Supplementary Materials

Mechanistic Insights into Specificity, Activity and Regulatory Elements of the RGS-containing Rho-specific Guanine Nucleotide Exchange Factors p115, PDZ-RhoGEF (PRG) and Leukemia-associated RhoGEF (LARG)

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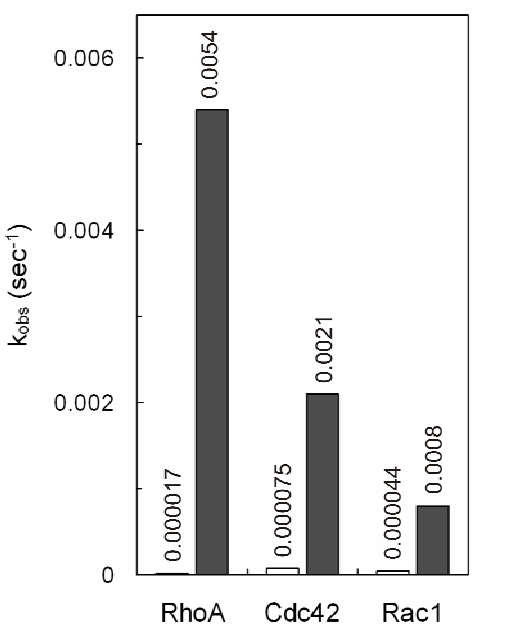
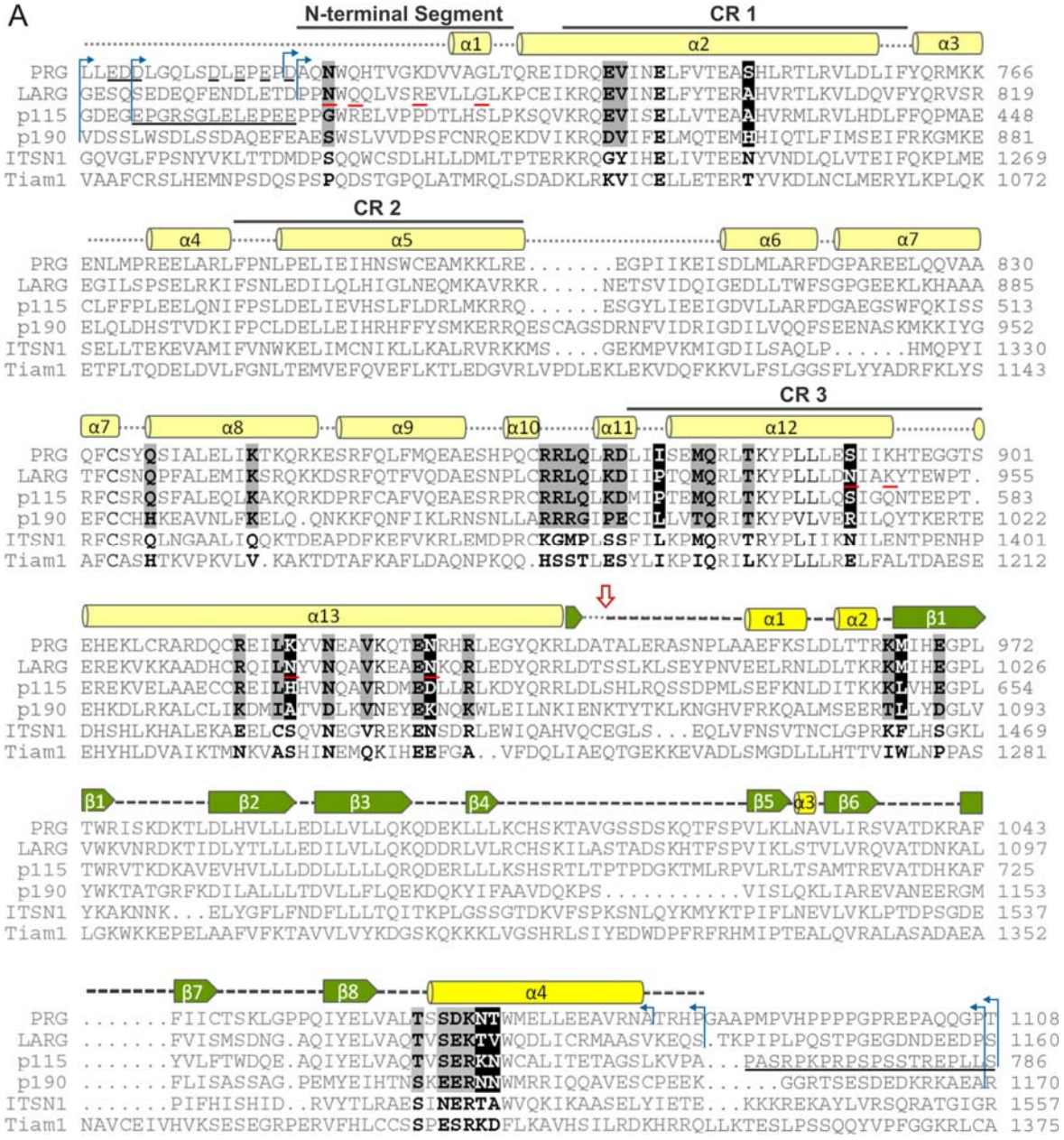


Fig. S1 Nucleotide exchange activity of RhoGEFs. The DH-PH domain of p115, Asef and Tiam1 catalyzes specifically the mantGDP exchange reaction of RhoA, Cdc42 and Rac1, respectively (conditions are decribed in Fig. 2; white bars: no GEF; black bars: GEF added).


A
N-terminal Segment
CR 1
CR 2
CR 3
α1
α2
α3
α4
α5
α6
α7
α8
α9
α10
α11
α12
α13
β1
β2
β3
β4
β5
β6
β7
β8
PRG LLEDDLGQLSDLEPEPDAQNWQHTVGKDVVAGLTQREIDRQEVINELFVTEASHLRTLRVLDLIFYQRMKK 766
LARG GESQSEDEQFENDLETDPPNWQQLVSREVLLGLKPCEIKRQEVINELFYTERAHVRTLKVLDQVFYQRVSR 819
p115 GDEGEPGRSGLELEPEEPPGWRELVPPDTLHSLPKSQVKRQEVISELLVTEAAHVRMLRVLHDLFFQPMAE 448
p190 VDSSLWSDLSSDAQEFEAESWSLVVDPSFCNRQEKDVIKRQDVIFELMQTEMHHIQTLFIMSEIFRKGMKE 881
ITSN1 GQVGLFPSNYVKLTTDMDPSQQWCSDLHLLDMLTPTERKRQGYIHELIVTEENYVNDLQLVTEIFQKPLME 1269
Tiam1 VAAFCRSLHEMNPSDQSPSPQDSTGPQLATMRQLSDADKLRKVICELLETERTYVKDLNCLMERYLKPLQK 1072
PRG ENLMPREELARLFPNLPELIEIHNSWCEAMKKLRE.....EGPIIKEISDLMLARFDGPAREELQQVAA 830
LARG EGILSPSELRKIFSNLEDILQLHIGLNEQMKAVRKR.....NETSVIDQIGEDLLTWFSGPGEEKLKHAAA 885
p115 CLFFPLEELQNIFPSLDELIEVHSLFLDRLMKRRQ.....ESGYLIEEIGDVLLARFDGAEGSWFQKISS 513
p190 ELQLDHSTVDKIFPCLDELLEIHRHFFYSMKERRQESCAGSDRNFVIDRIGDILVQQFSEENASKMKKIYG 952
ITSN1 SELLTEKEVAMIFVNWKELIMCNIKLLKALRVRKKMS....GEKMPVKMIGDILSAQLP.....HMQPYI 1330
Tiam1 ETFLTQDELDVLFGNLTEMVEFQVEFLKTLEDGVRLVPDLEKLEKVDQFKKVLFSLGGSFLYYADRFKLYS 1143
PRG QFCSYQSIALELIKTKQRKESRFQLFMQEAESHPQCRRLQLRDLITSEMQRLTKYPLLLESIIKHTEGGTS 901
LARG TFCSNQPFALEMIKSRQKKDSRFQTFVQDAESNPLCRRLQLKDIIPTQMQRLTKYPLLLDNIAKYTEWPT. 955
p115 RFCSRQSFALEQLKAKQRKDPRFCAFVQEAESRPRCRRLQLKDMIPTEMQRLTKYPLLLQSIGQNTEEPT. 583
p190 EFCCHHKEAVNLFKELQ.QNKKFQNFIKLRNSNLLARRRGIPECILLVTQRITKYPVLVERILQYTKERTE 1022
ITSN1 RFCSRQLNGAALIQQKTDEAPDFKEFVKRLEMDPRCKGMPLSSFILKPMQRVTRYPLIIKNILENTPENHP 1401
Tiam1 AFCASHTKVPKVLV.KAKTDTAFKAFLDAQNPKQQ.HSSTLESYLIKPIQRILKYPLLLRELFALTDAESE 1212
PRG EHEKLCRARDQCREILKKYVNEAVKQTENRHRLEGYQKRLDATALERASNPLAAEFKSLDLTTRKMIHEGPL 972
LARG EREKVKKAADHCRQILNYVNQAVKEAENKQRLEDYQRRLDTSSLKLSEYPNVEELRNLDLTKRKMIHEGPL 1026
p115 EREKVELAAECCREILHHVNQAVRDMEDLLRLKDYQRRLDLSHLRQSSDPMLSEFKNLDITKKKLVHEGPL 654
p190 EHKDLRKALCLIKDMIATVDLKVNEYEKNQKWLEILNKIENKTYTKLKNGHVFRKQALMSEERTLLYDGLV 1093
ITSN1 DHSHLKHALEKAEELCSQVNEGVREKENSDRLEWIQAHVQCEGLS...EQLVFNSVTNCLGPRKFLHSGKL 1469
Tiam1 EHYHLDVAIKTMNKVASHINEMQKIHEEFGA..VFDQLIAEQTGEKKEVADLSMGDLLLHTTVIWLNPPAS 1281
PRG TWRISKDKTLDLHVLLLEDLLVLLQKQDEKLLLKCHSKTAVGSSDSKQTFSPVLKLNAVLIRSVATDKRAF 1043
LARG VWKVNRDKTIDLYTLLLEDILVLLQKQDDRLVLRCHSKILASTADSKHTFSPVIKLSTVLVRQVATDNKAL 1097
p115 TWRVTKDKAVEVHVLLLDDLLLLLQRQDERLLLKSHSRTLTPTPDGKTMLRPVLRLTSAMTREVATDHKAF 725
p190 YWKTATGRFKDILALLLTDVLLFLQEKDQKYIFAAVDQKPS.....VISLQKLIAREVANEERGM 1153
ITSN1 YKAKNNK...ELYGFLFNDFLLLTQITKPLGSSGTDKVFSPKSNLQYKMYKTPIFLNEVLVKLPTDPSGDE 1537
Tiam1 LGKWKKEPELAAFVFKTAVVLVYKDGSKQKKKLVGSHRLSIYEDWDPFRFRHMIPTEALQVRALASADAEA 1352
PRG .....FIICTSKLGPPQIYELVALTSSDKNTWMELLEEAVRNATRHPGAAPMPVHPPPPGPREPAQQGPT 1108
LARG .....FVISMSDNG.AQIYELVAQTVSEKTVWQDLICRMAASVKEQS.TKPIPLPQSTPGEGDNDEEDPS 1160
p115 .....YVLFTWDQE.AQIYELVAQTVSERKNWCALITETAGSLKVPA...PASRPKPRPSPSSTREPLLS 786
p190 .....FLISASSAG.PEMYEIHTNSKEERNNWMRRIQQAVESCPEEK.....GGRTSESDEDKRKAEAR 1170
ITSN1 .....PIFHISHID..RVYTLRAESINERTAWVQKIKAASELYIETE...KKKREKAYLVRSQRATGIGR 1557
Tiam1 NAVCEIVHVKSESEGRPERVFHLCCSSPESRKDFLKAVHSILRDKHRRQLLKTESLPSSQQYVPFGGKRLCA 1375

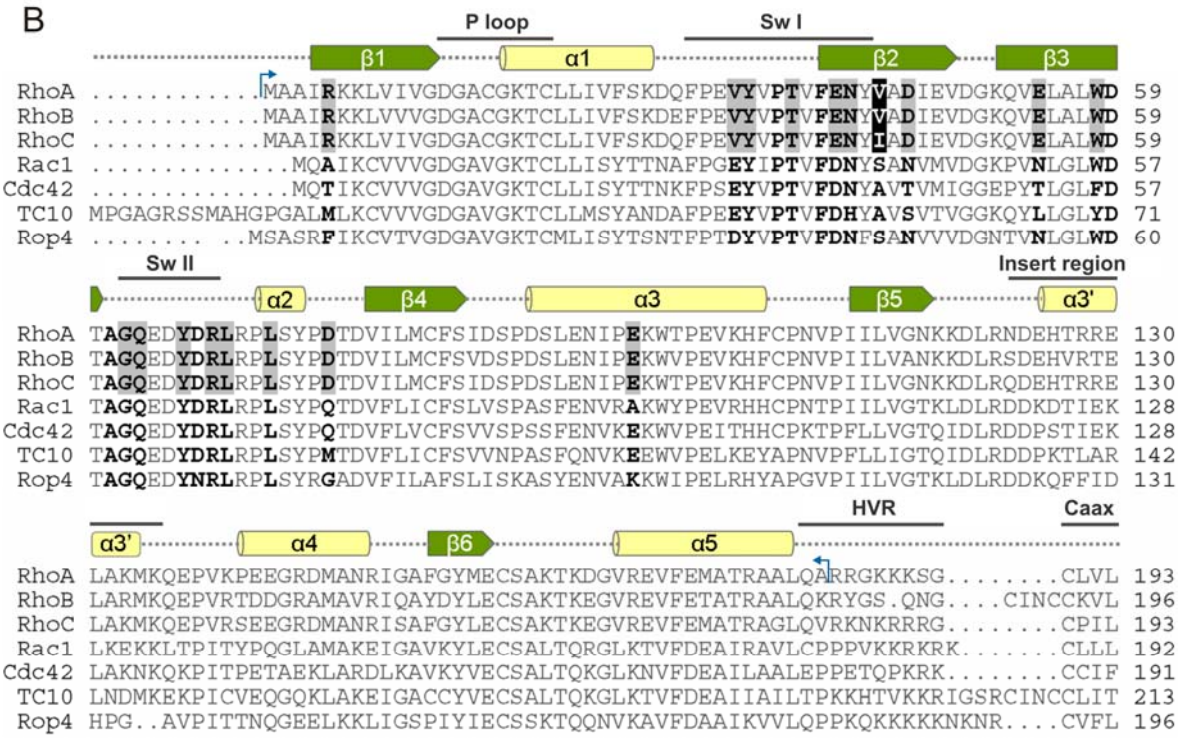
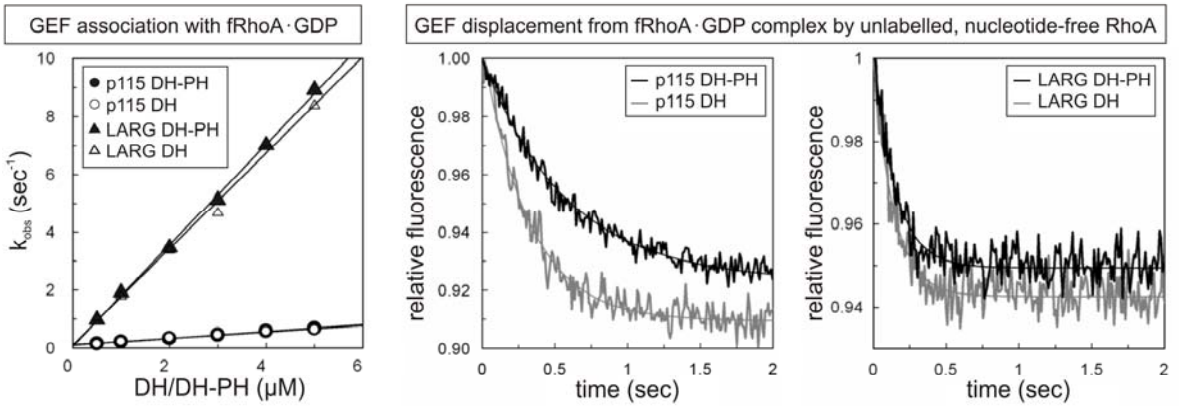


Fig. S2 Multiple sequence alignment of DH-PH-containing GEFs (A) and Rho GTPases (B). The DH-PH domains of Tiam1 (RacGEF), ITSN1 (Cdc42GEF) and the Rho specific GEFs p190, p115, LARG and PRG along with full length TC10, Cdc42, Rac1, RhoC, RhoB and RhoA are used to highlight and to discuss the specificity determining residues based on the crystal structures of RhoA in the complex with DH-PH of PRG (16)) (PDB ID 1XCG) and of LARG (17)) (PDB ID 1X86). All bold residues (**X**, **X**, **X**) are involved in the RhoA/DH-PH interaction. Black residues in bold with a grey background (**X**) are conserved and important in determining the specificity of the RhoA/DH-PH interaction. White residues in bold and black background (**X**) are variable and involved in assigning specificity. **X** and **X** are selected in Fig. 7 to discuss the biochemical data in this study. The conserved signatures of the Rho GTPases are highlighted: P loop, switch I, switch II, hypervariable region (HVR) and the prenylation site (CaaX). Conserved regions within the DH domain (CR1, CR2, CR3) and the N-terminal segments are shown. Black lines indicate the termini of p115 DH-PH that are truncated in DH-PHc and DH-PHcn of p115. The polypeptide backbone is shown as a dashed line and the secondary structure elements (alpha-helices and beta sheets) are illustrated based on the crystal structures of RhoA in the complex with the DH-PH of PRG (16)). An arrow (⇩) indicate the C-terminus of the DH and the N-terminus of the PH domains and blue arrows (↱) indicate the respective N-terminal and C-terminal ends of the proteins used in this study. Amino acids underlined in red in LARG may be responsible for the highly efficient exchange activity of LARG and PRG versus p115 and p190. Amino acids underlined in black at the N-terminus of PRG DH domain has been shown to have inhibitory effects on the PRG exchange activity (37).



<u>Fig. S3</u> Real-time monitoring of the RhoGEF interactions with the fluorescently labelled GDP-bound RhoA (fRhoA). Kinetics of the association between fRhoA and the DH and DH-PH domains of LARG and p115, respectively, at the left panel clearly revealed differential binding properties of the two RhoGEFs. Observed rate constants (k_{obs}) of the association curves obtained at increasing DH and DH-PH concentrations were calculated by single exponential fitting. The association rate constants (k_{on}) of fRhoA·GDP-binding to the DH and DH-PH proteins were calculated from the linear regression of the k_{obs} values plotted against the concentrations of the DH-PH (closed symbols) and the DH (open symbols) domains of LARG (triangle) and p115 (circles). The DH-PH (10 μM) dissociation from the fRhoA·GDP complex (middle and right panels) was measured in a displacement experiment in the presence of excess amounts of unlabelled, nucleotide-free RhoA (20 μM). The dissociation rate constant (k_{off}) was determined by an exponential fit of the data. The dissociation constant (K_d) was calculated from the kinetic parameters of dissociation and association reactions by the equation: $K_d = k_{off} / k_{on}$.