

## Rac and Rho Regulation of Focal Adhesion Kinetics

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Rac and Rho are small GTPases critical in regulating focal adhesion (FA) formation and maturation in cell migration. FA maturation is characterized by mechanically and/or biochemically induced growth of small focal complexes into FA. We hypothesize that growth/maturation is mediated by altering the balance of on and off rates of key structural proteins that bind to and dissociate from FA. To test this hypothesis, we analyzed the dynamics of GFP-fusion proteins in mouse embryo fibroblasts (MEF) cells using TIRF and FRAP. The size and FA turnover rates were assessed by TIRF microscopy and the turnover times of individual proteins within FA were measured with FRAP. We focused on paxillin, thought to be a key regulator of adhesion maturation. Rac and Rho activity were modulated by transfection with constitutively active (CA) and dominant negative mutants. As is established, CA Rac produced small focal complexes and the CA Rho produced elongated mature FA. Rac and Rho affected the recovery rates of paxillin-GFP fluorescence after photobleaching in FA. The recovery curves are well fit by the sum of two exponentials, indicating two characteristic times for paxillin interaction with FA. The long time recovery ( $t_{1/2} \sim 50$  s) was independent of Rho and Rac conditions, however, the initial recovery was affected by Rho activation. In control cells, the short time paxillin recovery occurred with a  $t_{1/2} \sim 1.5$  s, while for CA Rho conditions, paxillin recovered much faster ( $t_{1/2} \sim .2$  s). Thus, Rho promotes rapid dissociation of a portion of adhesion-associated paxillin. In addition, constitutive Rho activity caused anisotropy in the fluorescence recovery – the intensity filled in centripetally within the bleached zone. The complex kinetics of paxillin within FA are consistent with the established complexity of binding interactions and phosphoregulation for paxillin. These results suggest that Rho may be key in FA maturation.