Analysis of molecular interactions between focal adhesion proteins talin and vinculin using FRET

A.-K. Born, A. Bruinink, K. Maniura
Laboratory for Materials-Biology Interactions,
Empa, Swiss Federal Laboratories for Materials Testing & Research,
Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland

Introduction: The development of cell based sensors as well as new material concepts for medical applications will be greatly advanced by tools that allow online life monitoring of cellular processes. Cell adhesion and generation of force on the extracellular matrix (ECM) play an important role for cell viability, migration and differentiation[1]. Accumulating evidence indicates that cells can not only sense but respond to physical parameters such as substrate rigidity and to topographical as well as structural features of the ECM.
Primary sites of adhesion are formed between integrin receptors and the underlying substratum. Within the cell, integrin receptors bind to a large number of proteins such as talin, paxillin and actin. Binding of talin head to the intracellular integrin domains cause integrin activation followed by accumulation of talin in focal contacts. Talin rod contains up to eleven vinculin binding sites that are buried in its native, un-stretched structure[2].
The recruitment of vinculin to focal adhesions sites is force dependent. Tensile forces that are applied to newly formed adhesion sites cause stretching of the talin rod thereby activating talin's vinculin binding sites. In the cytoplasm vinculin is present in an auto-inhibited state but binding to the talin rod causes vinculin activation. Vinculin containing focal adhesions are capable of exerting migration forces.
Fluorescence resonance energy transfer (FRET) microscopy offers the capability to study the fate and function of biomolecules in living cells[3]. Using CFP and YFP as the donor-acceptor pair for FRET we study the molecular interaction of talin and its binding partner vinculin in focal adhesions. Responses of talin-vinculin interaction to mechanical properties of the underlying substrate are examined by culturing cells on fibronectin-coated polyacrylamide substrates with varying rigidity.

Methods: For FRET measurements we generated several constructs in which the yellow fluorescent protein is inserted in close proximity to vinculin binding sites of talin rod. As the binding sites for talin are located in the N-terminal vinculin head domain we positioned CFP in front of vinculin. These FRET constructs were used for nucleofection of human fibroblasts.
Polyacrylamide gels with varying rigidities were prepared following the protocol by Pelham and Wang[4]. Fluorescence localization of FRET constructs was monitored by confocal laser scanning microscopy.

Results: Human fibroblasts transfected with the fluorescently-labelled vinculin or talin showed the expected accumulation of fluorescence signal at focal adhesion sites. The correct localisation of both proteins was confirmed by staining against endogenous talin or vinculin, suggesting that both tagged proteins are correctly synthesized. On rigid substrates, transfected cells were well spread whereas on more flexible substrates cells become less well spread. Cells transfected with a control vector and analysed for FRET by the acceptor depletion method showed that our setup allows FRET determinations.

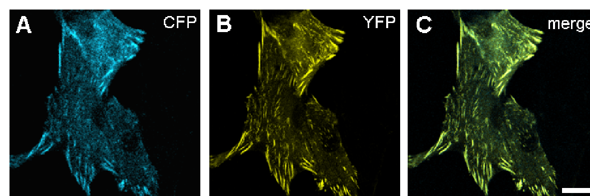

Fig. 1: Transfection of human fibroblasts with control vector pCFP-YFP-vinculin. [Scale bar 20 µm](A) CFP and (B) YFP are expressed as a fusion protein together with vinculin. (C) Cells showed the expected accumulation of both fluorescent proteins in focal adhesion sites.

Conclusions & Outlook: Our primary results suggest that transfection of human cells with our fluorescently labelled reporters is efficient and therefore qualified for FRET measurements and determination of interaction between focal adhesion proteins talin and vinculin in cells. The FRET technique allows testing the ability of cells to form adhesion complexes on a material surface and dynamics associated with this process. It has therefore been chosen as the tool of choice to evaluate cell-material interactions at the molecular level. We intend to study cells on substrates allowing the determination of local forces and plan to correlate these with vinculin-talin interaction as determined by FRET.

References:

1. Watt, F.M., Jordan, P.W., and O'Neill, C.H. *Cell Shape Controls Terminal Differentiation of Human Epidermal Keratinocytes.* Proc Natl Acad Sci USA, 85, 5576, 1988.
2. Gingras, A.R., Ziegler, W.H., Frank, R. Barsukov, I.L., Roberts, G.C.K., Critchley, D.R. and Emsley, J. *Mapping and Consensus Sequence Identification for Multiple Vinculin Binding Sites within the Talin Rod.* JBC, 280, 37217, 2005.
3. Sekar, R.B., and Periasamy, A. *Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations.* JBC, 160, 629, 2003
4. Pelham, R.J., Wang, Y.-I. *Cell locomotion and cell adhesion are regulated by substrate flexibility.* Proc Natl Acad Sci USA, 95, 13661, 1997

Acknowledgements: We thank Viola Vogel for stimulating discussion about talin structure and Vesa Hytönen for sharing his ideas for development of talin FRET constructs.

The present study is supported by the European Commission through the specific targeted research project CellForce (Contract N°: NMP4-CT-2005-016626) and CCMX Matlife.