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Dynamic and hierarchical reorganization of integrin-associated molecular complexes in nascent adhesions.

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An intricate network of putative molecular interactions comprises cell-matrix adhesions. Most of these interactions are inferred from co-immunoprecipitation, often using over expressed components. However, few have been demonstrated or characterized functionally in living cells, due to challenges in capturing highly regulated and transient associations that may form and evolve in adhesions. Our study uses high-resolution and image-based fluorescence fluctuation microscopy to map the formation and stoichiometry of integrin-associated molecular complexes in the nascent adhesions that populate the

leading edge of migrating cells. We focus on putative integrin activating (kindlin and talin) and actinlinking (talin, vinculin and □-actinin) molecules and show that all molecules are present in nascent adhesions as soon as they are visible; however, they form integrin containing complexes hierarchically, at different times and with variable stoichiometry within the adhesion itself. These observations suggest a working model for nascent adhesion assembly, whereby transient complexes containing ~ 3 a-actinin and integrin molecules help nucleate nascent adhesions. Subsequently, integrin complexes form that contain kindlin in a 1:1 ratio, but not talin. Talin localizes to adhesions independently of the integrinkindlin complex, and associates with vinculin in molecular complexes. However, once the nascent adhesion has formed, myosin II activity promotes talin association with the integrin-kindlin complex in a 2:1 stoichiometry, suggesting that each talin molecule cross-links two integrin-kindlin complexes. Finally, as adhesions mature into larger structures, talin increases to a 1:1 ratio with integrin and kindlin; whereas the ratio of vinculin to the other molecules continues to increase. In summary, using novel fluorescence fluctuation microscopy, we show that hierarchical adhesion assembly and reorganization accompanies adhesion formation.