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Roscoff (France), 24-28 septembre 2011

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

RESUME DU RAPPORT

Conférence Jacques Monod intitulée :
Analyses moléculaires de l'organisation et du remodelage des membranes
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Résumé français à venir

29 septembre 2011 - Décès de Barbara WINSOR

Nous avons appris la terrible nouvelle du décès de Barbara WINSOR au lendemain de la conférence Jacques Monod qu'elle avait co-présidée avec Harvey Mc Mahon à Roscoff fin septembre 2011 sur le thème "Molecular basis for membrane remodelling and organization".

Ceux qui ont eu la chance d'interagir avec Barbara sur le plan scientifique ont pu apprécier son dévouement à la science et aux autres. Pour cette conférence de Roscoff, dont elle a été la cheville ouvrière et pour laquelle elle a déployé une énergie qui ne pouvait nous laisser penser qu'elle usait ses dernières forces, Barbara a été présente jusqu'au bout, donnant aux autres et notamment son sourire.

Le site : http://www.endocytosis.org/F-BAR_proteins/JMConference/barbara_winsor.html donne la parole à ceux qui l'ont côtoyée.

Nous nous joignons à eux pour témoigner de la grande estime que la communauté avait pour Barbara, et apporter à sa famille notre compassion et nos plus cordiales pensées.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Molecular basis for membrane remodelling and organization

Roscoff, September 24-28, 2011

PREFACE

It is with sadness that I come to write a report on a conference where my co-chair, Barbara Winsor, has passed away just after the conference finished. In some way this conference can be dedicated to her memory and the success of this conference is a reflection of her abilities and insights. In the words of one participant "this led her work to be ahead of its time…. only in Roscoff did some of the ideas that she had planted...start to return to her as the fruits offered at the meeting". Barbara has made a largely unrecognised contribution at the interface of the fields of membrane dynamics and cytoskeleton dynamics, when she found the Arp2/3 complex has a role in yeast endocytosis, connecting these two fields together. Tributes and thoughts in memory of Barbara can be found at:
http://www.endocytosis.org/F-BAR_proteins/JMConference/barbara_winsor.html

CONFERENCE AIMS

The conference was born out of the recognition that membranes are actively shaped by proteins and the cytoskeleton, and that these shapes underlie function. To get to grips with this we aimed to bring together experts in fields/disciplines: from biophysics, biochemistry, cell biology and medicine. The conference sought to understand membrane dynamics and the factors underlying shape in the following areas: cell shape, organelle shape, vesicle shaping (formation), and vesicle fusion. We thus needed to have a strong emphasis on the interplay between membranes and the cytoskeleton and the various ways in which membrane binding and cytoskeletal proteins may possibly influence/control membrane shape changes. We also invited those involved in the study of major physiological membrane remodelling process (e.g. embryo morphogenesis and platelet production) to complement the more basic science approaches of others.

SUMMARY AND PROCEEDINGS

Many of our speakers and delegates commented that this was the best conference they ever attended. This was the first time that such a conference has been held at this interface and there were many talks exploring how membranes are shaped. There were a wide variety of mechanistic explanations, ranging from the view that virtually all proteins that come in contact with membranes are sensitive to their morphology, to the view that specific proteins (owing to their intrinsic properties) have either the ability to change local shape or to react to local membrane shape. These ideas have major implications given the wide variety of processes that membranes control (ranging from signalling pathways to transcription). These implications were clear from the presentation of those who studied disease, although a lot of work remains to be done to explore the implications. It was amazing how both biophysics of membrane and the medical implications of mutations in individual proteins are coming to the

same conclusions, and the combination points to a synergistic future where both disciplines come together to inform both basic science and therapeutics. It was interesting how the parameters measured in disease are often to do with signalling, and it was clear to the audience that the changes taking place in disease may very well have a deeper molecular explanation buried in membrane morphogenesis.

ORGANIZATIONAL ASPECTS

The participants were very appreciative of the level of organization given by Dominique Lidoreau, which contributed enormously to the smooth running of the conference. The food was especially good, with meal times being relaxed and a great time to carry on discussions and to mix. The kitchen staff provided a panoply of carefully crafted dishes, always served with a smile. The chef accommodated all the special requests and did a fantastic job.

The poster sessions were very well attended and many of the posters really deserved to be presented as talks. It was unfortunate that the room for posters was so isolated from the eating area and the meeting area, as otherwise conversations started at posters may have continued during coffee times or after lunch with the aid of the displayed posters. We wondered if it might be possible to display posters in a lab area close to the lecture theatre in future, or in the foyer area close to where we had lunch.

The talks all went smoothly, with expert help from Alain who set up the computers and constantly made sure pointers and equipment worked well. We also noted the willingness to accommodate the extended discussion times and his general helpfulness to all.

The conference had 91 participants with speakers and participants coming from the USA (11), Japan (1), Russia (2) Singapore (1), Canada (1) and Europe. The conference coincided with the major European conference on Endocytosis in Crete. The two conferences running in parallel likely did not damage us but we should try to avoid this in the future.

SUMMARY OF SPEAKERS AND DISCUSSIONS

Details of conference according to subject area rather than according to position in the programme: In the programme we tried to make sure that sessions had a specific focus but that there was still a mix of techniques presented.

The conference started with a plenary talk supported by EMBO and given by Prof James E Rothman (Yale) on the molecular basis of membrane fusion. Jim is responsible for the SNARE hypothesis for the control of membrane fusion and based on very recent data from his lab he developed the general principle of how Complexins might control SNARE proteins through the formation and coordinated disruption of a lattice-like arrangement. The energetics of membrane fusion was discussed by several participants with a general recognition that the opening of a fusion pore may be the most energy demanding step. We observed that in some experiments the fusion pore opening was transient (Josh Zimmerberg, NIH), and that membrane tension - the proteins responsible for modulating this remain to be defined/discovered - appeared to play a role in the kinetics of opening (Francois Darchen, University of Paris).

To complement the focus on fusion we also had a number of talks on the mechanics of vesicle endocytosis. Quantitative live-cell imaging was applied to clathrin-mediated endocytosis to

demonstrate the modular mature of the pathway (David Perrais, Bordeaux). Gene-editing was applied by Alexandre Grassart (Berkeley) to get around the problems of overexpressing fluorescently tagged protein in mammalian cells and to approach a more quantitative analysis. This is a technique that will find wide application outside this area. Nathalie Sauvonnet (Institut Curie) and Winfried Roemer (Freiberg) then reminded us that not all cargo is internalized in clathrin-coated vesicles. Nathalie dissected the components necessary for the internalization of the IL2 receptor while Winfried showed how *Pseudomonas aeruginosa* and Shiga toxin B can promote their own internalization by influencing the clustering of lipids that prefer a particular curvature. There has been much emphasis over the last few years on the mechanism of cargo selection and coat formation, yet there is as yet little understanding of how the membranes are shaped and how this influences cargo selection and budding. We heard two detailed morphological studies in yeast on actin/clathrin-dependent endocytosis. One study by John Briggs (EMBL) showed how a dynamic process could be imaged by correlative fluorescence/electron microscopy in a stage specific manner giving detailed information about molecular markers and their positioning in the endocytic process. This is likely a technique that will have much broader applications. The results were largely in agreement with another electron microscopy study presented by Maribel Geli Fernandez (Spain), with issues remaining as to the precise role that actin plays in membrane shaping and scission.

Consistent with their direct membrane remodelling action the proteins of the BAR-domain containing superfamily where very much at the centre of the conference. We heard from Pietro DeCamilli (Yale), Britta Qualmann (Jena, Germany) and Oleg Shupliakov (Stockholm, Sweden) on the role and mechanism of action of several BAR-domain proteins during synaptic vesicle recycling. Recent advances on the role of other members of the superfamily during vascular development, osteoclast formation, thrombocytopenia and tumorigenesis was further presented by Stephanie Oess (Frankfurt, Germany), Richard Stanley (New York, US), Seth Corey (Chicago, US) and Peter Greer (Kingston, Canada).

We had many experts in the field of actin dynamics with special emphasis on the role membranes may play in directing actin polymerization. We heard a fascinating story from Matthias Geyer (Dortmund, Germany) on the crystal structure of a formin complex showing a membrane interaction surface that would fit that found at the tips of membrane protrusions where the protein is normally localized. Thus there may well be a very simple relationship between actin polymerization/bundling and these proteins, and the question now to be addressed would be how to localize these proteins in cells. Bruno Goud (Paris, France) showed how Rab GTPases can recruit some myosins, other actin factors. Marie-France Carlier (Gif-sur-Yvette, France) presented an elegant and novel *in vitro* assay for monitoring actin polymerisation on beads or giant unilamellar vesicle (GUV) promoted by cellular factors, while a number of speakers (Giorgio Scita, Milan and Pierre-Francois Lenne, Marseille) addressed this interface in cells or in organisms, including a talk from Pontus Aspenstrom (Sweden) who is one of the fathers of the field of membrane shaping and dynamics.

A very important issue in membrane trafficking is how membrane remodelling takes place at the level of separating one compartment from a parent (membrane fission). This remodelling is simply the reverse of the membrane shape changes that take place during fusion, except that the driving forces must be different. Misha Kozlov (Tel Aviv) gave us the first clues of this using elastic membrane theory to explain how the process can take place in absence of mechanoenzymes. The precise mechanism was challenged by Aurelian Roux (University of Geneva) and Josh Zimmerberg (NIH) who had assays for membrane scission where they

observed that direct energy input at the level of GTP hydrolysis was needed. We heard from Rainer Beck (EMBL) how membrane scission can happen with small G-proteins, a very unexpected mechanism, and even more unexpected we heard how epsin proteins may play a vital role in membrane scission (Emmanuel Boucrot, Cambridge). We also heard from Oli Daumke (MDC, Germany) on his structure of the mechanochemical membrane scission molecule dynamin. This structure has just been published as an article in Nature and he also presented another novel structure, that of the antiviral protein MxA, allowing him to suggest how a conformational change accompanying GTP hydrolysis could lead to membrane fission. We had several posters presentation on disease mutations in dynamin and thus it was of interest how theses map onto the new structure. Finally we had a presentation on EHD proteins, which are ATPases similar to dynamin. Richard Lundmark (Sweden) was able to show that one of these proteins operates in caveolin-dependent pathways in cells, with great implication for how these might control the mechano-sensitivity and signalling pathways in cells. Miguel Angel del Pozo (Spain) took up this line in his analysis of the invasive nature of cancer cells and the role the caveolin 1 may play. Shiro Suetsugu (Tokyo, Japan) presented how PKC-mediated phosphorylation regulates the function of the F-BAR protein Pacsin2 during caveolin-dependent events. Tying these themes together we heard from Tom Rappoport (Harvard Medical School, US) who showed how the GTPase Altastin is involved in membrane fusion (not fission) of ER tubules *in vitro* and *in vivo*. We were left wondering what gives rise to the directionality (fission versus fusion) in many of these events.

We had a number of talks on molecular motifs that influence membrane curvature. These talks covered curvature sensing motifs; amphipathic helices (Bruno Antonny, CNRS), lipid anchors or transmembrane receptors (Dimitrios Stamou, Denmark) and BAR domains (Patricia Bassereau, Curie Institute). We also heard molecular details of how amphipathic helix may also drive shape changes (Ralf Langen (California) and how BAR domains can make a whole range of curvatures (Pekka Lappalainen, Finland).

Viral fusion and the role of protein insertion domains was shown by Leonid Chernomordik (NIH) and this was challenged by Felix Rey (CNRS) with his new structure of a cell-cell fusion protein. A mechanism was suggested but I think we agree that there needs to be further work. Winfried Weissenhorn (Grenoble) showed how coated viruses used host cell proteins to exit from cells. Many of these proteins influence both membrane shape and topology changes. This area was nicely extended by the work of (Stephane Meresse, Marseille) who showed us how the bacterium *Salmonella* can use a combination of host cell proteins and effector proteins to generate tubular compartments in cells supporting the infection process.

Finally, dynamics shape changes occur in synapses on stimulation where vesicles undergo membrane fusion to release transmitter content. The role of the curvature effector protein Doc2 was presented by Alexandre Groffen (Netherlands) where it plays an important role in spontaneous vesicle fusion in our brains. There was some discussion as to why we need this form of transmission. Synaptic transmission at ribbon synapses was highlighted in the talk of Saaid Safieddine (CNRS) who showed how hearing loss in patients results from mutations in another calcium sensor and membrane curvature effector protein family, the otoferlins.

In summary we were immersed in the frontiers of how membranes are influenced by proteins and how membranes in turn influence cellular processes. We came home with the understanding that membranes hold many of the secrets of life.

RECOMMENDATIONS

Many people referred to this conference as being the best they had ever attended. This is likely because the conference *did achieve its aims* of attracting participants and speakers from all over the world who were experts in their respective areas but who were branching into the emerging field of membrane dynamics. The newness of the field is reflected in the large number of new investigators who were main speakers at the conference. There were many new techniques presented and it was clear there is much creativity at work in this field. There was a unanimous vote from the participants to attempt to hold another meeting in approximately 2-3 years time to bring together the diverse interests and allow cooperativity promote discovery. Given the untimely death of our vice-president, the participants accepted Bruno Antonny (CNRS Valbonne, France) as the next President and Pekka Lappalainen (University of Helsinki, Finland) was voted as the next Vice-President. We thank the CNRS for their support and strongly recommend a repeat conference to them.