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RhoD localization and function is dependent on its GTP/GDP-bound state and unique N-terminal motif

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ABSTRACT

The atypical Rho GTPase RhoD has previously been shown to have a major impact on the organization and function of the actin filament system. However, when first discovered, RhoD was found to regulate endosome trafficking and dynamics and we therefore sought to investigate this regulation in more detail. We found that exogenously expressed RhoD in human fibroblasts localized to vesicles and the plasma membrane and that the active GTP-bound conformation was required for the plasma membrane localization but not for vesicle localization. In contrast to the GTPase deficient atypical Rho GTPases, which have a stalled GTPase activity, RhoD has an elevated intrinsic GDP/GTP exchange activity, rendering the protein constitutively active. Importantly, RhoD can still hydrolyze GTP and we found that an intact GTPase activity was required for efficient fusion of RhoD-positive vesicles. RhoD has a unique N-terminal extension of 14 amino acid residues, which is not present in the classical Rho GTPases RhoA, Cdc42 and Rac1. Deletion of this N-terminal motif often leads to clustering of RhoD positive vesicles, which are found accumulated at the peripheral membrane border. In addition, the number of vesicles per cell was increased manifold, suggesting that the N-terminal motif has an important regulatory role in vesicle dynamics.

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