

P006 Uncoupling of inhibitory and shuttling functions of rhoGDIs
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Rho guanine-nucleotide dissociation inhibitor (rhoGDIs) are postulated to regulate the activity and the localization of small G proteins of the Rho family by a shuttling process involving extraction of Rho from donor membranes, formation of inhibitory cytosolic Rho/rhoGDI complexes, and delivery of Rho to target membranes. However, the role of rhoGDIs in the site-specific membrane targeting or extraction of Rho is still poorly understood.

We investigated the *in vivo* functions of two mammalian rhoGDIs: the specific rhoGDI-3 and the well studied rhoGDI-1 after structure-based mutagenesis. We thus identified two sites in the rhoGDIs, forming conserved interactions with their Rho target, and whose mutation results in the uncoupling of inhibitory and shuttling functions of rhoGDIs *in vivo*.

Remarkably, these rhoGDI mutants were detected at Rho-induced membrane ruffles or protrusions, where they co-localized with RhoG or Cdc42, likely identifying for the first time the site of extraction of a Rho protein by a rhoGDI *in vivo*. We propose that these mutations act by modifying the steady-state kinetics of the shuttling process regulated by rhoGDIs, such that transient steps at the cell membranes now become detectable. They should provide valuable tools for future investigations of the dynamics of membrane extraction or delivery of Rho proteins and their regulation by cellular partners.