MOLECULAR BASIS FOR MEMBRANE REMODELLING AND ORGANIZATION

ANALYSES MOLECULAIRES DE L'ORGANISATION ET DU REMODELAGE DES MEMBRANES

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Conference Report

Rapport sur la Conférence

- **Résumé**

Cette conférence est la troisième conférence Jacques Monod sur la dynamique et le remodelage des membranes et elle est à ce jour la seule conférence européenne sur ce sujet, en dehors d'un workshop FEBS (qui a lieu à en général en Grèce, à Spetsès) qui s'intéresse à des aspects "plus lipidiques". La première CJM sur ce sujet a eu lieu en 2011 et s'intitulait "Analyses moléculaires de l'organisation et du remodelage des membranes". En 2013, le prix Nobel de médecine fut attribué à J. Rothman, R. Schekman et T. Südhof, pour leurs travaux pionniers sur le trafic vésiculaire et sur la biologie moléculaire des membranes en général, confirmant l'intérêt du sujet. Après le succès de la première CJM sur les membranes, une seconde fut organisée en 2014 sur les "Analyses moléculaires de l'organisation et du remodelage des membranes" qui eut encore plus de succès que la première. Pendant toutes ces dernières années, l'essor des microscopies haute-résolution, optiques ou électroniques et des approches interdisciplinaires de la biologie cellulaire ont permis d'élargir la compréhension de l'organisation de la membrane et de son remodelage à différentes échelles spatiales et temporelles. Nous avons travaillé à intégrer de nouveaux aspects de la dynamique des membranes dans notre programme qui n'avaient pas été abordés dans les conférences précédentes, en particulier des exposés de physiciens des membranes présentant une vision plus intégrée des flux membranaires à l'échelle de la cellule entière ou couplés à des flux d'actine, ou bien une session sur la communication inter-cellulaire. Notre programme a été organisé pour montrer la façon dont la mise en forme et l'organisation membranaire jouent un rôle in vivo, en partant de l'échelle moléculaire puis à des échelles mésoscopiques jusqu'à des niveaux plus intégrés au-delà de la cellule unique à la fin du programme.
Nous eûmes finalement 28 conférenciers invités, 10 français, 11 Européens, et 7 non Européens, et 75 participants (26 Français, 36 Européens, et 6 non Européens) dont 17 thésards. Nous avons eu quelques annulations tardives d'orateurs/trices que nous avons pu remplacer, à part un seul (G. Salbreux). Nous pensons que nous aurions pu avoir encore plus de participants ou de demandes en adaptant le calendrier de mise en ligne des conférences CJM. En pratique, c'est compliqué d'organiser une conférence CJM début Avril. En effet, la règle étant jusque-là de mettre toutes les conférences en ligne en même temps pour une année calendaire, la conférence a été mise en ligne mi-novembre 2016 pour une date limite d'inscription au 6 Janvier 2017, donc moins de 2 mois, avec les fêtes de fin d'année au milieu. De plus, compte tenu des règlements administratifs, la période de fin et début d'année est très compliquée d'un point de vue comptable, ce qui complique énormément le versement des soutiens par les sponsors. Néanmoins, le sujet de la conférence a été suffisamment attractif pour avoir un nombre très raisonnable de participants.
La conférence s'est déroulée classiquement sur 3 jours et demi du lundi soir au vendredi matin, avec 2 séances très animées le soir, et une visite à l'île de Batz par un beau soleil le mercredi après-midi. Il nous semble que les exposés des conférenciers invités ou sélectionnés ont été de niveau équivalent à celui des meilleures conférences internationales sur ce sujet et nous les en remercions tous. Nous avons eu d'excellents retours des participants et des orateurs.

- **Summary of the talks**

Keynote lecture (sponsored by EMBO)
Petra Schwille (MPI, Martinsried, Germany) gave a very interdisciplinary opening lecture on the peculiar role of membranes for driving the assembly and positioning of the bacterial dividome. Her talk beautifully demonstrated that physical concepts, reconstituted membrane systems and microfabrication can be instrumental in deciphering biological mechanisms. She also set the tone of this meeting bringing some physics flavor into questions related to membrane remodelling and organization.

Session 1: Membrane organization-1
This session was mainly focused on molecular aspects of membrane organization or fission. **Anne-Claude Gavin** (Heidelberg, Germany) started by presenting systematic approaches for deciphering complex arrays of lipid/protein interactions. She focused in particular on Osh proteins involved in PS transfer and finally showed with a liposome-microarray-based assay (LIMA) how sphingolipids can enhance affinity for PH-domain proteins for PiP2. **Carmen Valente** (Naples, Italy) demonstrated that a lipid transformation from LysoPA into PA, triggered by BARS binding to a TGN complex containing kinases and Arf, can lead to Golgi membrane scission. **Thomas van Zanten** (Bangalore, India) showed evidence of a very exciting mechanism for the formation of protein nanodomains (GPI-anchored proteins, or trans-membrane proteins such as integrins) on the outer leaflet of the plasma membrane: the active formation of actomyosin asters on the cytosolic leaflet drives clustering of the

proteins on the opposite side through a coupling that engages PS on the cytosolic side, cholesterol and lipids with long fully saturated chains (GPI) on the outer leaflet. This part of the session ended with **Noemi Jiménez-Rojo** (Geneva, Switzerland) who presented a new strategy for screening kinases' genes that affect sphingolipids levels. She showed an example with the transcription regulator BRD3, which, when KO, affects the levels of different lipids (ceramides, glycolipids and glycerophospholipids) while increasing the oxidative stress, showing the importance of lipid homeostasis to preserve cell viability.

Session 2: Membrane organization-2

The second session on membrane organization started with 2 talks on non-vesicular exchange of lipids between organelles. Contact sites (CS) and Lipid droplets (LD) have been only recently discovered. How CSs functionally work to exchange lipids between organelles and the consequences on lipid homeostasis are still under debate. Moreover, the detailed mechanism for the formation of LDs is not described yet. We had a beautiful presentation by **Bruno Antonny** (Nice, France) on the mechanism of the coordinated exchange of cholesterol and Pi(4)P between TGN and ER, orchestrated by OSBP at contact sites and by the PI4-kinase PI4IIIβ that he called the "ferry-bridge model" . He also showed that perturbation of the kinase leads impressive travelling waves across the TGN. **Pedro Carvalho** (Oxford, UK) presented a surprising interplay on the biogenesis of Lipid Droplets and of peroxisomes from the ER membrane, with Seipin and Pex30 having redundant functions for the LDs and peroxisomes, respectively. The 2 next talks dealt with the role of membrane shape in the localization of trans-membrane membrane proteins. **Kenneth Madsen** (Copenhagen, Denmark) with biophysical approaches demonstrated that the nature of the amphipathic helices of class A GPCRs determines how much these receptors are curvature-sorted and thus internalized. Finally, with a nice combination of AFM correlated with dSTORM, **Pierre-Emmanuel Milhiet** (Montpellier, France) has presented his recent results showing the local enrichment of the tetraspanins CD9 and CD81 at the HIV curved budding sites and the additional role of Gag Zn fingers in this recruitment.

Session 3: Membrane sculpting-1

Membrane shaping by proteins is traditionally a core topic of the conference since this process is totally ubiquitous in cells; we had 3 sessions on it with very prominent researchers in this area. The first session was devoted to the fission proteins, dynamin and ESCRTs and to structural and theoretical approaches. We started **Jenny Hinshaw** (NIH, Bethesda, USA) who presented very new super resolution data at 4.6 Å which was achieved on different conformational changes of dynamin, using the most advanced equipment in cryoEM. This revealed new structural features and the precise localization of the PH domain on the membrane. Next, **Markus Deserno** (Pittsburgh, USA) continued with a minimal theoretical modeling of the dynamin filaments. He has first considered a continuum mechanical model based on a classical representation of the membrane but with a saddle shape and a bound elastic helical filaments of fixed length, next a coarse-grained representation of both and calculated filament and membrane shape that minimize the system. Eventually, **Winfried Weissenhorn** (Grenoble, France) has presented structural results on proteins involved in enveloped virus budding, either M1 from influenza C virus or ESCRT-III that contributes to scission HIV-1 bud (CHMP2A, CHMP3 and the ATPase Vps4). He showed the capacity of M1-C to form tubular invaginations when polymerizing on the membranes of giant liposomes, but confirmed that it is not sufficient for triggering scission. The structural basis of CHMP2A, CHMP3 and Vps4 that assemble at the neck of the bud was presented. And he eventually presented hypothesis on ESCRT-dependent versus ESCRT-independent virus release mechanism.

Session 4: Membrane sculpting-2

John Briggs (Cambridge, UK) presented his most recent work on the structure of coat assemblies obtained with cryoEM tomography, with a particular focus on COP1. The most impressive part of this top-of the art talk was a COPI reconstruction from *in vivo* images with precisely the same structure as the *in vitro* reconstituted one, but containing natural cargoes inside. This *tour-de-force* obviously heralds a new era of structural biology directly inside cells. Next, **Olivier Daumke** (Berlin, Germany) described an how the proteins Mic60, Mic10 and Mic 9 of the mitochondrial contact site and cristae organization (MICOS) altogether coordinate their shaping capabilities and are able to simultaneously bind at the neck of the cristae at the inner mitochondrial membrane and tether it the outer membrane. Next, a new membrane scission mechanism, relevant at least during some clathrin-independent endocytic events, was introduced by **Patricia Bassereau** (Paris, France). It is called "friction-driven scission" and involves BAR-domain proteins scaffolding the neck of a growing tubules. Using

reconstitution on membrane nanotubes and a theoretical model, she showed that the friction between the scaffold and the membrane can lead to membrane lysis when the tubule is mechanically elongated. The two last talks of the session were about scission by ESCRT-III complexes. This process is currently a hot topic in membrane dynamics with many animated but still unsolved debates on the mechanism. First, with fluorescence light sheet microscopy and CLEM, **Simona Migliano** (Innsbrück, Austria) presented an impressive quantitative work on the *in vivo* recruitment and assembly of ESRT-III proteins on endosomes and the final inverse budding. She demonstrated unambiguously that the ATPase Vps4 is recruited shortly after Snf7 (CMP4 in humans) and is absolutely required for scission. She was even able to count the number of molecules at the different stages! Eventually, **Aurelien Roux** (Geneva, Switzerland) showed the *in vitro* dynamical assembly of Snf7 followed in real time on a supported membrane by high speed AFM. He proposed a new hypothesis where Vps4 induces an instability in this filament that can eventually lead to constriction by relaxation of the mechanical stresses. He also showed data from the Gerlich group confirming the Vps4 turnover is absolutely required for abscission. He ended his talk by presenting a new membrane tension sensor.

Session 5: Membrane sculpting-3

This session was globally devoted to the proteins that shape organelles. **Tom Rapoport** (Boston, USA) initiated this session by revealing the first successful assay for the reconstitution of ER architecture with purified proteins and liposomes. This purely reconstituted system recapitulates very well in the presence of GTP the network architecture of the ER, even the 3 way-junctions, or sheets depending on the addition of Atlastin or Lunapark. This presentation was followed by **Bruno Goud** (Paris, France), who showed that Rab6 is involved in key trafficking events at the TGN, such as fission of membrane tubules at hot spots of the TGN, and is also a general regulator of the post-Golgi traffic between TGN and plasma membrane where it targets vesicles at hot spots of exocytosis. **Richard Lundmark** (Umeå, Sweden) continued with a presentation on the role of protein EHD2 in the stabilization of the caveolae in an ATP-dependent manner that allows partial insertion of the protein in the membrane at the neck of the caeolae. ATP hydrolysis triggers EHD2 release and the consecutive destabilization of the caveolae. **Josh Zimmerberg** (NIH, Bethesda, USA) ended the session with impressive cryoEM tomograms and phase contrast EM images showing the unexpected existence of membrane edges near a hemifusion diaphragm between Influenza viral-like particles and liposomes. These edges are stabilized by activated HA.

Session 6: Membrane-cytoskeleton interactions-1

Since cell membranes are most of the time in close apposition or directly connected to cytoskeleton filaments, and this aspect was not very developed in the previous 2 meetings, we have devoted 2 sessions on membrane-cytoskeleton interactions. The physicist **Raphael Voituriez** (Paris, France) first introduced the current physical understanding of the different cortical actin flows that occur in the different modes of cell migration and the role of friction. He next emphasized the role of macropinocytosis at the front edge for the locomotion of dendritic cells. The next talk from **María Isabel Geli** (Barcelone, Spain) revealed a completely unexpected function of the contact site OSBP proteins Osh2 and Osh3 in the scission of endocytic vesicles in yeast. With Time Resoled EM, she showed that these proteins bridge the myosin 1 Myo5 and the Vamp protein Scs2 in the ER membrane, which initiate an actin dependent membrane invagination and eventually promotes vesicle scission. This very novel discovery on the involvement of CS proteins might well bring new insight on the role of actin in endocytosis a don endocytosis in general. The last talk by **Cédric Delevoye** (Paris, France) demonstrated that BLOC-1, myosin 6, actin and the kinesin KIF13A have to cooperate to form and scission tubular structures from recycling endosomes that eventually mature into melanosomes.

Session 7: Membrane-cytoskeleton interactions-2

In this session, the discussion about the interaction of cell membranes with the cytoskeleton was continued. **Stephane Meresse** (Marseille, France) discussed how bacterial effector proteins derived from Salmonella harness the cytoskeleton to induce membrane tubules. He presented his latest research about the SifA effector protein showing that it interacts via SKIP with kinesin. PipB2 is also required for the recruitment of kinesin to the vacuole. *In vitro*, OipB2 is sufficient to recruit kinesin to the membrane and to induce membrane tubules in a GUV based system. **Pekka Lappalainen** (Helsinki, Finland) concluded this session by discussing how the plasma membrane lipids regulate actin polymerization. He showed that actin binding proteins display drastically different affinities for $PI(4,5)P_2$ and there fine tune actin polymerization and its recruitment to the cell membrane.

Session 8: Membrane trafficking pathways-1

This session was opened by **Harvey McMahon** (Cambridge, UK), who presented the latest research of his lab on the connection between membrane curvature and Parkinson's disease. α-synuclein is recruited to the mitochondrial membrane via its unstructured N-terminal domain. This recruitment is PINK1-dependent and can result in vesiculation of mitochondria. The mitochondrial localization is abolished by several Parkinson's disease associated mutations suggesting that synuclein might be involved in a mitochondrial quality control pathway. **William Prinz** (NIH, Bethesda, USA) continued by giving further insights into lipid droplet biogenesis. Lipid droplets are lens-like structures in the ER. In FIT deficient cells the lipid droplets are surrounded by the ER membrane. He further showed that FIT proteins promote budding of lipid droplets from the ER and that nascent lipid droplets originate from Pex30 positive domains. **Joshua Lees (**Yale University, USA) talked about their new data on lipid homeostasis at membrane contact sites, and in particular about the role of the TMEM24 protein at ER-plasma membrane contact sites. TMEM24 tethers and these two membranes in a Ca^{2+}-dependent and phosphorylation regulated manner. It's SMP domain dimerizes and transports PI, possibly for $PI(4,5)P_2$ generation. TMEM24 sustains Ca^{2+} pulsativity and is required for full insulin secretion. **Ludger Johannes** (Paris, France), the last speaker of this session, presented his findings on the CLIC/GEEC pathway, which is a clathrin independent pathway of endocytosis. He showed that galectin-3 oligomerises, binds to glycosylated extracellular cargo material and induces membrane budding. Galectin-3 interacts with certain integrins preferentially in their closed conformation and colocalises with these proteins. These interactions were shown to stimulate endocytosis of integrins in a process requiring BAR domain proteins and dynamin.

Session 9: Membrane trafficking pathways-2

The presentation of the latest insights into membrane trafficking pathways was continued by **Volker Haucke** (Berlin, Germany). In his talk, he gave insights into the spatiotemporal control of endocytosis by phosphoinositides. He showed that there is a transition from $PI(4,5)P_2$ to $PI(3,4)P_2$ during clathrin-mediated endocytosis at the plasma membrane. When $PI(3,4)P_2$ synthesis is abolished clathrin coats assemble but no neck constriction occurs. An effector protein of $PI(3,4)P_2$ is SNX9, which binds this lipid, is localized to necks and is required for their constriction. In the following talk by **Lars Langmeyer** (Osnabrück, Germnay), an *in vitro* reconstitution system recapitulating rab GTPase conversion was presented. Rab conversion is required for the maturation of organelles, for example along the endo-lysosomal pathway and it was shown that Rab GDI confers strict GTP dependence to membrane fusion in a vacuolar fusion assay. The topic of **Ira Milosevic's** (Göttingen, Germany) talk was again clathrin mediated endocytosis. Her lab used endophilin knockout cells, in which uncoating is delayed to isolate clathrin coated vesicles. This experimental trick allowed her to show that the presence of the clathrin coat inhibits vesicle acidification by negatively regulating the vATPase. She further showed that the vATPase snuggly docks into the clathrin coat. **Stefano Vanni** (Fribourg, Switzerland), a bioinformatician, presented his latest data from a computational modelling approach to understand lipid droplet biology. In particular, he showed how the properties of different ER lipids control membrane tension and thereby formation and stability of lipid droplets.

Session 10: Autophagy

This session was opened by **James Hurley** (Berkeley, USA), who presented a reconstitution system for the studying membrane deformation and session by the ESCRT proteins. These proteins are required for autophagy and other endo-lysosomal trafficking pathways. ERSCRT-III proteins and the Vps4 ATPase were encapsulated into GUVs together with caged ATP. Uncaging of ATP occurred with an UV laser. This system allowed his group to propose that the ERSCRT-III proteins and Vps4 are sufficient for membrane scission and that the ATP activity of Vps4 is required for this event. **Sharon Tooze** (London, UK) presented a surprising connection between the centrosome and autophagy. She showed that GABARAPs, which shuttle between the centrosome, the cytoplasm and the autophagic membrane interact with the centriolar satellite marker PCM1. PCM1 was also shown to localize to the site of autophagosome formation. The session was concluded by **Carsten Sachse** (Heidelberg, Germany), who presented insights into the Atg18 protein. Atg18 is a peripheral membrane proteins. At acidic pH it forms helical oligomers. Cryo-electron microscopy showed that in this form the lipid binding sites of the protein are buried in one of the interfaces and therefore Atg18 cannot bind the membrane. In the presence of $PI(3,4)P_2$ the oligomers disassemble allowing membrane association. **Sascha Martens** (Vienna, Austria) presented their most recent insights into how the autophagic cargo receptor p62 assembles ubiquitinated cargo material into larger structures that subsequently become templates for the initiation and growth of the isolation membrane.

Session 11: Cellular Organization

The topic of this session was how the cellular machineries are coordinated within the cell. In the first talk **Marino Zerial** (Dresden Germany) presented their results about a class of coiled-coil membrane tethers. Using the endosomal EEA1 as model he showed this protein undergoes an entropic collapse to bring two rab5 positive endosomes into close contact and thereby to aid SNARE-dependent fusion. Many coiled-coil tethers may function in a similar manner. In the following presentation **Pierre Sens** (Paris, France) showed a biophysical and mathematical approach to understand intro-Golgi transport. His results suggest that the control of domain size by enzymatic activity plays an important role in regulating exchange within compartmentalized organelles such as the Golgi apparatus and membranous organelles in general. **Mathew Good** (Philadelphia, USA) discussed the role of cell membranes as boundaries. He focussed on experimental approaches to recapitulate these boundaries for reconstitution experiments in order to obtain mechanistic insights into these processes. Sphingomyelin metabolism and Golgi function was the subject of the final talk of this session, given by **Felix Campelo** (Barcelona, Spain). Employing a blend of computational modelling and cell biological experiments allowed him to propose that short chain sphingomyelin reduces the lateral organisation of the Golgi apparatus and inhibits Golgi function such as Golgi export, glycosylation and Golgi morphology. A hypothesis was put forward proposing that sphingomyelin metabolism regulated the structure and function of the Golgi.

General discussion and prospect

After this session, a general discussion about the future of the conference occurred. It was generally agreed that the format of this unique and exiting conference should be kept. The overall quality of the science was considered outstanding and everyone appreciated the small size of the conference promoting discussion and interactions between the participants at all levels. It was voted that **Ludger Johannes** (Paris, France) and **Anne-Claude Gavin** (Heidelberg, Germany) should serve as future Chair and Vice-Chair, respectively.

The evening was concluded with the fantastic and cheerful seafood banquet at the Gulf Stream Hotel.

Session 12: Cell-cell communication

The last session of this inspiring conference dealt with the question of how cells communicate with each other at cell-cell contact sites. It was opened by **Chiara Zurzolo** (Paris, France), who presented her results on so-called tunnelling nanotubes, which are tubular connections between cells. These structures are also used by prions to spread from cell to cells. Focussing on the formation of nanotubes she showed that filopodia and tunnelling nanotubes are formed by distinct cellular machineries. **Pascal Therond** (Nice, France) presented further insights into Hedgehog signalling. Hedgehog is lipidated and secreted morphogen. It is secreted by vesicles that bud off the plasma membrane in an ESCRT dependent manner. He further presented his findings on how hedgehog is trafficked *in vivo* employing Drosophila and mice as model organisms. Last but not least, **Isabel Guerrero** (Madrid, Spain) talked about the lab's recent results on hedgehog signalling in flies. Interestingly, this signalling occurs at cytonemes but these adopt synapse-like structures similar to the structures used by neurons to mediate cell-cell communication.