Regulation of Integrin Clustering: Models and Experiments

Erik S. Welf[1], Ulhas P. Naik[2], and Babatunde A Ogunnaike[3]

Short Abstract — **Integrins and integrin clustering during cell adhesion and migration play a central role in cell survival and function, yet we currently lack a framework for systematically characterizing the effects of integrin activation states on cluster formation and related signaling. Here, we use computational and experimental techniques to investigate the connections between integrin properties, integrin clustering, and integrin signaling; our results suggest that integrin properties and extra-cellular matrix properties combine to determine how cells control adhesion and migration processes.**

Integrins are transmembrane proteins that bind to extra-cellular matrix (ECM) proteins to attach cells and transfer signals bi-directionally across the cell membrane. Integrin signaling influences cell growth, differentiation, death, adhesion and migration [1], and a variety of pathologies have been linked to problems with integrin function [2]. At points of contact between cells and the ECM, integrins form clusters known as focal adhesions. Within focal adhesions. integrin clusters facilitate intra-cellular signaling and cell adhesion by providing a platform for protein interactions leading to protein phosphorylation and cytoskeletal attachment [3].

Integrin clusters continuously form and disperse in migrating cells, and numerous kinases, phosphatases, and adaptor molecules dynamically regulate focal adhesion signaling. We use carefully designed experiments and computational modeling to describe and understand integrin clustering and signaling.

We have previously described a reaction-diffusion model that represents integrin clustering as a function of specific integrin properties [4]. This model is used to explore ways in which integrins and the ECM modulate integrin cluster properties such as cluster shape and size. These integrin cluster properties may then be related to adhesion strength [5] and cell migration speed through the physics that underlie force transduction by multiple coordinated bonds. Our results indicate that certain combinations of integrin properties and ECM properties lead to integrin clusters that show more potential for efficient force transduction than other combinations. For example, patterned ECM surfaces and fast integrin clustering result in integrin clusters capable of transferring adhesive forces more effectively through specific spatial organization of integrins. By incorporating elements of integrin cluster turnover into our model, we also show how ECM patterns and integrin properties affect cell migration by controlling integrin cluster remodeling.

To understand how cells regulate integrin clustering, we developed an experimental system to measure integrin cluster size, and show that lower concentrations of ECM lead to larger integrin clusters. This phenomenon may be caused by integrin signaling related to ECM ligand density. A key signaling protein, focal adhesion kinase (FAK), forms one of the initial links between integrins and intracellular signals [6]. The function of FAK is two-fold: sense the extent of integrin clustering, and regulate cluster size [7, 8]. To examine the pivotal role of FAK in integrin cluster regulation, we measure FAK phosphorylation as a function of ECM concentration. By comparing differences in the magnitude and dynamics of FAK phosphorylation between different ECM ligand densities, we develop insight into integrin cluster size regulation via intracellular signals from FAK as a function of ECM properties.

By combining experimental and computational approaches, we show how integrin clustering can control cell adhesion and motility, and how integrin clustering may be regulated by a combination of ECM properties and integrin signaling.

[1]Department of Chemical Engineering, University of Delaware, Newark, Delaware. E-mail: eswelf@udel.edu

[2]Department of Biological Sciences, University of Delaware, Newark, Delaware. E-mail: unaik@udel.edu

[3]Department of Chemical Engineering, University of Delaware, Newark, Delaware. E-mail: ogunnaike@udel.edu

REFERENCES

[1] Wozniak MA et al. Focal adhesion regulation of cell behavior. (2004) *Biochim Biophys Acta* **1692**, 103-119.

[2] Wehrle-Haller B and Imhof BA, Integrin-dependent pathologies. (2003) *J Pathol* **200**, 481-487.

[3] Miyamoto S et al. Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. (1995) *J Cell Biol* **131**, 791-805.

[4] Welf ES, Naik UP, and Ogunnaike BA (in Review) A diffusion-reaction model for integrin clustering. *Biophys J.*

[5] Ward, M.D., and D.A. Hammer (1993) A theoretical analysis for the effect of focal contact formation on cell-substrate attachment strength. *Biophys J.* **64**, 936-959.

[6] Mitra SK, Hanson DA, and Schlaepfer DD (2005) Focal adhesion kinase: In command and control of cell motility. *Nature Rev Mol Cell Biol* **6**, 56-68.

[7] Shi, Q and Boettiger DA (2003) A novel mode for integrin-mediated signaling: tethering is required for phosphorylation of FAK Y397. *Mol Biol Cell.* **14**, 4306-4315.

[8] Sieg DA, Hauck CR and Schlaepfer DD (1999) Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. *J Cell Sci* **112**, 2677-2691