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Assemblage coordonné de l'actine et des microtubules dans la motilité et la morphogenèse

Actin and microtubule cytoskeleton in cell motility and morphogenesis: An integrated view

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Assemblage coordonné de l'actine et des microtubules dans la motilité et la morphogenèse

Roscoff, 26-30 mai 2015

La Conférence Jacques Monod « Assemblage coordonné de l'actine et des microtubules dans la motilité et la morphogenèse » (président Marie-France Carlier, CNRS Gif-sur-Yvette, vice-président Thomas Surrey, Francis Crick Institute, Londres) s'est tenue à Roscoff du 26 au 30 mai 2015. Elle a rassemblé 33 orateurs invités, experts de niveau international (10 US, 1 Japon, 11 européens, 11 Français), et 82 participants (35 FR, 12 DE, 11 US, 10 UK, 3 NE, 2 ES, 1 CH, 2 IT, 2 IN, qui ont présenté leurs travaux sous forme de posters (66) et communications orales sélectionnées (12); en outre, 4 éditeurs de Nature Reviews in Mol. Cell. Biol., Nature Cell Biology, Journal of Cell Science, EMBO Journal, ont participé à la Conférence.

La régulation de la dynamique des filaments d'actine et des microtubules est au cœur d'un très grand nombre de processus biologiques vitaux tels la division cellulaire ou la migration des cellules normales et pathologiques. L'auto-assemblage de l'actine et de la tubuline est responsable de la formation de systèmes motiles auto-organisés comme le fuseau mitotique, l'anneau contractile dans la cytocinèse ou les protrusions membranaires dynamiques par lesquelles les cellules explorent et communiquent avec le monde extérieur. Les polymères du cytosquelette orchestrent donc la majorité des fonctions vitales des cellules, qui font elles-mêmes l'objet de nombreux congrès de biologie cellulaire. Afin de proposer un programme original, séminal à long terme et susceptible de fédérer les communautés actine et tubuline, Thomas Surrey et moi-même avons souhaité privilégier les études mécanistiques des réactions moléculaires qui gouvernent la fonction biologique des filaments d'actine et des microtubules et leur coordination, et leur évolution vers des systèmes reconstitués biomimétiques. Cette orientation a permis de présenter les travaux qui représentent les avancées majeures dans le domaine par l'originalité conceptuelle, les progrès technologiques et les modèles mathématiques.

La Conférence a bénéficié en outre du concours efficace et sans faille de Nathalie Babic et ses coéquipiers projectionnistes, d'un temps ensoleillé et d'une restauration réjouissante.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Actin and microtubule cytoskeleton in cell motility and morphogenesis: An integrated view

Roscoff, May 26-30, 2015

GENERAL ASPECTS

This Conference on cytoskeleton dynamics aimed at presenting the molecular mechanisms at work in the motile processes organized by the assembly dynamics of actin filaments and microtubules, using high resolution structural biology, bulk solution and single polymer, single molecule kinetics, reconstituted systems, novel cell imaging methods and mathematical modeling. Additional emphasis was given to the mechanisms that support the coordinated functions of actin filaments and microtubules, which is an emerging trend. The Conference lasted 4 days from Tuesday evening to Saturday morning included. It was financially supported mainly by CNRS. Outside sponsors included EMBO (support of one keynote lecture, Vic Small) and the Company of Biologists (support of travel for students and a second keynote lecture, Tim Mitchison), private companies Cytoskeleton, Safas-Monaco, and Cairn. The length of the talks was restricted to 25 min (invited speakers) and 15 min (selected short talks) including 5 min discussion. Two large enough poster sessions (33 posters each) of two hours were organized. This format made enough time available for discussions and facilitated exchanges between participants and the leaders in the field. Ten sessions of oral communications were organized focusing on the following topics : the structural bases of the regulation of self-assembly ; the mechanisms of nucleation and polarized growth of actin filaments and microtubules ; the mechanical aspects of assembly-promoted movement *in vitro* and *in vivo* ; the molecular processes at work in cell migration and cell division ; the reconstituted coordinated actions of various actin arrays and of actin and microtubules together, and the combination of self-assembly and contractility processes in motile functions ; finally molecular processes at the origin of more integrated motile systems (axonal growth cone, clathrin-mediated endocytosis, regulation of cilia functions, contractile waves in developing embryo…). A keynote lecture was first given Tuesday evening by Tim Mitchison (Harvard Medical School) on the use of biochemistry and quantitative mass spectrometry to analyze the structure and dynamics of meiotic spindles in Xenopus eggs, to elucidate how a large cell divides. A second keynote lecture was given on Wednesday evening by Vic Small (IMBA, Vienna) on the mechanisms that support lamellipodium extension, from molecules to models. Part of the Thursday afternoon was devoted to an excursion to the Batz Island or free time.

At the end of the Conference, the proposal was made to all participants to possibly renew this conference in 3 years, Thomas Surrey being then the new chair assisted by a new french co-chair. Participants were encouraged to write to Thomas Surrey regarding this possibility.

In addition to positive comments made by 40% of the participants in the questionnaire, the chair and co-chair received personal congratulations from several speakers and participants in the days that followed the end of the meeting.

SCIENTIFIC ASPECTS

Session 1 « Control of self-assembly: chemical and structural aspects » (chair Dorit Hanein) brought together experts on high resolution cryoelectron microscopy of large protein assemblies. Eva Nogales (Lawrence Berkeley Labs) showed cryoEM structures of microtubules at 3.5 Angstroems resolution with various bound nucleotides which establish the structural basis for dynamic instability caused by GTP hydrolysis triggering strain of the microtubular lattice via a conformational change in the α-tubulin. The structures observed with bound end-tracking proteins (EBs) shed light into the mechanism by which these proteins track the + ends of microtubules and affect both catastrophes and rescues. These isssues were also addressed in a complementary fasion by Denis Chrétien (Rennes) using nanogold-labeled EBs to visualize the GTP cap. Jan Löwe (MRC LMB, Cambridge) similarly showed high resolution structures of bacterial ParM filaments whose self-assembly develops pushing forces to segregate plasmids, while in contrast TubZ filaments treadmill with minus-end tracking TubRC, pointing to a DNA pulling mechanism used by the TubZRC system.
Finally Andrew Carter (MRC LMB, Cambridge) presented the cryo-EM structure of the dynactin complex at 4 Angstroems resolution (just out in Nature two weeks earlier) and gave a clear demonstration of the rationale for the unique organization of the 23 subunits in this large complex and its stabilization by the D2 adaptor coiled coil structure. This first prestigious session set the note very high for the subsequent ones.

Sessions 2 and 4: The two big sessions (2 and 3, chairs Dyche Mullins and Thomas Surrey) that followed addressed the mechanisms of nucleation and polarized assemblies of actin and microtubules. Single molecule measurements using fluorescent formin and capping protein performed by Jeff Gelles (Brandeis university) showed that these two proteins, thought to compete for binding the barbed ends of actin filaments, were bound transiently simultaneously to the filament end, allowing faster dissociation of one by the other (a poster by Shekhar et al., Carlier lab, provided similar data). David Kovar (Chicago) showed that profilin, by being required for formin-induced actin bundles while it is not required for but inhibits assembly of branched filaments, acts as an orchestrator of the contribution of each meshwork to motile activities of the cell. John Hammer (NIH, Bethesda) gave an elegant demonstration of the regulation of filament barbed end growth by Capping protein cycling between myotrophin V1-bound (inactive) and CARMIL-bound (locally active) forms. Louis Reese (Erwin Frey's lab, LMU Munich) revisited the physics of actin nucleation pioneered by Fumio Oosawa-san, taking into account the finite number of monomers in a restricted volume. Michael Way then showed novel evidence for isoforms of ArpC1 and ArpC5 subunits of Arp2/3 complex as regulators of the kinetic parameters and morphologies of branched actin arrays elicited by vaccinia virus. On the microtubule side, similar concepts were developed by Johanna Roostalu (Surrey's lab, Crick Institute, London) using single molecule-TIRF assays, regarding the mechanism of nucleation of microtubules via the Ran

pathway in meiosis and mitosis, via the synergy between TPX2, a new nucleator, and the polymerase chTOG. Hugo Arellano-Santoyo (from David Pellman lab, Harvard Medical School) used a powerful mutagenetic approach to elucidate the mechanism by which Kip3, the yeast kinesin-8, processively depolymerizes microtubules. Carlos Sanchez (IRB, Barcelona) demonstrated a general function of the gammaTURC microtubule nucleating complex cooperating with augmin in a module of broad occurrence in non centrosomal locations.
Two talks given by MF Carlier (CNRS Gif-sur-Yvette) and Dyche Mullins (UCSF San Francisco) closed this session. MFC shed new light on the regulation of filament barbed end growth by competitive binding of profilin, barbed end capping proteins, barbed end trackers and barbed end branching machineries. RD Mullins revealed new functions of nuclear actin assembly in DNA repair mediated by Spire-Formin2 in response to DNA damage.

Session 4 (Mechanics in assembly and cell movement, chair Jim Sellers, NIH Bethesda) addressed the mechanical properties that result from actin assembly, and how assembly processes are affected by external forces at various scales, *in vitro* and *in vivo*. Using 3D super resolution microscopy, Jennifer Lippincott-Schwarz (NIH, Bethesda) first gave an enlightening view of the mechanics underlying the cycles of protrusion and retraction of ruffles, coordinated by the rearward contractile activity of the lamella, organized in sarcomeric units. Naoki Watanabe (Kyoto) developed new powerful imaging methods for single molecule live cell imaging. On the *in vitro* side, two talks were given by Guillaume Romet-Lemonne (Carlier lab, CNRS, Gif-sur-Yvette and Institut Jacques Monod, Paris) and by Peter Bieling (UCSF and Berkeley University). Using microfluidics-assisted TIRF microscopy, GRL showed how the kinetics of formin-induced processive actin assembly were enhanced by pulling forces applied to barbed end-bound formin, providing insight into the mechanism of the processive cycle, while PB used first AFM to monitor the forces that are produced by assembly of actin, then demonstrated that regulation of actin assembly in branched networks operates in response to applied load, indicating that chemistry is coupled to mechanics in actin assembly. The details of this coupling will require deeper biochemical and structural understanding of molecular processes at work.

Session 5 (Cell migration, chair Vic Small) was in large part devoted to mechanisms responsible for establishment of polarity and self-organization of the cytoskeleton. The session was opened by Sandrine Etienne Manneville (Institut Pasteur Paris) who showed, using a wound healing assay, that intermediate filament network cooperate with microtubules in polarization of cells. Sasha Bershadsky (Weizmann Institute, Rehovot and Mechanobiology Institute, Singapore) gave a spectacular demonstration, in cells growing on circular micropatterns, of the switch from a radial to a chiral pattern of the actin cytoskeleton, driven by the sliding of contractile transverse fibers along the radial fibers. Thus the inherent chirality of the actin filament may be at the origin of left-right asymmetry in higher scale embryonic processes. Mathieu Piel (Institut Curie, Paris) designed new tools to address the effects of confinement on cell migration, and monitor these at the molecular scale using live cell imaging. Cells induced to migrate through small gaps of controlled size used site-directed actin assembly to squeeze their nucleus. Finally Jim Sellers showed that due to the co-polymerization of several functionally different paralogs of non-muscle myosin II in

minifilaments, and additional incorporation of inactive myosin 18 in these minifilaments, different populations of myosin II filaments co-exist in the same cells and may be differently regulated, which questions the validity of the conclusions regarding the function of myosin II when they rely on imaging methods using GFP-tagging. This piece of work thoughtfully indicated that the devil can be in the details and refined biochemistry should more often inspire live cell imaging experiments.

Session 6 (Cell division, chair Tim Mitchison) presented first a broad coverage by Anne Paoletti (Institut Curie, Paris) of the signaling pathways that specify the division plane in budding yeast, by a feedback loop (Cell Geometry Network) involving the membrane bound Cdr2 kinase and antagonistic gradient of Pom1 kinase. Then Tarun Kapoor (Rockefeller university, NY) demonstrated by an *in vitro* approach using TIRF microscopy how the mitotic kinesin-5 regulates spindle geometry and the length of microtubule overlap by generating pushing and braking forces ; Buzz Baum (UCL, London) focussed on the coordination between actin and microtubules during mitosis, a central topic in the conference ; Peter Lenart (EMBL, Heidelberg) used the large transparent starfish oocyte to elucidate the molecular mechanism that causes the cortical contraction wave at meiosis, triggered by recruitment of non-muscle myosin II. Evidence was obtained for a gradient of cdk1 at the origin of the wave. Fred Chang (Columbia university, NY) did not talk about cytokinesis as anticipated, but rather presented a complete functional analysis of the regulation of microtubule dynamics *in vivo* in fission yeast by a set of 8 +TIPS proteins. A mutagenetic approach specified the function of all proteins in nucleation, growth and dynamic instability.

Session 7 (chair Carolyn Moores) and **Session 8** (chair Mathieu Piel) were devoted to the functional analysis, reconstitution and modeling of highly integrated complex systems.
Gijsje Koenderink (AMOLF, Amsterdam) presented elegant single molecule experiments aiming at understanding the basis for coordinated behavior of actin filaments and microtubules, observed long ago for instance in the dynamics of focal adhesions. She demonstrated how the tip of microtubules targets actin filaments using +TIPs proteins that also bind actin filaments and bundles, with different mutual influences, actin bundles guiding microtubule growth and microtubules pulling on individual actin filaments. Zoher Gueroui (ENS Paris) used functionalized paramagnetic nanoparticles of adequately calibrated size to establish a gradient of RanGTP in the mouse oocyte, as an example of spatial control of cellular organization. Another method to spatially control actin assemblies and organize contractile networks was presented by Laurent Blanchoin and Manuel Théry (CEA, Grenoble and Paris). Micropatterned surfaces mimicking the shape of living cells and initiating assembly of dendritic actin meshworks growing perpendicular to the coverslip were shown to generate cytoskeletal architectures allowing to demonstrate that mechanical connections between actin structures by crosslinkers have to be established to maintain the symmetry of the contracting networks. François Nedelec presented a mathematical model to explain the generation of force by insertional actin polymerization in endocytosis and demonstrate that the force is higher than the one expected from polymerization of individual filaments, concluding that it is the pressure generated at the interface between the membrane and the dense actin gel that operates in yeast endocytic patches. Two *in vivo* biological systems were then presented. Rut Carballido-Lopez (INRA, Jouy-en-Josas) elegantly showed that the

bacterial actin MreB determines the shape of the wall by assembling patches that are moved at the surface of the membrane by cell wall synthesis. TIRF microscopy was used to analyze the dynamics of MreB in the processive movement of patches and elucidate the molecular connection between cell wall synthesis and MreB assembly. Another level of complex connection between actin assembly and contractility was presented by Jan Faix (Hanover, DE) using *Dictyostelium* as a model. In this amoeba, an mDia1-related formin, ForA, was shown to cooperate with myosin II-dependent contractility to support efficient migration in 2D-confined environments.

In **session 9** chaired by Jennifer Lippincott-Schwarz and Michael Way were discussed, in continuation with the previous sessions, how molecular processes are integrated in motile functions.
Anna Akhmanova gave a vibrant introduction to the general principles that regulate dynamic instability using a pharmacological approach, and new +TIP proteins. Then Klemens Rottner (Braunschweig, DE) brought a detailed quantitative evaluation of the relative contributions of various formins of the FMNL family to lamellipodium protrusion. Remarkably, deletion of these formins reduces the rate of protrusion but does not affect the rate of actin assembly at the leading edge. Ed Giniger (NIH, Bethesda) showed exciting data on another paradigm system for actin-based motility, the growth cone from the axons of developing *Drosophila* wing. High resolution cell imaging reveals the main features of axon extension, in particular initial formation of fine extensions without lamellipodium, followed by stabilization and filling of the space between the fine extensions. Then Christien Merrifielfd (CNRS, Gif-sur-Yvette) presented kinetics of the subsequent elementary steps in endocytosis with very high time resolution, focussing on the role of actin assembly and the action of its regulators. The cases of clathrin-mediated endocytosis and of EGFR-endocytosis were compared. Guillaume Charras (UCL, London) studied the coordination between adhesion and protrusion during cell migration, by monitoring chemotactic migration of neutrophils in microchannels. The controlled geometry triggers well-defined sub-cellular morphologies generated by actin assembly driven by two different « nucleators », a front slab and a side adhesive network which compresses the front network and mechanically interacts with it to enhance protrusion synergistically. In a system of higher scale of complexity displayed by *C. elegans* developing embryo, François Robin (from Ed Munro lab, Chicago) analyzed, using single molecule in vivo imaging and single particle tracking, the sequence of the events that lead to contractile waves and demonstrated that pulses of activation of Rho-1 precede accumulation of F-actin, myosin and anillin. Thus kinetic analysis again provides insight into the potential underlying molecular mechanisms.

The last session, chaired by Manuel Théry, started with two entertaining talks by physicists Nicolas Minc (Institut Jacques Monod, Paris) and Zvonimir Dogic (Brandeis University). Nicolas Minc discussed how the chemical-mechanical interplay between yeast wall rigidity and Cdc42-dependent polarity caps controls the definition of the first polarity axis. On a more molecular note, and following the early bioinspired studies from Leibler's lab with Nedelec and Surrey, Zvonimir Dogic showed how macromolecular crowding facilitates the formation of microtubule bundles which self-organize, in the presence of kinesin clusters, into active

gels that display, at a macroscopic scale, sliding movements and self-organized invaginations that evoke some developmental processes.
Finally Carsten Janke (Institut Curie, Orsay) gave a fascinating overview of the multiple translational modifications of tubulin, from the functional characterization of the enzymes that catalyze polyglutamylation, polyglycylation and detyrosination, to the complex and unsuspected functional consequences of these modifications in neurodegeneration, in ciliogenesis including formation of the primary cilium, and in mitosis. This exciting talk brought a bright ending to the Conference.

CONCLUDING REMARKS

The meeting was characterized by outstanding and timely contributions, equally found in main and short talks. A large proportion of presented works consisted of unpublished data. The goal to bring together the members of the actin and microtubule communities was successfully achieved, as judged by the 4-days full engagement of the whole audience in animated discussions and questions at the end of each talk. The poster sessions too were very lively and interactive. A good balance was maintained between scientific issues and technology advances developed to answer new questions. Multidisciplinarity was effective, with experimentalists and theoreticians mingling well in discussions. All participants enjoyed the charm of Roscoff and the sea shore. All aspects of management of the conference and the food were more than satisfactory, thanks to the expert care of Nathalie Babic and her colleagues. The early closing time of the bars in Roscoff at this season was deplored, which may be seen as a sign of the lively exchanges at the end of the day.