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RESEARCH CONFERENCES

ESF-EMBO Symposium

Emergent Properties of the Cytoskeleton: Molecules to Cells

3-8 October 2010

Hotel Eden Roc, Sant Feliu de Guixols, Spain

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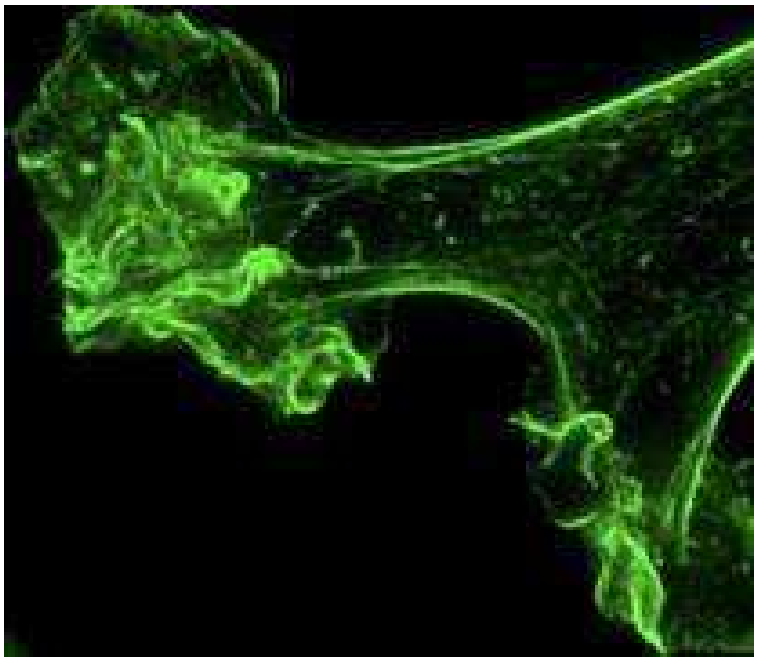

Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

The focus of the meeting was to discuss recent developments in our understanding of the cytoskeleton in cells. All cells contain a cytoskeleton, which provides a structural framework for the cell, controlling its shape and how it adheres to adjacent cells, and provides internal tracks, used to transport organelles, proteins, mRNA and so on, to the right place. Many of the new findings presented had exploited new technological advances, including modeling of experimental data, imaging, and in vitro approaches, to understand various features of the cytoskeleton and its cellular role.

One approach is to take individual components, mix them together in solution, find out what types of cytoskeletal assemblies result, and how this depends on the component parts, rather like finding out how individual components of a car engine work to understand how the engine works. This approach is now becoming a finely developed art, and exciting new data from several researchers showed how with just a few components, a cytoskeleton can be produced which mimics that in living cells. Analysing these stripped down structures provides novel insight into which components are required and when to generate characteristic cytoskeletal architectures seen in vivo.

Forces are important in biology, and several researchers at the meeting showed new results on how forces applied to cytoskeleton are converted into changes in cell shape and movement during processes such as embryo development, and used in cell adhesion. Particularly intriguing was the demonstration that when immune cells (T-cells) meet antigen-presenting cells, signals are generated that stop the T-cells from migrating, by influencing cytoskeletal structure, and lead to strong adhesion between these two cell types to form the immunological synapse.

Forces are also developed by motor proteins that walk along the cytoskeleton in cells, with myosins walking on actin filaments, and dyneins and kinesins walking along microtubules. There are about 40 different types of myosin and about the same of kinesin in humans alone; determining how all these motors work, how they are regulated, and how they are adapted for their cellular roles is a major task, and new ideas about a variety of motors, and how they might work were presented, as well as how motors and the cytoskeleton work in a wide range of organisms from the malarial parasite to the fungal pathogen Ustilago maydis, as well as the more commonly studied mammalian systems.

In summary, the wide variety of approaches, and systems used have led to new understanding about the emergent properties of the cytoskeleton, which were showcased beautifully at the meeting.

☒ I hereby authorize ESF – and the conference partners to use the information contained in the above section on 'Conference Highlights' in their communication on the scheme.

Scientific Report

Executive Summary

(2 pages max)

The original plan for this meeting was that it would cover the complexity, emergent properties and systems approaches concerning the cytoskeleton. We planned to bring together scientists from the more traditional cell biology research area with those from biophysics and chemistry, for cross-fertilization of ideas across disciplines. The scientific scope was to include subjects from passive mechanical properties of the cytoskeleton, to how the cytoskeleton acts as tracks for motors and the behaviour of single molecules that move along these tracks, as well as how the cytoskeleton and motors are regulated inside cells. We planned that it would cover emerging and state-of-the-art techniques used to investigate the properties of these molecules in vitro ranging from biochemical approaches to optical trapping and AFM. One goal was that the meeting would showcase how experimental work is increasingly integrating what we know from studies of isolated molecules to how macromolecular assemblies interact and function in cells, providing a molecular understanding for complex systems. We also anticipated that the conference would attract a significant number of young researchers, that there would be a strong European bias, and that there would be an overall gender balance of attendees about 50:50. We expected to obtain additional funding from a variety of sources to support the attendance of young researchers at the meeting, in addition to the grant from ESF/EMBO.

Overall, the meeting was a great success and our original goals were accomplished. 9 female and 12 male speakers accepted invitations to speak and thus 42% of the speakers were from the minority gender. We received 110 applications for the meeting, of which 102 were accepted. The standard of the applications to the meeting was in general very high, and this really added to the content of the meeting, as we were able to select a large number of applicants for short talks, all of which were of very high quality. We selected a further 31 speakers from the applications for short talks, of which 42% were from the minority gender (13 female and 18 male speakers). 48% of the participants in the conference were female, and 49% were under the age of 36. Over half (51%) of the budget was allocated to support young scientists to attend the meeting. The applicants were based in 22 different countries, of which 15 were ESF member countries. Attendees also came from the USA (20%), although many of these were European in nationality, Israel (6%), and smaller numbers from Mexico, Japan, Australia, Russia and the Ukraine. The three largest contingents from ESF member states were from France (13%), Germany (12%) and the UK (19%).

We were successful in raising additional funds, to a total of 9,000 Euros. This included funds from the Developmental Hybridoma Studies Bank ($500), the Abercrombie fund (£500), the Company of Cell Biologists, Journal of Cell Science (£3000), the European Biophysical Association (2600 Euros), Springer (£1000), and the Royal Microscopical Society (£1000). We used the majority of this additional money to provide additional funds to support young researchers, and just over half of our total budget was used to support this group of attendees. The money from the Abercrombie funded was used to part fund the invited speaker Klemens Rottner, and the money from the Royal Microscopical Society was used to part fund the first session on Thursday morning, in which the majority of the talks included imaging. Cytoskeleton kindly provided 400 Euros towards two prizes for the best short talks, Nature Cell Biology and EMBO provided a prize of a years' subscription for the two best posters. Some of the speakers will write a short review to be published in the Journal of muscle research and cell motility (published by Springer), early in the new year. Embo Reports will publish a meeting report, to be written by Meg Titus.

The meeting was organized into 7 sessions (see summary below), in which there was a mixture of invited speakers (30 minutes each) and speakers from the abstracts (15 minutes each). We also ran two poster sessions on Monday and Tuesday evening, and a general discussion and 'look forward' session on Wednesday evening, all of which were well attended, and stimulated much discussion.

Scientific Content of the Conference

(1 page min.)

- *Summary of the conference sessions focusing on the scientific highlights*
- *Assessment of the results and their potential impact on future research or applications*

The conference was organized into 6 scientific sessions as follows

Session 1: Myosin Structure and Function

This session focused on myosins, and provided new insight into how these motors work, how they might organize their tracks and recognise different actin structures. Michelle Peckham described the diversity of myosins, and the potential role of single alpha helices in extending the 'canonical' lever of myosins. Anne Houdusse focused on myosin 6, which is the only myosin motor that moves to the pointed end, rather than the barbed end of actin, and how structural studies suggest a potential mechanism for this movement. She also talked about the myosin ATPase, and how, from a structural perspective, the phosphate is released from the motor. Claudia Veigel talked about the novel properties of actin purified from the malarial parasite, and the relationship between force, stepping and strain for motors as they walk along actin. Justin Molloy demonstrated how myosin has the capacity to orient and organize actin filaments in vitro, and suggested that it may be able to act similarly in vivo.
In short talks, Jim Sellers discussed the binding of Drosophila myosin 7 associated protein (DMAP) to myosin 7, and how this protein regulates the cellular activity of myosin 7, Meg Titus revealed how Myosin 7 is important for chemotaxis in Dictyostelium, and how this requires two tail domains (Myosin Tail homology 4, and the Four point one, Radixin Moesin domain). Dan Mulvihill and Matthew Lord both talked about how myosin activity is regulated in yeast, the role of tropomyosin in regulating myosin activity and the function of acetylation of tropomyosin on its activity.

Session 2: **Actin Dynamics and Organisation I**

These two sessions (sessions 2 and 3) focused on actin organization in cells, and how proteins that bind to actin organize actin filaments in cells. Marie-France Carlier began the session by discussing tropomyosin binds to actin filaments, its preference for non-branched filaments, and a novel approach to investigating actin polymerisation using a microfluidic system, with seeds bound onto a surface, using flow to align actin filaments, and TIRF microscopy to measure rates of polymerisation. Jan Faix demonstrated how cofilin is more efficient in severing actin filaments in fascin-crosslinked bundles with cofilin, by generating more barbed ends, than in single actin filaments using his state-of-the art in vitro assays.
In short talks, Jonathon Terman explained how the protein MICAL, involved in semaphoring-plexin mediated actin reorganisation in vivo, disrupts parallel actin organisation, and decreases actin bundling. Thomas Iskratsch explained how specific residues in the muscle formin (FHOD3) determine whether or not this protein is recruited to the sarcomere in cardiac muscle. Jennifer Gallop showed how she could initiate actin filament growth on supported lipid bilayers in novel in vitro assays, and demonstrated the time sequence of recruitment for the various actin binding proteins required to form filaments in this system. Metello Innocenti explained his ideas about how formins work in lamellipodia and filopodia, whether they are involved in capping or elongation, Sawako Yamashiro compared the tropomodulins Tmod 3 and T mod 4 and their different interactions with actin in the sarcomere and Peter Gunning finished up the session with a reminder of how little we understand about the different tropomyosins in non-muscle cells in different tissues and in different animals, their potential roles in cancer, and possible therapies directed at specific tropomyosin isoforms.

Session 3: Actin Dynamics and Organisation II
Klemens Rottner began this session by showing that cortactin is not essential for actin assembly, but might be involved in activation of Arp2/3. He went on to explain his recent results on the roles of formins in cell migration and filopodial formation. Anne Ridley focused on T-cell migration and the importance of RhoA, and the origin of 'stop moving' signals generated by antigen presenting cells that inhibit T-cell migration. Pekka Lappalainen highlighted the roles of the variety of tropomyosins in cells, about which very little is known, showing the different localisations of the different tropomyosin isoforms. He talked about how myosin II is recruited to stress fibres and discussed the possibility that there are different types of stress fibres in cells.
In short talks, John Heuser showed electron micrographs of actin structures and cells, which led to vigorous discussion on whether branched filaments seen in EM studies are genuine or artefactual. Rhoda Hawkins showed how mathematical modeling could provide insight into cell polarisation with yeast polarity formation as a model system. Keren Kinneret demonstrated how the optical traps could be used to apply forces to cells and to pull out

tethers, in order to investigate strength of cell adhesions. Florian Huber showed how actin organisation in vitro could be investigated in droplets under different ionic conditions. Evelyn Bloch-Gallego discussed the importance of migration of neurones in generating folds in the cerebellum, the role of netrin-1 in axon guidance and the differential roles of actin and tubulin in this migration, and regulation by Rac (rho GTPase expression pattern).

Session 4: Forces and Cell Adhesion in Biology

This session gave us an overview on how forces are important in generating cell movement particularly in tissues in vivo. Pierre-Francois Lenne showed how forces emerge during the dynamics of tissue formation, about cell movement in Drosophila embryos, and how polarized recruitment of myosin 2 generates anisotropy of tension, which is important in cell intercalation. Sasha Bershadsky discussed how focal adhesions contain over 100 different proteins, the importance of myosin 2 in maturing focal adhesions, and the force sensing behaviour of these structures.

In short talks, Antonio Schepis, explained the role of adherens junctions in gastrulation in the zebrafish. Di Jiang and Bo Dong, using a range of fluorescently tagged proteins, showed how notochords form in the worm Ascidia, leading onto the formation of the intracellular vacuole in notochord cells, and the importance of actin filaments in cell morphological changes. Ronen Zaidel-Bar, discussed how ventral enclosure in C. elegans is followed by elongation, and how Bar-domain containing proteins are important for altering the morphology of cells at cell-cell junctions, which contribute to the rate of closure. Pere Roca-Cusachs described how he could use magnetic tweezers to pull on beads attached to the surface of cells, and the differential roles of talin and alpha actinin in force sensing/force generation in focal adhesions.

Session 5: Force Generation and Cellular Transport

The focus of this session was to show how molecules in involved in sensing and responding to tension are involved in force generation. Mathias Rief explained how the protein myomesin, in the M-line of muscle, cross-links thick filaments in adjacent muscle sarcomeres. He investigated the unfolding of this protein using Atomic Force Microscopy, and showed how the elasticity of short single alpha helices in myomesin are important for its elasticity. Christoph Schmidt showed how he could use beads trapped in matrices (e.g. actin filaments in solution) to investigate the properties of the cross-linked network of filaments, and how this changes in the presence of myosin. Patricia Bassereau demonstrated she investigates lipid deformation in vesicles using micropipettes and optical tweezers. She discussed how motors such as kinesin deform artificial liposomes to cause tubulation in vitro, sorting of lipids in vesicular membranes and how this is important for fission of vesicles.

In short talks, Julien Husson described the immunological synapse between T-cells and antigen presenting cells, and how he uses micro-pipette based techniques to investigate this interaction. He then went on to show how different cell-membrane markers (CD18 and CD3) had differential effects on this interaction. Ross Rounsevell discussed the properties of an acto-myosin network organised onto giant unilamellar vesicles to understand how actin networks work in the cortex of cells. Stephanie Misery-Lenkei described the roles of Rab GTPases at the Golgi, their role in vesicle fission, and how myosin 2 is also involved in this process. Valeria Piazza explained a novel imaging technique called multi-isotope imaging mass spectroscopy, which has allowed her to investigate protein turnover in inner ear cells in the cochlea, demonstrating the high stability and low turnover rate of these structures. Eliza Morris showed how she uses magnetic field gradients to pull on beads in an entangled actin network, to investigate how vesicles might move through networks in vivo. David Richmond explained his novel approach for generating small asymmetric vesicles using microfluidic jetting, in which proteins can be inserted into the inner leaflet of the vesicle, in order to reconstitute exocytic vesicles in vitro.

Sessions 6 and 7: Microtubules and Motors I and II

The last two sessions focused on microtubular organization in cells, and how motors use microtubules as tracks to drive cellular processes. Anna Akhmanova introduced us to the fast growing ends of microtubules, the many binding partners of EB1, a protein associated with the growing ends and how they combine to regulate the dynamics of MT growth. Gero Steinberg showed how the cytoskeleton in Ustilago Maydis, a fungal pathogen, is different to that of mammalian cells, and how myosin and kinesin co-operate to deliver vesicles to the tips of these cells. Carolyn Moores explained how some kinesins depolymerise their microtubule tracks, similarities and differences between these and other kinesins, and hints from structural studies about how depolymerising kinesins manage to depolymerise microtubules. Peter Rosenthal showed how high resolution cryo-EM tomography has provided new insight into the structure of Weibel Palade bodies in endothelial cells, and how von-Willebrand factor might be stored in these structures. Stan Burgess showed us his new dynein structure, as obtained from Cryo-EM, and how this motor might work in vivo. Takashi Ishikawa demonstrated how dynein complexes are organised in the flagellum, and how they may generate flagellar bending. Erika Holzbaur introduced her ideas on how dynein and kinesin on the same cargo co-operate to generate movement, with estimates of the numbers of motors involved, and went on to show how dynein may tether the ends of microtubules to the cell membrane.

In short talks, Itushi Minoura explained how the single headed processive kinesin, Kif1a, recognises binding sites on the microtubule track in order to move along its track. Johanna Roostalu discussed how the homotetrameric kinesin, Kinesin 5, (Cin8) in budding yeast moves along microtubules in vitro and how this is linked to its various roles in mitosis. Isabelle Palacios described how kinesin and dynein are involved in generating polarity in oocytes during oogenesis. Leah Gheber also talked about kinesin 5 in mitosis and its important role in assembly and maintenance of the bipolar spindle, as well as movement of the spindle during anaphase, and how this is regulated by phosphorylation. Ligon Lee explained how tubulin modifications are linked to the change from 2D to 3D polarity in MDCK epithelial cells in vitro. David Pastre discussed how small cationic molecules such as spermidine and spermine, which are essential for cell growth and proliferation, appear to be able to affect microtubule dynamics in vitro.

A particular highlight from the meeting was the wealth of innovative new techniques that are being developed and used to understand the cytoskeleton and its properties. These included techniques to understand how motors work on their tracks in vitro, the variety of in vitro techniques being used to generate a cut-down cytoskeleton in vitro, in order to understand how the individual components work, including the assembly of actin and myosin onto artificial vesicles and lipid bilayers, the use of optical tweezers to measure forces produced by motors in a variety of systems, and the wide range of imaging techniques used by many presenters. In vivo, there were clear advances in the understanding of how cells use forces to generate morphological changes. There were new developments into our understanding of how proteins generate and respond to forces, for example the response of myomesin to force in muscle, and how membrane proteins generate tight connections between cells, such as in the T-cell system. There were good examples of how mathematical modeling is making a valuable contribution to our understanding of cellular processes. Finally, there were key advances in our knowledge about how different motors work, and how they co-operate in generating forces and/or movement, such as the links between dynein and kinesin on microtubules, and how motor proteins such as kinesin can deform vesicles as well as traffic them along microtubules, were presented. Overall the new science presented in all the talks and the posters was very exciting, and showed that while considerable progress has been made in our understanding of the emergent properties of the cytoskeleton, there is still much to learn.

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

Overall, the topics covered by the speakers, the format of the sessions, with many short talks, and opportunities for speakers, both invited and abstract presenters to show their latest, unpublished work, was very well received, and many attendees reported forming new potential collaborations on the basis of the work presented and their discussions.

There was a lot of interest at the meeting on forces in biology, how they compare, how they are exerted and the range of experimental approaches used to understand and measure forces. Future developments are likely to include combining techniques, merging fields, collaborations between physicists and biologists, and synthetic biology, in order to discover what proteins do, how to make them do it, and to do it correctly. As a follow up to this meeting, it was suggested that one or more workshops on emerging techniques, including ones presented at the meeting, but including additionally chemical tools, probes etc would be useful in a future meeting.

It was also suggested that clear links between cell biology, and developing tools for treatments and diagnosis are now becoming evident. This area was briefly covered in the meeting, particularly in the talk by Peter Gunning, and should become an area of even more importance in the next 5-10 years, with an even greater focus on translational research and clinical studies.

An area that we would have like to have covered in the meeting was the use of super-resolution imaging to understand cytoskeletal organization, and this was highlighted in our discussion session. This approach is likely to become more widely used in the next 5-10 years, and is certainly an emerging topic that should be included in future meetings.

Chemo-mechanical signaling, mechanosensing, cell-cell communication, quantitative understanding of the cell (modality), in silico mathematical modeling were all approaches that were highlighted as emerging areas of research. The interactions between study of the basic properties of molecules, how molecules are organized and function in cells, and how these are involved in cellular organization in the formation of tissues were all identified as important areas of research, covered in the meeting, which will continue to be areas of interest in the future.

Other areas of cytoskeletal research that are starting to generate new and interesting results are our understanding of the roles of intermediate filaments, and the nuclear cytoskeleton, not covered in the meeting, but which could be covered in a future meeting, along with the bacterial cytoskeleton, and the ways in which pathogens exploit the cytoskeleton for cell invasion and infectivity.

There was general consensus that another meeting should be organized in 3 years time, and volunteers were identified to organize this next meeting included Stan Burgess, Dan Mulvihill, Meg Titus, and Anne Houdusse.

- Is there a need for a foresight-type initiative?

There is much potential for collaboration between basic cell biologists and physicists/mathematical modelers, that was already in evidence at the meeting, and which would strongly benefit from a foresight-type initiative.

Atmosphere and Infrastructure

▪ *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

The location was excellent, relatively straightforward to get to, and a really beautiful place to hold a meeting. The food was very good, and the staff were very friendly and helpful, and keen to help us solve any problems that we had. The structure of the meeting meant that there was lots of time for discussion and networking, particularly over lunch and in the break in the day after lunch, and again over dinner and during the poster sessions, which was much appreciated by the participants. There was a very positive feeling to the meeting, with much discussion and excitement over the science, and I think this is reflected in results of the questionnaire filled out by participants at the end of the meeting. Our particular thanks go to Anne Blondeel-Oman, who was very efficient, and provided us with excellent support as organizers, prior to and during the meeting.