

# 14th Meeting of the European Cytoskeleton Forum

*Roy Quinlan and Alan Prescott*

*The 14th meeting of the European Cytoskeleton Forum was organised by Claudina Rodrigues-Pousada, Helena Soares and Luisa Cyrne and held in Oeiras, a coastal resort north of Lisbon, Portugal. Sun, sea and sangria – who said science isn't fun?*

The meeting included a special symposium to celebrate Klaus Weber's 60th birthday and to mark the extensive contribution he has made to cell biology and particularly to all aspects of the cytoskeleton. It was the pioneering work of Klaus and Mary Osborn that demonstrated the potential of immuno fluorescence microscopy to cell biology and allowed us to visualise the cytoskeleton in cells for the first time. Immunofluorescence microscopy is still one of the most important tools to modern cell biology, especially now with the advent of GFP-tagged proteins to follow protein dynamics and localisations in living cells, a theme that was strongly represented in the meeting.

Another dominant theme was the interaction between the different cytoskeletal elements, intermediate filaments, microtubules and actin. Although recently the Weber laboratory has concentrated on the evolution of cytoplasmic intermediate filament proteins from their lamin ancestors, the integration of the different cytoskeletal elements is a well-versed theme in the Weber lab as seen by the breadth of publications in all things cytoskeletal. The identification of lamin-like members of the cytoplasmic intermediate filament family in early chordates was the theme of Klaus's opening talk to the Forum. This 'explosive' beginning was appropriately followed by a party at the Oeiras Gunpowder Factory!

The first session had a 'Weber' theme in that the speakers were associated with Klaus's laboratory and ranged in topics from nuclear compartments and

splicing events (**Joan Steitz**; Yale) to ERM proteins (**Tony Bretscher**; Cornell). **Paul Matsudaira** (MIT) discussed results that implicated the actin-binding protein fimbrin in an association with vimentin at focal adhesions. These examples illustrate other dominant themes of the meeting: the importance of the cytoskeleton in signal transduction and the developing interest in the cross-talk between different elements of the cytoskeleton.

From a technical point of view it is clear that the use of GFP fusion proteins in living cells are revealing the way the cytoskeleton integrates into the machinery of the whole cell. A pioneer in this area has been **Bob Goldman** (Chicago) and his talk focussed on the dynamic nature of the nuclear lamins and their association with sites of DNA replication. **Harald Herrmann** (Heidelberg) has used a novel approach to probe nuclear structure and internal compartments, by engineering the expression of vimentin, a cytoplasmic intermediate filament protein, to the nucleus. With this approach, he has begun to examine the nature of the 'interchromosomal domain'. Surprisingly, whether as filaments or as aggregates, vimentin in the nucleus had little effect on the organisation of other nuclear compartments and also failed to disrupt normal mitosis.

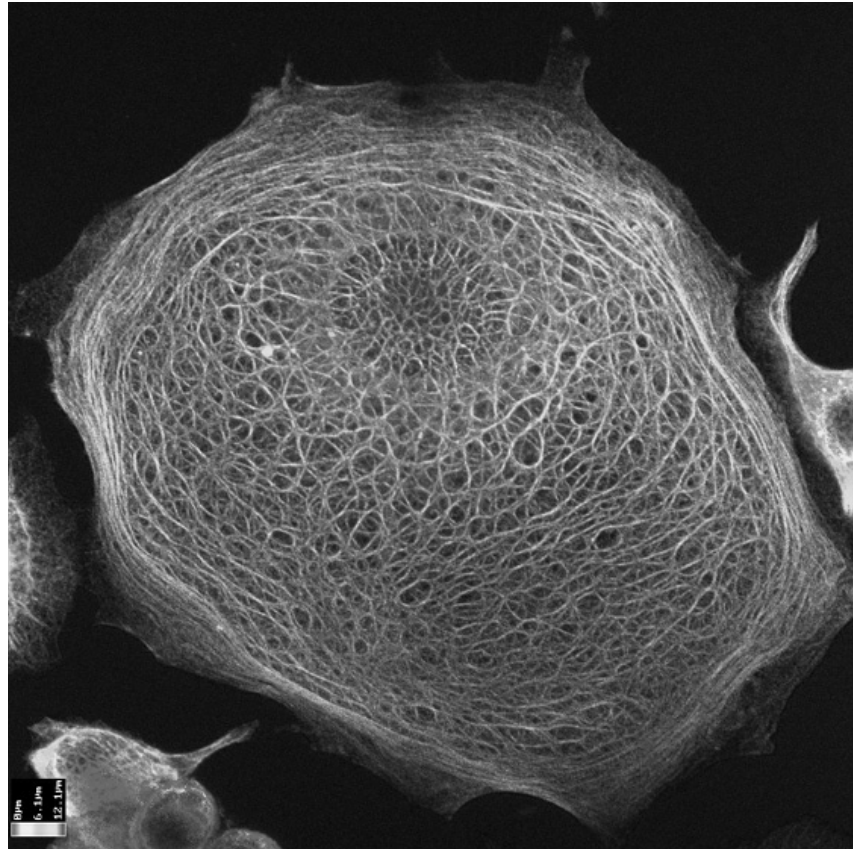
**Roland Foisner** (Vienna) described the different roles of LAP2 $\alpha$  and  $\beta$  in the nuclear lamina assembly and their associations with lamins and chromatin. It was one of four talks from the Biocentre in Vienna (**Wiche, Propst and Eger**) that explored the interacting partners of cytoskeletal proteins (plectin, MAP1A and B and  $\beta$ -catenin respectively) and their role in determining function.

A number of talks described experiments aimed at dissecting the  $\beta$ -catenin signalling pathway: **Hans Clevers** (Utrecht) showed that members of the

TCF/LEF family, that are only expressed in lymphoid cells in the adult, are critical effectors of  $\beta$ -catenin signalling during early development. **Avri Ben-Ze'ev** (Israel) also showed that the  $\beta$ -catenin/LEF-1 complex could be important in colon cell transformation through an interaction with cyclin D1. **Jurgen Behrens** (Berlin) identified a new  $\beta$ -catenin binding protein, conductin, that promotes  $\beta$ -catenin degradation.

The role of the ERM (Ezrin-Radixin-Moesin) family of proteins in the regulation of the actin cytoskeleton and their association with tumour suppressor proteins were covered in talks by **Tony Bretscher** (Cornell), **Richard Lamb** (London) and **Alexis Gautreau** (Paris). Richard Lamb collaborating with Alan Hall described a new application of the Trojan horse principle to study protein function. This involved the localised inactivation of tagged proteins and their associated complexes in one region of the cell by the release of free-radicals generated by the laser-activation of the fluorochrome-tag on the protein ('Chromophore Assisted Laser Inactivation'). He used this technique to explore the role of hamartin (TSC1) in actin reorganisation. Hamartin was identified from 2-hybrid assays as a protein that interacts with merlin, the tumour suppressor protein in the ERM family.

The link between tumour suppressor activity and the cytoskeleton was a recurring theme and was addressed by talks from **Kathrin Schülter** (Braunschweig) and **Wolfgang Deppert** (Hamburg). **Anne Ridley** (London) covered how the signals coming from the Rac/Rho GTPases modulate the actin-plasma membrane interaction during such actin-driven processes as cell spreading and migration.



This human epithelial cell (MCF7) has been stained with anti-HSP27 antibodies. Note the cytoskeletal pattern of the staining which from other studies (Perng et al.1999 J Cell Sci, 112, 2099-2112) we know corresponds to the keratin intermediate filament network. These data indicate that small heat shock proteins are normally associated with intermediate filaments in growing, unstressed cells. The fluorescence signal has been coded for depth.

Migration has traditionally been studied using the Bowden chamber (2 chambers separated by a filter); however, Anne described a novel chamber designed by Graham Dunn (London) that allows the migration of microinjected cells in response to cytokines to be video recorded. This has allowed Anne to inject dominant negative proteins of the Rho family of GTPases in order to determine which of the family members are important for chemotaxis. Cdc42, for instance, is not necessary for macrophage migration but is required for chemotaxis in response to CSF-1. This process also requires PI3K activity as shown by using both drug and neutralising antibodies.

More links between the cytoskeleton and cell signalling pathways are being discovered and was

## MEETING REPORT

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reflected in the attention these areas received at the meeting. The Rho GTPase family's interaction with the actin cytoskeleton has already been mentioned and **Tomasek** (Oklahoma) presented data to suggest that Rho is involved in myofibroblast contraction through regulation of myosin light chain phosphatase activity rather than calcium acting on the kinase as is the case in smooth muscle cells. **Leterrier** (France) implicated cAMP and PIP2 in the regulation of the weak interactions between microtubules mediated by MAP2. **Clive Lloyd** (Norwich) gave a flowing talk on plant microtubules and their role in plant growth, cell wall deposition as well as plant MAPs and the features that they share with animal cell equivalents. The **Joël Vandekerckhove** (Ghent) presentation was a tour de force in mass spectrometry and proteomics to identify those actin-binding proteins involved in *Listeria* motility. Clearly this is a rich vein of research of which we will hear more at the next meeting.

Two video-enhanced talks, one from **Andrew Matus** (Basel) and a second from **Vic Small** (Austria), confirmed (if you needed confirmation!) that using Green Fluorescent Protein fusions is definitely the method of choice when it comes to looking at dynamic cell processes. Using GFPs with two different fluorescent tags fused to actin and MAP2, the Matus lab looked at the cytoskeleton in dendritic spines of neurones. These spines undergo rapid shape changes that are actin-driven when unstimulated but become stabilised upon synaptic excitation. This suggests that these spines may be the molecular basis of neuronal plasticity and possibly the site of action of anaesthetics rather than the microtubule cytoskeleton of the dendrites which the MAP2 probe showed to be somewhat static. This idea is not difficult for cytoskeleton-minded people to accept and this talk was certainly one of the high points in the meeting.

The Small talk was anything but, and concentrated on cytoskeletal dynamics during cell movement using fish fibroblasts as the model system. These cells were recorded with fluorescently labelled tubulin and GFP-tagged zyxin to visualise the formation of focal contacts during progression. This dual labelling protocol demonstrated the requirement for

microtubules to 'mature' focal contacts. During the post-video discussions Paul Matsudaira pointed out that the inhibitor BDM which is sometimes touted as a specific inhibitor of the actin/myosin system is in fact a rather non-specific modifier of Arginine residues and should therefore be used with caution!

The GFP technology was also exploited by **Alison North** (Manchester) to demonstrate that both intermediate filaments and their associated cell-cell junctions, namely desmosomes, are dynamic structures. Using GFP-desmoglein and Cy3-conjugated desmoplakins Alison has shown that junction precursors are associated with the keratin intermediate filaments in sub-confluent or low calcium MDCK cells and these precursors migrate to the cell periphery to assemble the desmosomes. These junctions remain in a constant state of flux and are not at all static. There really is no substitute for 'seeing' in order to 'believe'!

The approach of using two fluorescently conjugated proteins to study protein dynamics has led to the use of fluorescence ratio imaging to demonstrate the close association of two proteins in the same structure. **Benny Geiger** (Israel) has used this technique to show that there are at least two types of focal contact in cells, classical focal adhesions rich in vinculin, paxillin and phosphotyrosine and tensin-rich fibrillar adhesions. The fibrillar adhesions are dynamically associated with the focal contacts and move towards the cell centre during maturation in an actomyosin dependent fashion.

The importance of substrate adhesion to cells was emphasised by two other talks by **Alan Horwitz** (Virginia) and **van de Water** (Leiden). Horwitz showed that the integrin ratio determined the decision to proliferate or differentiate in myoblasts, mediated via paxillin/MAPK/FAK signalling network. van de Water showed that loss of focal adhesion in kidney epithelial cells by treatment with the neurotoxin DCVC led to apoptosis via effects on the phosphorylation status of FAK, paxillin and the adducins. The implication from these experiments is that perturbation of the actin cytoskeleton during

apoptosis would reorganise the focal contacts and prevent cell adhesion and thus decrease the chance of the cell being able to reverse the apoptotic process.

Among the many cytoskeleton-associated proteins discussed at the meeting, the involvement of protein chaperones in the correct folding of the cytoskeletal polymer sub-units and in coordinating dynamic interactions of the three filament systems was timely. Talks by **Sally Lewis** (New York), **Julie Grantham** (London), **Katja Siegers** (Glasgow), **Helena Soares** (Oieras) and **Christophe Ampe** (Ghent) revealed the crucial role for chaperonins and their cofactors in actin and tubulin folding. Soares (Lisbon) showed that the chaperonin complex was present in the ciliate *Tetrahymena* suggesting that it has co-evolved with actin and tubulin even to the extent that chaperonin gene expression is regulated by anti-microtubule drugs such as colchicine and taxol. Christophe Ampe's group (Ghent) have identified actin and tubulin residues necessary for interaction with the chaperonin complex and have suggested that one of the cofactors, prefoldin, exposes these residues during the formation of the folding complex.

Chaperones are not only important in folding cytoskeletal monomers but are also involved in the regulation of polymer interactions. This was demonstrated in a talk by **Paul van den IJssel** from our group in Dundee. He showed data from a recently discovered human mutation in  $\alpha$ B crystallin, a member of the small heat shock family of chaperones, which causes DRM (desmin related myopathy) and cataract, both characterised by inappropriate intermediate filament bundling. *In vitro* the mutant chaperone is unable to prevent filament-filament interactions that lead to gel formation and induced bundling of the filaments when included in filament assembly assays.

This illustrates the diversity of topics covered at the Cytoskeleton Forum from animal cells to *Drosophila* to plant cells and from the cytoskeleton to the nucleoskeleton. This report has inevitably not been able to acknowledge everyone who took part in this successful meeting. It is commendable that many young scientists attended and contributed, but this has

always been a feature of these meetings. The poster sessions were lively and are clearly an important aspect in the success of the meeting. This is the place to foster an interest in the cytoskeleton, because this meeting is not just for the aficionados of the cytoskeleton. After all, this topic touches all aspects of cell behaviour. See you next year in Ghent!

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