Revealing the three dimensional architecture of focal adhesion components to explain Ca^{2+}-mediated turnover of focal adhesions

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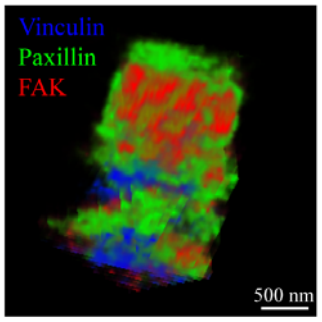
Focal adhesions (FAs) are large, dynamic protein complexes located close to the plasma membrane. Degradation, or turnover, of FAs is a major event at the trailing edge of a migratory cell, and is mediated by Ca^{2+}/calpain-dependent proteolysis and disassembly. Recent studies have proven nanoscale protein organization in FAs using three-dimensional (3D) super-resolution fluorescence microscopy [1]. This revealed the existence of a vertical FA core region of ~40 nm, consisting of multiple protein-specific strata between integrins and actin: a membrane-apposed integrin signaling layer, an intermediate force transduction layer, and an uppermost actin-regulatory layer. Many intricate molecular machines may contribute to the complexity of FA composition and dynamics. Therefore, the 3D construction of a FA, and interactions between FA molecules, play an important role in the regulation of FA dynamics and cell migration. We investigated how Ca^{2+} influx induces cascades of FA turnover in living cells. Images obtained with a total internal reflection fluorescence microscope (TIRFM) showed that Ca^{2+} ions induce different processes in the FA molecules focal adhesion kinase (FAK), paxillin, vinculin, and talin. Three mutated calpain-resistant FA molecules, FAK-V744G, paxillin-S95G, and talin-L432G, were used to clarify the role of each FA molecule in FA turnover. Vinculin was resistant to degradation and was not significantly affected by the presence of mutated calpain-resistant FA molecules. In contrast, talin was more sensitive to calpain-mediated turnover than the other molecules. Three-dimensional (3D) fluorescence imaging and immunoblotting demonstrated that outer FA molecules were more sensitive to calpain-mediated proteolysis than internal FA molecules. Furthermore, cell contraction is not involved in degradation of FA. Taken together, these results suggest that Ca^{2+}-mediated degradation of FAs was mediated by both proteolysis and disassembly. The 3D architecture of FAs is related to the different dynamics of FA molecule degradation during Ca^{2+}-mediated FA turnover.



[1] P. Kanchanawong, G. Shtengel, A.M. Pasapera, E.B. Ramko, M.W. Davidson, H.F. Hess, C.M. Waterman, "Nanoscale architecture of integrin-based cell adhesions," *Nature* **468,** 580-584 (2010).