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

Shu-Jing Chang, Ying-Chi Chen, Chi-Hsun Yang, Soon-Cen Huang, Ho-Kai Huang, Chun-Chun Li, Hans I-Chen Harn, Wen-Tai Chiu*

Department of Biomedical Engineering, National Cheng Kung University
No. 1, University Rd., Tainan 701, Taiwan.
E-mail: wtchiu@mail.ncku.edu.tw

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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.