Arul Sakkaravarthi Sahadevan

Dr. sc. hum.

Regulation of the RGS-domain containing RhoGEFs PDZ-RhoGEF and LARG through dimerization and interactions with Diaphanous-related formins

Geboren am 01.07.1979 in Erode, INDIA

Diplom der Fachrichtung Master of Science in Life Sciences (Specialization in Microbiology) from Bharathidasan University, Tamil Nadu, India.

Promotionsfach: Pharmakologie

Doktorvater: Prof. Dr. Stefan Offermanns

Leukemia associated RhoGEF (LARG) and PDZ RhoGEF belong to the family of RGS domain containing guanine nucleotide exchange factors for RhoA. The RGS domain provides a functional motif by which Gα12 and Gα13 can bind and regulate the activity of these RhoGEFs, establishing a link between heterotrimeric G proteins and RhoA. The Diaphanous related formin Dia1 is a RhoA effector that possesses conserved functions in actin cytoskeleton dynamics exerted through their FH2 domain to control cytokinesis, SRF transcriptional activity and cell motility. The dormant conformation of the DRF Dia1 is maintained by intra-molecular association of its regulatory N-terminus to the Diaphanous auto-regulatory domain (DAD), which is relieved following binding to activated RhoA.

The present work shows that LARG and PDZ RhoGEF undergo homo and hetero dimerization through their C-terminal region. Deletion of the C-terminus or N-terminus regions of these RhoGEFs results in RhoA activation and an increase in their ability to activate Serum Response Factor (SRF)-mediated transcription. These findings suggest the existence of a mechanism controlling the activity of LARG and PDZ RhoGEF, which involves conformational changes and or homo-and hetero-dimerization through their inhibitory C-terminus.

Using purified proteins, our work shows that Dia1 binds to LARG through RhoA-dependent release of Dia1 auto-inhibition. A dominant negative form of LARG inhibits Dia1-induced RhoA

activity, indicating the involvement of LARG in RhoA activation via Dia1. The FH2core domain directly binds to the regions at the C-terminus of LARG, which is responsible for GEF oligomerization. FH2core binding to LARG interferes with the C-terminal conformational changes, to stimulate LARG GEF activity *in vitro*.

Our results reveal a novel signaling module by which Dia1, in addition to its role as a downstream RhoA effector, can function upstream of RhoA. This is achieved by direct interaction and thereby activation of the RhoA guanine nucleotide exchange factor LARG. This constitutes a positive feedback loop.