Reelin modulates cytoskeletal organization by regulating Rho GTPases

Jost Leemhuis1,2,* and Hans H. Bock2,3,*

1Department of Pharmacology; 2Center for Neuroscience; 3Department of Medicine II; Albert-Ludwigs-University; Freiburg, Germany

Key words: ApoE receptor, PI3K, Cdc42, Rac1, RhoA, cofilin, N-WASP, actin cytoskeleton, growth cone motility, radial glia, filopodia, golgi, polarization, axonal branching, neuronal vesicle transport

Abbreviations: Akt, v-AKT murine thymoma viral oncogene homolog; Apoer2, apolipoprotein E receptor 2; Blbp, brain lipid-binding protein; Cdc42, cell division cycle 42; Crk, v-CRK avian sarcoma virus CT10 oncogene homolog; CrkL, Crk-like; Dab1, disabled-1; Dcx, doublecortin; DHR, dock homology region; ER, endoplasmic reticulum; GABA, gamma-aminobutyric acid; GEF, guanine nucleotide-exchange factor; GFP, green fluorescent protein; GM130, golgi matrix protein of 130 kDa; GSK3, glycogen synthase kinase 3; GTP, guanosine triphosphate; hGFAP, human glial fibrillary acidic protein; IQGAP, IQ motif-containing GTPase-activating protein; LIMK, LIM domain kinase; Lis1, lissencephaly 1; LKB1, liver kinase B1; Map1b, microtubule-associated protein 1b; n-WASP, neuronal wiskott-aldrich syndrome protein; PACAP, pituitary adenylate cyclase-activating polypeptide; PAK, p21-activated kinase; PI3K, phosphatidylinositol-3-kinase; Rac, ras-related C3 botulinum toxin substrate; RhoA, Ras homolog gene family member A; SFK, Src family kinases; Stk25, serine/threonine protein kinase 25; STRAD, STE20-related adaptor protein; VIP, vasoactive intestinal peptide; Vldlr, very low-density lipoprotein receptor; WAVE, WASP family verprolin-homologous protein

The correct positioning of postmitotic neurons in the developing neocortex and other laminated brain structures requires the activation of a Reelin-lipoprotein receptor-Dab1 signaling cascade. The large glycoprotein Reelin is secreted by Cajal-Retzius pioneer neurons and bound by the apolipoprotein E receptor family members Apoer2 and Vldlr receptor on responsive neurons and radial glia. This leads to the tyrosine phosphorylation of the cytoplasmic protein Disabled-1 (Dab1) by non-receptor tyrosine kinases of the Src family. Various signaling pathways downstream of Dab1 connect Reelin to the actin and microtubule cytoskeleton. Despite this knowledge, a comprehensive view linking the different cell-biological and biochemical actions of Reelin to its diverse physiological roles not only during neurodevelopment but also in the maintenance and functioning of the adult brain is still lacking. In this review, we discuss our finding that Reelin activates Rho GTPases in neurons in the light of other recent studies, which demonstrate a role of Reelin in Golgi organization, and suggest additional roles of Cdc42 activation by Reelin in radial glial cells of the developing cortex.

Introduction

Reelin, a conserved extracellular glycoprotein, controls the migration and laminar arrangement of neurons during the development of the neocortex, hippocampus, cerebellum and spinal cord. When newborn postmitotic neurons have reached their final position, Reelin promotes the maturation of dendrites and dendritic spines. In reeler mice, which have a naturally occurring mutation that makes them Reelin-deficient, cortical neurons migrate abnormally, resulting in an inversion of the cortical laminar organization, with later-born neurons remaining in the deeper layers of the cortex (reviewed in ref. 1–3).

Reelin binding to the lipoprotein receptors very-low-density lipoprotein receptor (Vldlr) and the apolipoprotein E receptor 2 (Apoer2) induces the Src family kinase (SFK)-mediated tyrosine phosphorylation of the adaptor protein Disabled-1 (Dab1).4-7 Hence, disruption of Dab1, absence of both lipoprotein receptors Apoer2 and Vldlr or simultaneous inactivation of the Src family kinases (SFKs) Src and Fyn leads to a reeler-like phenotype.8-11 The phosphorylation of Dab1 activates, besides other signaling pathways, class I phosphatidylinositol-3-kinase (PI3K).12-14 However, despite growing insights into the signaling cascades activated by Reelin in responsive neurons, the cellular mechanisms of its action on neuronal positioning and development are still poorly understood.15

Rho GTPases as Phosphatidylinositol-3-Kinase Effectors during Neurodevelopment

The large number of PI3K effectors creates a complex signaling web downstream of PI3K activation.16 Among the key players linking PI3K activity to specific cellular responses17 are small GTPases of the Rho family, which regulate the cytoskeletal and membrane rearrangements required for cell movement.18,19 Small GTPases act as molecular switches, cycling between an active, GTP-bound and an inactive, GDP-bound form.20,21
Furthermore, Reelin increases neuronal transport vesicle formation and redirects neuronal vesicle transport into small neurites, the prospective dendrites (Fig. 1A). Cdc42, but not RhoA or Rac1, localizes in part to the Golgi apparatus, together with its targets N-WASP, IQGAP and the Golgi vesicle coat protein and coatomer (reviewed in ref. 26 and 27). Cdc42- and N-WASP-controlled actin polymerization regulates Golgi-to-ER transport and thus is a central event of the multiple steps of vesicle trafficking. The differential subcellular localization and activity of Cdc42 and the Par complex are critical for axon specification.29,30 By activating Cdc42 via Apoer2 at the tips of all neurites in stage-II neurons, Reelin seems to interfere with axon-dendrite differentiation.24

Reelin, Neuronal Polarization and Golgi Organization

The members of the Rho-family Rac, RhoA and Cdc42 are essential regulators of many cellular processes during neural development.22,23

**Rho GTPases Link Reelin Signaling to the Neuronal Cytoskeleton**

In order to investigate cellular effects of Reelin, we employed time-lapse microscopy of cultured primary neurons and found an increased motility of distal neurites.24 Using this cellular effect as a readout we identified the Rho GTPase Cdc42 as a novel cellular effector of Reelin signaling. Our results indicate that Reelin and Rho GTPase-mediated signaling cascades interact during neuromorphogenesis to increase filopodia formation and growth cone motility (Fig. 1A). The activation of Cdc42 by Reelin requires signaling through Apoer2, Dab1 and PI3K (Fig. 1B). In addition, Reelin-induced localized Rac1 activation might participate in Reelin’s effects on growth cone motility. Furthermore, Reelin increases neuronal transport vesicle formation and redirects neuronal vesicle transport into small neurites, the prospective dendrites (Fig. 1A). Cdc42, but not RhoA or Rac1, localizes in part to the Golgi apparatus, together with its targets N-WASP, IQGAP and the Golgi vesicle coat protein and coatomer (reviewed in ref. 26 and 27). Cdc42- and N-WASP-controlled actin polymerization regulates Golgi-to-ER transport and thus is a central event of the multiple steps of vesicle trafficking. The differential subcellular localization and activity of Cdc42 and the Par complex are critical for axon specification.29,30 By activating Cdc42 via Apoer2 at the tips of all neurites in stage-II neurons, Reelin seems to interfere with axon-dendrite differentiation.24

Of note, a recent study demonstrated that Reelin-Dab1 signaling antagonizes the action of the protein kinase LKB1 on
neuronal polarization,\textsuperscript{31} LKB1, whose effect on cell polarization is controlled by its interaction with the pseudokinase STRAD,\textsuperscript{32} was shown to form a complex involving the serine/threonine kinase Stk25 and the Golgi matrix protein GM130, which regulates Golgi morphology and axon specification in neurons. Interestingly, the kinase activity of Stk25 was dispensable for mediating these effects.\textsuperscript{33} Thus, Reelin seems to have prominent roles in the regulation of the Golgi apparatus and the direction of neuronal vesicle transport, thereby participating in the axon determination of maturing neurons. Importantly, it has been shown that the Golgi protein GM130 can also form a complex that includes Cdc42.\textsuperscript{33} Future studies will have to address how Cdc42 activation by Reelin relates to the antagonizing effect of Reelin on LKB1-STRAD-Stk25-GM130 signaling.

**Reelin and Neuropeptidergic Signaling Converge on the Level of Rho GTPases**

In the adult brain, Reelin is mainly localized to GABA-containing peptidergic interneurons.\textsuperscript{34} The functional relevance of this colocalization, which implies a cosecretion under physiological or pathophysiological conditions, is largely unknown. We could demonstrate that Reelin functionally interacts with the peptidergic VIP/PACAP38-receptor system to increase axonal branch formation.\textsuperscript{24} As VIP-mediated Rho-kinase inhibition\textsuperscript{35,36} induces the elongation of dendrites and axons by stabilizing microtubules,\textsuperscript{37} these results demonstrate that Reelin and a neuropeptidergic system can cooperate to promote neuronal development by inducing axonal branching. In addition, these findings pinpoint the influence of the tubulin cytoskeleton in mediating Reelin’s effect on neurormorphogenesis, which is also targeted by other effectors of the Reelin-Dab1 signaling cascade, including the Tau kinase GSK3beta,\textsuperscript{12} Lis1,\textsuperscript{38} and Map1b.\textsuperscript{39}

**Putative Functions of Cdc42 in Reelin-Dependent Spine Morphogenesis and Synapse Formation**

In addition to its prominent role in controlling neuronal positioning in developing brain structures, Reelin is also involved in the development of dendrites\textsuperscript{40,41} and promotes spine morphogenesis and synapse formation.\textsuperscript{42,43} Given the known function of Rho GTPases in the growth and remodeling of dendrites and synaptogenesis (reviewed in ref. 22, 44 and 45), which require extensive remodeling of the neuronal cytoskeleton, it is tempting to speculate that Cdc42 cooperates with other Reelin effector molecules such as the adapter proteins Crk and CrkL,\textsuperscript{46} Akt and mTor signaling\textsuperscript{44} to regulate dendrite morphogenesis. An additional level of complexity is added to this signaling network by the interaction of Reelin and Notch signaling,\textsuperscript{47} which cooperates with Rho GTPases in the specification of dendrite morphology (reviewed in ref. 48). Defects in dendrite and spine morphology and a reduction in synapse number are observed in many neuropsychiatric diseases,\textsuperscript{49} and both have been connected to alterations in Reelin signaling as well as to defects in Rho GTPase function (reviewed in ref. 22 and 50). We propose that the activation of Cdc42 by Reelin\textsuperscript{24} links the observation that defects in Rho GTPase function and Reelin signaling both contribute to morphological defects leading to neurological and psychiatric disorders.

**Role of Cdc42 Activation in Radial glia**

A recent report demonstrated that Cdc42 is required for maintaining the polarized morphology of the cortical radial glial scaffold.\textsuperscript{30} Live imaging of radial glial cells in embryonic cortical slice cultures revealed dynamic inter-radial glial interactions involving transient filopodia-like radial glial protrusions and highly motile radial glial leading edges resembling neuronal growth cones. By in utero electroporation of a dominant-negative Cdc42-GFP construct under control of a radial glial-specific Blbp promoter it was demonstrated that polarized Cdc42 activation was necessary for maintaining radial glial morphology and dynamics. Crossing mice carrying floxed \textit{Cdc42} alleles with \textit{bGFP-Cre} transgenic mice expressing Cre recombinase under control of the human \textit{GFAP} promoter resulted in the conditional ablation of Cdc42 in radial progenitor cells and led to cortical layering defects and neuronal ectopias. Defects in axonogenesis were also observed.\textsuperscript{51} This phenotype resembles but is not identical to that of mice lacking essential components of the Reelin signaling cascade, probably because Cdc42 integrates the input of additional ligand-receptor signaling systems. Moreover, it should be noted that the use of radial glial-specific promoters will not prevent neuronal Cdc42 inactivation, since the vast majority of neurons is derived from radial glial progenitors.\textsuperscript{52} To clearly distinguish the separate effects of radial glial vs. neuronal Cdc42 ablation and their relative contributions to the observed lamination defect, the comparison with mice lacking Cdc42 specifically in neurons, e.g., by using a \textit{Dcx-Cre} transgenic mouse line, might be helpful. However, in light of the data generated by Yokota and colleagues, it seems likely that at least some of the effects of Cdc42 on radial glial morphology and dynamics are mediated by Reelin, whose receptors and intracellular effectors have been described to be expressed in radial glial cells of cortical structures.\textsuperscript{53,54}

**Perspective**

At first sight it might seem surprising that so many different cellular effects of Reelin are modulated by a single Rho GTPase. However, one has to bear in mind that Rho-GTPases are embedded in different effector-domain complexes depending on the contextual situation. Selectivity can be achieved by engaging different guanine nucleotide-exchange factors (GEFs). GEFs are responsible for the activation of Rho-family GTPases in response to various extracellular stimuli, and regulate diverse downstream cellular responses in neurons such as migration, morphogenesis and axonal pathfinding.\textsuperscript{55} DbI-related GEFs represent the largest family of direct activators of Rho GTPases in humans. In addition, atypical Rho-GEFs that contain Dock homology regions (DHR-1 and DHR-2) are expressed in a variety of tissues, including the nervous system and achieve spatial and temporal restriction of Rho GTPase-signaling.\textsuperscript{56} It remains to be determined which of these GEFs are activated by Reelin and lipoprotein
receptor-mediated signaling and how they preferentially couple to different downstream effector pathways, thereby mediating Reelin's different effects on the actin and microtubule cytoskeleton via Rho GTPases. The fine tuning and signal integration of these partially convergent or divergent pathways downstream of the Rho GTPase effectors is fundamental for correct neuronal development.

Acknowledgements

The authors acknowledge financial support by the Deutsche Forschungsgemeinschaft (DFG) and the Forschungskommission der Medizinischen Fakultät Freiburg. We wish to thank Michael Frotscher for continuous support and Lutz Hein for critical reading of the manuscript.

References

1. Frotscher M. Role for Reelin in stabilizing cortical architecture. Trends Neurosci 2010; 33:407-14.
2. Rice DS, Curran T. Role of the reelin signaling pathway in central nervous system development. Annu Rev Neurosci 2001; 24:1005-39.
3. Tisier F, Goiffinet AM. Reelin and brain development. Nat Rev Neurosci 2003; 4:496-505.
4. Arnaud L, Balfil BA, Forster E, Cooper JA. Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. Curr Biol 2003; 13:15-17.
5. Bock HH, Herz J. Reelin activates Src family tyrosine kinases in neurons. Curr Biol 2003; 13:18-26.
6. D’Arcangelo G, Homayouni R, Keshvara L, Rice DS, Sheldon M, Curran T. Reelin is a ligand for lipoprotein receptors. Neuroreport 1999; 24:471-9.
7. Fuchs E, Tseffon M, Howell BW, Goiffinet A, Mumber MC, Cooper JA, et al. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. Neuron 1999; 24:481-95.
8. Howell BW, Green TL, Cooper JA. Mouse disabled-1 (mDab1): a Src binding protein implicated in neuronal development. EMBO J 1997; 16:121-32.
9. Kuo G, Arnaud L, Kronstad-O’Brien P, Cooper JA. Absence of Fyn and Src causes a reeler-like phenotype. J Neurosci 2005; 25:8578-86.
10. Sheldon M, Rice DS, D’Arcangelo G, Yonishi H, Nakajima K, Mikishova K, et al. Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. Nature 1997; 387:730-3.
11. Tseffon M, Gorstadt M, Hesseberger T, Shelton JD, Stocker T, Wun NF, et al. Reeler/disabled-1 disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 1999; 97:689-701.
12. Befter U, Morfini G, Bock HH, Reyna H, Brady ST, Mumby MC, Cooper JA, et al. Phosphatidylinositol-3-kinase and Akt to Control development. EMBO J 1997; 16:121-32.
13. Schwamborn JC, Puichel AW. The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. Nat Neurosci 2004; 7:923-9.
14. Matsuki T, Matthews RT, Cooper JA, van der Brug MP, Cookson MR, Hardy JA, et al. Reelin and sqkt2 have opposing roles in neuronal polarization and dendritic Golgi development. Cell 2010; 143:826-36.
15. Zeziqui E, Filippini BM, Deak M, Alessi DR, van Aalten DM. Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. Science 2009; 326:1707-11.
16. Kodani A, Kresistenis I, Huang L, Sutterlin C, GM130-dependent control of Cdc42 activity at the Golgi regulates centrosome organization. Mol Biol Cell 2009; 20:1192-200.
17. Posold C, Impagnatiello F, Uzunov DP, Costa E, Guidoitt A, et al. Reelin is preferentially expressed in neurons synthesizing gamma-aminobutyric acid in cortex and hippocampus of adult rats. Proc Natl Acad Sci USA 1998; 95:3212-6.
18. Herz E, Fischer C, Meyer DK, Leemhuis J, Vassar R. J A, et al. Reelin regulates the development and synaptogenesis of the layer-specific entohippocampal connections. J Neurosci 1999; 19:1345-58.
19. Negishi M, Karoh H. Reelin family GTPases and dendrite plasticity. Neuroscientist 2005; 11:137-91.
20. Tada T, Sheng M. Molecular mechanisms of dendritic spine morphogenesis. Curr Opin Neurobiol 2006; 16:95-101.
21. Hashimoto-Tori K, Torii M, Sarkisian MR, Bartley CM, Shien J, Radlfer E, et al. Interaction between Reelin and Notch signaling regulates neuronal migration in the cerebral cortex. Neuron 2008; 60:273-84.
22. Redmond L, Ghosh A. The role of Notch and Rho GTPase signaling in the control of dendritic development. Curr Opin Neurobiol 2001; 11:111-7.
23. Kaufmann WE, Moser RW. Dendritic anomalies in disorders associated with mental retardation. Cereb Cortex 2000; 10:981-91.
24. Fureni SH. Reelin glycoprotein: structure, biology and roles in health and disease. Mol Psychiatry 2005; 10:251-7.
25. Yokota Y, Tone TY, Stanaco A, Kim WY, Rao S, Snider WD, et al. Cdc42 and Gsk3 modulate the dynamics of radial glial growth, inter-radial glial interactions and polarity in the developing cerebral cortex. Development 2010; 137:4101-10.
26. Anthony TE, Klein C, Fishell G, Heintz N. Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 2004; 41:881-90.
27. Hartfuss F, Forster E, Bock HH, Hack MA, Leprince P, Iskuj MM, et al. Reelin signaling directly affects radial glial morphology and biochemical maturation. Development 2010; 137:4597-609.
28. Füster E, Tielich A, Saum B, Weiss KH, Johansen C, Gräus-Porta D, et al. Reelin, Disabled 1 and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. Proc Natl Acad Sci USA 2002; 99:13178-83.
29. Rosman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol 2005; 6:167-70.
30. Core NE, Vuori K. GEF what? Dock180 and related proteins help Rac to polarize cells in new ways. Trends Cell Biol 2007; 17:383-93.
31. Ng J, Luo L. Rho GTPases regulate axon growth through convergent and divergent signaling pathways. Neuroreport 2004; 15:779-93.