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Paxillin Aggregation, Interactions and Dynamics During Adhesion Assembly and Disassembly by Correlation Spectroscopic Methods

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Cell-matrix adhesions comprise an intricate signaling network of protein complexes that form and disassemble at specific cellular sites during cell migration. While paxillin is an important adapter protein that regulates adhesion turnover and cell migration, the organization and associations of paxillin in adhesions and the cytosol have not been quantitatively characterized in terms of the degree of protein aggregation and lifetimes of the aggregates. We have combined image correlation spectroscopy (ICS), single point-fluorescence correlation spectroscopy (FCS), and scanning-FCS techniques to elucidate paxillin-EGFP in live CHOK1 cells interactions at adhesions and in the cytosol. Moreover, we have also obtained photon counting histogram (PCH) data from temporal fluctuations observed from ICS to determine the cluster size of paxillin at in adhesions and the cytoplasm. We observe multiple diffusion constants for paxillin at different stages during the assembly and disassembly process that occurs at the leading and trailing edges of adhesions. The values range from 2.52 ± 0.75 $\mu m^2/s$ to 0.0019 ± 0.0009 $\mu m^2/s$ suggesting that the dynamics of the associations in these regions are quite complex. Our results from the PCH analysis indicate that paxillin interacts with other proteins as a monomer in the cytosolic regions near adhesions and forms small self-aggregates at the adhesions. The slower diffusing components in the vicinity of the adhesions suggest paxillin forms aggregates with other proteins before assembling at the adhesion. From the scanning-FCS data, we determined that the growth of these adhesions to occur in the time scale of several seconds. This study shows that paxillin resides in different aggregated structures during the recruitment and disassembly processes. Supported by the Cell Migration Consortium, PHS SUB UV GC10988, and PHS 5 P41-RRO3155, and GM23244.