

Super-resolution microscopy: a window for integrin spatiotemporal and mechanical regulation

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Focal adhesions (FAs) are adhesive structures linking the cell to the extracellular matrix (ECM) and constitute molecular platforms for biochemical and mechanical signals controlling cell adhesion, migration, differentiation and survival. Integrin transmembrane receptors are core components of FAs, connecting the ECM to the actin cytoskeleton. Intracellular protein talin, which directly binds to the cytoplasmic tail of β -integrins, is considered as the main integrin activator. More recently, the protein kindlin, which also binds to β -integrin, was demonstrated to be also a critical integrin activator. Kindlin is considered to be a co-activator as it regulates the level of talin-induced integrin activation. However, the molecular basis for this cooperation between kindlin and talin during integrin activation is still unknown.

Most studies focusing on kindlin functions have been carried in vitro or in suspended cells. Here we combined PALM microscopy with single protein tracking to decipher the role and behavior of kindlin during key molecular events occurring outside and inside FAs at the plasma membrane and leading to integrin activation, as we have done previously for talin (Rossier et al., 2012). We found that β 1-integrins with a point mutation inhibiting binding to kindlin show reduced immobilization and enrichment inside FAs but to a lower extent compared to talin-binding defective integrin. This suggests that talin could still induce integrin activation without kindlin, but less efficiently, supporting that kindlin is facilitating activation by talin. Importantly, we found that kindlin2, which is enriched inside FAs, displayed free diffusion at the plasma membrane outside and inside FAs, whereas talin is directly recruited to FAs from the cytosol. To determine the molecular basis of kindlin membrane recruitment and diffusion, we use point-mutated kindlin variant known to decrease binding to integrins (kindlin2-QW). Kindlin2-QW mutant displayed increased membrane diffusion showing that free diffusive kindlin2 are not associated with freely diffusing integrins. In contrast, deletion of the kindlin pleckstrin homology (PH) domain suppressed the membrane recruitment and diffusion of kindlin. We assessed the functional role of kindlin membrane diffusion in cell spreading and FAs formation in kindlin1/kindlin2 double KO cells. Those experiments demonstrated that kindlin2 membrane recruitment and diffusion are crucial for integrin activation during cell spreading and FA formation. Kindlin membrane diffusion may increase its probability to encounter integrins at the plasma membrane, priming their final activation by talin in FAs.

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