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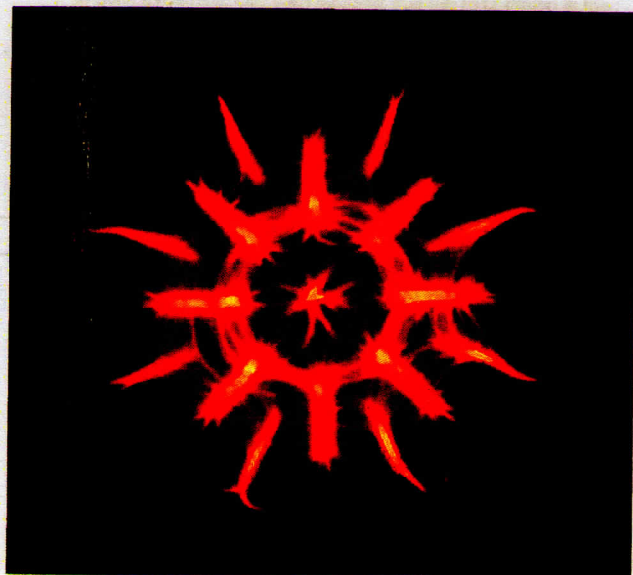
## Programme & Abstracts

A Biochemical Society Focused Meeting

# European Cytoskeletal Forum - Cell Adhesion and Migration

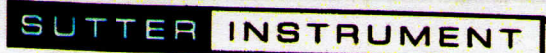
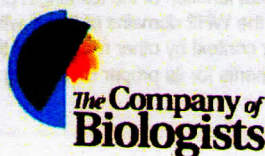
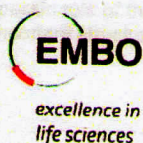
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**P077** Basement membrane laminin  $\alpha 5$  is required for vascular smooth muscle contraction

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Blood pressure is regulated at the level of resistance arteries (100-300  $\mu$ m diameter) through active contraction and dilation, which is controlled by the contractile state of vascular smooth muscle cells (vSMCs). Individual vSMCs are encased in a basement membrane (BM) that connects to the contractile apparatus via cellular receptors and to the extracellular matrix, thereby, interconnecting adjacent cells. Laminins are the major functional component of the basement membrane, and isoforms that contain the  $\alpha 4$  and  $\alpha 5$  chains are the predominant ones found in vascular BMs. Through the use of tissue-specific KO mice, we address the roles of these laminin chains in the function of resistance arteries. *Ex vivo* functional studies showed that arteries from mice lacking laminin  $\alpha 5$  in perivascular cells (p-Lama5<sup>-/-</sup>) have an impaired ability to contract but have normal dilation. Data suggests that this is due to the upregulated expression of contractile machinery proteins:  $\alpha$ -smooth muscle actin, caldesmon, and calponin, as shown by immunofluorescence staining. Mesenteric artery vSMCs have been shown to express integrins  $\alpha v\beta 3$ ,  $\alpha 5\beta 1$  and  $\alpha 7\beta 1$ , but not  $\alpha 6\beta 1$ , and immunofluorescence studies indicated that cells lacking laminin  $\alpha 5$  have an upregulated expression of integrin  $\alpha 5\beta 1$ , suggesting a role for this integrin receptor in laminin  $\alpha 5$  signaling. Atomic force microscopy studies revealed that laminins  $\alpha 4$  and  $\alpha 5$  have opposing roles on vSMC cytoskeletal stiffness. Whether laminin  $\alpha 5$  affects vSMC contraction by solely affecting the contractile machinery or whether it also affects cell-cell communication is currently being investigated.

Notes:

**P078** Biochemical activities of the Wiskott-Aldrich syndrome homology region 2 domains of Sarcomere Length Short protein

**Mónika Tóth<sup>1</sup>, Péter Gaszler<sup>1</sup>, Andrea Vig<sup>1</sup>, Ede Migh<sup>2</sup>, József Mihály<sup>2</sup> and Beáta Bugyi<sup>1</sup>**

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*Drosophila melanogaster* Sarcomere Length Short (SALS) is a recently identified Wiskott-Aldrich syndrome protein homology 2 (WH2) domain protein involved in skeletal muscle thin filament regulation. SALS was shown to be important for the establishment of the proper length and organization of sarcomeric actin filaments. Here we present the detailed characterization of the biochemical activities of the tandem WH2 domains of SALS (SALS-WH2). Our results revealed that SALS-WH2 binds both monomeric and filamentous actin and shifts the monomer : filament equilibrium towards monomeric actin. In addition, SALS-WH2 can bind to but fails to depolymerize phalloidin-, or jasplakinolide-bound actin filaments. These interactions endow SALS-WH2 with two major activities in the regulation of actin dynamics: SALS-WH2 sequesters actin monomers into non-polymerizable complexes and enhances actin filament disassembly by severing, which is modulated by tropomyosin. We also show that profilin does not influence the activities of the WH2 domains of SALS in actin dynamics. In conclusion, the tandem WH2 domains of SALS are multifunctional regulators of actin dynamics. Our findings suggest that the activities of the WH2 domains do not reconstitute the presumed biological function of the full-length protein. Consequently, the interactions of the WH2 domains of SALS with actin must be tuned in the cellular context by other modules of the protein and/or sarcomeric components for its proper functioning.

Notes:

*Gaszler Péter*

**P079** The CT region of DAAM has a supporting role in FH2-mediated actin dynamics regulation

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DAAM is a DRF formin protein essential for the regulation of actin cytoskeleton dynamics in diverse biological processes. The conserved FH1-FH2 domains of DAAM catalyse actin nucleation and processively mediate filament elongation. These activities of FH2 are autoregulated by the N- and C-terminal regions. The C-terminal DAD-CT of formins was suggested recently to directly regulate actin dynamics, as well. We analysed the role of DAD-CT region of *Drosophila* DAAM in its interaction with actin by *in vitro* biochemical and *in vivo* genetic approaches. We found that DAD-CT can bind actin and enhance the nucleating activity of FH2, through the CT region. Despite of being able to bind actin, DAD-CT does not significantly influence actin assembly in the absence of the FH2 domain. Analysis of the I732A loss-of-function mutation in FH2 and FH2-DAD-CT revealed that DAD-CT can compensate for the compromised actin-binding of the FH2 domain. However, the actin nucleation, elongation and barbed end capping activity of the FH2 domain cannot be restored by the presence of DAD-CT. Consistently, *in vivo* data show that the CT region is not essential, yet contributes to DAAM-mediated filopodia formation in primary neurons. In conclusion, DAAM DAD-CT possesses an FH2-dependent function in actin dynamics. Our data suggest that the FH2 region is the core actin-interacting element of DAAM and DAD-CT has only a supporting role in actin nucleation, presumably through stabilisation of nucleation intermediers.

Notes:

*Gaszler Péter*

**P080** Active invadopodia of mesenchymally migrating cancer cells contain both  $\beta$  and  $\gamma$  cytoplasmic actin isoforms

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Invadopodia are actin-rich protrusions formed by mesenchymally migrating cancer cells. They are mainly composed of actin, actin-associated proteins, integrins, proteases and proteins of signaling machineries. These protrusions display focalized proteolytic activity towards the extracellular matrix. It is well known that polymerized (F-)actin is present in these structures, but the nature of the actin isoform has not been studied before. We here show that both cytoplasmic actin isoforms,  $\beta$ - and  $\gamma$ -actin, are present in the invadopodia of MDA-MB-231 breast cancer cells cultured on a 2D-surface, where they colocalize with the invadopodial marker cortactin. Invadopodial structures formed by the cells in a 3D-collagen matrix also contain  $\beta$ - and  $\gamma$ -actin. We demonstrate this using isoform-specific antibodies and expression of fluorescently-tagged actin isoforms. Additionally, using simultaneous expression of differentially tagged  $\beta$ - and  $\gamma$ -actin in cells, we show that the actin isoforms are present together in a single invadopodium. Cells with an increased level of  $\beta$ - or  $\gamma$ -actin, display a similar increase in the number and size of invadopodia in comparison to control cells. Moreover, increasing the content of both actin isoforms also increases invasion velocity. The work presented here forms the basis to use the actin antibodies in combination with the tagged-actins as tools to study invadopodia and to establish a possible specific role of the cytoplasmic actin isoforms in these biologically important structures.

Notes:

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