The many faces of the guanine-nucleotide exchange factor trio

Jos van Rijssel and Jaap D. van Buul*
Department of Molecular Cell Biology; Sanquin Research and Landsteiner Laboratory; Academic Medical Center; University of Amsterdam; Amsterdam, the Netherlands

Keywords: Trio, Rac1, RhoG, lamellipodia, Rho-GEF
Submitted: 03/22/12
Revised: 06/14/12
Accepted: 07/09/12
http://dx.doi.org/10.4161/cam.21418
*Correspondence to: Jaap D. van Buul; Email: j.vanbuul@sanquin.nl

Small Rho-GTPases are enzymes that are bound to GDP or GTP, which determines their inactive or active state, respectively. The exchange of GDP for GTP is catalyzed by so-called Rho-guanine nucleotide exchange factors (GEFs). Rho-GEFs are characterized by a Dbl-homology (DH) and adjacent Pleckstrin-homology (PH) domain that serves as enzymatic unit for the GDP/GTP exchange. Rho-GEFs show different GTPase specificities, meaning that a particular GEF can activate either multiple GTPases or only one specific GTPase. We recently reported that the Rho-GEF Trio, known to be able to exchange GTP on Rac1, RhoG and RhoA, regulates lamellipodia formation to mediate cell spreading and migration in a Rac1-dependent manner. In this commentary, we review the current knowledge of Trio in several aspects of cell biology.

Introduction

The Rho-GEF Trio was originally identified in 1996 as a binding partner of the transmembrane tyrosine phosphatase LAR. Trio is a large protein of 350 kD that harbors three domains with putative enzymatic activity, hence the name Trio. Trio encodes two Dbl-homology-Pleckstrin-homology (DH-PH) Rho-GEF units with different specificities. The N-terminal DH-PH unit (TrioD1) mediates GDP to GTP exchange on Rac1, RhoG and RhoA, regulates lamellipodia formation to mediate cell spreading and migration in a Rac1-dependent manner. In this commentary, we review the current knowledge of Trio in several aspects of cell biology.

Isoforms of Trio

Several isoforms of both Kalirin and Trio have been identified. For both proteins, a single gene is responsible for the expression of Trio and Kalirin. However, due to alternative splicing and the use of different promoters, several isoforms are formed. Kalirin-7 (also known as Duo), -9 and -12 are expressed in the brain and differ in length at their C-terminus. Several Trio isoforms, Trio A, B and D, are strongly expressed in the brain and during development, whereas Trio C, also known as Solo/Trio8, is exclusively expressed in the cerebellum. All these splice variants include the N-terminal SEC14 and spectrin-repeat domain and a C-terminal serine/threonine kinase domain (Fig. 1). Using DomPred Protein Domain Prediction Server (freely available at http://bioinf.cs.ucl.ac.uk/dompred) and based on the protein sequence, we predict that Trio has nine spectrin-repeats at the N-terminus. Shortly after the discovery of Trio, a closely related protein was identified that was named Kalirin. Trio and Kalirin share 68% nucleotide and 65% amino acid sequence identity, but whereas Trio is ubiquitously expressed, Kalirin expression is mainly confined to the central nervous system.

Surprisingly, the N-terminal GEF unit is almost identical between the two proteins, showing 92% homology at the protein level, whereas the C-terminal GEF unit shows 67% homology.

Commentary to: van Rijssel J, Hoogenboezem M, Wester L, Hordijk PL, Van Buul JD. The N-terminal DH-PH domain of Trio induces cell spreading and migration by regulating lamellipodia dynamics in a Rac1-dependent fashion. PLoS One 2012; 7:e29912; PMID:22238672; http://dx.doi.org/10.1371/journal.pone.0029912
Our recent Fig. 1 Trio, Whether the 80 Rho-GEF members that comprise For Kalirin, it is Research by the group of Der 28 This In addition, Thus, the Filamin may 6,29,30 and the F-actin Fig. 1 such as In contrast, The levels of However, Thus, the N-ter C-terminal GEF unit only and is found in patients with adult T-cell leukemia.15

Trio and Regulatory Mechanisms

As mentioned above, Trio is a large protein that harbors next to the two GEF and kinase domains several other domains that may be involved in protein or lipid interaction. Up to now, the mechanisms by which the individual domains of Trio are activated and the functional consequences of this for Trio as a single protein are unclear. In this section we will discuss the potential contribution of phosphorylation, inter- and intra-molecular interactions and presence of two GEF domains with different specificities.

Trio and phosphorylation. Our recent work suggests that during cell spreading Trio is activated upon the engagement of integrins, in particular integrin β1, since in our studies the cells were plated on fibronectin-coated surfaces in serum-free conditions.16 Research by the group of Der showed that for the exchange factor Vav1, tyrosine phosphorylation by Lck is crucial for its GEF function in vitro.17 However, although Trio harbors several tyrosine residues, it is not known if tyrosine phosphorylation is required for Triomediated GTP exchange.

Medley and colleagues showed that the kinase domain of Trio, known to interact with LAR, is constitutively phosphorylated on tyrosine residues.6 The levels of phosphorylation were further increased when FAK was co-expressed. Trio interacted with FAK through two distinct regions: the SH3-Ig-like region and the serine/threonine kinase domain (Fig. 1). The authors furthermore showed that upon phosphorylation, Trio shifted to a more detergent-insoluble fraction.6 This indicates that Trio tyrosine phosphorylation may trigger its interaction with the actin cytoskeleton. However, it remains unclear if FAK affects the GEF activity of Trio.

Trio interaction partners. Although the molecular mechanism of the activation of the TrioD1 GEF domain is thus far unclear, its ability to activate Rac1 was shown to be regulated by interactions with several proteins. Rac1 activation by Trio is negatively regulated by interactions with the F-actin capping protein CARMIL,18 the motor protein Myosin II19 and the F-actin binding protein Tara.20 In contrast, association of the integral membrane protein Kidins220/ARMS (kinase-D-interacting substrate of 220 kDa/ankyrin repeat-rich membrane spanning) with the spectrin-repeats of Trio was demonstrated to promote Rac1 activation.21 In addition, the F-actin cross-linker protein Filamin interacted with the PH-domain of the TrioD1 GEF unit and was required for TrioD1-induced membrane dynamics.22 In another study by Bellanger and colleagues, they showed that the PH domain of TrioD1 is involved both in regulating the catalytic activity of TrioD1 and in determining the sub-cellular localization of its associated DH domain.23 Thus, the N-terminal PH domain of Trio may serve as a cytoskeletal targeting signal. Since Rac1 has also been shown to bind to the actin-binding protein Filamin,24–26 Filamin may function as a scaffold for Trio-mediated Rac1 activation in a similar manner as has been reported for the interaction of filamin with the GEF Vav2.26

Trio and the SEC14 domain. Trio, together with the Rho-GEFs Kalirin and Dbs, are the only Rho-GEFs in the family of > 80 Rho-GEF members that comprise a SEC14 homology domain (Fig. 1).27 Proteins encompassing a SEC14 domain are widely expressed in plants, yeast, invertebrates and mammals, suggesting that this domain is highly conserved.28 SEC14 domains, also known as CRAL-TRIO domains, were shown to mediate the interaction between proteins and specific phospholipids,8,29,30 such as PtdIns, PtdCho, PtdSer and a number of different phosphorylated forms of PtdIns. Work by the group of Whitehead showed that removal of the SEC14 domain of Dbs induced Dbs distribution to the periphery of the cell, whereas full length Dbs was found in peri-nuclear regions and co-localized with Golgi markers.31 Thus, the SEC14 domain in Rho-GEFs may promote membrane targeting and thereby determine local Rho-GEF activity. The authors furthermore showed that the SEC14 homology domain forms intracellular contacts with the PH-domain of Dbs. Next, they showed that these contacts must be released to achieve full transformation activity by Dbs.31 Whether the SEC14 domain of Trio plays a similar regulatory role in the activity of the N-terminal DH-PH domain of Trio is unknown.

Trio and the spectrin-repeats. Spectrin-repeats are three-helix bundle structures that occur in many different proteins, either as single copies, for Dbs, or in tandem repeats, for Trio and Kalirin.32 These repeats can act in a structural way, by coordinating cytoskeletal interactions with high spatial precision, as well as an intermediary for interactions with several regulatory proteins.32 For Kalirin, it is known that the spectrin-repeats bind to

Figure 1. Schematic representation of the structure of Rho-GEFs Trio and Kalirin. Trio and Kalirin both express two DH-PH units (green/red) and a serine-kinase domain (yellow). Both DH-PH units are flanked by a SH3 domain (lime/rose). Trio and Kalirin harbor a SEC14 domain (royal blue) and spectrin-repeats (sky blue) at the N-terminus.
Thus, Rho-GEFs such as it is likely that the Mice deficient for Using biochemical and cortical actin in ArT-20 cells. Recently, a second protein Disrupted-in-Schizophrenia 1 (DISC1) is found to bind to the first part, i.e., the amino-half, of the spectrin-repeat of Trio. Interestingly, by binding of DISC1 to Trio, the N-terminal GEF domain is relieved from intramolecular inhibition and able to activate Rac1.

Neubrand and colleagues showed that Kidins220/ARMS binds to the spectrin-repeat of Trio. The authors additionally showed that Kidins220/ARMS can also bind to Kalirin. They hypothesized that Kidins220/ARMS mediates the cellular distribution of Trio in neuronal cells.

As with the SEC14 domain, the relevance of the Trio spectrin-repeat is still largely unclear, although the study of Chen and coworkers suggest that the repeats may bind to the first GEF domain and thereby preventing GTPase activation. To fully understand its working mechanism, future studies are required.

**Trio and its two GEF domains.** Trio and its close relative Kalirin are unique in that they can activate both Rac1 and RhoA with two separate GEF units within the same molecule. Since overexpression of a full-length Trio construct induces primarily Rac1- and RhoA-dependent phenotypic changes, it is likely that the activation of RhoA by the TrioD2 GEF domain is tightly regulated within the Trio molecule. Indeed, the PH-domain that is adjacent to the TrioD2 DH-domain was shown to negatively regulate RhoA activation. This auto-inhibition was shown to be relieved by binding of the Gαq-subunit of heterotrimeric G-proteins to a C-terminal extension of the PH-domain resulting in RhoA activation.

However, it remains unclear why Trio expresses two catalytic GEF domains that target small GTPases with apparent antagonistic downstream effects, i.e., RhoA and Rac1. At this point one can only speculate about their function. It is broadly accepted that Rho-GEFs determine local activity of downstream GTPases. Therefore, it may be that the activity of Rac1 and RhoA are required at the same location but not at the same time. Recently, it became clear that Rac1 and RhoA are both activated at the leading edge of a migrating cell. Using biochemical techniques for Rac1 and RhoA revealed that first RhoA and then Rac1 is activated. In such a situation, it may be efficient to have only one GEF present with two distinct GEF domains that can activate both GTPases in a spatially and temporally coordinated manner. However, future experiments are needed to prove such hypothesis.

For Kalirin, it has been reported that Kalirin7 localizes to postsynaptic densities (PSD), where it is tyrosine phosphorylated by EphB2 tyrosine kinase receptor. Although the phosphorylation does not affect the GEF activity, it does change the distribution of Kalirin and thereby changes its mode of action. The Rho-GEF Trio may be regulated in a similar way. Debant and colleagues indicated that Trio may be phosphorylated on serine and threonine residues. However, it is unclear if changes in phosphorylation status of Trio affect its distribution or activity. Another interesting feature of Kalirin is its ability to bind specifically to iNOS, preventing dimerization of iNOS, resulting in inhibition of iNOS activity. This is an example of Kalirin acting as a scaffold protein. The group of Debant showed that Trio targets Filamin in order to regulate the actin cytoskeleton. Thus, Rho-GEFs such as Trio and Kalirin cannot only act as proteins with enzymatic activity but may also be used by other proteins for correct cellular targeting.

**Trio and Neuronal Development.**
Upon the finding that UNC-73, an important regulator of axon guidance during nervous system development in C. elegans, is an ortholog of mammalian Trio, several studies followed demonstrating a role for Trio in axon guidance and neuronal development in Drosophila and mammals. Trio was shown to mediate Rac1 and/or RhoG activation during neuronal growth cone migration and axon guidance downstream of several guidance receptors, including the Netrin receptor, Notch, Sax-3/

ROBO and the NGF receptor. In addition, Trio was demonstrated to be an essential regulator of skeletal muscle development. Mice deficient for Trio died between embryonic day E15.5 and birth and showed, besides aberrant organization of the hippocampus and olfactory bulb, defects in secondary myogenesis. Trio was later demonstrated to interact with M-cadherin and to regulate myoblast fusion by mediating Rac1 activation downstream of M-cadherin engagement. These latter findings indicate that Trio may also be involved in regulating cell-cell contacts.

**Trio and Cancer.**
Seipel and coworkers have shown that the N-terminal GEF domain of Trio induces migration in 3T3 fibroblast cells and promotes anchorage-independent growth. Our recent data underscore these findings. Together with the knowledge that Rac1 is involved in transformation and tumor progression, these data suggest that the ability of Trio to activate Rac1 and induce cell migration is linked to tumor progression.

Trio was found to be highly expressed in glioblastoma, breast tumors, soft tissue sarcomas and urinary bladder tumors. In addition, Trio levels are also significantly increased in breast cancer patients with poor predictive outcome. Moreover, Sallha and coworkers showed that reduction of Trio using siRNA perturbed the migration capacity of glioblastoma cells in vitro. These studies show that the expression of endogenous Trio is increased in several types of cancer, but do not clarify if Trio activity is also hampered.

Tgt, an alternative splice variant encoding only the DH domain of the TrioD2 GEF unit of Trio, was identified in patients with adult T-cell leukemia and was demonstrated to induce cell transformation and tumor formation, indicating that also the C-terminal GEF domain of Trio may potentially regulate cancer progression.

Although Trio may potentially be an interesting target in anti-tumor therapy, it remains to be proven if increased protein levels or mutations indeed lead to changes in Trio activity.
leukocytes. Upon analysis of Trio expression in different types of leukocytes, we were unable to detect endogenous, full-length Trio protein in freshly isolated neutrophils, monocytes and naive lymphocytes (Fig. 2). Interestingly, we did detect Trio in several leukemic cell lines of both myeloid and lymphoid origin, correlating Trio expression also with leukemic cancers (Fig. 2). Trio was also detected in immature dendritic cells that were differentiated from primary monocytes (Fig. 2).

The expression of Trio in cell lines does not prove that Trio is involved in leukemia. However, it is an intriguing hypothesis that Trio expression is increased in these cell types and may promote the exchange rate on Rac1, RhoG and/or RhoA. Future studies will be required to show if Trio is a potential regulator of leukemic cell migration.

Acknowledgments

We thank Dr. P.L. Hordijk for critically reading the manuscript. J.D. van B. is supported by the Dutch Heart Foundation (grant no. 2005T039), LSBR fellowship (fellowship no. 1028) and NWO Veni grant 916.76.053. J. van R. is supported by AMC Research B.V.

References

1. Debant A, Serra-Pages C, Seipel K, O’Brien S, Tang M, Park SH, et al. The multidomain protein Trio binds the LAR transmembrane tyrosine phosphatase, contains a protein kinase domain, and has separate rac-specific and rho-specific guanine nucleotide exchange factor domains. Proc Natl Acad Sci USA 1996; 93:5466-71; PMID:8643598; http://dx.doi.org/10.1073/pnas.93.11.5466
2. Bellanger JM, Lazaro JB, Driot JL, Fernandez A, Lamb N, Debant A. The two guanine nucleotide exchange factor domains of Trio link the Rac1 and the RhoA pathways in vivo. Oncogene 1998; 16:147-52; PMID:9464532; http://dx.doi.org/10.1038/sj.onc.1210153
3. Blangy A, Vignal E, Schmidt D, Debant A, Gautier-Rouviere C, Fort P, Trio GEF1 controls Rac and Cdc42-dependent cell structures through the direct activation of rhoG. J Cell Sci 2000; 113:729-39; PMID:1074/jbc.M300277200
4. Seipel K, Medley QG, Kedersha NL, Zhang XA, O’Brien SP, Serra-Pages C, et al. Trio amino-terminal guanine nucleotide exchange factor domain expression promotes actin cytoskeleton reorganization, cell migration and anchorage-independent cell growth. J Cell Sci 1999; 112:1825-34; PMID:10341202
5. Medley QG, Serra-Pages C, Iannotti E, Seipel K, O’Brien SP, et al. The trio guanine nucleotide exchange factor is a RhoA target. Binding of RhoA to Trio requires to show if Trio is a potential regulator of leukemic cell migration.

Trio and Leukocytes

Since Trio controls spreading and migration of HeLa cells, its role in these processes may potentially also be extrapolated to other types of migratory cells, such as

**Figure 2.** Trio expression in leukocytes and leukemic cell lines. Trio (350 kDa) protein expression in primary peripheral blood mononuclear cells (PBMC; lane 1), naïve lymphocytes (lane 2), neutrophils (lane 3) and monocytes (lane 4), followed by HL60, HL60 differentiated to neutrophil-like with 1.3% (v/v) DMSO, U937 cells, Jurkat and immature dendritic cells. Approximately 100,000 cells were loaded per lane. Actin (40 kDa) was used as a control for equal sample loading.

8. Saito K, Taurzi L, Mustelin T. The lipid-binding SEC14 domain. Biochim Biophys Acta 2007; 1771: 719-26; PMID:17448729; http://dx.doi.org/10.1016/j.jbabip.2007.02.010
9. Alam MR, Caldwell BD, Johnson RC, Darlington DN, Mains RE, Eipper BA. Novel proteins that interact with the COOH-terminal cytosolic routing determinants of an integral membrane peptide-processing enzyme. J Biol Chem 1996; 271:28636-46; PMID:8910496; http://dx.doi.org/10.1074/jbc.271.45.28636
10. Alam MR, Johnson RC, Darlington DN, Hand TA, Mains RE, Eipper BA. Kalirin, a cytosolic protein with spectrin-like and GDP/GTP exchange factor-like domains that interacts with peptidylglycine alpha-amidating monooxygenase, an integral membrane peptide-processing enzyme. J Biol Chem 1997; 272: 12667-75; PMID:9139772; http://dx.doi.org/10.1074/jbc.272.25.12667
11. Colomer V, Engelender S, Sharp AH, Duan K, Cooper JK, Lanahan A, et al. Huntington-associated protein 1 (HAP1) binds to a Trio-like polypeptide, with a rac guanine nucleotide exchange factor domain. Hum Mol Genet 1997; 6:1519-25; PMID:9285789; http://dx.doi.org/10.1093/hmg/6.9.1519
12. Portales-Casamar E, Briançon-Marjollet A, Fromont S, Triboulet R, Debant A. Identification of novel neuronal isoforms of the Rho-GEF Trio. Biol Cell 2000; 112:1825-34; PMID:10341202
13. Colomer V, Engelender S, Sharp AH, Duan K, Cooper JK, Lanahan A, et al. Huntington-associated protein 1 (HAP1) binds to a Trio-like polypeptide, with a rac guanine nucleotide exchange factor domain. Hum Mol Genet 1997; 6:1519-25; PMID:9285789; http://dx.doi.org/10.1093/hmg/6.9.1519
14. Sun YJ, Nishikawa K, Yuda H, Wang YL, Osaka H, et al. Solo/Trio8, a membrane-associated protein kinase domain, and has separate rac-specific and rho-specific guanine nucleotide exchange factor domains. Proc Natl Acad Sci U S A 1996; 93:5466-71; PMID:8643598; http://dx.doi.org/10.1073/pnas.93.11.5466
15. Yoshizuka N, Morinchi R, Mori T, Yamada K, Hasegawa S, Mueda T, et al. An alternative transcript derived from the trio locus encodes a guanosine nucleotide exchange factor with mouse cell-transforming potential. J Biol Chem 2004; 279:43998-4004; PMID:15308664; http://dx.doi.org/10.1074/jbc.M406082200
16. van Rijssel J, Hooogboezem M, Wester L, Hordijk PL, Van Buul JD. The N-terminal DH-PH domain of Trio induces cell spreading and migration by regulating lamellipodia dynamics in a Rac1-dependent fashion. PLoS One 2012; 7:e29912; PMID:22238672; http://dx.doi.org/10.1371/journal.pone.0029912
17. Abe K, Whitehead IP, O’Bryan JP, Der CJ. Involvement of NH2-terminal sequences in the negative regulation of Vav signaling and transforming activity. J Biol Chem 1999; 274:30410-8; PMID:10521148; http://dx.doi.org/10.1074/jbc.274.43.30410
18. Vanderzalm PJ, Pandey A, Hurwitz ME, Bloom L, Hurwitz HR, Garriga G. C. elegans CARMIL negatively regulates UNC-73/Trio function during neuronal development. Development 2009; 136: 1201-10; PMID:19244282; http://dx.doi.org/10.1242/dev.026666
19. Lee CS, Choi CK, Shin EY, Schwartz MA, Kim EG. Myosin II directly binds and inhibits Dbl family guanine nucleotide exchange factors: a possible link to Rho family GTPases. J Cell Biol 2010; 190:663-74; PMID:20713598; http://dx.doi.org/10.1083/jcb.201003059
20. Yano T, Yamazaki Y, Adachi M, Okawa K, Fort P, Uji M, et al. Tars up-regulates E-cadherin transcription by binding to the Trio RhoGEP and inhibiting Rac signaling. J Cell Biol 2011; 193:319-32; PMID:21482718; http://dx.doi.org/10.1083/jcb.201009100
21. Yano T, Yamazaki Y, Adachi M, Okawa K, Fort P, Uji M, et al. Tars up-regulates E-cadherin transcription by binding to the Trio RhoGEP and inhibiting Rac signaling. J Cell Biol 2011; 193:319-32; PMID:21482718; http://dx.doi.org/10.1083/jcb.201009100
21. Neubrand VE, Thomas C, Schmidt S, Deban A, Schiavo G. Kidins220/ARMS regulates Rac1-dependent neurite outgrowth by direct interaction with the RhoGEF Trio. J Cell Sci 2010; 123:2111-23; PMID: 20519585; http://dx.doi.org/10.1242/jcs.064095

22. Bellanger JM, Astier C, Sardet C, Ohya Y, Stossel TP, Deban A. The Rac1- and RhoG-specific GEF domain of the Abelson tyrosine kinase remodels cytosolic actin. Nat Cell Biol 2000; 2:888-92; PMID: 11416652; http://dx.doi.org/10.1038/35046533

23. Bellanger JM, Estrach S, Schmidt S, Briançon-Marjollet A, Zaugani O, Fromont S, et al. Different regulation of the Trio Dbl-Homology domains by their associated PH domains. Biol Cell 2003; 95:625-34; PMID:14720465; http://dx.doi.org/10.1042/jbc.2003.10.002

24. Ohta Y, Suzuki N, Nakamura S, Hartwig JH, Stossel TP. The small GTPase RaLa targets filamentin to induce filopodia. Proc Natl Acad Sci U S A 1999; 96:2122-8; PMID:105051605; http://dx.doi.org/10.1073/pnas.96.5.2122

25. Onj Y, Choi JS, Lee JY, Yu KR, Kh Sh, Cho Y, et al. Filamin B serves as a molecular scaffold for type I interferon-induced c-Jun NH2-terminal kinase signaling pathway. Mol Cell Biol 2008; 28:1516-30; PMID:18812578; http://dx.doi.org/10.1091/mbc.E08-05-0576

26. Délle-Pérez B, Martínez VG, Lacaça-Salavert C, Figuetas A, Shapiro SS, Takatufu T, et al. Filamin B plays a key role in vascular endothelial growth factor-induced endothelial cell motility through its interaction with Rac-1 and Vav-2. J Biol Chem 2002; 277:10727-32; PMID:11846571; http://dx.doi.org/10.1074/jbc.M002325420

27. Rossman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide exchange factors. Nat Rev Mol Cell Biol 2005; 6:167-75; http://dx.doi.org/10.1038/nrm1578

28. Aravind L, Neuwald AF, Ponting CP. Sec14p-like superfamily and mechanisms for crosstalk between lipid metabolism and lipid signaling. Trends Biochem Sci 2000; 25:773-782; PMID:10768665; http://dx.doi.org/10.1016/s0968-9504(00)81406-3

29. Costenko EV, Mahon GM, Cheng L, Whitehead IP. The Sec14 superfamily: a structural platform for cytoskeletal dynamics visualized in living cells. Nat Rev Mol Cell Biol 2011; 12:749-56; PMID:21248344; http://dx.doi.org/10.1038/nrm3121

30. Kanekura K, Aravind L, Ponting CP. A cell active chemical GEF inhibitor selectively targets the Trio/Rho/Galphaq Rac1 signaling pathway. Chem Biol 2009; 16:587-60; PMID:19549603; http://dx.doi.org/10.1016/j.chembiol.2009.04.012

31. Schiavo G. Kidins220/ARMS regulates Rac1-dependant neurite outgrowth by direct interaction with the Galphaq-specific GEF domain of the Abelson tyrosine kinase. Mol Biol Cell 2000; 11:148-60; PMID:15746383; http://dx.doi.org/10.1016/S1073-8584(00)2271290

32. Eisenberg D, Gallo M, Robert C, Segal RA, et al. Differentiation and translation of the Rac GTPase is required for cell and growth cone migration in C. elegans. Cell 1999; 97:593-9; PMID:10378580; http://dx.doi.org/10.1016/s0092-8674(00)01413-5

33. Steven R, Kubiseski TJ, Zheng H, Kulkarni S, et al. The Abelson tyrosine kinase, the Trio GEF and the GEF1 domain of Trio. Adv Cancer Res 2002; 84:329-72; PMID:11833522; http://dx.doi.org/10.1016/S0065-230X(02)40003-9

34. Sillitio B, Tran NL, Chan A, Wolf A, Nakada M, Rutka JT, et al. The guanine nucleotide exchange factors Trio, Ezrin and Vav1 mediate the invasive behavior of glioblastoma. Am J Pathol 2008; 173:1828-38; PMID:19083876; http://dx.doi.org/10.2353/ajpath.2008.080043

35. Alam MR, Stevenson TC, Johnson RC, Back N, Abraham B, Mains RE, et al. Signaling mediated by the cytosolic domain of peptide-dehydrogenase alpha-amidating monooxygenase. Mol Biol Cell 2001; 12:629-44; PMID:11251076

36. Chen SY, Huang PH, Cheng HJ. Disrupted-in-Schizophrenia 1-mediated axon guidance involves TRIO-RAC-PAK small GTPase pathway signaling. Proc Natl Acad Sci U S A 2011; 108:5861-6; PMID:21422296; http://dx.doi.org/10.1073/pnas.1018218108

37. Estrach S, Schmidt S, Döring S, Penna A, Blangy A, Fort P, et al. The Human Rho-GEF trio and its target GTPase RhoG are involved in the NGF pathway, leading to neurite-outgrowth. Curr Biol 2002; 12:307-12; PMID:11846571; http://dx.doi.org/10.1016/s0960-9822(02)00658-9

38. Rojas RJ, Yeho ME, Gerishiub G, Kawano T, Kozasa T, Sendhe J. Galpahq directly activates p63RhoG and Troio via a conserved extension of the Dbl homology-associated pleckstrin homology domain. J Biol Chem 2007; 282:29201-10; PMID:17606614; http://dx.doi.org/10.1074/jbc.M704582200

39. Williams SL, Luz S, Charlie NK, Vettel C, Ailion M, Coccio C, et al. Trio’s Rho-specific GEF Domain is the missing Galpah q effector in C. elegans. Genes Dev 2002; 16:2731-46; PMID:17942708; http://dx.doi.org/10.1101/gad.1521907

40. Kraynov VS, Chamberlain C, Bokoch GM, Schwartz MA, Slabbaugh S, Hahn KM. Localized Rac activation and Trio in vivo Rac activity during myoblast fusion. Mol Biol Cell 2007; 28:2314-23; PMID:18212043; http://dx.doi.org/10.1128/MCB.00998-07

41. Peng YJ, He WQ, Tang J, Tao T, Chen C, Gao YQ, et al. Trio is a key guanine nucleotide exchange factor coordinately regulating the migration and morphogenesis of granule cells in the developing cerebellum. J Biol Chem 2010; 285:24834-44; PMID:20516607; http://dx.doi.org/10.1074/jbc.M109.096537

42. Varamshlay LL, De Martes C, Gineger E. Molecular separation of two signaling pathways for the receptor. Notch. Dev Biol 2008; 313:556-57; PMID:18062953; http://dx.doi.org/10.1016/j.ydbio.2007.10.030

43. Song JK, Gineger E. Noncanonical notch function in motor axon guidance is mediated by Rac1 GTPase and the GEF1 domain of Trio. Dev Dyn 2011; 240:324-32; PMID:21246649; http://dx.doi.org/10.1002/dvdy.22525

44. Charrasse S, Comunale F, Dorlet P, Portales-Casamar R, Deban A, Gauthier-Rouvire C, Gindre I, Orbi M, Fromont S, et al. Trio mediates neratin-1-induced Rac1 activation in axon outgrowth and guidance. Mol Cell Biol 2008; 28:2314-23; PMID:18212043; http://dx.doi.org/10.1128/MCB.00998-07

45. Rabiner CA, Mains RE, Eipper BA. Kalirin: a dual Rho GTPase exchange factor trio mediates axonal extension. Neuron 2000; 26:119-31; PMID:10798997; http://dx.doi.org/10.1016/s0896-6273(00)50125-7

46. Liebl EC, Forshoeof DL, France LS, Sample SH, Hess JE, Cowger JA, et al. Dosage-sensitive, reciprocal genetic interactions between the Ab class tyrosine kinase and the putative GEF trio reveal trio’s role in axon pathfinding. Neuron 2008; 26:1670-1718; PMID:17978836; http://dx.doi.org/10.1016/j.neuron.2008.02.027

47. Schiavo G. Kidins220/ARMS regulates Rac1-dependant neurite outgrowth by direct interaction with the Galphaq-specific GEF domain of the Abelson tyrosine kinase. Mol Biol Cell 2000; 11:148-60; PMID:15746383; http://dx.doi.org/10.1016/j.cell.2009.04.012
62. Lane J, Martin TA, Mansel RE, Jiang WG. The expression and prognostic value of the guanine nucleotide exchange factors (GEFs) Trio, Vav1 and Tiam-1 in human breast cancer. Int Semin Surg Oncol 2008; 5:23; PMID:18925966; http://dx.doi.org/10.1186/1477-7800-5-23

63. Sosa MS, Lopez-Haber C, Yang C, Wang H, Lemmon MA, Busillo JM, et al. Identification of the Rac-GEF PRex1 as an essential mediator of ErbB signaling in breast cancer. Mol Cell 2010; 40:877-92; PMID:21172654; http://dx.doi.org/10.1016/j.molcel.2010.11.029

64. Adamowicz M, Radwimmer B, Rieker RJ, Mertens D, Schwarzbach M, Schraml P, et al. Frequent amplifications and abundant expression of TRIO, NRD2, and IRX2 in soft tissue sarcomas. Genes Chromosomes Cancer 2006; 45:829-38; PMID:16752383; http://dx.doi.org/10.1002/gcc.20343

65. Zheng M, Simon R, Mirlacher M, Maurer R, Gasser T, Forster T, et al. TRIO amplification and abundant mRNA expression is associated with invasive tumor growth and rapid tumor cell proliferation in urinary bladder cancer. Am J Pathol 2004; 165:63-9; PMID:15215162; http://dx.doi.org/10.1016/S0002-9440(10)6275-0

66. Yamada K, Moriguchi R, Mori T, Okazaki E, Kohno T, Nagayasu T, et al. Tgat, a Rho-specific guanine nucleotide exchange factor, activates NF-kappaB via physical association with IkappaB kinase complexes. Biochem Biophys Res Commun 2007; 355:269-74; PMID:17292329; http://dx.doi.org/10.1016/j.bbrc.2007.01.147

67. Bouquier N, Fromont S, Zezh JC, Auziol C, Larrousse P, Robert B, et al. Aptamer-derived peptides as potent inhibitors of the oncogenic RhoGEF Tgat. Chem Biol 2009; 16:391-400; PMID:19389625; http://dx.doi.org/10.1016/j.chembiol.2009.02.006