

Cellular mechanosensing: Analysis of molecular interactions between fluorescent-tagged focal adhesion proteins talin and vinculin

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INTRODUCTION: The development of new material concepts for medical applications as well as cell based sensors will be greatly advanced by tools that allow on-line monitoring of cellular processes. Processes depending on mechanosensing of cells such as cell spreading and generation of force on the extracellular matrix (ECM) play an important role for cell viability, migration, proliferation and differentiation. Primary sites of adhesion are formed between integrin receptors and the underlying substratum. These initial adhesions consist of a large number of linker proteins such as talin. The talin rod contains up to eleven vinculin binding sites (VBS) whereas five VBS are located within fragment H1-H12 [1]. Binding of the talin head to intracellular integrin domains cause activation of integrins followed by rapid accumulation of talin in focal contacts. Recruitment of vinculin to cell adhesion sites, however, is force dependent. Intramolecular interactions between the head and tail domains retain vinculin in an inactive closed conformation and prevent binding to talin and actin and hence focal adhesion formation. When mechanical forces are applied to adhesion complexes talin becomes stretched which leads to unfolding of talin rod into helix sub-bundles and activation of VBS. Vinculin switches to an open, activated conformation that can now interact with VBS within the talin rod. The number of exposed VBS is defined by applied forces and force-exposure time of talin [2]. FRET microscopy using the CFP-YFP pair is a powerful technique that enables the visualization of protein interactions, protein conformations and biochemical status inside living cells.

The goal of our study is to investigate the molecular interaction of talin and its binding partner vinculin in focal adhesions.

METHODS: For FRET measurements we generated several constructs in which yellow fluorescent protein is inserted in close

proximity to vinculin binding sites within the talin rod. As the binding sites for talin are located in the N-terminal vinculin head domain CFP was positioned in front of vinculin. These FRET constructs were used for nucleofection of human fibroblasts followed by confocal laser scanning microscopy to monitor the fluorescence localisation of fusion proteins.

RESULTS: Our first results showed that transfection of cells with the different fluorescently-labelled vinculin or talin variants is efficient and results in the expected accumulation of fluorescence signal at focal adhesion sites. The correct localisation of both proteins was individually confirmed by immunohistochemical staining against talin or vinculin, suggesting that both tagged proteins are correctly synthesized and localized. First cotransfection experiments of both fluorescently labelled proteins resulted in colocalisation promising FRET to be observable.

DISCUSSION & CONCLUSIONS: Cell adhesion to surfaces is strongly influenced by substrate topography, chemical properties as well as mechanical cues. Therefore our FRET-based approach for online monitoring of cell adhesion state seems to be an attractive tool for studying newly developed biomaterials.

REFERENCES:

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