Title
Paxillin at focal adhesions: Scanning fluorescence correlation spectroscopy

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Abstract

Cellular adhesions are important for cell migration during development, neuronal network formation, wound healing and cancer metastasis. The attachment of integrins to the extracellular matrix generates an attachment point, or adhesion, which cells can use to generate a traction point that the cell can use in order to migrate. This adhesion causes an array of signaling processes where cytosolic factors can be targeted to the adhesion, the actin cytoskeleton can reorganize and other downstream processes important for migration are activated. Amongst focal adhesion proteins, paxillin is known to play a regulatory role as an adaptor protein. A variety of methods including image correlation spectroscopy (ICS), have been used to evaluate these types of protein interactions in order to analyze protein aggregation states. ICS is best suited for spatial and temporal measurements for detecting slowly diffusing molecules. We have used a unique experimental approach, two-photon excitation scanning fluorescence correlation spectroscopy (S-FCS), which expands the capability of ICS by simultaneously measuring fast and slow diffusing molecules while retaining spatial information. We have measured the concentration and molecular interactions of EGFP-paxillin in vivo (CHO cells) at focal adhesions and in the cytosolic environment using S-FCS. The results indicate two populations corresponding to freely diffusing and bound paxillin at focal adhesion points. In the cytosol, only free EGFP-paxillin is detected. It is interesting to note that the "free" paxillin diffuses more slowly than expected for a protein of its size suggesting it complexes with other proteins in the cytosol before it binds to adhesions. ... [truncated at 250 words]