Final Report

Jacques Monod Conference "Actin and Microtubule Cytoskeleton: Bridging Scales from single molecules to tissues"

Roscoff, 8-12 May 2017

organised by

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Overview

The Jacques Monod conference "Actin and Microtubule Cytoskeleton: Bridging Scales from single molecules to tissues" took place in Roscoff, 8-12 May 2017. It was the second Jacques Monod conference bringing the two research fields studying the actin and microtubule cytoskeleton together (two years after the first conference). This time the conference was organised by Thomas Surrey, The Francis Crick Insitute, London, UK (chair) and Laurent Blanchoin, Biosciences & Biotechnology Institute of Grenoble, France (vice-chair); it attracted a large number of 135 applications of which only 89 could be accepted for registration (due to limitations of the size of the venue), resulting in a total number of 118 conference participants, including the invited speakers and the two organisers. 27 invited speakers (9 from France, 9 from other European countries, 9 from USA, Canada and Japan) gave excellent oral presentations (25 min including discussion or 35 min for the keynote lecture). 13 speakers were additionally selected from poster abstracts (4 from France, 5 from other European countries and 4 from USA and Singapore) and gave excellent short talks (15 min including discussion). Most speakers presented significant amounts of unpublished results. 37 postdoctoral scientist and 21 PhD students were among the participants and had the chance to directly interact with leaders in their fields throughout the meeting. Apart from the two journal editors (Curr. Biol., J. Cell Sci.) who were attending this conference, all other participants presented their work in one of the two very lively poster sessions (2 hours each).

In addition to the generous support provided by CNRS, the conference profited from additional sponsorship provided by EMBO, the Company of Biologists, the Grenoble Alliance for Integrated Structural Biology, Nikon, Zeiss, Roper Scientific, Cytoskeleton and Alvéole. Local support and the logistic organisation by Nathalie Babic was outstanding, the technical staff responsible for projections was reliable, and the excellent CNRS restaurant contributed to the very good atmosphere of the meeting. At the end of the conference, in response to the positive feedback during the meeting, the participants were encouraged to contact the current vice-chair if they are interested in organising a Jacques Monod conference on a similar research theme in the future to further develop the momentum of this very successful conference.

La conférence Jacques Monod "Le cytosquelette d’actine et de microtubules: de la molécule au tissu" a eu lieu à Roscoff du 8 au 12 mai 2017. C'était la deuxième conférence Jacques Monod permettant la rencontre de ces deux domaines de recherche (deux ans après la première conférence). La conférence a été organisée par Thomas Surrey, Institut Francis Crick, Londres, Royaume-Uni (président) et Laurent Blanchoin, Institut des Biosciences et Biotechnologie de Grenoble, France (vice-président). Cette conférence a eu un large succès puisque nous avons reçu 135 résumés de participants potentiels dont seulement 89 ont pu être acceptés, en raison de la capacité de la salle de conférence. Au total, 118 scientifiques ont participé à cette conférence. 27 conférenciers invités (9 français, 9 d'autres pays européens, 9 des USA, du Canada et du Japon) ont donné des séminaires de très hauts niveaux, particulièrement appréciés par la quantité de données non-publiées présentées. 13 intervenants ont été choisis parmi les résumés d'affiches (4 français, 5 d'autres pays européens et 4 en provenance des États-Unis et de Singapour). La moitié des participants étaient des stagiaires post doctorants ou étudiants en

thèse. Cette conférence leur a permis d'interagir directement avec les leaders dans leurs domaines. Outre le soutien généreux fourni par le CNRS (via la Conférence Jacques Monod), la conférence a profité de parrainages supplémentaires fournis par EMBO, la Société des Biologistes, le labex GRAL, Nikon, Zeiss, Roper Scientific, et Alvéole. Le soutien local et l'organisation logistique par Nathalie Babic ont été remarquables. L'excellent restaurant du CNRS a contribué à la très bonne ambiance de la réunion. À la fin de la conférence, en réponse au retour très positif que nous avons eu, les participants ont été encouragés à contacter le Vice-président s'ils souhaitent organiser une conférence Jacques Monod sur un thème de recherche similaire afin de développer davantage l'élan de cette conférence très réussie.

Scientific Scope

The aim of this conference was to bring together researchers from the two research fields of the actin and microtubule cytoskeleton, two fields that currently begin to merge, but are still largely separated, making this a timely conference theme. Participants enjoyed hearing about experimental research covering a range of model organisms (budding yeast, fission yeast, C. elegans, Drosophila, Arabidopsis, mouse, cultured human cells) and also about theoretical work aiming to explain experimental data in a quantitative manner. A broad range of topics was covered: cell migration, spindle assembly and function, cytokinesis, and cytoskeletal reorganisations important for cell differentiation and development. The organisation of the sessions of the conference intended to illustrate how research at different scales of complexity is required to address questions about the complex nature of cytoskeleton function. The presented work covered studies ranging from high-resolution structural biology to imaging in living organisms. It was noticed by the participants that a strength of the conference was a focus on mechanistic understanding with a good representation of biochemistry, quantitative cell biology and theoretical modelling.

Scientific Programme

The scientific programme consisted of one keynote lecture at the beginning of the meeting followed by seven thematic sessions. Some minor adjustments to the programme were made shortly before and after the programme was printed due to some last minute cancellations. An updated programme reflecting all changes was distributed to all participants on the first day of presentations. This report reflects the final programme with the talks being summarised in the order as presented at the conference.

The conference started with an EMBO-sponsored **keynote lecture** given by Tom Pollard, Yale University, giving a comprehensive overview of what is currently understood about cytokinesis in fission yeast, budding yeast and animal cells. This talk summarised the contents of a review article to be published in the Journal of Cell Science. The talk was organised around nine questions regarding the composition of the cytokinetic ring and the mechanisms of ring assembly, constriction and disasssembly. The point was made that information from biochemistry, quantitative cell biology and mathematical modelling needs to be combined for a comprehensive understanding. Areas of open questions and hence research opportunities were pointed out. This lecture nicely defined the level of understanding that researchers aim to achieve in the cytoskeleton field.

Session I, "Mechanistic insight from high-resolution structures" (chaired by Bruce Goode, Brandeis University) began with a talk given by Michael Steinmetz, Paul Scherrer Institute in Villigen. He presented unpublished work on a new motif that mediates recruitment of proteins to growing microtubule ends by EB1 proteins. The mode of interaction of this motif with the EB1 C-terminal domain was compared to the previously identified SxIP motif and CAP-Gly domains that can also interact with EB1, and the possibility for differential regulation of the various motifs to differentially direct proteins to microtubule plus ends was discussed. Gregory Voth, University of Chicago, presented theoretical studies explaining how an entropic

spring model can explain the inhibition of formin activity by mechanical forces. Lukas Kapitein, University of Utrecht (swapping slots with Andrew Carter, LMB Cambridge) showed recent and largely unpublished experiments investigating the question how cargoes are selectively targeted to either axons or dendrites in neurons. Using a combination of optogenetics, super-resolution microscopy, cell extraction with motility assays on the cytoskeleton of extracted cells, he showed that kinesin-1 cargos are excluded from dendrites as a consequence of this motor having a preference for a more stable subset of microtubules pointing towards the cell body in dendrites. Gregory Alushin, Rockefeller University, presented a new cryo-EM structure of myosin-VI bound to an actin filament. Comparing structures obtained in different nucleotide states, movements in the myosin motor domain and also in the actin filament could be visualised. Furthermore, new data on the effect of force production on the structure of actin filaments were presented using an EM-adapted 'gliding assay', remarkably indicating that force may induce defects in the actin filament structure. Thomas Müller-Reichert, Technical University Dresden, ended this session, showing impressive EM tomography reconstructions of mitotic spindles in C. elegans embryos, showing that many kinetochore microtubules do not directly connect to the centrosomes suggesting that minus ends of kinetiochore microtubules can release from centrosomes after nucleation and growth and then form indirect centrosome links via other microtubules.

Session II, "Biochemical and mechanical control of cytoskeleton dynamics (I)" (chaired by Tom Pollard, Yale University) began by a talk given by Jan Faix, Medical University Hannover, who presented studies of a new actin filament nucleating and bundling protein from the parasitic nematode Brugia malayi investigating its biochemical activity using overexpressions of fragments and full-length protein in mammalian cells and using TIRF microscopy based in vitro assays with purified proteins. Antoine Jegou, Institut Jacques Monod, addressed the question of how the geometry of actin filaments is established in filopodia focusing on the role of actin bundling (zippering) by fascin, using TIRF microscopy assays with purified proteins. Jim Sellers, National Institute of Health (NIH) in Bethesda, presented electron microscopy and TIRF microscopy studies investigating the biochemistry of non-muscle myosin minifilaments A and B. Using recombinant protein assemblies, they investigated the mechanism of processive motility of these minifilaments, revealing that assemblies of ~30 non-processive myosins can be processive in agreement with duty ratio measurements of these myosins. Higher order assemblies were also reconstituted, possibly shedding light on the mechanism of higher order arrangements of stress fibers in cells. Klemens Rottner, University of Braunschweig (replacing David Kovar), presented new experiments investigating the role of FMNL2/3 formins for the function of cell protrusions such as lamellipodia. Interestingly, knockout cells that were generated using genome editing technology were found to have slower protrusion growth rate, although the actin assembly rate was unchanged. Protrusions in these mutants produced less force, indicating that these formins are important for mechanical stability of the lamellipodium. Gary Brouhard, McGill University in Montreal, then ended this session by addressing the question of why microtubules with different protofilament numbers can be found in different cell types. The focus was here on C.elegans tubulin that can form microtubules with 11 (instead 13) protofilaments in vivo. Cryo-

electron microscopy of microtubules assembled from this tubulin in vitro demonstrated that their overall lattice structure was similar to microtubules grown from mammalian tubulin. Nevertheless these microtubules displayed strongly increased dynamicity when studied in TIRF microscopy-based in vitro assays, an interesting difference to mammalian microtubules, raising the question how structural details of the microtubule lattice affect the dynamicity of microtubules. The scientific programme of this day ended with a lively poster session in which 37 posters were presented.

Session III, "Biochemical and mechanical control of cytoskeleton dynamics (II)" (chaired by Iva Tolic, Ruder Boskovic Institute in Zagreb). Johanna Roostalu, The Francis Crick Institute in London, presented unpublished work exploring the design principles by which molecular motors organise dynamic microtubules in space, using fluorescence microscopy-based in vitro assays with purified proteins. She demonstrated that two prominent mitotic microtubule-crosslinking kinesins can organise microtubules either into contractile networks that can form asters or into percolated networks of extensile bundles. The choice of architecture depended on microtubule and motor concentrations and the kinetic properties of the motors and the dynamic properties of microtubules. This work has implications for the function of these motors during spindle assembly. Isabelle Arnal, Institute of Neurosciences in Grenoble, continued the session presenting TIRF microscopy-based in vitro reconstitutions dissecting the molecular mechanisms of how the neuronal microtubule stabilising protein tau interacts with and affects microtubules and actin filaments. The different parts of tau responsible for microtubule bundling versus microtubule stabilisation and actin binding and various phosphorylation sites affecting these functions were characterised using mutant constructs in elegant experiments, partly in the presence of both types of dynamic cytoskeletal filaments. Bruce Goode, Brandeis University, then addressed the topic of the combinatorial control of barbed actin filament kinetics, suggesting that at barbed actin filament ends a comparably complex protein interaction network exists controlling its kinetic properties as is well established for microtubule plus ends. New TIRF microscopy-based in vitro reconstitutions were presented providing new insight into the multiple activities of Spire (nucleation, formin displacement, severing, slow-down of depolymerisation) and the partly antagonistically acting protein Twinfilin that has an overall destabilising activity on the barbed end. Olivia de Roure, University Paris Diderot, addressed the question of force production by Arp2/3-dependent actin networks, directing the attention towards endocytosis in budding yeast. A new high-throughput assay allowing force measurements was presented that was based on the reconstitution of an actin network around magnetic colloidal beads. Using networks assembled from extracts from yeast mutants and from purified proteins, Sac 6 was shown to be a main crosslinker responsible for controlling network elasticity, however the data also showed that other proteins will still need to be identified for a complete understanding of network elasticity. Manuel Théry, Institut Universitaire d'Hematologie in Paris, then presented an EMBO-sponsored YIP lecture. His lab used in vitro reconstitutions with purified proteins and experiments in cultured mammalian cells to investigate the phenomenon of microtubule 'self-repair'. Evidence was presented for tubulin exchange on the time scale of several minutes in the microtubule lattice, possibly where lattice defects may exist. He proposed that the

occurrence of defects might well have wide implications for microtubule mechanical and dynamic properties. Isabelle Sagot, IBGC Bordeaux, ended this very mechanistically oriented session with the largely unexplored question of how cellular architecture is remodelled when cells enter the quiescent state. They observed that in budding yeast, actin reorganises into localised bodies and microtubules organised into long stable nuclear bundles with kinetochores at their tip, while telomeres form hyperclusters. Dynein/dynactin-dependent bundle stability was shown to be important for survival during quiescence, implying that the observed changes in cell architecture are fundamentally important.

Session IV, "Cytoskeleton interactions in the presence of membrane confinement" (chaired by Jim Sellers, NIH) was started by Cécile Sykes, Institut Curie Paris, who reported in vitro reconstitution experiments of a contractile cortex at liposome membranes. She demonstrated how the presence of a biomimetic actomyosin cortex alters membrane properties. This could lead to membrane deformations in which the membrane was either 'pulled' or 'pushed' resulting in the formation of tubular structures that are reminiscent of endocytotic structures or filopodia, respectively. Gregory Giannone, Interdisciplinary Institute for Neurosciences in Bordeaux, showed experiments in which single molecules of WAVE and Arp2/3 were imaged in the lamellipodium of cultured migrating cells, suggesting a model in which mechanical feedback influences biochemical reactions in the lamellipodium. After a break offering the opportunity to visit the Island of Batz, Andreas Bausch, Technical University of Munich, continued the session showing in vitro reconstitution experiments in which self-organising systems consisting of stabilised microtubules and crosslinking motor proteins encapsulated in liposomes formed dynamic nematic phases on the confining membrane. Morphologies and dynamics of the observed patterns could be explained using concepts derived from liquid crystal theory. He also reported unpublished data of the formation of membrane invagination driven by actin assembly at the inner membrane of large vesicles. Mohan, Balasubramanian, Warwick University, presented studies of cytokinetic ring dynamics in fission yeast protoplasts, demonstrating that the cytokinetic ring is indeed the force producing entity and not the surrounding cell wall. Using STORM microscopy, the morphology of the cytokinetic ring could be further characterised and it could be shown that ring closure coincides with ring disassembly by a mechanism that expels actin bundles from the ring at regions of highest curvature. Martin Lenz, University Paris Sud, then gave the second EMBO-sponsered YIP talk, turning the attention towards theoretical studies aiming at quantitatively understanding the conditions under which actomyosin systems can either contract or expand, using a one-dimensional theory of actin bundles held together by mixtures of crosslinking motors and static crosslinkers. His theory demonstrated the importance of the relative motor versus filament growth kinetics for the self-organisation of different bundled structures that then determine whether bundles contract or expand. Martin Loose, IST Vienna, presented biochemical work addressing the functioning of the bacterial cytoskeleton. He demonstrated that reconstituted FtsZ/FtsA filaments on supported lipid bilayers can form dynamic, treadmilling ring-like structures with defined orientation that display a diameter similar to the width of bacteria in agreement with their function during bacterial cytokinesis. Shiqiong Hu, Mechanobiology Institute in Singapore, presented very recently published work

where she characterised in unprecedented detail a regular sarcomer-like structure of stress fibres in migrating cells using SIM microscopy of a variety of proteins that are part of these fibres. She demonstrated that both actin turnover and myosin activity are required for the establishment of these sarcomere-like structures, having profound implications on the mechanism of stress fiber self-organisation and mechanical function. Hélène Bouvrais, IDGR Rennes, presented technically sophisticated work combining careful confocal fluorescence microscopy imaging and advanced quantitative data analysis to study the interaction of dynamic microtubule ends with the cell cortex in the C. elegans embryo during cell division. This work is important to understand the mechanism of asymmetric spindle positioning in this organism. She demonstrated that different types of dynamic microtubule contacts can be identified that either contribute to dynein-dependent pulling or microtubule polymerisation-dependent pushing on the spindle apparatus. The scientific programme ended on this day with a presentation given by Ivana Gasic, Harvard Medical School, who presented unpublished work investigating the 'microtubule integrity response' of cells using a combination of perturbation of the stability of the microtubule cytoskeleton by drugs followed by mass spectroscopy to identify proteins whose expression levels are changed as response to an increased or decreased free tubulin pool, demonstrating that several tubulin genes are the target of regulatory control.

Session V, "Cell division in different cell types" (chaired by Isabelle Sagot, Bordeaux), began with a talk given by Phong Tran, Institut Curie Paris, who addressed the topic of nuclear migration during mating in fission yeast. Using live cell imaging and mutagenesis, he demonstrated that efficient nuclear migration depends on two minus-end directed motor proteins, dynein and kinesin-14, in this organism. He conceptualised the findings by noticing that nuclear migration in fission yeast can be seen as a combination of two mechanisms that are used exclusively either during migration of pronuclei in fertilised eggs or during spindle positioning in budding yeast. Chris Brownlee, UC Berkeley, continued the session, presenting largely unpublished work on the biochemical mechanism of spindle size and nuclear size scaling in Xenopus eggs and embryos. He discovered that importins have a critical role in sensing cell volume by the virtue of associating with the plasma membrane which has the capacity to deplete them from the cytosplam which in turn affects the activities of a number of NLS-containg proteins. Geoffrey Wasteneys, University of Bristish Columbia in Vancouver, presented the first talk of the conference about the cytoskeleton in plants, addressing the specific topic of the spatiotemporal organisation of microtubules inside plant cells. Using confocal fluorescence microscopy imaging of microtubules in plant root cells, he showed how the antagonistic activities of a microtubule polymerase and a kinesin control microtubule dynamics in this organism. He then demonstrated that the microtubule-associated protein CLASP is critical for spatial microtubule organisation, because it allows microtubules to grow around 'sharp cell edges'. Ana Carvalho, i3S Institute in Porto, addressed the question of the respective roles of formins and Arp2/3 for cytokinetic ring assembly and constriction in the C. elegans embryo. Depletion experiments combined with contractile ring imaging revealed clearly different roles of these two protein complexes for ring function. A model emerged stating that formin is essential for ring assembly and Arp2/3 is important for correct timing and

cortical stability. Marie-Emilie Terret, College de France in Paris, presented work addressing the role of cortex stiffness for spindle positioning in mouse oocytes, demonstrating that a cortex that is either too soft or too stiff causes defects in spindle migration towards the cortex. An imbalance of cortical forces also led to defects in chromosome alignment. Iva Tolic, Ruder Boskovic Institute Zagreb, then presented work addressing the role of the mechanical properties of kinetochore fibers and especially of central microtubule bridges connecting sister kinetochore fibres for spindle organisation and function in cultured human cells. STED and confocal microscopy was used to define the exact shape of these fibres in spindles. Laser cutting experiments were then used to demonstrate that inter-kinetochore fibre bridges do not only have an important function for metaphase spindle stability, but also for anaphase chromosome segregation. Emmanuel Derivery, LMB Cambridge, ended the session presenting studies in the context of asymmetric cell division during bristle development in Drosophila. He demonstrated that asymmetric segregation of endosomes in SOP cells is a consequence of an asymmetric antiparallel microtubule organisation in anaphase spindles in these cells. If this organisation is inverted artificially, segregation of endosomes is inverted, demonstrating how spindle morphology can have profound consequences for development.

Session VI, "Different forms of cell migration" (chaired by Cécile Sykes, Institut Curie), was started by Matthieu Piel, Institut Pierre-Gilles de Gennes in Paris, who addressed the topic of cell motility and actin network behavior in cells migrating through confined geometries. He demonstrated differential roles of formin/myosin-II and actin/Arp2/3 for speed of migration versus ability to squeeze through constrictions, respectively. Experiments were presented that indicated that mechanical deformation of the nucleus during migration through constrictions or by pressing on the cell has differential effects on gene expression. François Nédélec, EMBL Heidelberg (replacing Michael Sixt), then persented a theory explaining whether well-crosslinked filament networks with motile and static crosslinkers contract or expand. The analytical theory could predict behaviours observed in simulated networks, providing a general framework for understanding well-crosslinked network design. Alex Mogilner, NYU, then presented theoretical work with the aim to understand spreading of nuclei in differentiating myoblasts. In unpublished work, he used a theoretical screening approach to explorate the outcome of a large number of simulated force-balance models predicting the kinetics and outcome of nuclear distribution. The correct final outcome could be explained by two types of models, whereas correct kinetics required additional temporal regulation of short range repulsive and long-range attractive forces, making testable predictions for experiments. James Bear, University of North Carolina in Chapel Hill, ended the session by presenting work that investigated which actomyosin functions in cultured human cells are maintained in the absence of the Arp2/3 complex. Using microfluidic devices to produce well-defined stable chemical gradients, he demonstrated that both fibroblasts and macrophages can still migrate and perform chemotaxis in the absence of the Arp2/3 complex, although speed was reduced. In contrast, haptotaxis was completely inhibited in the absence of Arp2/3 activity, because ECM sensing by the lamellipodium depends on Arp2/3.

Session VII, "Cytoskeletal rearrangements during cell differentiation" (chaired by Emmanuel Derivery, LMB Cambridge) was started by Takashi Hasimoto, Nara Institute of

Science and Technology. He presented the second talk on the microtubule cytoskeleton in plants. He investigated cytoskeletal rearrangements during the stress response and demonstrated that phosphorylation of tubulin at an amino acid that is involved in a longitudinal contact is used by Arabidopsis to destabilise the microtubule cytoskeleton during stress response. He furthermore elucidated the role of the kinase/phosphatase pair mediating this response. The session was then paused for the second poster session in which 38 posters were presented, followed by the conference dinner. The session was continued on the next morning by Thomas Lecuit, Aix-Marseille University, who presented elegant live organism fluorescence microscopy imaging experiments of the dynamic actin cytoskeleton in Drospophila embryos studying the role of myosin-II-dependent contractility generating flows and pulses important for cell/cell rearrangements during morphogenesis. The conference then ended with a talk given by Andrew Carter, LMB Cambridge (swapped with Lukas Kapitein) who presented cryo-EM structures and TIRF microscopy experiments investigating the mechanism of human dynein autoinhibition. He presented high-resolution structural information about the contacts that form in the autoinhibited 'phi conformation' of dynein in the absence of dynactin and an adapter protein. Disrupting these contacts did indeed increase microtubule binding, but processivity still required the additional interaction with dynactin in order to change the geometrical arrangement of the motor heads to allow repeated stepping along the microtubule.

Conclusion

Overall, the conference achieved its goal of stimulating interactions between researchers studying different types of the cytoskeleton in different model organisms, using a variety of experimental as well as theoretical approaches. The participants enjoyed being exposed to a broad overview of the field, with a focus on quantitative, mechanistic work. Differences and similarities between actin and microtubule cytoskeleton functions were discussed, allowing in several cases more general conceptualisations. Scientific exchange was very productive, profiting also from sharing technical expertise since similar methods are used in both the actin and microtubule cytoskeleton research fields. The excellent atmosphere of the conference, which was certainly also promoted by the venue, the CNRS restaurant and the beautiful surroundings, encouraged many participants to present their latest unpublished work, something which was positively evaluated by several of the attendees during the meeting. Several participants expressed their hope that in the future another Jacques Monod conference of similar high quality could take place, bringing again researchers from different cytoskeleton research fields together. Options for potential organisers were discussed. Such a conference would be timely, given the increasing number of labs studying the crosstalk between different cytoskeletal systems, membranes and other cellular structures; this is certainly a research direction with the promise for profoundly promoting our mechanistic understanding of the functioning of healthy and diseased cells and organisms.