

**P004** Engagement of syndecan-4 is essential for adhesion-stimulated Rac1 activation and focal complex formation  
M.D. Bass, M. R. Morgan, Z. Mostafavi-Pour  
and M.J. Humphries  
*Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester M13 9PT*

Fibroblasts plated onto a recombinant ligand of integrin alpha5beta1 spread but fail to form vinculin-containing focal adhesions or fully organise actin into bundled stress fibres unless co-stimulated with a ligand of syndecan-4. Addition of a soluble syndecan-4 ligand to cells, already spread on an immobilised ligand of alpha5beta1, stimulates transient activation of Rac1 with a profile similar to that seen when cells spread on whole fibronectin. Rac1 activation coincides with the formation of nascent focal complexes in the pre-spread cells that act as the precursors of the syndecan-4-dependent adhesions reported previously. Significantly, cells adhering to a ligand of alpha5beta1 alone fail to exhibit the normal wave of Rac1 activation, as do syndecan-4-null fibroblasts when plated onto fibronectin, demonstrating that engagement of syndecan-4 is absolutely required for activation of Rac1 during spreading. Furthermore, expression of a C-terminal truncation mutant of syndecan-4, lacking the PDZ ligand, causes dysregulation of Rac1 activation during spreading on fibronectin, indicating that the cytoplasmic interactions of syndecan-4 influence GTP-loading. Recent reports have described the role of integrin engagement in recruitment of activated Rac1 to the membrane, and based on these results we propose that syndecan-4 synergises with alpha5beta1 to cause localised activation of Rac1 upon exposure of cells to a fibronectin matrix.