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**Analyses moléculaires de l'organisation et du remodelage des
membranes**
Molecular basis for membrane remodelling and organization

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Rapport sur la Conférence

Conference Report

Resumé

Il y a quelques années, il n'existait pas en Europe de conférence de haut niveau réunissant des scientifiques intéressés par la forme et la dynamique des membranes biologiques. Comment une vésicule se forme? Comment sont générés les tubes du reticulum? Quels sont les protéines et les lipides clés de la déformation membranaire, de la fission? Quels sont les principes des barrières de diffusion? Comment se forment les sites de contact entre compartiments et quelle est leur fonction? En 2011 eut lieu la première CJM « *analyses moléculaires de l'organisation et du remodelage des membranes* ». Elle remporta un franc succès et permit l'organisation d'une seconde édition plus étoffée en 2014. Cette fois ci nous dûmes refuser du monde, preuve d'un domaine de recherche en plein essor. Nous eûmes 26 conférenciers, 7 français, 11 Européens, et 8 non Européens, et 88 participants (36 Français, 41 Européens, et 11 non Européens). Cette seconde édition se distingua de la précédente par l'introduction de deux champs de recherche : la dynamique moléculaire pour mieux comprendre l'agencement des lipides à l'échelle moléculaire, et le métabolisme lipidique pour mieux cerner l'adaptation des protéines du remodelage aux compositions lipidiques différentes des compartiments. Enfin, cette deuxième édition fut couplée à la journée des trois prix Nobel 2013 à Paris (Rothman, Schekman, Südhof), récompensés pour leurs travaux pionniers sur le trafic vésiculaire et sur la biologie moléculaire des membranes en général.

General aspects

This conference was the second CJM on "molecular basis for membrane remodeling and organization". Compared to the first edition (2011), the number of attendees increased and reached the maximum limit of 115 people (about 125 people applied).

The main aim of this CJM is to gather scientists from different fields interested in membrane dynamics. At the very heart of this conference are biochemists, structural biologists and cell biologists who study proteins that shape membrane-bound organelles to induce various stable or dynamics structures (typically transport vesicles, tubules at the ER, inverted buds in multivesicular bodies...). BAR domains with their banana shape are best examples of such proteins. However, current studies illuminate the variability of the structures capable of deforming membranes. Furthermore, the field of membrane remodelling requires expertise beyond classical biochemical and cell biology approaches. In this second edition, we invited new speakers to cover other expertise, including molecular dynamics simulations, lipidomics analysis, and cryo EM. Because defects in membrane remodeling processes are also at roots of some diseases (e.g. viral infections, muscular disorders), several sessions included talks on the connections between membrane shaping mechanisms and pathological disorders.

The conference schedule was concentrated on three days (Sunday, Monday, Tuesday) and not three days and half compared to classical CJM. The reason for this was to make our program compatible with the Nobel prize conference held in Paris on Wednesday with Jim Rothman, Randy Schekman and Tom Südhof. We cooperated with the organizers of this event to favor attendance of the two meetings. A special bus was organized very early on Wednesday from Roscoff so that about 30 brave people could join Paris on time and use the seats that were reserved for them at the Collège de France.

The weather was uneven and the attendees could experience the pleasure of watching the scenic view of the Iles de Batz from the Roscoff shore as well as the difficulty in holding an umbrella during a rainy and windy afternoon. Thanks to the excellence of science presented, both by the invited speakers and the selected applicants, and because of the commitment of the CJM staff, the delicious food and the incomparable atmosphere of the small Brittany harbor, the conference went very well. The two poster sessions were full, lively and we had to ask the participants to shorten their final discussions to leave the room not after midnight. Feedback that we got from participants and speakers was very good, but of course the filled questionnaire should give a more impartial view. One attendee cancelled the day before because of sickness and it was too late to arrange a substitution.

Summary of the talks

Keynote lecture (sponsored by EMBO)

Reinhard Jahn (Max Planck, Goettingen, Germany) gave the opening lecture on membrane fission that was remarkable by the link made between old observations, most of them were probably unknown by the youngest attendees, and current observations. With these comparisons, he could

highlight the still challenging issues in this remodeling pathway (specificity, stalk formation, hemifission) and thus set the tone of this conference. The EM pictures shown during this talk are still in many heads.

Session I: Lateral membrane organization part I

Progresses in the area lateral membrane organization are slow due to the technical limitations in studying lateral membrane organization at a relevant scale (say < 100 nm). In addition, concepts such as rafts, which were initially appealing and stimulating, turned out to be too simplistic and not always based on firm grounds. The presentation of **Yves Barral** (ETH, Zürich, Switzerland) gave a refreshing view on lateral membrane organization by showing the striking change in membrane diffusion at the endoplasmic reticulum during asymmetric cell division in *S cerevisiae*. In brief, a diffusion barrier at the bud neck prevents the lateral homogenization of transmembrane proteins between the mother and the daughter cells. What is fascinating is the correlation of this new diffusion regime with the process of cellular aging: the non-inheritance of aging factors between the two cells depends on the establishment of the diffusion barrier. Most importantly for the audience, the key factor of this diffusion barrier is now unraveled: the formation of long chain sphingosines, which makes possible the assembly of a thick membrane region at the bud neck. The presentation of **Emmanuelle Bayer** (CNRS, Bordeaux, France) echoes back to that of Barral by showing accumulation of saturated lipid species in the plasmodesmata of plant cells, which also corresponds to a plasma membrane – cortical ER specialized region. The quality of the plasmodesmata preparation, as shown by electron microscopy, was noticed by the audience. The presentations of **Derek McCusker** (CNRS, Bordeaux, France) and of **David Albrecht** (King's College, London, UK) illustrate two complementary aspects of membrane lateral segregation. The former highlighted the importance of dynamics GTPase circuits to create lateral domains (with the small G protein Cdc42 and the negatively charged lipid PS), whereas the later discussed the role of the regularly spaced axon cytoskeleton on the establishment of diffusion barriers in neuronal cells and which requires state-of-the art imaging techniques (super-resolution and single particle tracking).

Session II: Metabolism and organelles

This new session was organized to illustrate the complexity of lipid chemistry at membrane interfaces for organelle remodeling and dynamics. Put differentially, we organized this session to underline the fact that membrane remodeling and dynamics are not just questions of protein scaffolds, but also of lipid packing. **Michael Schlame** (NYU, New York, USA) started the session by showing the puzzling enzymatic properties of Tafazzin, an acyltransferase that is responsible for changing the acyl chains of cardiolipin, the characteristic lipid of the mitochondrial internal membrane. On the one hand, tafazzin seems very promiscuous in its ability to swap the acyl chains of various phospholipids and lysophospholipids. On the other hand, tafazzin is responsible for the enrichment of mitochondrial cardiolipin in linoleic acid. Using a reconstitution approach the dilemma was solved by showing that tafazzin is very sensitive to the physical state of membranes, notably curvature, which tends to amplify its ability to introduce linoleic acid into cardiolipin. **Cathy Jackson** (Institut Jacques Monod, Paris, France) presented another example of a protein highly sensitive to the physical state of membrane interfaces: GBF1, the guanine nucleotide exchanger for Arf1 at the Golgi 'jumps' from this organelle to lipid droplets through amphipathic sequences. The mechanism at the origin of this translocation remains elusive, but this work suggests that GBF1 is a master regulator for directing the small G protein Arf1 towards two types of lipid interfaces: bilayers (e.g. Golgi cisternae) and monolayers (lipid droplets). Using other proteins with characteristic amphipathic sequences, the Jackson lab dissects the physicochemical mechanisms at the origin of the specific adsorption of proteins onto lipid droplets vs classical bilayer-bound organelles. **Frédéric Pincet** (Ecole Normale Supérieure, Paris, France) addressed the role of Arf1 on lipid droplets and suggested that this protein and its associated coat (COPI) regulate membrane tension at the lipid droplet surface, thereby facilitating the subsequent enzymatic work of proteins that consume the triacyl core. This talk was remarkable by the fact that it was based mostly on physical principles. **Hideo Shindou** (Tokyo, Japan) concluded this session by showing huge progresses in the identification of the various acyl transferases at the origin of phospholipid acyl chain diversity in membranes. Not only the repertoire of these enzymes is now almost complete in model organisms, but also some of their function can be assigned (e.g. formation of saturated species for the lung surfactant).

Session III: Membrane bending: different mechanisms - different approaches – part I

This session was a 'classics' in the sense that it was led by three prominent researchers in the field of BAR domain-containing proteins. However, their presentations showed new lines of research

and unanticipated findings. **Vinzenz Unger** (Northwestern University, USA) presented the diversity of BAR domain scaffolds onto model membranes by Cryo EM on model membranes. This illustrates that, despite a common principle (the 'banana' shape), not all BAR domain lattices are the same. They display remarkable differences in side-side contacts and overall patterns, suggesting different functions, dynamics and stability. **Patricia Bassereau** (Institut Curie, Paris, France) presented a new type of reconstitution aimed at dissecting the recognition of concave surfaces by I-BAR domains. This assay is a tour de force because it requires inversion of the classical geometry: instead of adding BAR domain proteins at the surface of giant vesicles and tubes, the protein is trapped inside the GUV, allowing the authors to study its partitioning onto the GUV and tube inner leaflet. The preference of the I-BAR domain for negative curvature was directly demonstrated. Last, **Gregory Voth** (University of Chicago, USA) presented molecular dynamics simulations aimed at capturing the processes of membrane curvature sensing and generation by BAR domains at different time and dimension scales. This process is highly dependent on protein density and results in membrane deformations of various shapes, illustrating the potency of BAR domain-containing proteins to drive different membrane shaping mechanisms (tubule, vesicles, fission...). A linear aggregation model of BAR domains was presented as well as the effect of membrane tension on BAR interactions.

Session IV: Membrane contact sites

A few years ago, membrane contact sites were barely evoked in meetings on molecular membrane biology. With the identification of proteins involved in these contacts as well as progresses in multi-color live cell imaging, these sites are now intensely studied owing to their importance in lipid homeostasis. They allow membrane compartments to exchange lipids and maintain their characteristic composition. After a previous demonstration that the contact sites between the ER and the mitochondria marks the sites of mitochondria fission, **Gia Voeltz** (University of Colorado, Boulder, USA) presented a new set of live cell experiments showing that the ER tubules also mark the sites of endosome fission. The parallel between the two findings is striking and suggests a general and decisive advantage of membrane contact sites for organelle division (lipid exchange, Calcium, curvature?). With **Karin Reinish** (Yale University, USA), we moved to one of the first structure of a protein involved in membrane contact sites: the extended synaptotagmin, which shows a striking dimer interface, able to extract lipids, and C2 domains attached to long flexible linkers. This architecture is suggestive of at least two potential modes of membrane apposition, which were discussed with the audience. Moreover, the speaker showed a first reconstitution of lipid transfer catalyzed by this protein using liposomes and time resolved assays. These types of assays have been used extensively by **Guillaume Drin** (CNRS, Valbonne, France), who studies lipid exchange by Osh proteins. His previous work suggested coupling between forward sterol transport and retrograde transport of PI(4)P by Osh4. Using a new assay to follow the displacement of PI(4)P in real time, he demonstrated that this coupling is tight and stoichiometric. Furthermore, new data on Osh6 suggest that the counter-exchange of PI(4)P is a general mechanism by which Osh proteins transport specific lipids (PS in the case of Osh6). He concluded that PI(4)P gradients provide the metabolic energy to direct the transfer of lipids across organelles. The cell biology work of **Christopher Stefan** (University College London, UK) further underlines the importance of PI metabolism in the maintenance of the contact sites between the ER and the plasma membrane.

Session V: Autophagy

The formation of the autophagosome is a fascinating event from the point of view of membrane remodeling because of the unusual shape of the membrane (the cup shape of the phagophore), the unresolved origin of the membrane (ER, mitochondria, ERGIC...), a unique lipid modification event (formation of an Atg8-PE covalent complex) and the involvement of specific proteins involved in membrane deformation, lipid sensing, lipid modification and cargo sorting. **James Hurley** (UC Berkeley, USA) presented the first structure of the class III phosphatidylinositol 3-kinase complex I (PI3KC3-C1) that functions in early autophagy. The complex was determined at 27 Å by single-particle EM, and showed a V-shaped architecture. Subunits could be fitted in this structure. **Tom Melia** (Yale University, USA) focused on another aspect of phagophore maturation: the formation of the covalent Atg8-PE adduct. Previous reconstitution experiments suggested that this reaction requires a much higher amount of PE than what is found in the phagophore membrane. The Melia lab solved this dilemma by showing that the reaction is strongly favored by membrane curvature. Owing to an amphipathic helix sensitive to lipid packing defects, Atg3 can work at a much lower concentration of PE. **Sascha Martens** (Max Perutz Lab, Vienna, Austria) presented reconstitutions that recapitulate the mechanism of

selective autophagy by which Atg8-PE recognizes cargo-Atg19 complexes for membrane engulfment. Protein-decorated beads were used to mimic the cargo whereas GUVs were used to mimic the phagophore. He also presented a semi in vitro system with mammalian cells and beads. It is remarkable that so many steps in autophagy are known in such details. **Aliaksandr Khaminets** (Goethe Univ, Frankfurt, Germany) concluded the session by presenting an autophagy –regulated mechanism for ER recycling that is based on the reticulon-like protein FAM134B. In the absence of this protein, the ER shows unusual expansions in neurons, resembling the clinical symptoms of patients with FAM134B mutations.

Session VI: Membrane sorting and budding

Coat intermediates in membrane traffic have two functions: to deform the lipid bilayer and to select proteins ('cargo') for encapsulation and further transport. However, the balance between these two activities is not always clear-cut. The sorting endosome is a main sorting membrane platform where transmembrane proteins have two fates: when sorted into intraluminal vesicles, they are ultimately degraded into the lysosome; when sorted into tubes budding from the endosomes, they are recycled to the plasma membrane or the trans golgi. **Peter Cullen** (University of Bristol, UK) presented new data on this second pathway: the role of SNX-3, a sorting nexin that is atypical by the lack of BAR domain and that works in tandem with a lipid flippase, which may contribute to lipid asymmetry and thereby curvature. Two pathways are presented: SNX-BAR leading to tubules, and SNX3 and clathrin leading to vesicles. **Christien Merrifield** (CNRS, Gif sur Yvette, France) is one of the few experts able to follow in live cells all steps of clathrin coat assembly and cargo selection using various fluorescence constructs and imaging tricks. He showed examples of coupling between cargo capture and coat assembly and other examples where this coupling is more loose, suggesting a broad range of cargo fate. He also discussed the difference between flat and curved clathrin regions. Last, **Mihaela Anitei** (TU Dresden, Germany) and **Stéphanie Miserey-Lenkei** (Institut Curie, Paris, France) presented new cell biology and reconstitution data on the various mechanisms by which the trans golgi network (TGN) is physically deformed and further cut by molecular motors, cytoskeleton elements (actin) and dedicated BAR-domain containing proteins.

Session VII: Endocytosis

This session is another 'classics': no membrane deformation mechanism has been more studied than the budding of endocytic vesicles. However, this session turned out to be one of the most original as it was marked by unexpected findings. **Ludger Johannes** (Institut Curie, Paris, France) and **Harvey McMahon** (MRC Cambridge, UK) presented independent experiments revealing that a large number of endocytic events not only bypass the clathrin coat (which was already known) but also are driven by the concerted action of the BAR-domain protein endophilin and dynamin. By its rapidity, this pathway might explain some very fast endocytosis events in neurons. Other aspects such as the involvement of actin is reminiscent of the key role of the cytoskeleton in yeast endocytosis. This aspect was heavily discussed by **Marko Kaksonen** (EMBL, Heidelberg, Germany) in his presentation on the role of the clathrin adaptors Sal2 and Ent1, which are representative of Anth and Enth domains. They are not redundant, formed highly organized structures on membranes and both are needed for fission.

Session VIII: Lateral membrane organization, part II

Daniel Fletcher (UC Berkeley, USA) showed a further striking example of the power of membrane reconstitution: mimicking the budding of a virus using a simple GUV and the M1 component of influenza A. M1 domains grow from the base and are attached to the negatively charged lipid PS. **Sebastian Barg** (Uppsala Univ, Sweden) presented an opposite top-bottom approach to decipher the stepwise assembly of the exocytic machinery in live cells. **Siewert Marrink** (Univ of Groningen, The Netherlands) showed one of the most comprehensive attempts to mimic in coarse grain simulations the lipid complexity of the plasma membrane using not less than 60 different lipid species, asymmetrically distributed between the two membrane leaflets. This approach is invaluable for addressing some key issues in membrane domain organization (size, asymmetry, time scale, coupling between the two leaflets).

Session IX: Endo-lysosomal pathways

This title of this session (with a plural) was deliberately vague to fit various talks. It turned out to be one peak of the conference. This was largely due to the talks of **Aurélien Roux** (Univ of Geneva, Switzerland) and **Adam Frost** (UCSF, USA), who presented new and unexpected data on ESCRTIII. The

ESCRTIII filaments are a late event in the formation of luminal vesicles on endosomes and pose key issues about membrane deformation. ESCRTIII is very different from classical protein coats, although both structures are capable of generating vesicles. First, ESCRTIII displays a spiral shape. Second it drives a deformation that projects outside the protein complex. Adam Frost presented the structure of conical ESCRTIII spirals as deduced from Cryo EM: the cone structure is suggestive of a mechanism of membrane deformation, but the electrostatics between the outer and inner faces of the cone is opposite to what was expected for a structure capable of interacting with negatively charged membranes. The talk of Aurélien Roux drove some exclamations. For the first time, we could see the assembly process of a membrane-deforming complex thanks to the use of Live Atomic Force Microscopy (AFM). Beautiful ESCRTIII spirals formed and then, at high density, deformed into polygonal structures. The analysis of the speed of ESCRTIII spiral formation, together with their lateral deformation allows the authors to determine the stiffness of the spiral and to suggest a way by which it could escape from the planar geometry and lead to membrane budding. **Frédéric Boal** (Inserm, Univ of Toulouse, France) and **Christian Ungermann** (Univ of Osnabrueck, Germany) concluded this session by presenting the key role of an overlooked phosphoinositide (PI(5)P) in the endolysosomal pathway and a first link between the small G protein Rab7 and metabolic control at the vacuole through the novel effector Ivy1, which is found in small patches of negative curvature.

Session X: Plasma membrane mechanics

The shape and mechanics of the plasma membrane is obviously not only a question of protein coats and BAR domains. In large part the main factor explaining the shape of the plasma membrane is the underneath actin cytoskeleton. Using biological perturbations, quantitative imaging and theory, **Ewa Paluch** (MRC, University College London, UK) presented recent investigations to study the contractile instabilities of the cell cortex. **Shiro Suetsugu** (NAIST, Japan), who is well known for his work on BAR domains (and actually was initially invited for this topic), presented very recent data on the interaction of the ankyrin repeat domain of a channel, TRPV4, and the plasma membrane phosphoinositide (PIP2). Structural and functional studies identify a key role of this interaction in normalizing the channel activity of TRPV4, further highlighting the key role of PIP2 in plasma membrane identity. Last, **Richard Lundmark** (Umea Univ, Sweden) presented a most recent analysis on the interaction between cavin and caveolin, the main component of caveolae, which controls plasma membrane tension. Cavin 3 was shown to compete with cavin 2 for the cavin 1 coat and to decrease the lifetime of caveolae.

Session XI: Membrane bending: different mechanisms - different approaches – part II

Britta Qualmann (Jena Univ, Germany) studies the function of BAR domain proteins through an integrated approach (Gene knockout and RNAi in individual neurons and morphological and functional characterization). Her previous work revealed a role of syndapin 1 in presynaptic features. Now the authors show that syndapin 1 is also involved in post-synaptic functions, notably by interacting with postsynaptic scaffold components and with factors that govern the actin cytoskeleton. Membrane-associated Syndapin 1 appears as a main anchoring point for organizing distinct neuronal membrane areas. **Emmanuel Lemichez** (Inserm, Nice, France) is an expert in cell responses to pathogens. We invited him because he uncovered a few years ago a fascinating membrane remodeling event, the formation of transcellular tunnels through RhoA inhibition upon cell infection by *Staphylococcus aureus* factors. These tunnels are 'repaired' by I-BAR domains. He presented the role of the actin cytoskeleton and myosin in this repair mechanism. **Daniel Levy** (Institut Curie, Paris, France) is an expert in the structure of transmembrane proteins by CryoEM. The advantage of this technique is that it can give a fair idea of not only the protein structure but also of the shape of the surrounding membrane. He showed examples of proteins whose intrinsic shape drive membrane deformation in a cycle manner. This triggered discussions on the role of the hydrophobic matrix in damping these morphological changes.

Session XII: Membrane fission

This last session was devoted to one of most fundamental events of membrane remodeling: fission to give two separate membranes. Fission is notoriously difficult to study because of the very transient nature of fission intermediates. **Oliver Daumke** (Max-Delbrück-Centrum Berlin, Germany) presented his last structural data (structure of dynamin 3 tetramer) suggesting a general model for dynamin right hand helix and membrane fission. It includes complex intra and intermolecular movements in the dynamin molecule and in the dynamin spirals. He also discussed the capital roles of

some dynamin amino-acids, thereby introducing the talk of **Ya-Wen Liu** (National Taiwan Univ, Taiwan), who studied dynamin mutants responsible for myopathies. She has set up a comprehensive analysis of these mutants, from biochemical assays (GTPase, fission) to functional studies. Her results nicely complement the structural studies of the Daumke lab and the meeting serves as a go-between for the two laboratories, which conducted their studies in an independent manner. **Chris Williams** (Univ of Groningen, The Netherlands) presented his recent data on a dynamin-like protein Dnm1 and its partner Pex1 involved in peroxisome fission. He presented some mutants deficient in fission. **Bruno Antony** (CNRS, Valbonne, France) concluded this session by presenting a combinatorial approach using biochemical reconstitutions, physical measurements on cells, and molecular dynamic simulations to study the facilitation of membrane fission by polyunsaturated lipids.

Session XIII: Final discussion

During this session led by **Pekka Lappalainen** (Univ of Helsinki, Finland), the participants came back to all topics discussed at the meeting, notably whether the new approaches that were introduced in this second edition (molecular dynamics, lipidomics, autophagy, ESCRT, contact sites) fit well with the objective of the meeting. The feedback on the balance between these approaches was good. **Patricia Bassereau** and **Sascha Martens** were elected as president and vice president, respectively, and were encouraged to present another exciting program for a third CJM on 'molecular basis for membrane remodeling and organization' in 2017. The three-year period seems well adapted to the topics. Everybody then ran to the Gulf Stream Hotel to enjoy the fantastic seafood dinner.