Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics*, 18, 142.

Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics*, 33, 3461-3467.

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res*, 46, D649-D655.

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology*, 14, e1005968.

Reactome database release: 82

This document contains 9 pathways and 12 reactions (see Table of Contents)
The Roundabout (ROBO) family encodes transmembrane receptors that regulate axonal guidance and cell migration. There are four human Robo homologues, ROBO1, ROBO2, ROBO3 and ROBO4. Most of the ROBOs have the similar ectodomain architecture as the cell adhesion molecules, with five Ig domains followed by three FN3 repeats, except for ROBO4. ROBO4 has two Ig and two FN3 repeats. The cytoplasmic domains of ROBO receptors are in general poorly conserved. However, there are four short conserved cytoplasmic sequence motifs, named CC0-3, that serve as binding sites for adaptor proteins. The ligands for the human ROBO1 and ROBO2 receptors are the three SLIT proteins SLIT1, SLIT2, and SLIT3; all of the SLIT proteins contain a tandem of four LRR (leucine rich repeat) domains at the N-terminus, termed D1-D4, followed by six EGF (epidermal growth factor)-like domains, a laminin G like domain (ALPS), three EGF-like domains, and a C-terminal cysteine knot domain. Most SLIT proteins are cleaved within the EGF-like region by unknown proteases (reviewed by Hohenster 2008, Ypsilanti and Chedotal 2014, Blockus and Chedotal 2016). NELL2 is a ligand for ROBO3 (Jaworski et al. 2015).

SLIT protein binding modulates ROBO interactions with the cytosolic adaptors. The cytoplasmic domain of ROBO1 and ROBO2 determines the repulsive responses of these receptors. Based on the studies from both invertebrate and vertebrate organisms it has been inferred that ROBO induces growth cone repulsion by controlling cytoskeletal dynamics via either Abelson kinase (ABL) and Enabled (Ena), or RAC1 activity (reviewed by Hohenster 2008, Ypsilanti and Chedotal 2014, Blockus and Chedotal 2016). While there is some redundancy in the function of ROBO receptors, ROBO1 is implicated as the predominant receptor for axon guidance in ventral tracts, and ROBO2 is the predominant receptor for axon guidance in dorsal tracts. ROBO2 also repels neuron cell bodies from the floor plate (Kim et al. 2011).

In addition to regulating axon guidance, ROBO1 and ROBO2 receptors are also implicated in regulation of proliferation and transition of primary to intermediate neuronal progenitors through a poorly characterized cross-talk with NOTCH-mediated activation of HES1 transcription (Borrell et al. 2012).

Thalamocortical axon extension is regulated by neuronal activity-dependent transcriptional regulation of ROBO1 transcription. Lower neuronal activity correlates with increased ROBO1 transcription, possibly
mediated by the NFκB complex (Mire et al. 2012).

It is suggested that the homeodomain transcription factor NKX2.9 stimulates transcription of ROBO2, which is involved in regulation of motor axon exit from the vertebrate spinal code (Bravo-Ambrosio et al. 2012).

Of the four ROBO proteins, ROBO4 is not involved in neuronal system development but is, instead, involved in angiogenesis. The interaction of ROBO4 with SLIT3 is involved in proliferation, motility and chemotaxis of endothelial cells, and accelerates formation of blood vessels (Zhang et al. 2009).

**Literature references**

Blockus, H., Chédotal, A. (2016). Slit-Robo signaling. *Development, 143*, 3037-44.

Hohenester, E. (2008). Structural insight into Slit-Robo signalling. *Biochem Soc Trans, 36*, 251-6.

Geng, JG., Dietrich, UM., Wang, L., Bicknell, R., Esko, JD., Zhang, B. (2009). Repulsive axon guidance molecule Slit3 is a novel angiogenic factor. *Blood, 114*, 4300-9.

Jaworski, A., Tong, RK., Gildea, HK., Tom, I., Gonzalez, LC., Koch, AW. et al. (2015). Operational redundancy in axon guidance through the multifunctional receptor Robo3 and its ligand NELL2. *Science, 350*, 961-5.

Ypsilanti, AR., Chédotal, A. (2014). Roundabout receptors. *Adv Neurobiol, 8*, 133-64.

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| Date       | Action         | Author         |
|------------|----------------|----------------|
| 2008-09-05 | Authored, Edited | Garapati, P V. |
| 2009-08-18 | Reviewed       | Kidd, T.       |
| 2017-06-23 | Edited, Revised | Orlic-Milacic, M. |
| 2017-07-31 | Reviewed       | Jaworski, A.   |
| 2017-08-04 | Edited         | Orlic-Milacic, M. |
Regulation of expression of SLITs and ROBOs

Location: Signaling by ROBO receptors

Stable identifier: R-HSA-9010553

Expression of SLIT and ROBO proteins is regulated at the level of transcription, translation and protein localization and stability. LIM-homeodomain transcription factors LHX2, LHX3, LHX4, LHX9 and ISL1 have so far been implicated in a cell type-dependent transcriptional regulation of ROBO1, ROBO2, ROBO3 and SLIT2 (Wilson et al. 2008, Marcos-Mondejar et al. 2012, Kim et al. 2016). Homeobox transcription factor HOXA2 is involved in transcriptional regulation of ROBO2 (Geisen et al. 2008). Transcription of SLIT1 during optic tract development in Xenopus is stimulated by FGF signaling and may also involve the transcription factor HOXA2, but the mechanism has not been established (Atkinson-Leadbeater et al. 2010). PAX6 and the homeodomain transcription factor NNX2.2 are also implicated in regulation of SLIT1 transcription (Genethliou et al. 2009). An RNA binding protein, MSI1, binds ROBO3 mRNA and promotes its translation, thus increasing ROBO3 protein levels (Kuwako et al. 2010). A poorly studied E3 ubiquitin ligase ZSWIM8 promotes degradation of ROBO3 (Wang et al. 2013). ROBO1 protein half-life is increased via deubiquitination of ROBO1 by a ubiquitin protease USP33 (Yuasa-Kawada et al. 2009, Huang et al. 2015). Interaction of SLIT2 with DAG1 (dystroglycan) is important for proper localization of SLIT2 at the floor plate (Wright et al. 2012). Interaction of SLIT1 with a type IV collagen COL4A5 is important for localization of SLIT1 to the basement membrane of the optical tectum (Xiao et al. 2011).

Literature references

Rao, Y., Kinoshita-Kawada, M., Wu, JY., Yuasa-Kawada, J. (2009). Deubiquitinating enzyme USP33/VDU1 is required for Slit signaling in inhibiting breast cancer cell migration. Proc. Natl. Acad. Sci. U.S.A., 106, 14530-5.

Ma, L., Leung, H., Lyon, KA., Leahy, DJ., Wright, KM., Ginty, DD. (2012). Dystroglycan organizes axon guidance cue localization and axonal pathfinding. Neuron, 76, 931-44.

Brunet, JF., Rijli, FM., Pasqualetti, M., Geisen, MJ., Chédotal, A., Di Meglio, T. et al. (2008). Hox paralog group 2 genes control the migration of mouse pontine neurons through slit-robo signaling. PLoS Biol., 6, e142.

Dodd, J., Shafer, B., Lee, KJ., Wilson, SI. (2008). A molecular program for contralateral trajectory: Rig-1 control by LIM homeodomain transcription factors. Neuron, 59, 413-24.

Zhu, L., Wen, P., Liu, J., Kong, R., Wu, JY., Chen, X. et al. (2015). USP33 mediates Slit-Robo signaling in inhibiting colorectal cancer cell migration. Int. J. Cancer, 136, 1792-802.

https://reactome.org
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| 2017-06-27 | Authored| Orlic-Milacic, M. |
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The full length SLIT proteins are secreted and, when not bound to ROBO receptors, are indirectly associated with the plasma membrane via the extracellular matrix proteins. These full length SLITs undergo posttranslational modification and proteolytic processing to generate an N-terminal fragment (SLIT2-N) and a corresponding C-terminal fragment (SLIT2-C). SLIT2 is cleaved within the EGF repeats, between EGF5 and EGF6, by unknown proteases. Cleavage of SLIT proteins is evolutionarily conserved, although the molecular biological significance is unknown. The N-terminal fragment of SLIT2 stimulates growth and branching of dorsal root ganglia (DRG) axons, and this activity is opposed by un-cleaved SLIT. The stimulation of axon branching is mediated by ROBO receptors. Additional functional differences between the full-length and N-terminal forms have been discovered in their abilities to repel different populations of axons and dendrites. Finally, SLIT can attract migrating muscles in the fly, and also human endothelial cells, both via ROBO receptors (Brose et al. 1999, Wang et al. 1999).

SLIT C-terminal fragments may transduce signaling independently of ROBO receptors and Neuropilins (semaphorin receptors) by directly binding to Plexin A1 (Delloye-Bourgeois et al. 2015).

Followed by: Glypican-1 (GPC1) binds SLIT2

Literature references

Henzel, W., Brose, K., Arnott, D., Goodman, CS., Kidd, T., Wang, KH. et al. (1999). Biochemical purification of a mammalian slit protein as a positive regulator of sensory axon elongation and branching. Cell, 96, 771-84.

Henzel, W., Brose, K., Bland, KS., Arnott, D., Goodman, CS., Kidd, T. et al. (1999). Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell, 96, 795-806.
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| Date       | Action     | Author/Editor       |
|------------|------------|---------------------|
| 2008-09-05 | Authored, Edited | Garapati, P V.     |
| 2009-08-18 | Reviewed   | Kidd, T.            |
| 2017-06-23 | Edited     | Orlic-Milacic, M.   |
| 2017-07-31 | Reviewed   | Jaworski, A.        |
SLIT2 and both its natural cleavage products bind glypican-1 (GPC1), a glycosyl phosphatidyl inositol (GPI) anchored heparan sulfate proteoglycan (HSPG), through its C-terminus. Besides glypican-1, other HSPGs may also be involved in SLIT2 binding. GPC1:HSPG is important for high affinity binding of SLIT to its receptor and for the repulsive activity of SLIT. SLIT-ROBO signaling strictly requires binding to heparan sulfate. HSPGs may also modulate the extracellular distribution or stability of SLIT proteins (Ronca et al. 2001, Zhang et al. 2004).

**Preceded by:** Proteolytic processing of SLIT

**Literature references**

Andersen, JS., Margolis, RU., Paech, V., Ronca, F. (2001). Characterization of Slit protein interactions with glypican-1. *J Biol Chem*, 276, 29141-7.

Zhang, F., Linhardt, RJ., Margolis, RU., Ronca, F. (2004). Structural determinants of heparan sulfate interactions with Slit proteins. *Biochem Biophys Res Commun*, 317, 352-7.

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| 2017-06-23 | Edited            | Orlc-Milacic, M.      |
| 2017-07-31 | Reviewed          | Jaworski, A.          |
SLITs bind keratan sulfate

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-9010815

**Type:** binding

**Compartments:** plasma membrane, extracellular region

SLIT2 is expressed by corneal epithelial cells and able to bind to keratan sulfate, which is part of the corneal stroma extracellular matrix. This interaction may influence corneal nerve growth cone penetration. SLIT3 may also interact with keratan sulfate, as well as ROBO receptors ROBO1 and ROBO2 (Conrad et al. 2010).

**Literature references**

Zhang, Y., Conrad, AH., Conrad, GW., Tasheva, ES. (2010). Proteomic analysis of potential keratan sulfate, chondroitin sulfate A, and hyaluronic acid molecular interactions. *Invest. Ophthalmol. Vis. Sci.*, 51, 4500-15.

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ROBOs bind keratan sulfate

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-9014812

**Type:** binding

**Compartments:** plasma membrane, extracellular region

ROBO receptors ROBO1 and ROBO2 interact with keratan sulfate through their extracellular regions (Conrad et al. 2010).

**Literature references**

Zhang, Y., Conrad, AH., Conrad, GW., Tasheva, ES. (2010). Proteomic analysis of potential keratan sulfate, chondroitin sulfate A, and hyaluronic acid molecular interactions. *Invest. Ophthalmol. Vis. Sci.*, 51, 4500-15.

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SLIT2 ligand forms a complex with the ROBO1 receptor (Brose et al. 1999). The SLIT family consists of three members that are all expressed in the ventral midline (floor plate) of the neural tube. SLIT1 is predominantly expressed in the nervous system whereas SLIT2 and SLIT3 are also expressed outside the nervous system.

SLIT proteins are the ligands for the ROBO receptors. In humans, there are four ROBO genes: ROBO1, ROBO2, ROBO3 and ROBO4. The extracellular domain of ROBO comprises five Ig domains and three FN domains except for ROBO4 (two Ig + two FN). Ig1 and Ig2 domains of ROBO1 and ROBO2 are highly conserved and are important for SLIT binding. The concave face of SLIT's second LRR domain accommodates the Ig1 and Ig2 domains of ROBO1 and ROBO2. ROBO3 does not bind SLITs (Camurri et al. 2005, Mambetisaaeva et al. 2005, Zelina et al. 2014, Jaworski et al. 2015). SLIT binding with ROBO4 is controversial as the interaction is weak and it has been observed using the in-vitro methods (Wang et al. 1999, Brose et al. 1999, Piper et al. 2003, Andrews et al. 2007).

Binding of secreted (cleaved) SLIT2 to ROBO1 and ROBO2 is involved in fasciculation (bundling) of motor axons, which facilitates axon pathfinding and muscle innervation (Jaworski and Tessier-Lavigne 2012).

Followed by: SLIT2:ROBO1 binds CXCR4, Ena/VASP proteins bind ROBO1:SLIT2 complex

Literature references

Henzel, W., Brose, K., Arnott, D., Goodman, CS., Kidd, T., Wang, KH. et al. (1999). Biochemical purification of a mammalian slit protein as a positive regulator of sensory axon elongation and branching. Cell, 96, 771-84.

Henzel, W., Brose, K., Bland, KS., Arnott, D., Goodman, CS., Kidd, T. et al. (1999). Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell, 96, 795-806.

Piper, M., Little, M. (2003). Movement through Slits: cellular migration via the Slit family. Bioessays, 25, 32-8.

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ROBO2 binds SLIT2

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-9010898

**Type:** binding

**Compartments:** plasma membrane

**Inferred from:** Robo2 binds SLIT2 (Homo sapiens)

ROBO2 receptor binds to SLIT2 ligand (Brose et al. 1999, Nguyen Ba-Charvet et al. 2001).

**Editions**

| Date       | Action               | Author            |
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| 2017-06-26 | Authored, Edited     | Orlic-Milacic, M. |
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Based on studies in mice, SLIT3 can bind to both ROBO1 and ROBO2 receptors (Mommersteeg et al. 2013). The interaction between SLIT3 and ROBO2 contributes to targeting of axons of olfactory sensory neurons (Cho et al. 2012).

**Editions**

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| 2017-06-26 | Authored, Edited | Orlic-Milacic, M. |
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Commissural axons project to the floor plate, attracted by the interaction of their DCC receptors with Netrin-1 (NTN1) produced by floor plate cells (Dickson and Gilestro 2006) and radial glia (Dominici et al. 2017, Varadarajan et al. 2017). Once an axon enters the floor plate, it must be efficiently expelled on the contralateral side. A switch from attraction to repulsion allows commissural axons to enter and then leave the CNS midline. Based on studies in Xenopus neurons and by yeast two hybrid screens, it is observed that the attractive response of axons to netrins is silenced by activation of ROBO. SLIT bound ROBO binds to DCC, preventing it from transducing an attractive response to netrin. The sensitivity of axons to the repulsive action of SLIT does not only depend on repulsive SLIT receptors (ROBO1 and ROBO2), but is also influenced by expression of ROBO3, a SLIT receptor that suppresses the activity of ROBO1 and ROBO2. Upon crossing the midline, commissural axons downregulate expression of ROBO3 and increase expression of ROBO1/ROBO2 (reviewed by Dickson and Gilestro, 2006). Two transcript variants of ROBO3, ROBO3.1 and ROBO3.2 are considered to play different roles in midline crossing. ROBO3.1 is expressed in the pre-crossing and crossing commissural axons, while ROBO3.2, generated by alternative splicing, is expressed after midline crossing and thought to block midline re-crossing (Chen et al. 2008). In addition to SLITs, a secreted ligand NELL2 also acts as an axonal guidance cue that, by acting through ROBO3 receptors, helps to steer commissural axons to the midline. Both ROBO3.1 and ROBO3.2 can bind to a secreted ligand NELL2. Pre-crossing commissural axons, which express ROBO3.1, are repelled by NELL2. Post-crossing axons, which express ROBO3.2 are not repelled by NELL2 (Jaworski et al. 2015).

**Literature references**

Varadarajan, SG., Phan, KD., Kania, A., Novitch, BG., Kong, JH., Panaitof, SC. et al. (2017). Netrin1 Produced by Neural Progenitors, Not Floor Plate Cells, Is Required for Axon Guidance in the Spinal Cord. *Neuron*, 94, 790-799.e3.

Gilestro, GF., Dickson, BJ. (2006). Regulation of commissural axon pathfinding by slit and its Robo receptors. *Annu Rev Cell Dev Biol*, 22, 651-75.

Puiggros, SR., Rama, N., Rappeneau, Q., Moreno-Bravo, JA., Mehlen, P., Dominici, C. et al. (2017). Floor-plate-derived netrin-1 is dispensable for commissural axon guidance. *Nature*, 545, 350-354.
Jaworski, A., Tong, RK., Gildea, HK., Tom, I., Gonzalez, LC., Koch, AW. et al. (2015). Operational redundancy in axon guidance through the multifunctional receptor Robo3 and its ligand NELL2. *Science, 350*, 961-5.

Gore, BB., Ma, L., Long, H., Tessier-Lavigne, M., Chen, Z. (2008). Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. *Neuron, 58*, 325-32.

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Besides being involved in axon repulsion during neuronal system development, SLIT-ROBO signaling is also involved in dendrite branching. Based on studies in mice, SLIT1 triggers cortical dendrite branching by activating receptors ROBO1 and/or ROBO2. ROBO effector NCK2 is needed for SLIT1-mediated dendrite branching (Round and Sun 2011).

**Literature references**

Sun, H., Round, JE. (2011). The adaptor protein Nck2 mediates Slit1-induced changes in cortical neuron morphology. *Mol. Cell. Neurosci.*, 47, 265-73.

**Editions**

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Orlic-Milacic, M. Jaworski, A.
Ena/VASP proteins bind ROBO1:SLIT2 complex

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-376140

**Type:** binding

**Compartments:** plasma membrane, cytosol

**Inferred from:** Ena/VASP binds Robo1:Slit2 complex (Drosophila melanogaster)

Ena/VASP proteins (ENAH, EVL and VASP) are required in part for ROBO's repulsive output. Ena/VASP proteins are drawn as effectors downstream of Robo signaling via a direct interaction with ROBO. ROBO's CC2 (LPPPP) motif is the consensus binding site for the EVH1 domain of Ena/VASP proteins.

The Ena/VASP family of proteins has a universal role in control of cell motility and actin dynamics. These proteins consist of an N-terminal EVH1 domain, a central proline rich region, which acts as a ligand for the actin monomer binding protein Profilin (PFN), as well as several SH3 domain-containing proteins, such as ABL, and a C-terminal EVH2 domain involved in oligomerization and F-actin binding (Bashaw et al. 2000).

**Preceded by:** ROBO1 binds SLIT2

**Followed by:** Recruitment of Profilin by Ena/VASP proteins

**Editions**

| Date       | Action       | Author/Editor               |
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| 2008-09-05 | Authored, Edited | Garapatii, P V.           |
| 2009-08-18 | Reviewed     | Kidd, T.                   |
| 2017-06-26 | Edited       | Orlic-Milacic, M.          |
| 2017-07-31 | Reviewed     | Jaworski, A.               |
Recruitment of Profilin by Ena/VASP proteins

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-428534

**Type:** binding

**Compartments:** plasma membrane, cytosol

Ena/VASP proteins (ENAH, EVL1 and VASP) enhance actin filament elongation via the recruitment of profilin:actin complexes to the tips of spreading lamellipodia. Profilin (PFN1 or PFN2) binds to the central proline rich domain of an Ena/VASP protein (Bashaw et al. 2000).

**Preceded by:** Ena/VASP proteins bind ROBO1:SLIT2 complex

**Literature references**

Pawson, T., Bashaw, GJ., Goodman, CS., Kidd, T., Murray, D. (2000). Repulsive axon guidance: Abelson and Enabled play opposing roles downstream of the roundabout receptor. *Cell, 101*, 703-15.

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| Edition     | Action          | Author(s)          |
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| 2008-09-05  | Authored, Edited| Garapati, P V.     |
| 2009-08-18  | Reviewed        | Kidd, T.           |
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| 2017-07-31  | Reviewed        | Jaworski, A.       |
Rho family GTPases, including RAC1, RHOA, and CDC42, are ideal candidates to regulate aspects of cytoskeletal dynamics downstream of axon guidance receptors. Biochemical and genetic studies have revealed an important role for CDC42 and RAC1 in ROBO repulsion. ROBO controls the activity of Rho GTPases by interacting with a family of SLIT/ROBO-specific GAPs (SrGAPs) and Vilse/CrossGAP. SrGAPs inactivate CDC42 and Vilse/CrossGAP specifically inactivates RAC1.

It was recently implicated that SRGAP3 may inactivate RAC1 downstream of SLIT1-activated ROBO2, which promotes neurite outgrowth in mammalian dorsal root ganglion (DRG) neurons (Zhang et al. 2014).

**Editions**

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| 2008-09-05 | Authored, Edited| Garapati, P V.    |
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| 2017-06-26 | Edited          | Orlic-Milacic, M. |
| 2017-07-31 | Reviewed        | Jaworski, A.      |
A low level of RAC1 activity is essential to maintain axon outgrowth. ROBO activation recruits SOS, a dual specificity GEF, to the plasma membrane via Dock homolog NCK (NCK1 or NCK2) to activate RAC1 during midline repulsion.

**Editions**

| Date       | Action     | Author/Editor |
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| 2008-09-05 | Authored, Edited | Garapati, P V. |
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| 2017-06-26 | Edited     | Orlic-Milacic, M. |
| 2017-07-31 | Reviewed   | Jaworski, A.  |
Role of ABL in ROBO-SLIT signaling

Location: Signaling by ROBO receptors

Stable identifier: R-HSA-428890

Compartments: plasma membrane

ABL (ABL1 or ABL2) plays a dual role in the ROBO pathway. As a key enzymatic component in the signaling pathway, ABL supports repellent signaling (by recruiting the necessary actin binding proteins) and also feeds back on the receptor (by down regulating through phosphorylation) to adjust the sensitivity of the pathway.

ABL cooperates with multiple effectors, including the actin binding protein Capulet (Capt) and Orbit/MAST/CLASP, suggesting that ABL simultaneously coordinates the dynamics of two major cytoskeletal systems to achieve growth cone repellent guidance.

Literature references

Kalil, K., Dent, EW. (2004). Hot +TIPS: guidance cues signal directly to microtubules. Neuron, 42, 877-9.

Rusch, J., Engel, U., Lee, H., Sheard, K., Van Vactor, D., Scherrer, S. (2004). The microtubule plus end tracking protein Orbit/MAST/CLASP acts downstream of the tyrosine kinase Abl in mediating axon guidance. Neuron, 42, 913-26.

Rusch, J., Wills, Z., Baum, B., Van Vactor, D., Bikoff, J., Perrimon, N. et al. (2002). A Drosophila homolog of cyclase-associated proteins collaborates with the Abl tyrosine kinase to control midline axon pathfinding. Neuron, 36, 611-22.

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| 2008-09-05 | Authored, Edited | Garapati, P V. |
| 2009-08-18 | Reviewed | Kidd, T.          |
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ROBO1 receptor, activated by SLIT2, binds to MYO9B and inhibits its RHOA GAP activity. SLIT2-ROBO1 signaling thus results in increased RHOA activity, which is thought to negatively regulate invasiveness of lung cancer cells (Kong et al. 2015). ROCK-mediated signaling and phosphorylation of the myosin regulatory light chain (MLRC) downstream of activated RHOA is needed for SLIT-mediated axon pathfinding in cranial motor neurons (Murray et al. 2010).

**Literature references**

Nie, Y., Li, X., Zhu, L., Fan, D., Wen, P., Liu, J. et al. (2015). Myo9b is a key player in SLIT/ROBO-mediated lung tumor suppression. *J. Clin. Invest.*, 125, 4407-20.

**Editions**

| Date       | Actions         | Author(s)       |
|------------|-----------------|-----------------|
| 2017-06-26 | Authored, Edited| Orlic-Milacic, M.|
| 2017-07-31 | Reviewed        | Jaworski, A.    |
SLIT2:ROBO1 binds CXCR4

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-8986258

**Type:** binding

**Compartments:** plasma membrane

SLIT2-activated ROBO1 receptor can form a complex with a G-protein coupled receptor (GPCR) CXCR4 (Prasad et al. 2007), resulting in downregulation of CXCR4 signaling (Prasad et al. 2004, Prasad et al. 2007). Formation of the complex between ROBO1 and CXCR4, which involves the CC3 motif of ROBO1, does not interfere with CXCR4 binding to its ligand, CXCL12 (Prasad et al. 2007). SLIT-ROBO signaling may also downregulate CXCR4 expression (Marlow et al. 2008). Downregulation of CXCL12-CXCR4 signaling is thought to contribute to SLIT-ROBO-mediated inhibition of cell migration (Prasad et al. 2004, Prasad et al. 2007, Marlow et al. 2008).

**Preceded by:** ROBO1 binds SLIT2

**Literature references**

Ganju, RK., Wu, J., Qamri, Z., Prasad, A. (2007). Slit-2/Robo-1 modulates the CXCL12/CXCR4-induced chemotaxis of T cells. *J. Leukoc. Biol.*, 82, 465-76.

**Editions**

| Edition   | Author(s)               | Reviewer          |
|-----------|-------------------------|-------------------|
| 2017-06-26| Authored, Edited        | Orlic-Milacic, M. |
| 2017-07-31| Reviewed                | Jaworski, A.      |
ROBO1 binds FLRT3

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-9010231

**Type:** binding

**Compartments:** plasma membrane

**Inferred from:** Robo1 binds Flrt3 (Mus musculus)

Based on studies in mice, ROBO1, activated by SLIT1 binding, forms a complex with FLRT3. This interaction involves the intracellular domains of FLRT3 and ROBO1. FLRT3 is a member of the fibronectin leucine-rich repeat transmembrane protein family. The interaction of FLRT3 and ROBO1 in the presence of SLIT1 increases Netrin-1 attraction of thalamocortical axons by increasing the amount of DCC receptors at the plasma membrane via an unknown mechanism that may involve PKA activation (Leyva-Diaz et al. 2014).

**Editions**

| Date       | Action       | Author          |
|------------|--------------|-----------------|
| 2017-06-26 | Authored, Edited | Orlic-Milacic, M. |
| 2017-07-31 | Reviewed     | Jaworski, A.    |
AKAP5 (also known as AKAP79 in humans and Akap150 in mice) is an A-kinase anchoring protein which is able to bind to ROBO receptors ROBO2 and ROBO3.1, an isoform of ROBO3, by interacting with their cytoplasmic tails. The interaction was originally detected between endogenous proteins from the mouse brain lysates. AKAP5 can recruit protein kinase A (PKA), protein kinase C (PKC) and protein phosphatase PP2B to ROBO2. AKAP5-mediated recruitment of PKC to ROBO3.1 leads to phosphorylation of ROBO3.1 by PKC. Functional implications of AKAP5 interaction with ROBO receptors are not known (Samelson et al. 2015).

**Literature references**

Gore, BB., Nygren, PJ., Zweifel, LS., Colledge, M., Scott, JD., Dell'Acqua, ML. et al. (2015). A-kinase Anchoring Protein 79/150 Recruits Protein Kinase C to Phosphorylate Roundabout Receptors. *J. Biol. Chem.*, 290, 14107-19.

**Editions**

| Date       | Author(s)           | Edition |
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| 2017-06-26 | Authored, Edited    | Orlic-Milacic, M. |
| 2017-07-31 | Reviewed            | Jaworski, A. |
ROBO1 binds NRP1

**Location:** Signaling by ROBO receptors

**Stable identifier:** R-HSA-9010951

**Type:** binding

**Compartments:** plasma membrane

**Inferred from:** Robo1 binds Nrp1 (Mus musculus)

Based on studies in mice, ROBO1 binds semaphorin receptor NRP1. Other NRP1-binding proteins, such as NRP2, Plexin A1 and Plexin A2, also co-immunoprecipitate with ROBO1, but it is thought that the direct interaction involves ROBO1 and NRP1 only. ROBO1 binds to NRP1 via Ig1 and Ig2 domains in the extracellular region of ROBO1. Interaction with ROBO1 may increase the stability of NRP1 and NRP1-associated proteins, or increase their abundance at the plasma membrane. Semaphorins direct the migration of cortical interneurons, and mice deficient in Robo1 function show reduced responsiveness of cortical interneurons to semaphorins (Hernandez-Miranda et al. 2011).

**Editions**

| Date       | Action          | Author/Editor  |
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| 2017-07-31 | Reviewed        | Jaworski, A.   |
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