

FHOD1 is a member of the subfamily of Diaphanous-related formins, which are activated by Rho GTPase mediated release of their intramolecular autoinhibition. The expression of an activated FHOD1 mutant induces the formation of thick stress fiber bundles in cells. However, the cellular relevance of this effect as well as molecular mechanisms leading to FHOD1 mediated stress fiber formation are not well understood. The aim of the present study was to characterize the molecular function of FHOD1 in the dynamic reorganization of the actin cytoskeleton and the role of formin mediated actin dynamics in actin-based cellular processes.

RNAi-mediated depletion of endogenous FHOD1 as well as expression of constitutively active FHOD1 mutants revealed distinct functions of the formin during the generation of different stress fiber types in U2OS cells. Overall, depletion of FHOD1 lead to massive defects in the formation of actin stress fibers in the leading edge which often resulted in the entire collapse of the actin filament network. These striking defects were accompanied by decreased protrusion dynamics at the cell front as well as perturbed turn over of focal adhesions, ultimately leading to significantly decreased cell migration.

Detailed studies of stress fiber dynamics showed that active FHOD1 leads to the accumulation of contractile ventral stress fibers due to enhanced formation of transversal arcs. In contrast, growth of non-contractile dorsal fibers was inhibited in cells expressing active FHOD1. Localization studies revealed distinct localization patterns of FHOD1 along the induced contractile stress fibers suggesting a direct function of the formin in the generation of these structures. Additionally active FHOD1 colocalized partially with Myosin at contractile stress fibers, whereas both proteins were absent along non-contractile dorsal fibers. These findings indicate a possible interplay of both proteins in the process of transversal arc generation. The FHOD1 mediated enhanced formation of transversal arcs, which are primarily formed by fusion and bundling of short actin filaments, along with the inhibited growth of dorsal stress fibers indicate a potential dual molecular function of the formin as a capping and actin bundling protein.

In summary, the present work provides fundamental molecular knowledge of FHOD1 function and upstream regulatory mechanisms in the organization of the actin cytoskeleton. Thus, the presented findings offer a critical basis for future work to deepen our understanding of formin mediated actin-based dynamic processes.