1 and Parl1 are regulating apoptosis by controlling cytochrome c release: Parl1 cleaves OPA-1 and this soluble form participates in the maintenance of *cristae* morphology and cytochrome c compartmentalization.

Rab GTPases and Organelle Identity

Rab GTPases have received a lot of attention in recent years because of their key function in organelle identity, maintenance and maturation. Indeed, several independent efforts are now underway to understand the regulation of this class of molecules and the mechanisms involved in vesicle maturation. The hierarchical organization of Rabs in the endocytic pathway is relatively well established, but the exact mechanisms involved in the maturation of endosomes along the endocytic pathway are still poorly understood.

The laboratory of **Marino Zerial** (Dresden, Germany) characterized recently the molecular events taking place during transport from early endosomes to late endosomes. They showed that Rab5 accumulated in endocytic structures containing endocytic cargo, e.g., low-density lipoprotein receptor, resulting in homotypic fusion of early endosomes during cargo internalization and concentration. Furthermore,

when these Rab5-positive structures translocated to the perinuclear region, a conversion from Rab5 to Rab7 occurred, consistent with cargo progression from early to late endosomes (25). Compartmentalization of receptors in specific membrane domains is also emerging as particularly important during signal transduction. Signaling endosomes are now thought to act as key platforms in the regulation of intracellular signaling. Zerial and collaborators unraveled the role of Rab5 endosomes in the transduction of the EGF signal. Marino Zerial presented data showing that EGF can be internalized into two distinct populations of Rab5 structures that differed in their signaling capacity: early endosome antigen (EEA1)-Rab5 endosomes allowed internalization and maturation toward late endosomes, whereas anaplastic large cell lymphoma (APPL1)-Rab5 structures were involved in the signal transduction of EGF (26). Moreover, in order to identify the molecules regulating the endocytic sorting, Marino Zerial performed high-content genome-wide RNAi screening coupled to high-throughput microscopy-based assays using automated image analyses and bioinformatics. This approach will be highly informative because it allows the simultaneous tracking of hundreds of endocytic structures and the acquisition of numerous parameters such as endosome size and motility.

Dynein-mediated axonal transport is of particular importance in neurotrophin signaling and neuronal survival. **Giampietro Schiavo** (London, UK) reported on the molecular characterization of neurotrophin carriers and on the role of the small GTPases Rab5 and Rab7 in the regulation of the axonal retrograde transport in primary neurons. Schiavo and coworkers took advantage of an atoxic fragment of the *Tetanus* neurotoxin, a bacterial toxin that is retrogradely transported in axons in endocytic structures shared by neurotrophins and their receptors. By using this toxin coupled to magnetic beads, neurotrophin carriers were isolated from embryonic motor neurons and analyzed by mass spectrometry and Western blotting, revealing the presence of both Rab5 and Rab7. Live imaging of motor neurons expressing GFP-tagged versions of Rab5 and Rab7 has shown the presence of these GTPases on stationary (Rab5) and retrograde axonal carriers (Rab7). In particular, Rab7 controlled the onset of axonal retrograde transport of these carriers as the impairment of its activity completely blocked the retrograde transport of all neurotrophin carriers (26). These observations are of particular importance because the control of motor-protein-driven transport by Rab GTPases is emerging as a key feature in vesicle trafficking and membrane remodeling. Furthermore, human disorders affecting the nervous system have been shown to be caused by because of mutations of Rabs and their regulators, highlighting the crucial role of this family of proteins in the maintenance of cellular homeostasis and neuronal survival.

A new regulator of Rab7 function in *C. elegans*, SAND-1, was identified by **Anne Spang** (Basel, Switzerland) and coworkers. Based on observations of similar phenotypes after RNAi of Rab7 and SAND-1, they argued for a functional link between this newly characterized protein and the Rab7. They showed a delay in the sorting through the endosomal pathway in the SAND-1 mutant *or552,* and consequently, the yolk proteins were not able to reach the yolk granules. However, in *or552* mutants, the lysosomes were still labeled with Lysotracker, indicating that lysosome biogenesis *per se* was not impaired and therefore arguing for a defect earlier in the endocytic pathway. Enlarged structures positive for both early and late endosome markers were observed in SAND-1 −/− cells. This observation led Anne Spang and coworkers to hypothesize that early to late endosome progression could be impaired in the *or552* mutant. Indeed, a GFP version of a yolk protein, vitellogenin (VIT)-2, accumulated in these large granules, and a Texas Red-labeled BSA was found in the same structures. Finally, the endosomal membrane localization of Rab7 was impaired in the *or552* mutant, confirming the regulatory role of SAND-1 for Rab7 function in the progression from early to late endosomes (28).

Francis Barr (Martinsried, Germany) presented a study on the Rab GTPase-activating protein (GAP) family of proteins. His laboratory used biochemical and cell biology approaches to identify the GAPs for the 60 human Rabs. To this end, they searched the human genome for proteins that contain Tre2/Bub2/Cdc16 domains that are characteristic of Rab GAPs, and retrieved 40 proteins. These proteins were then screened against all human Rabs to detect physical interactions, and GAP assays with recombinant proteins were subsequently performed to confirm the relevance of these interactions. Barr and coworkers then focused on Golgi morphology defects caused by Rabs and their GAPs. They found Rab1, Rab33 and Rab43 as key actors in this process. Indeed, a dominant-negative form of Rab43 was able to cause Golgi fragmentation and to block the trafficking of Shiga toxin from the plasma membrane to the Golgi apparatus. Using their biochemical approach, they found RN-Tre as a RabGAP for Rab43. Because Rab GAPs are still poorly understood, any effort undertaken to characterize them could provide valuable information regarding mechanisms of membrane remodeling.

Membrane Dynamics and Autophagy

Autophagy is the major degradative pathway in the cell together with the proteasome pathway. Macroautophagy induced by starvation in yeast and the stress-induced autophagy in mammalian cells are both non-specific, in contrast to organelle-specific autophagy. In all cases, a double-membrane vesicle is formed and traps the cytoplasmic constituents and organelles. The central question in autophagy is what the identity of the donor membrane is for the formation of autophagosomes. The answer might come from the study on trafficking of autophagy gene 9 (Atg9), the only transmembrane autophagy protein identified so far.

To find out how autophagosomes are formed, **Fulvio Reggiori** (Utrecht, The Nederlands) found that, in contrast to most of the Atg proteins, which are restricted to the preautophagosome structure (PAS), Atg9p cycled between the mitochondria and the PAS in the yeast *S. cerevisiae*. This recycling, dependent on the Atg proteins Atg2 and Atg18, required a functional early secretory pathway (29). In addition, he showed that Atg9p sorting from mitochondria was dependent on the ER-mitochondria network as well as on the integrity of the actin cytoskeleton (29). He presented experiments showing that Atg9 was more likely to be in a compartment in close proximity to the mitochondria. However, using the split-ubiquitin system, he found that Atg9p did not interact with mitochondrial proteins, such as Tom5p, or with the ER proteins, Ubc6p and Sec62p. The compartment from which Atg9p cycles to reach the PAS remains to be identified, as most of the tested Golgi apparatus, early or late endosome protein markers do not localize to it. The identification of this compartment will provide new insights about the donor membrane for the formation of autophagosomes.

Sharon Tooze (London, UK) presented results regarding the topology as well as the intracellular localization of the mammalian homologue of the *S. cerevisiae* Atg9p protein, mAtg9, recently identified in her laboratory. At steady state, mAtg9 localized mainly in a juxta-nuclear region that corresponded to late Golgi membranes and in peripheral structures that corresponded to late endosomes (30). Upon autophagy induction, the juxta-nuclear pool of mAtg9 dispersed throughout the cytoplasm in a reversible manner. The mAtg9 was present in the outer and inner membranes of autophagosomes and colocalized with the autophagosomal marker Atg8p/MAP-LC3. These results suggested that mAtg9 could cycle between the TGN and the endosomes in mammalian cells. The mAtg9 seems to have a different behavior than the *S. cerevisiae* Atg9p. The cycling of mAtg9 was dependent on UNC51-like kinase (ULK)1, the mammalian homologue of the yeast protein Atg1p. In yeast, Atg9p is restricted to the PAS in *atg1*Δ cells. In contrast, in ULK1-depleted cells, mAtg9 was blocked in the TGN. These data led to the hypothesis that in mammalian cells, the Golgi membrane could be the donor membrane for the autophagosome formation.

In her lecture, **Ana Maria Cuervo** (New York, USA) provided us with an overview as well as the latest finding of the work of her laboratory on chaperone-mediated autophagy (CMA). This mechanism does not required formation of vesicles or membrane invagination, but occurs by translocation of soluble proteins from the cytoplasm through the lysosomal membrane. This pathway is activated during stress such as prolonged starvation, oxidative stress or exposure to toxic compounds. In starvation, CMA is induced after macroautophagy has taken place upon a prolonged starvation. This is a selective pathway for substrate proteins containing a KFERQ-like motif, which is recognized by the cytosolic chaperone Hsc70. The lysosomal receptor in this process is lysosomal-associated membrane protein (LAMP)2A, and the rate of CMA is directly dependent on the level of LAMP2A at the lysosomal membrane, as the binding of the substrate to this receptor is the rate-limiting step in CMA (31). In a recent work, they identified a dynamic sub-compartmentalization of LAMP2A in the lysosomal membrane, which underlies the molecular basis for the regulation of LAMP2A function in CMA (32). LAMP2A localized in different lysosomal membrane microdomains that were detergent resistant. Upon CMA activation, LAMP2A exited these microdomains and multimerized (33). The CMA was then regulated by the LAMP2A degradation rate at the lysosomal membrane, which was linked to its lysosomal microdomain localization. They observed that cholesterol depletion increased CMA activity, whereas cholesterol addition decreased CMA activity. In addition, changes with age in the lipid composition of the lysosomal membrane were responsible for the declined CMA during aging (33). Lysosomal membrane lipid composition therefore appeared to be crucial for the downregulation of the resident proteins.

Conclusion

Although most of the presentations reported basic research, a few talks gave an outlook on possible applications in medicine. At this point, we would like to mention Richard Scheller (San Franscico, USA). He is a cell biologist studying the molecular mechanisms of exocytosis and is also the executive Vice President of Research in the company Genentech. He described very recent results by his company: they 'armed' antibodies specific against cancer cells with toxins and, using a systematic approach, improved this relatively old idea from many different angles. Now they are in the last phase of clinical trials and testing these antibodies on patients, giving hope for a possible new treatment of cancer very soon.

The extremely interesting talks and posters presented gave a great overview of the latest research in membrane dynamics. In the past two decades, many major players of membrane traffic pathways have been identified and characterized. Research in this field is now focused on understanding the fine regulation of the activities of these major players. In particular, the cross talk between lipids and proteins involved in membrane traffic has just begun to be investigated and remains to be further explored. For these reasons, we look forward to the next meeting and for more exciting reports to come.

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