**Integrin Clusters of 100nm Form Early Adhesions and Depend on Talin**

Rishita Changede1, Xiaochun Xu1, Michael P Sheetz1,2 1Mechanobiology Institute, National University of Singapore, Singapore. 2Department of Biological Sciences, Columbia University, New York, NY 10027, USA

Clustering of matrix-activated integrins is a key step in the formation of matrix adhesions but the size and nature of this cluster formation is poorly understood. To analyze the cellular but not the matrix factors controlling clustering, we used supported lipid bilayers (SLB) with fluid, lipid-linked Arg-Gly-Asp (RGD) ligands. Fibroblasts bound to the fluid RGD surfaces and formed integrin clusters that were motile and saturated in fluorescence intensity. When these clusters were analyzed at the nanometer scale using photoactivated light microscopy (PALM), clusters of ~100nm in diameter were observed, with ~50 integrin molecules per cluster. Surprisingly, similar clusters formed on RGD-glass surfaces, indicating that integrins cluster around activated integrins independent of ligand mobility. Actin played an important role in organization of these clusters. Further, depletion of Talin1 and 2 dramatically inhibited cluster formation; but expression of either Talin1 head or rod domains restored nearly normal clusters. Upon analysis, talin head clusters displayed active movements like full-length talin whereas talin rod clusters aggregated through an actin-dependent process. Thus, from PALM analyses of integrin clusters on supported bilayers, we suggest that early integrin adhesions form from 100 nm clusters of activated integrins; and clusters depend upon talin head and/or rod but are independent of ligand mobility or external traction forces.