Rho small GTPase regulates the stability of individual focal adhesions: a FRET-based visualization of GDP/GTP exchange on small GTPases

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RhoA and Rac1 are small GTPases primarily involved in cytoskeletal remodeling. Many biochemical studies have suggested that they are also key organizers of cell-substrate adhesion. Recently, fluorescence resonance energy transfer (FRET)-based indicators have been developed to visualize RhoA and Rac1 activity in living cells [Yoshizaki et al., J. Cell Biol. 162, 223 (2003); Pertz et al., Nature 440, 1069 (2006)]. These indicators use one of the interactions between RhoA (Rac1) and the RhoA (Rac1)-binding domain of their effector proteins. However, distribution of RhoA activity in single cells has not yet been observed with micrometer-scale resolution. Here, we employed an approach that detects GDP/GTP exchange on small GTPases by using FRET from YFP-fused small GTPases to a fluorescent analogue of GTP, BODIPY(TR)-GTP. This approach allowed us to visualize confined localization of active (GTP-bound forms of) RhoA and Rac1 in individual focal adhesions. Activated RhoA accumulated in immobile and long-lived focal adhesions but was not evident in unstable and temporary adhesions, while activated Rac1 was observed at every adhesion. Our results suggest that RhoA is the major regulator determining the stability of individual cell adhesion structures.

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