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Review Article

# The functions of Reelin in membrane trafficking and cytoskeletal dynamics: implications for neuronal migration, polarization and differentiation

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Reelin is a large extracellular matrix protein with relevant roles in mammalian central nervous system including neurogenesis, neuronal polarization and migration during development; and synaptic plasticity with its implications in learning and memory, in the adult. Dysfunctions in reelin signaling are associated with brain lamination defects such as lissencephaly, but also with neuropsychiatric diseases like autism, schizophrenia and depression as well with neurodegeneration. Reelin signaling involves a core pathway that activates upon reelin binding to its receptors, particularly ApoER2 (apolipoprotein E receptor 2)/LRP8 (low-density lipoprotein receptor-related protein 8) and very low-density lipoprotein receptor, followed by Src/Fyn-mediated phosphorylation of the adaptor protein Dab1 (Disabled-1). Phosphorylated Dab1 (pDab1) is a hub in the signaling cascade, from which several other downstream pathways diverge reflecting the different roles of reelin. Many of these pathways affect the dynamics of the actin and microtubular cytoskeleton, as well as membrane trafficking through the regulation of the activity of small GTPases, including the Rho and Rap families and molecules involved in cell polarity. The complexity of reelin functions is reflected by the fact that, even now, the precise mode of action of this signaling cascade *in vivo* at the cellular and molecular levels remains unclear. This review addresses and discusses in detail the participation of reelin in the processes underlying neurogenesis, neuronal migration in the cerebral cortex and the hippocampus; and the polarization, differentiation and maturation processes that neurons experiment in order to be functional in the adult brain. *In vivo* and *in vitro* evidence is presented in order to facilitate a better understanding of this fascinating system.

## Overview

The aim of this review is to explain how the reelin signaling pathway is involved in neurogenesis, neuronal migration, polarization and differentiation with emphasis in the brain cortex and to a lesser extend in the hippocampus. For simplicity, the discussion will not consider in role of reelin in other relevant neuronal systems such as the cerebellum. The main focus is on the intracellular pathways triggered by reelin that have an impact on cytoskeleton dynamics and membrane trafficking, and are dependent on different small GTPases, especially from the Rho, Rap, Rab and Arf families. Our work also includes possible new targets and regulators of this pathway in order to broaden our understanding of the complex roles of this system. Despite focusing our work on reelin, it is worth mentioning that there are many other molecules, receptors and signaling pathways that play fundamental roles in brain development and neuronal function that are not considered in this review.

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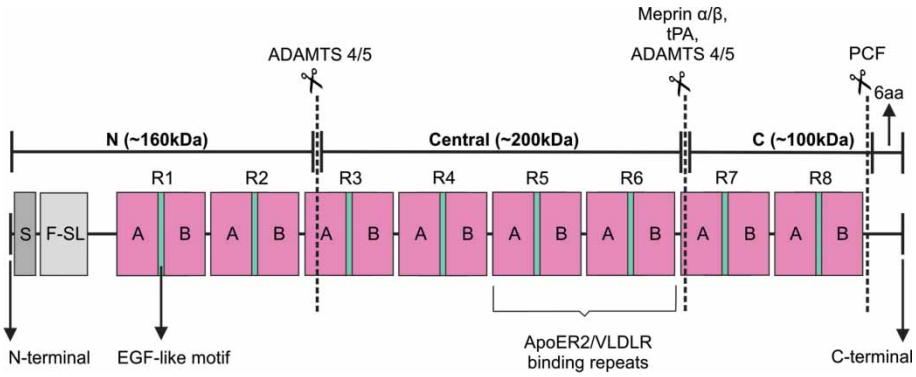


**Figure 1. Squematic representation of reelin structure and proteolytic processing.**

Reelin is a glycoprotein with a molecular mass of ~460 kDa (~390 kDa in the absence of N-glycosylation). The N-terminal region consists of a signal peptide (S) and an F-Spondin-like domain (F-SL). The full-length reelin has eight 'reelin repeats' (R) of ~350–390 aminoacids (R1–8) where each repeat has two related subrepeats A and B, separated by an EGF-like motif of ~35 amino acids. The C-terminal domain has a positively charged sequence. Several proteases that cleave reelin at different positions are indicated (as scissors) in the figure. Reelin binds ApoER2 and VLDLR through the R5–6 repeats.

# Introduction

## Reelin and its signaling pathway

Reelin is an extracellular glycoprotein, which is secreted by Cajal–Retzius cells during embryonic brain development and by GABAergic interneurons in the adult brain [1–4]. The full-length reelin is about 460 kDa and has a signal peptide, an F-spondin-like domain, eight reelin repeats (R1–R8) and a positively charged sequence at the C-terminus (Figure 1). Reelin is processed by several proteases including ADAMTS 4 and 5 (A disintegrin and metalloproteinase with thrombospondin motifs 4 and 5) [5,6], Meprin α and β [7] and proteases of the proprotein convertase family (PCF) [8] among others. The proteolytic processing of reelin gives rise to different fragments, including the N-terminal fragment of ~160 kDa, a central fragment of ~200 kDa and a C-terminal fragment of ~100 kDa [6], as well as others partially proteolysed forms: one of 380 kDa formed by the N-terminal plus the central fragment and a 300 kDa form that lacks the N-terminal region (Figure 1). Reelin fragments have different biological activities; the N-terminal region has been associated with aggregation [9], but it is also required for full activity of reelin [10,11]. Similarly, the C-terminal region is important to stabilize the functional reelin homodimer [6] and for full signaling [12,13]. The central fragment, the most relevant in cortical plate development [14], is responsible for receptor binding [15] and for the formation of a cysteine-dependent homodimer [16].

Homozygous loss of reelin in humans causes lissencephaly, a condition characterized by the absence of normal convolutions in the cerebral cortex and cerebellar hypoplasia. A similar phenotype is caused by mutations in the *LIS1* (Lissencephaly type 1) gene [17]. Mice that carry a mutation in the reelin gene, called *reeler* mice, display several defects in brain development such as inversion of cortex cell layers, neuronal invasion of the external cortical layer named marginal zone (MZ), malposition of neurons throughout the hippocampus, small and/or lack of foliation of the cerebellum and evident ataxia [18–20]. In addition, mutations in the reelin gene, as well as a decrease in reelin mRNA (messenger RNA) expression, are associated with autism [21–23], mental retardation [24,25], schizophrenia and bipolar disorder [2,26]. Some patients with cortical dysplasia (associated with epilepsy) exhibit an increased number of Cajal–Retzius cells, pointing to reelin as a risk factor for this disease [27]. On the other hand, the hippocampal Cajal–Retzius cells from patients with epilepsy have decreased expression of reelin, a condition that correlates with dispersion of the granule cells in the dentate gyrus [28]. Reelin also has roles in neurogenesis in the cortex, hippocampus and cerebellum [29,30]. In addition, defects in the reelin signaling pathway are associated with impairments in dendrite and synapse formation, which are reflected in learning and memory deficits [18,31–36]. Therefore, the participation of reelin in the control of neuronal migration, differentiation and function has been broadly studied and confirmed using different experimental strategies. Many of the cellular responses triggered by reelin have a direct impact on the regulation of cytoskeleton dynamics and membrane trafficking. However, as we will discuss later, it is still a

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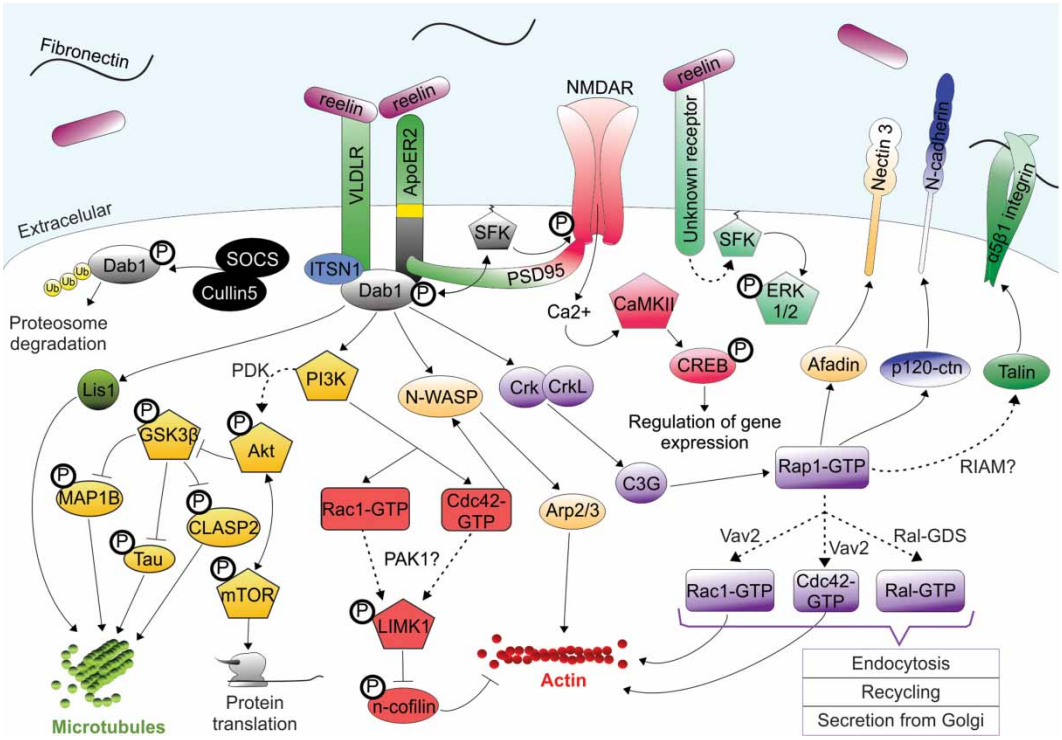


**Figure 2. Reelin signaling pathway.**

Binding of reelin to its receptors, VLDLR and ApoER2, triggers the canonical pathway that requires the recruitment of the adaptor protein Dab1 to the NPxY motif in the cytoplasmic domains of ApoER2 and VLDLR and its phosphorylation mediated by SFK. pDab1 leads to the recruitment or activation of different molecules. One is Crk/CrkL, which by inducing Rap1 activation plays a role in the insertion of cell–cell adhesion proteins at the plasma membrane. Other Rap1 effector proteins, including GTPases of the Rho family and Ral, resuls in the regulation of cytoskeleton dynamics and membrane trafficking. pDab1 also activates PI3K, leading to two different pathways, the activation of Akt with different downstream targets that promote protein translation and neuronal microtubule dynamics and function, and the ApoER2, dependent activation of Cdc42 and Rac1 that regulate actin dynamics via LIMK1-dependent inhibition of *n*-cofilin. Reelin allows the stabilization of VLDLR/Dab1 interaction via ITSN1. Also, pDab1 interacts with Lis1/Pafah1β1 resulting in microtubule stabilization. Reelin's role in synaptic transmission requires pDab1 activation of SFK that in turn phosphorylates NMDAR, increasing influx of $Ca^{2+}$, inducing the activation CaMKII and, in this way, CREB phosphorylation. ERK1/2 also can be activated in response to reelin and SFK but independently of Dab1 (a non-canonical pathway). Finally, pDab1 is down-regulated by a mechanism that involves its ubiquitination by SOCS proteins and Cullin5 and subsequent proteasome degradation.

matter of debate exactly how this protein and the signaling pathway triggered in the responding cells can explain the phenotype of humans with mutation(s) in genes associated with reelin signaling or the phenotype of the *reeler* animals.

The best-characterized reelin receptors belong to the Apolipoprotein E receptor family and are ApoER2 (apolipoprotein E receptor 2)/LRP8 and VLDLR (very low-density lipoprotein receptor) [37]. The interaction of reelin with its receptors triggers the canonical-signaling pathway shown in Figure 2 that includes the activation of the Src family of tyrosine kinases (SFK), specially Fyn but also Src [38], which phosphorylate the intracellular membrane-bound adaptor protein Dab1 [37]. Reelin signaling includes the participation of the scaffold protein Intersectin 1 (ITSN1), associated with Down Syndrome, as a relevant component that binds to VLDLR and Dab1 [39]. Dab1 also binds to the NPxY motif in the cytoplasmic domain of the receptors [40–43]. pDab1, in turn, recruits several intracellular proteins and therefore regulates different signaling pathways. When Dab1 is phosphorylated on tyrosines 220 and 232, it predominantly activates the Crk pathway [44,45]. The activation of the Crk family of adaptor proteins gives rise to the Dab1–Crk/CrkL–C3G (C3 glomerulopathy)–Rap1 (Ras-related protein 1) pathway. C3G is an activator of the small G-protein Rap1

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[46,47] that plays fundamental roles in neuronal adhesion to fibronectin, via the regulation of α5β1 integrin expression on the cell surface; and in cell–cell interactions through the regulation of Nectins and N-cadherin trafficking. In addition to the Crk pathway, pDab1 (phosphorylated in residues 185, 198/200) activates PI3K (phosphatidylinositol-3-kinase) [44,45], which plays important roles in several neuronal process during development and also in adult stages [48]. The function of PI3K is relevant to the reorganization of the actin cytoskeleton by activating Cdc42 (cell division control protein 42 homolog) and Rac1 (Ras-related C3 botulinum toxin substrate 1). These two small GTPases of the Rho family have important roles in cell migration and neuronal differentiation [49]. In the reelin signaling pathway, LIMK1 (LIM motif-containing protein kinase 1) is one of the downstream targets of these GTPases. This protein phosphorylates the actin severing protein *n*-cofilin (in serine 3) and inactivates it [50]. It also phosphorylates the neural Wiskott–Aldrich syndrome protein (N-WASP) that regulates the actin-polymerizing activity of Arp2/3 (actin-related protein 2/3) [49]. Reelin also activates Akt (Ak thymoma), via PI3K–PDK1, inducing its phosphorylation on threonine residue 308 [51]. On one hand, active Akt regulates and inactivates glycogen synthase kinase 3β (GSK3β) [52] and has therefore an impact on microtubule dynamics via regulation of tau phosphorylation [40,52] and of the cytoplasmic linker-associated protein 2 (CLASP2) [53]. On the other hand, Akt activates the mTOR (mammalian target of rapamycin) complex, the mammalian target of rapamycin. This activation is associated with dendritic outgrowth [54]. At the level of the synapse, reelin signaling, also via Dab1 and Src, regulates the activity and subunit composition of *N*-methyl-D-aspartate (NMDA) receptors (NMDARs) [55,56], activates both $Ca^{2+}$/calmodulin-dependent kinase II (CaMKII) [57,58] and the extracellular signal-regulated kinases (ERK) 1/2 [59], and regulates trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPA-R) at the postsynaptic site [60,61] as well the secretion of neurotransmitters at the presynaptic site [62,63].

## General aspects of cortex development and neuronal migration

Proper positioning of migratory neurons during development is a pivotal process for the formation of laminated brain structures such as the neocortex, hippocampus and cerebellum in mammals. The extracellular matrix protein reelin has a central role in this process. During cortex development in the mouse embryonic brain, immature neurons are generated from radial glial (RG) cells in the ventricular zone (VZ). At approximately embryonic day 11 (E11), the first cohort of excitatory neurons exits the VZ giving rise to a transient layer called preplate (PP) just beneath the pia mater. Axons from PP cells and from the thalamus establish the intermediate zone (IZ)/subventricular zone (SVZ) in between the VZ and the PP [64,65]. The second cohort of neurons form the cortical plate (CP) at around E13 splits the PP into two layers resulting in the external layer MZ, composed of a monolayer of Cajal–Retzius cells that secrete reelin (future layer I) and a deeper layer named subplate (SP) zone (Figure 3A) [64,66]. Subsequent cohorts of neurons migrate radially along RG fibers through their predecessors towards the pial surface to reach the top of the CP. This progressive and systematic process is defined as 'inside-out' patterning of the six cortical layers [67].

There are three different modes of migration. First the multipolar, characterized by neurons randomly changing direction and their rate of migration [68]; second, the somal translocation, that begins after the multipolar cells are polarized into a bipolar shape, is characterized by the shortening of the leading process that remains attached to the outer surface while the soma progressively advances towards the pial surface [69]. The third mode of migration is locomotion characterized by bipolar neurons moving towards the pial surface using RG as scaffold [70–72]. At the end of migration, neurons suffer an RG detachment and terminal somal translocation that requires previous anchoring of the apical dendrite to the MZ. The migration and layering process ends at about E17.5 in mice (Figure 3B) [73]. The laminated cortex in the adult is recognized by its six layers plus the white matter (Figure 3C) [74].

## The roles of the cytoskeleton and membrane trafficking in neuronal migration and differentiation

Migratory neurons experiment an active cytoskeletal remodeling that includes actin filaments and microtubules. In the bipolar migrating neuron, the centrosome and the nucleus are relocated for migration to occur in the correct direction towards the MZ [75,76]. The nucleus is surrounded by microtubules, and microtubule bundles that emanate from the centrosome are present at the leading process [75–77]. The centrosome and the nucleus enter into the leading process (the future apical dendrite) in a 'two stroke' cycle [75]. At the beginning of the cycle, the centrosome is pulled by microtubules into a dilation formation. This movement is followed by

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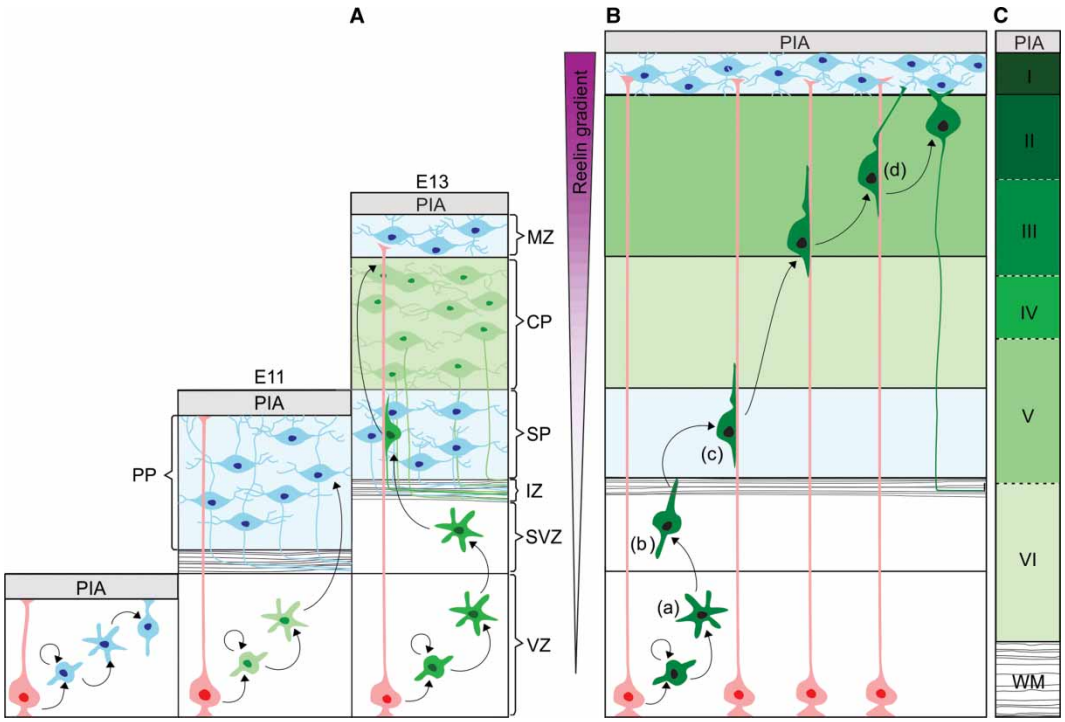


**Figure 3. Phases and modes of migration during development of the mouse neocortex.**

(**A**) *Splitting of the preplate*: the first cohort of neurons (light-blue neurons), arising from asymmetric division of RG cells (pink cells), exits the VZ and forms the PP, which is then divided by the second cohort of neurons (light-green neurons) that migrate through the PP and give rise to the MZ of Cajal–Retzius cells and to the deeper layer corresponding to the SP. The axons of these neurons and the thalamic afferences establish the IZ and the future WM, just beneath the SP. (**B**) *Neuronal migratory modes during cortical formation*: after PP splitting, excitatory neurons move through the CP towards the MZ. (**a**) Immature neurons acquire a multipolar morphology in the SVZ. (**b**) Neurons in the IZ/SVZ acquire the bipolar morphology and undergo somal translocation migration. (**c**) The migration mode requires the RG fibers as scaffold for locomotion through the cortical plate. (**d**) As the bodies of neurons get closer to the MZ, they undergo detachment from RG cells followed by the anchoring of the leading processes to the Cajal–Retzius cells and they finally experiment terminal somal translocation. Reelin (depicted in purple) is secreted from Cajal–Retzius cells and diffuses through the neocortex. (**C**) *Establishment of the six-layered neocortex*. This architecture becomes evident in the adult. The colors of the layers represent the inside-out pattern of migration, with earlier born neurons (light-green) occupying lower layers (VI and V) and later born neurons (dark green), progressively occupying upper positions in layers IV, III and II.

a process known as nucleokinesis or nuclear translocation that uses the minus end-directed microtubule motor protein dynein. [75,78]. Suppression of dynein or of the microtubule-stabilizing protein, Lis1, affects centrosome and forward nuclear movement [79]. The actin cytoskeleton is also required for nuclear movement and involves F-actin and the myosin II motor (actomyosin). The trailing process (future axon), which retracts during locomotion, also depends on actomyosin to generate neuronal movement. On the other hand, the leading process has a crucial role in sensing guidance cues and requires a dynamic actin cytoskeleton. F-actin is present as stress fibers, which concentrate at the distal end of the neuronal leading process, establishing contact with adhesion molecules and forming focal adhesions. Actin stabilization at the leading process is relevant, for example, for allowing somal translocation when the migratory neuron arrives at the MZ. Focal adhesions are also assembled either below the nucleus or in the proximal area of the leading process close to the centrosome. Integrins are membrane proteins that form part of focal adhesions, interact with extracellular substrates and the cytoskeleton, and are therefore relevant participants in cell migration [80]. Focal adhesion dynamics is required during radial migration and includes cycles of integrin endocytosis and exocytosis for attachment/detachment to/from the substrate.

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After finishing migration, neurons develop a more complex dendritic arbor, and they differentiate and mature developing their dendritic spines in order to establish synaptic contacts with other neurons in different circuits. The maturation process also includes events of dendritic pruning and changes in the protein composition of the synapse. In general, many of these events take place at the end of the embryonic development, but some are also present in the adult brain, where they are responsible for the plasticity necessary in the processes of learning and memory. As expected, alterations in the pathways controlling any of these processes have been associated with diverse brain dysfunctions, including neurodevelopmental disorders and neurodegenerative diseases.

Similarly to migration, the processes associated with neuronal differentiation and maturation require extensive participation of the microtubular and actin based-cytoskeleton, as well as the intracellular membrane trafficking. Overall, these processes involve the participation of small GTPases of the Rho family that act as principal controllers of actin cytoskeleton dynamics, which also affect microtubule function [81]. The Rab and Arf (ADP ribosylation factor) families are also implicated, with roles in membrane trafficking in the exocytic and endocytic pathways for the regulation of signaling pathways and the availability of proteins in the plasma membrane [82], especially N-cadherin and integrins during migration, as will be discussed in the following sections.

# Reelin signaling in cortical development: neurogenesis, neuronal migration and polarization

This section will address how the reelin signaling pathway is associated with different events that occur during the development of the cerebral cortex and, to a lesser extent, also in the hippocampus.

## Neurogenesis

Besides reelin's recognized participation in neuronal migration and layer formation in the 'inside-out' pattern of cortical development, a relevant and previously unnoticed role of this protein corresponds to the regulation of neurogenesis in the neocortex, hippocampus and cerebellum. *Reeler* mice exhibit reduced neurogenesis in the hippocampus [29] and a low number of mitotic cells in the VZ [30]. RG cells extend from the VZ and orient towards the pial surface. These cells divide asymmetrically to generate the neuronal precursors while maintaining apical–basal polarity allowing newborn postmitotic neurons to migrate radially [83]. In the cortex, reelin increases the number of neuronal precursors at the VZ. The neurogenic effect of reelin is mediated by cross-talk with the Notch signaling pathway [30,84,85]. Reelin increases the Notch signaling fragment NICD (Notch intracellular domain) in the RG cells via ApoER2 and Dab1 [30,84]. In human neural progenitor cells, reelin-induced pDab1 interacts with NICD increasing its transcriptional activity and the formation of RG cells [86]. This relevant role of the reelin signaling pathway in the generation of the future migratory neurons could also determine the characteristics of these cells, such as the type of membrane proteins expressed on the surface and differential gene expression [87], which would affect their future behavior on the journey to their final destination.

## Neuronal migration from the SVZ to the IZ

After the formation of the CP [88], the early-born cortical neurons migrate in an RG-independent way to induce PP splitting and give rise the MZ and the SP (Figure 3A) [89]. These neurons will form cortical layer VI. Reelin is necessary for PP splitting since, in *reeler* mice, layer VI neurons fail to split the PP [90] and also lack the apical–basal orientation [91]. The ectopic injection of reelin partially rescues this phenotype by promoting neuronal nuclei orientation and Golgi polarization towards the pia (discussed below), allowing somal translocation and resulting in PP splitting [92]. Likewise, C3G-deficient mice have defects in cortical PP splitting showing a *reeler*-like phenotype [93].

Initially, newborn neurons in the VZ exhibit a bipolar shape that changes to a multipolar shape when they exit this zone to enter into the SVZ/IZ (Figure 3B) [75]. The mechanisms involved in this very early cell shape modification are not clear. Within the SVZ/IZ, multipolar neurons migrate randomly [65], and before exiting the IZ, they undergo a multi-to-bipolar transition, with a defined leading process and a trailing axon [65,94,95], and migrate by somal translocation (Figure 3B). The polarization process, that is required for the next migratory mode, starts with the emergence of the axon in a process finely controlled by the small GTPases Rac1 and Rho [96,97] (see also section Emergence, growth and branching of the axon) (Figure 4). Dysfunctions associated with the migratory step in the SVZ/IZ include the accumulation of multipolar neurons

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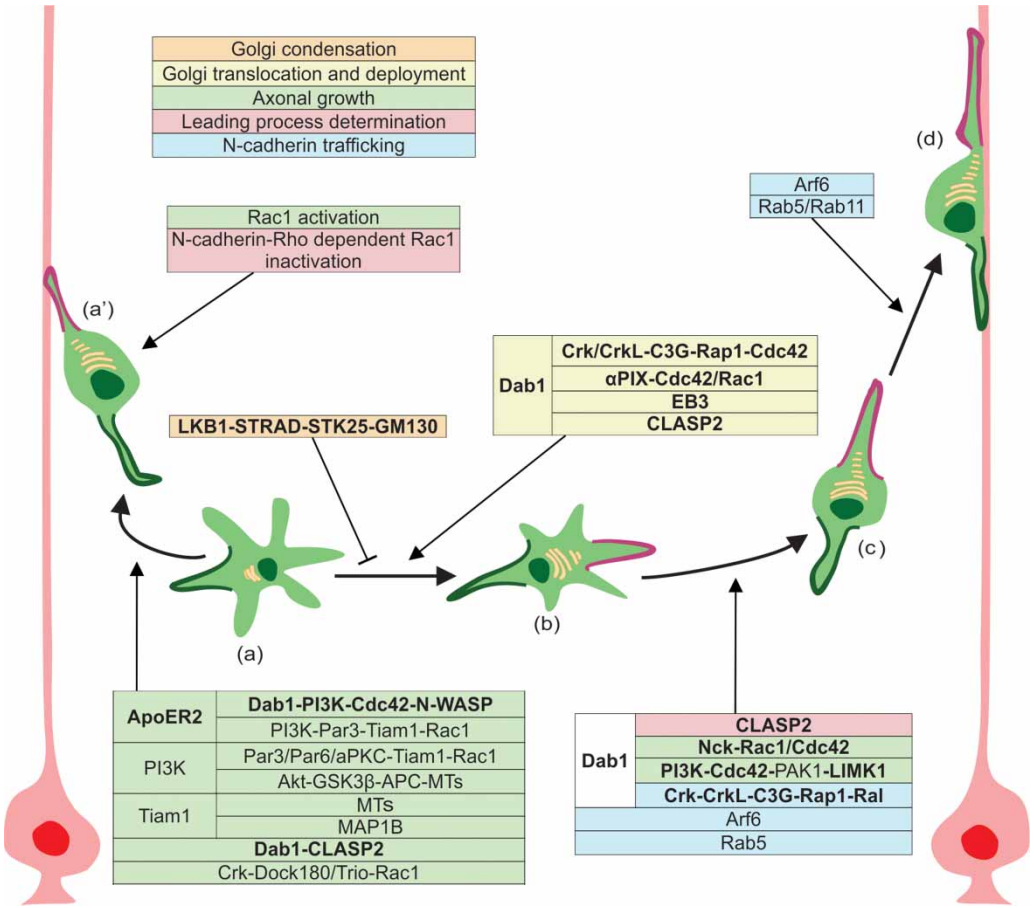


**Figure 4. Proposed roles for reelin in the SVZ/IZ.**

The multipolar to bipolar transition is a relevant process that takes place at the SVZ/IZ, in which immature neurons specify their axons and a leading process. Several processes take place in this area indicated at the left-top table; 'Golgi condensation' (orange box) and 'Golgi translocation and deployment' (yellow box) events are specially related to axonal specification and leading process determination, respectively; 'Axonal growth' (green box) indicates signaling pathways that specify and/or promote growth of a single axon; 'Leading process determination' (pink box) indicates signaling pathways playing a role in the determination and stabilization of the leading process and finally, 'N-cadherin trafficking' (light-blue box) depicts the molecules/pathways with roles in the cell surface expression of N-cadherin. The trafficking of N-cadherin has a direct impact in neuronal polarization and migration events. (**a**) Signaling pathways controlling axonal specification and growth in multipolar neurons (thick green-edge). (**b**) Golgi apparatus location specifies the axon and defines the leading process (opposite thick pink-edge). (**c**) Polarization also requires signaling/trafficking pathways controlling morphological changes, including the regulation of N-cadherin trafficking. (**d**) Bipolar neurons start locomotion migration by establishing the attachment between neuron and the RG. (**a**′) In a significant proportion of multipolar neurons, the axon emerges when the neuron is attached to RG via N-cadherin; this contact point activates Rho GTPase to restrict Rac1 activity specifically to the tip of the future axon. The pathways and molecules known to be controlled by reelin in these processes are shown in bold. For the rest of the proteins involved in the indicated processes, there is no direct evidence of reelin's role.

and the lack of polarization, and therefore the impairment to exit the upper IZ and to start radial migration. Here, reelin signaling is required to regulate several of the multiple changes that neurons undergo in this area, including neuronal polarization and their ability to exit the IZ. The predominant reelin receptor at this developmental stage is ApoER2 since an important subpopulation of early-born neurons deficient in this receptor remains localized nearby the IZ in a multipolar shape [98,99]. Recently, Hirota et al. [99] have also demonstrated the migration failure of early-born neurons from ApoER2 KO (knockout) mice, strongly suggesting that ApoER2 is involved in the somal translocation of the first cohort of excitatory neurons that move towards their

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final destination via glia-independent migration. The main reelin signaling axis in the SVZ/IZ corresponds to that involving the Dab1–Crk/CrkL–C3G–Rap1 pathway, where pDab1 is required for glia-independent somal translocation of early-born neurons [46]. Accordingly, conditional C3G-KO mice show that a large proportion of C3G silenced neurons have a multipolar shape, even at the time when neurons should be polarized [100], and they remain scattered in positions below the unsplitted PP [93]. The *in vivo* experiments performed by Jossin and Cooper using a dominant-negative version of Rap1 in neurons, resulted in a notable delay in neuronal migration from the lower IZ (SVZ/IZ) to the upper IZ and CP. Also, most of the Rap1-inhibited neurons remained multipolar, indicating that, downstream pDab1, Rap1 has a critical role in the polarization of postmitotic neurons [101]. Altogether, these observations indicate that reelin-induced C3G-mediated Rap1 activation may be required for early-born neuron polarization and for splitting the PP by somal translocation in an ApoER2- and Dab1-dependent manner.

Migration by somal translocation requires that the Golgi apparatus be oriented towards the pial surface. Polarization of the Golgi apparatus also determines which process will become the leading process required for radial migration and that will, in turn, evolve into the apical dendrite [83,102]. Initially, the Golgi apparatus is condensed and localized nearby the future axon (Figure 4a), but once this is specified, the Golgi is translocated to a perinuclear area that will determine the future leading process (Figure 4b). The change in Golgi orientation and deployment has been linked to the activity of Rap1 and Cdc42 [101]; however, another study did not find that connection, despite the fact the Dab1 was required [46]. The Stk25 kinase (STE20 family of serine–threonine kinases) participates in establishing cell polarity by interacting with STRADα (STe20-Related ADapter), an activator of the serine–threonine kinase LKB1 (liver kinase B1, also known as serine/threonine kinase 11 STK11). The LKB1–STRAD–Stk25 complex can interact with the *cis*-Golgi protein GM130, which regulates tethering of endoplasmic reticulum-derived vesicles with the Golgi [103]. The suppression of GM130, Stk25 or LKB1, by *in utero* electroporation at E14.5, causes Golgi dispersion in neurons examined at E17.5 [103], as well as the high percentage of neurons stalled in the IZ [104]. In contrast, overexpression of Stk25 causes Golgi condensation and multiple axons, a phenotype rescued by activating reelin signaling, which drives the deployment of the Golgi and the formation of a single axon [104]. As expected, the cytoskeleton is also important in the Golgi apparatus positioning in a process that is regulated by the activities of Cdc42 and Rac1 in response to reelin. Besides actin cytoskeleton regulation, these GTPases also control microtubule dynamics and the positioning of microtubule-associated Golgi vesicles in a αPIX/cool2- [a GEF (guanine nucleotide exchange factors) for Cdc42/Rac1] dependent manner [105]. It was recently shown that reelin-induced Golgi deployment and positioning as well as axonal growth are also under the control of the plus-end tracking protein (+TIPs) CLASP2. [53]. This protein is enriched in the Golgi apparatus as well in the centrosome, controlling microtubule nucleation and nucleokinesis. CLASP2 overexpression is associated with the development of multiple axons and also with Golgi condensation, while its down-regulation *in vivo* is linked to defects in neuronal polarization and migration. Most CLASP2 KD (knockdown) cells remain in the IZ and fewer are present in the IZ at P0 and, at P14, many postmitotic neurons remain in deeper layers [53]. Overall, this evidence indicates that neuronal polarization events, including the reorientation/deployment of the Golgi apparatus, and the appearance of the axon (discussed in detail in the next sections), are regulated by the reelin signaling pathway.

N-cadherin is a central plasma membrane protein with roles in neuronal migration and polarization in the SVZ/IZ. *In vivo* experiments of N-cadherin KD resulted in defective neuronal migration where two different subpopulations clearly accumulated, one in the CP and another in the IZ [106]. The localization of this membrane protein at the plasma membrane regulates cell–cell contacts [65], including the early interaction of the multipolar neuron with the RG, in a process required for the polarization of a group of multipolar neurons (Figure 4a′) [96]. N-cadherin trafficking and localization is controlled by active Rap1, via its downstream effectors such as Ral GTPases that regulate membrane trafficking and exocytosis, and also via Cdc42 and Rac1, which regulate cytoskeleton dynamics [101]. RalA or RalB silencing by shRNA (short hairpin RNA) *in utero* electroporation at E14.5 results in partial inhibition of neurons to enter the CP and the subsequent impact on RG-dependent migration, implicating Ral proteins in this phase of migration [101]. Moreover, Ral GTPases also activate the exocyst [107,108], a membrane tethering complex that has a fundamental role in membrane addition and also in neuronal migration during development. Suppression of exocyst function by expressing a dominant-negative form of the exocyst subunit Exo70 at E15 induces a significant accumulation of neurons in the VZ and IZ, at the time when the majority of them have reached the upper CP [109]. N-cadherin disturbances, associated with dysfunctions of Rap1, are dependent on reelin signaling since N-cadherin cell surface levels also appear decreased in cells expressing a dominant-negative form of VLDLR, VLDLRΔC (VLDLR

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lacking the C-terminal cytoplasmic tail) in the multipolar zone [101]. The endosomal small GTPases Rab5 and Rab11 also control N-cadherin surface expression and are required for correct neuronal migration [106]. A suppression of Rab5 induces the accumulation of round neurons in the IZ (Figure 4c). On the other hand, once neurons have become bipolar, both Rab5-dependent endocytosis and Rab11-dependent recycling pathways are required for the process of neuronal entrance into the CP, to contact the RG cells and to start their locomotion migration (Figure 4d). Therefore, reelin activation of Rap1 regulates N-cadherin localization at the plasma membrane [101], but is not known if reelin signaling controls Rab5 and Rab11 activities.

Arf6 is another relevant protein recently implicated in cortical neuronal migration and N-cadherin trafficking (Figure 4). This protein is a small GTPase that regulates a variety of neuronal functions such as membrane trafficking, neuronal polarization and axon/dendrites/spines formation [110–114]. TBC1D24 (Tre2–Bub2–Cdc16 1 domain, member 24), a protein encoded by a gene associated with epilepsy and cognitive deficits, down-regulates Arf6 activity in a direct manner promoting radial migration. In the rat embryo, the KD of TBC1D24 at E15 results in the accumulation of multipolar neurons at the IZ. This phenotype is rescued by a dominant-negative form of Arf6 (Arf6T27N), suggesting that the activation of Arf6 inhibits neuronal migration and corticogenesis [115]. These results were recently challenged by Yoshinobu Hara et al., who demonstrated that, in mice, Arf6 activity is required for N-cadherin recycling in order to promote multipolar–bipolar transition and exit from the IZ. These researchers found that the Arf6-KD phenotype is characterized by the cytoplasmic accumulation of N-cadherin, and neuronal migration impairment similar to what is found upon exocyst

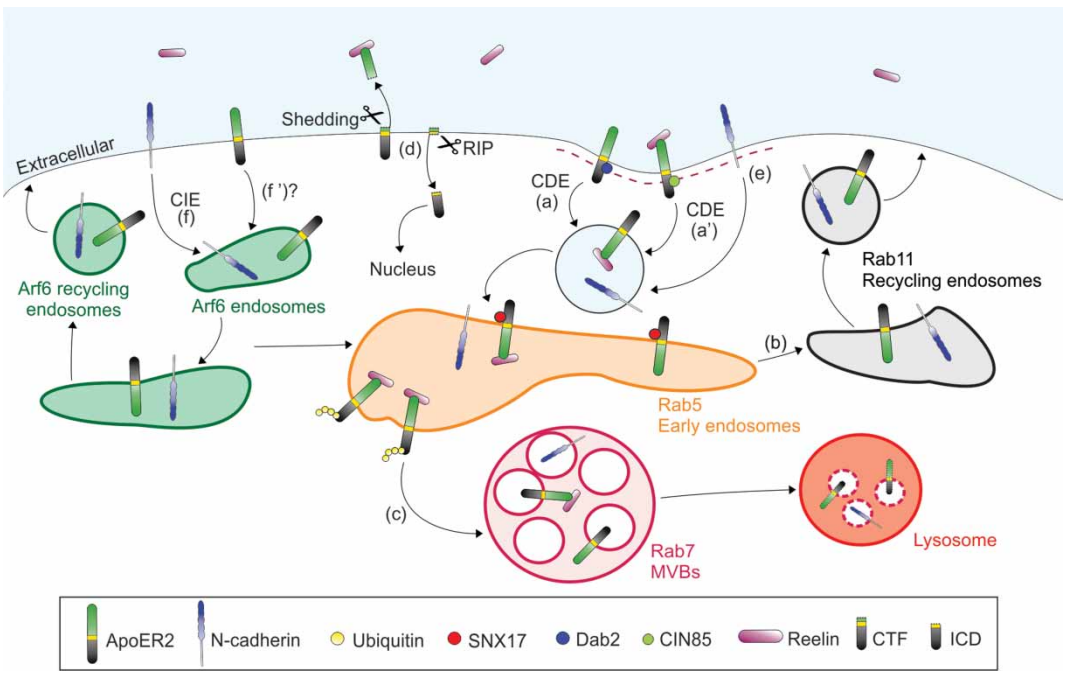


**Figure 5. Model of endocytic and recycling routes for ApoER2 and N-cadherin.**

The endocytosis/recycling and degradation of N-cadherin depend on Rab5, Rab11, Arf6 and Rab7 functions. ApoER2 endocytosis is Dab2 (**a**) or CIN85 (**a**′) dependent (constitutive or reelin-induced, respectively) and requires Clathrin (CDE). The receptor arrives at a Rab5 endosomal compartment (early endosomes) from which is sorted either to the recycling or to the degradation pathway. (**b**) Recycling to the plasma membrane is assisted by the endosomal adaptor protein SNX17, which binds the NPxY motif of the receptor. This recycling is either direct or via Rab11 recycling endosomes. (**c**) In the presence of reelin, ApoER2 would be ubiquitinated and degraded in lysosomes. (**d**) Reelin binding to ApoER2 also induces receptor 'shedding', resulting in a soluble extracellular fragment; and a carboxyl-terminal fragment or CTF that, as a γ-secretase complex substrate, gives rise to a soluble intracellular fragment (ICD). The ICD of ApoER2 goes to the nucleus and regulates gene expression. (**e**) N-cadherin could also follow a CDE and/or (**f**) Clathrin-independent endocytosis (CIE)–Arf6-dependent pathway, and then recycles (Rab11) or is degraded, via multivesicular bodies (MVBs), in lysosomes. (**f**′) It is possible that ApoER2 could also follow the Arf6 pathway.

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complex suppression [109] and consistent with the role of Arf6 in the activation of this complex [116]. The contrasting results of these two studies could be explained by differences in the experimental conditions. On the other hand, because the phenotype of Arf6 suppression was not fully rescued by N-cadherin overexpression, Arf6 might have other targets and/or regulate the trafficking of other cargos [116]. A similar situation has been observed in the cases of down-regulation of Rab5 and Rab11 as we mentioned before [106]. Here, we hypothesize that, besides N-cadherin, ApoER2 trafficking could be under the control of Rab 5, Rab11 and possibly of Arf6 (Figure 5). Hence, (1) ApoER2 traffics via Rab5 endosomes after clathrin-dependent endocytosis [117,118]; (2) the receptor enters into Rab11 endosomes to recycle to the plasma membrane, stimulated by the adaptor protein Sorting Nexin 17 (SNX17) [117]; (3) ApoER2-silenced neurons display a similar phenotype to Arf6-KD and Rab5-KD neurons, with accumulation of multipolar neurons in the IZ [99,106,116] and (4) ApoER2 is the main reelin receptor in the IZ [119].

The participation of reelin signaling in neuronal polarization and migration may also include other components and pathways. *In vitro* studies conducted in non-neuronal cells show a direct role of Dab1 on the actin regulatory protein N-WASP [120] as well as being dependent on PI3K and Cdc42 (Figure 2) [49]. In the context of neuronal migration, the pDab1–PI3K pathway leads to the inactivation *n*-cofilin [50] via LIMK in an ApoER2-dependent manner. PAK1 (p21-activated kinase) phosphorylates LIMK1 on threonine residue 508 increasing its activity on *n*-cofilin (Figure 2) [121,122]. PAK1 is a key effector of Rac1 and Cdc42 [121], known targets of reelin via ApoER2 [49,123,124]. *In vivo* experiments show that loss of PAK1 results in the accumulation of neurons with aberrant morphology in the IZ and deep layers (Figure 4) [125]. Regarding polarization, studies in primary cultures of hippocampal neurons show that PAK1 acts upstream of LIMK1, whose overexpression increases axon formation and enhances the accumulation of the polarity complex proteins Par3 and Par6 at growth cones via *n*-cofilin inhibition [126]. PAK1 also plays a role in neurite outgrowth and is regulated by Src kinases [126]. Therefore, in spite of lack of evidence on the role of PAK1 in the context of reelin signaling, it is very likely an element of the cascade. Rac1 has also a role in neuronal migration when activated via P-Rex1, a Rac GEF localized at the leading process of migrating neurons. *In utero* electroporation experiments using a dominant-negative form of Rac1 at E14 and evaluated at P0, results in inhibition of neuronal migration with neurons trapped in the IZ [127]. Similar results are observed when, instead of electroporating a Rac1 D/N form, a D/N form of P-Rex1 is expressed. However, besides the neurons not being able to exit the IZ under this condition, there is another group of neurons able to reach the layers beneath the MZ [115], indicating that the activity of Rac can be controlled by other GEFs. In addition, Tiam1 (T-cell lymphoma invasion and metastasis-inducing protein 1), a major Rac1-specific GEF, was recently found to be required for reelin-induced Schwann cell (SC) migration [110]. Tiam 1 interacts with polarity protein Par3, and this interaction is also necessary for reelin-induced SC migration [110]. Even though this evidence was found in a different cellular context, it is relevant considering that *in vivo* experiments show that Tiam1 silencing suppresses radial neuronal migration [114] while Par3 silencing results in loss of tissue polarity [128].

Regarding cytoskeletal dynamics, reelin also controls the microtubular network, which is relevant for cell migration. The microtubule-associated protein 1B (MAP1B) is required for cortical migration during development and its phosphorylation level is controlled in response to reelin in a pDab1-dependent manner (Figure 2) [129]. Reelin activation allows PI3K/Akt and p35/Cdk5 (p35 as a Cdk5 neuron-specific regulator) to control GSK3β activity and MAP1B phosphorylation [129–131]. In contrast with *reeler* mice, MAP1B-deficient animals do not show PP splitting defects (E10), but the SP displays migrating neurons that fail to settle properly into their correct final destination, indicating that MAP1B has a role in migration after PP splitting (from E13). MAP1B also regulates axonal growth by stimulating microtubule assembly and stabilization (Figure 4a) [132,133]. Other reelin-regulated processes that involve the microtubule cytoskeleton are discussed in the following sections.

## Locomotion: radial glial fiber-dependent migration

In the locomotion mode of migration, bipolar neurons move towards the cortical surface using the RG fibers as scaffold (Figure 3B). In this step, which accounts for the largest part of neuronal migration, bipolar neurons exiting the IZ migrate along the RG fiber until they anchor their leading process to the MZ. This 'inside-out migration' process is unique to mammals [134,135]. In the *reeler* mouse, the RG cells are affected and the defects in locomotion are mainly due to the RG scaffold [136]. Cortical RG cells, at about E14, display sparse, poorly packed and differentiated fibers where their extending feet towards the MZ are shorter and where some of them fail to form end foot along the pia mater [136].

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During locomotion, the control of N-cadherin surface expression in migrating neurons and in RG cells is required to establish N-cadherin-mediated adhesion complexes [106,137]. During locomotion, N-cadherin is internalized into Rab5 early endosomes, especially at the trailing edge of the neuron, and recycled through Rab11-dependent mechanisms at the leading edge [78,106,137]. Recently, it was demonstrated that reelin increases RG-neuron adhesion via N-cadherin in a transient manner [138], somehow implicating this signaling pathway in late-born neurons, in order to pass through the CP [138]. On the other hand, β1 integrin has a role in cell–matrix attachment and participates in cell migration. However, *in vivo* studies in mice with neuronal-specific β1-integrin suppression have failed to show alterations in cell layers, indicating that the expression of β1-integrin is not required for RG-guided migration [139,140]. In contrast, when β1 integrin is absent from both RG and migrating neurons, migration is impaired. However, the phenotype of these animals is not similar to *reeler* [139].

So far, most of the evidence does not support a determinant role of the reelin signaling pathway, specifically in neuronal locomotion. Early studies suggested that the reelin pathway elements Dab1–PI3K and Akt are required for the organization of the CP, although the downstream Akt effectors mTOR or GSK3β are apparently not involved [54,58]. Also, the migration defect seen in ApoER2 KO mice is partially overcome by activation of Akt [84]. However, it is not completely clear if reelin controls neuronal migration via the Akt pathway. Upstream of Akt is PDK1, which is required for speed regulation of neurons during bipolar locomotion by controlling the coupling of the nucleus with the centrosome [123]. PDK1 ablation results in layering defects distinct from those of *reeler* mutants, since only the upper-layer neurons show malpositioning. Other studies have discarded the participation of Dab1 and Rap1 in migratory cells during locomotion. *In vivo* experiments, using Dab1-deficient mice, showed that locomoting neurons are able to pass through their predecessors with no differences in migration speed with respect to the control group, and they reach the CP just beneath the MZ (where terminal somal translocation takes place) [46]. These results suggest that Dab1 is mainly required in the terminal translocation step [141] but not during locomotion [72]. Similar results were obtained from Rap1-silenced neurons [101]. The expression of Rap1GAP (GTPase-activating protein) in neurons shows that their locomotion through the CP occurs at similar speed than in wild-type neurons but they fail to form an apical dendritic tree at the top of the CP [101]. This evidence indicates that Rap1 is not pivotal during locomotion migration [101].

## Terminal somal translocation and stop migration signal

The terminal somal translocation, the last step in the journey of the migratory neuron that takes place and ends in the reelin-rich MZ, corresponds to the glial-independent movement that occurs immediately prior to migration arrest. For neurons that arrive at the MZ by locomotion mode, somal translocation also requires the detachment from the RG followed by extension and stabilization of their leading processes (Figure 6) [71]. It has been described that terminal somal translocation needs the degradation of N-cadherin in the soma of neurons via a Rab7-dependent pathway, as well as its stabilization at the leading process. Disruption of Rab7 is associated with neuronal positioning defects near the top of the cortical plate (Figure 6) [106].

Reelin, secreted locally by Cajal–Retzius cells, promotes adhesion, stabilization and branching of Dab1-expressing neurons, specifically their apical processes, in the MZ (Figure 6) [142]. Franco et al. demonstrated that terminal translocation is Dab1-dependent. Dab1-KO mice show defects in the polarization of the Golgi apparatus, retracted leading processes and are therefore unable to contact the MZ, indicating the need of Dab1 for neuronal positioning [46]. Dab1 is required for the reelin-induced activation of PI3K–Cdc42/LIMK–cofilin axis, resulting in the stabilization of actin at the leading process. The attachment of the leading process to the MZ allows terminal somal translocation and stop migration [50]. *Reeler* mice do not show increased phosphocofilin levels in the leading process of late-born migrating neurons arriving at the MZ [50]. Consistently with its role as an LIMK activator, PAK1 is also required for leading projections during neuronal migration and stop migration at the MZ [125].

In addition, the Dab1–Crk/CrkL–C3G–Rap1 pathway regulates the recruitment of cell–cell interaction molecules such as Nectins 1/3 (immunoglobulin-like adhesion molecules) and N-cadherin (Figures 2 and 6) [46,101,143,144]. The deleterious effects of Rap1GAP overexpression that decrease Rap1 activity can be partially rescued by N-cadherin overexpression. In contrast, the Dab1-KO migration phenotype cannot be rescued, indicating that Rap1-dependent N-cadherin expression in the plasma membrane is not the only mechanism acting downstream from Dab1 [46,101]. Cajal–Retzius cells in the MZ express Nectin 1, and somal translocating neurons express Nectin 3 [143]. The cytoplasmic domains of these nectins interact with the adaptor

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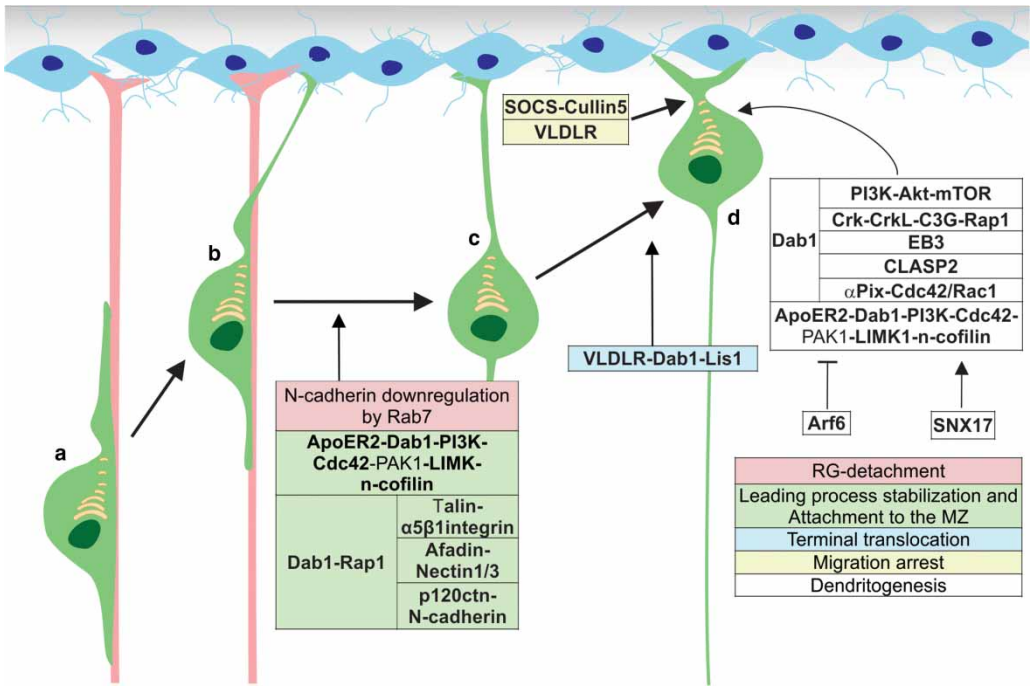


**Figure 6. Terminal translocation and migration arrest.**

Terminal translocation is reelin- and Dab1-dependent. Migrating neurons (in green) stop their movement by adhering their apical processes to the Cajal–Retzius cells (in light blue) in a sequence of events: (**a**) movement towards the reelin-rich area at the MZ. (**b**) Extension of the leading process and detachment from the RG (in pink), in a process that needs Rab7-dependent N-cadherin degradation. (**c**) ApoER2/Reelin-induced leading process stabilization results in the inactivation of *n*-cofilin. The leading process attaches to the Cajal–Retzius cells via Rap1 activation, resulting in the insertion of nectin 3, α5β1 integrin and N-cadherin at the plasma membrane, and the establishment of cell–cell and cell–matrix interactions. (**d**) The detachment of the neuron is followed by somal translocation, stop migration and the maturation process. Stop migration depends mostly on VLDLR and requires SOCS/Cullin5. Dendritic outgrowth, including branching, is part of the maturation process and involves several pathways and molecules known to be controlled by reelin. In addition, endosomal membrane trafficking proteins such as Arf6 and SNX17 (that have been associated with inhibition or stimulation of dendritogenesis in hippocampal neurons *in vitro*) could have a role in this process *in vivo*. Reelin-related processes are shown in bold.

protein Afadin [145,146], and the complex Nectin3/Afadin stabilizes the leading process in the MZ. Rap1 interacts with Afadin and with p120 catenin (p120ctn), facilitating the recruitment of N-cadherin to the adhesion sites of Nectin 3 [143,145,146]. On the other hand, the scaffold protein p120ctn prevents N-cadherin endocytosis and degradation in the apical process [147]. These results suggest that reelin signaling provokes the assembly of N-cadherin and Nectins adhesion by inducing the formation of the Afadin–Rap1–p120–ctn cytosolic complex in order to stabilize N-cadherin and Nectins at the cell surface. The Dab1–Crk/CrkL–C3G–Rap1 pathway also participates in terminal somal translocation leading to the inside-out activation of α5β1 integrins and, in this way, to the attachment of neuronal apical processes to fibronectin in the MZ (Figures 2 and 6) [144,148]. By forming a complex with RIAM (Rap1-interacting adaptor molecule) [149,150], Rap1 directly activates Talin, a protein that binds to the cytoplasmic NPxY motif of β1 integrin and to actin filaments [151] increasing integrin affinity for extracellular matrix proteins (Figure 2) [149]. Altogether, these mechanisms enable terminal translocation, the end of radial migration near the reelin-rich MZ and the formation of the cellular layer [143,144].

The control of neuronal migration by reelin also includes microtubule function and stability via the interaction of Dab1 with Pafah1b1 (platelet-activating factor acetylhydrolase 1b) or Lis1, a regulatory subunit of the Pafah1b complex (Figure 2) that is present in all migrating neurons during development of the human brain. Mutations in the *LIS1* gene associate with a reduction in the number of reelin-producing Cajal–Retzius

neurons [152]. Lis1 suppresses microtubule dynamics reducing microtubule catastrophe events by interacting with tubulin [153]. In addition, mammalian Lis1 is enriched in the centrosome, interacts with dynein and promotes nucleus–centrosome coupling in migratory neurons [154]. In response to reelin/VLDLR, Lis1 interacts with pDab1 when Dab1 is phosphorylated on Tyr198 and Tyr220, and both proteins colocalize in the perinuclear area [155]. The catalytic Pafah1b complex subunits (α1 and α2) bind to the NPxYL sequence of VLDLR (and not to ApoER2), promoting the interaction of pDab1 with Lis1 [156] and therefore allowing nuclear translocation (Figure 6) [155].

There are controversial studies showing different roles of reelin receptors in somal translocation and migration arrest. VLDLR is expressed mainly in the most superficial part of the CP, whereas ApoER2 is preferentially found in the lower part of the IZ but also beneath the MZ [104]. Hack et al. demonstrated that VLDLR-KO mice display overmigration of neurons in the MZ, consistent with a role of VLDLR in the signal for stopping migration. On the other hand, ApoER2 KO mice exhibit two accumulated populations of neurons, one in the upper part of the cortex right underneath the MZ and the other one trapped close to the VZ [83], consistent with the pro-migratory role of ApoER2 [83]. In contrast, the work of Trommsdorff et al. has shown that neurons from VLDLR-KO mice do not invade the MZ [46]. As reelin is secreted from Cajal–Retzius cells in the MZ, its full-length form might be present mainly in the MZ rather than in deeper areas where reelin fragments are probably present, instead of the full-length protein [145]. Of note, the C-terminal region of reelin is required for VLDLR binding, and mutant mice expressing reelin that lacks this region display overmigration of neurons in the MZ [146], similarly to VLDLR-KO mice [83]. These results may suggest that VLDLR is the reelin receptor necessary for stopping migration. Nevertheless, recent studies from Hirota et al. show that ApoER2 also has an important role in the last step of migration [84] in a process involving Rap1, α5 integrin and Akt. The overexpression of a constitutively active form of Rap, or of wild-type Akt, together with a constitutively active form of α5 integrin partially rescues overmigration of neurons into the MZ in the ApoER2 KO mice [84]. Therefore, it seems that ApoER2 and VLDLR are both relevant for stopping migration since the double KO mutant has a *reeler* phenotype, with invasion of neurons in the MZ. However, the contribution of both receptors seems to be different and, even now, is still unclear.

The migration arrest signal is not fully understood (Figure 6). Some evidence indicates that Dab1-mediated signaling needs to be rapidly down-regulated by a mechanism that involves the ubiquitination of pDab1 by the E3 ubiquitin ligase component Cullin 5, followed by its degradation by the proteasome system (Figure 2) allowing for proper termination of neuronal migration [157,158]. Down-regulation of Cullin 5, by in utero electroporation of its shRNA, blocks Dab1 degradation and induces overmigration of cortical neurons [157]. These results suggest that Dab1 degradation is essential for proper cortical development. Another study considers that the protein SOCS6 has an important role in the termination of reelin signaling [159]. SOC6 belongs to the suppressors of cytokine signaling (SOCS) family thought to act largely as negative regulators of cytokine or growth factor signaling. It has been proposed that SOCS6 and SOCS7 are absolutely required for PP splitting and inside-out cortical layer formation because double KO mice display cortical layer inversion similar to *reeler*. Individual mutants of SOCS6 or SOCS7 do not show this phenotype [159]. The degradation of Dab1 is under control of SOCS6 and SOCS7, which are required for Dab1 ubiquitination mediated by Cullin 5. However, recent evidence points to SOCS6 and Cullin5 as regulators of focal adhesion stability at the leading edge of migrating cells [160]. The mechanism involves turnover inhibition of the focal adhesion by sending phosphorylated Cas (adhesion disassembly promoting protein) to the ubiquitin proteasome degradation system via SOCS6/Cullin5 [160]. Even though this evidence was obtained in non-neuronal cells and in the absence of reelin, it indicates that the roles of SOCS6 and Cullin5 in migration could be broader than the control of Dab1 levels in neuronal migration. Therefore, the interpretation of a pro-migratory function of Dab1 under Cullin5 suppression should be considered with caution. Altogether, the current evidence suggests that the reelin signaling pathway is necessary for the terminal step of migration.

Reelin signaling is also relevant for dendrite development after arrest, and several pathways may operate at this stage (see also the section Dendritic development and refinement) (Figure 6). In neurons close to the MZ, reelin accelerates microtubule assembly and stabilization in the developing neuronal processes via the microtubule plus end-binding protein 3 (EB3) [161]. EB3 enrichment at the MZ is not evident in the *reeler* neocortex [161]. Interestingly, the localization of EB3 can be regulated by the microtubule-associated protein tau [162], which is also under the control of reelin signaling [40,52]. Another microtubule-interacting protein, CLASP2, has also a role in the regulation of neuronal differentiation and dendritic growth under reelin control, possibly due to its function in the control of Golgi positioning in the leading process [53].

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# Reelin signaling in the regulation of neuronal polarization and differentiation

Much of the knowledge regarding the basic determinants and steps of neuronal polarization comes from *in vitro* studies [163–166]. It is still a challenge to establish how the process of neuronal polarization occurs *in vivo*. In this scenario, it is also challenging to determine the role of different signaling pathways, including the one triggered by reelin, in this complex process. The use of *in vitro* models (primary culture neurons and brain slices and explants) plus the new tools for *in vivo* studies (*in utero* electroporation, different constitutive and conditional transgenic mice, cutting-edge microscopy) are making essential contributions to a better understanding of the process of neuronal polarization and differentiation. They are also important in analyzing the participation of different molecules with roles in cytoskeleton dynamics and membrane trafficking.

## Emergence, growth and branching of the axon

Central to the development of neuronal polarization are the processes of axonal specification and extension [167]. The different steps in neuronal polarization and the cellular machinery involved in this event have been extensively characterized thanks to the *in vitro* model of rodent-dissociated cortical and hippocampal neurons. The key step of polarization in the *in vitro* model is defined by the appearance of an axon emerging from one of the neurites, while the remainder neurites become the dendritic tree [164,166,168].

During the development of the neocortex, for some excitatory neurons, the polarization is defined by the emergence and outgrowth of the axon that takes place in the IZ [101,169], specifically at the level of the multipolar neurons [97]. These cells are characterized by the presence of actin-rich immature neurites with behaviors reminiscent of those of lamellipodia and filopodia in the growth cones of growing axons [170]. The emerging axon corresponds to the trailing process of the migratory bipolar neuron at the upper IZ [97]. Axonal specification and posterior extension depends on extrinsic [171,172] as well as on intrinsic factors. The actual information linking reelin and its signaling pathway with this developmental process is rather scarce, but there is evidence showing that, after birth, reelin regulates axonal growth and branching in the hippocampus, with *reeler* mice showing reduced branching [173]. In the studies of developing neocortex, the possibility that reelin regulates axon specification has been considered less important mainly because, as discussed in the previous section, the majority of reelin is present in the MZ [1]. However, recent evidence complements previous detection of reelin mRNA [37,174,175] with the presence of the reelin protein and ApoER2 in the lower part of the IZ, the cortical area enriched in multipolar neurons [119]. Studies performed on cortical development indicate that inhibition of Rap1 does not prevent cortical axogenesis [101], contrasting with the requirement of Rap1B for *in vitro* hippocampal axogenesis [176,177]. Recently, however, Shah et al. [100] demonstrated very elegantly, *in vitro* and *in vivo*, that the activity of Rap1, triggered by its GEF C3G, plays a fundamental role in axogenesis both in the cortex and in the hippocampus (Figure 4a). Although these authors did not test the role of reelin, many of the features of the neuronal migration defects they found resembled the *reeler* phenotype. More interestingly, the requirement of C3G for cortical axon formation is at a much earlier stage than for the hippocampus [100]. Moreover, the cortical and hippocampal inactivation of the reelin target Cdc42 leads to evident defects in axon formation in mice [178]. Similarly, decreased expression of CLASP2 affects reelin-induced effect on axonal growth [53]. Another pathway recently associated with axonal emergence of pyramidal neurons at the upper IZ is dependent on N-cadherin-mediated interaction between the migrating neurons and the RG [96]. In this case, the roles of Rap1 and C3G were not tested. However, the authors suggest that Rap1 could act upstream of N-cadherin expression and be activated by the axis Reelin–C3G–Rap1 and/or by Cdk5–RapGEF2 (Figure 4a′) [179].

Many pathways and proteins involved in axonal specification and extension share elements with reelin signaling. However, few studies have addressed the connection of reelin signaling with axonal growth. The Src family of kinases has a central role in the reelin pathway. They are activated by extracellular stimuli and regulate the function of small GTPases of the Rho family, including Rac1, via the activation of its GEFs Dock1/Dock180 and Trio [180,181]. Of note, both GEFs are involved in axonal growth [182]. Reelin signaling activates Dock1 via its interaction with Crk, which is recruited to the membrane by pDab1 [183], but this activation has not been studied in the context of axonal growth. PI3K is relevant for axonal specification [102,168] because in cultured hippocampal neurons its lipid product, phosphatidylinositol-3,4,5-triphosphate (PIP3), accumulates at the tip of the future axon [184]. PI3K is activated by extracellular signals mediated by cell surface receptors and Ras GTPases [185]. Not only PI3K but also Akt/PKB (protein kinase B) is present at the tip of the future axon,

and this is relevant for the local inactivation of GSK3β [186]. Inactivation of GSK3β allows unphosphorylated Adenomatous polyposis coli (APC) to stimulate microtubule assembly stabilizing their growing ends [187,188]. Reelin treatment of cortical and hippocampal neurons increases axonal extension and growth cone motility dependent on its receptor ApoER2 and on PI3K [189]. These effects require Cdc42, but do not require the inactivation of GSK3β [189]. Both Cdc42 and Rac1 activities have been associated with axon specification [176,190] and extension (Figure 4a) [190]. Moreover, deletion of Rac1 affects axon growth *in vivo* [191]. The activation of Cdc42 could also be downstream from Rap1 [176]. Interestingly, in the previously mentioned work by Xu et al. [96], *in vivo* and *in vitro* experiments show that axon emergence for a group of multipolar cells in their transition from multipolar to bipolar neurons depends on the activation of Rac1. This activation takes place at the opposite side of the N-cadherin-mediated contact of the neuron with the RG. At the contact site, that defines the leading process of the neuron starting its locomotion mode to the pia, there is a local activation of Rho GTPase that inhibits Rac1 (Figure 4a′) [96]. Another relevant element in axonal specification, associated with PI3K and Rho GTPases, is the Par (partitioning defective) complex [188]. Par3 and Par6 associate and colocalize with Cdc42 as well as with Rac1 during axonal specification [176] in a PI3K-dependent way [184]. The Par3–Par6–aPKC (atypical protein kinase C) complex can also activate Rac GTPase via the recruitment of its GEFs Tiam1 and STEF (Tiam2) that bind directly to Par3 [190,192]. Tiam1 is required for axon specification [193], and its overexpression induces the formation of multiple axons *in vitro* [190]. Tiam1 also links Rac1-mediated activation of actin dynamics with microtubules at the axonal growth cone [193]. Tiam1 interacts with microtubules in the brain [193], as well as with Map1B [194]. Could reelin signaling and the polarity complex be connected in some way, specifically to Par3 and Tiam1? *In vitro*, Par3 is phosphorylated by ERK2, and this event correlates with Par3 localization at the tip of the growing axon [195]. In this regard, it has been shown that reelin signaling activates ERK1/2 either in a non-canonical pathway in cortical neurons [59] or in a canonical Dab1- and PI3K-dependent pathway during development [196]. The reduction in Par3 using *in utero* electroporation at E13 dramatically decreases the polarization of multipolar neurons into a bipolar phenotype characterized by the emergence of the trailing process [195]. As already discussed, Par3/Tiam1 and reelin have been recently found to be connected to the process of SC migration in the peripheral nervous system mediated by ApoER2 [124]. Moreover, the positioning of Par3 at the leading process of the migratory SC could also be mediated by its direct binding to the ApoER2 cytoplasmic domain [124]. Therefore, we can speculate that, at the neuronal level, this system (ApoER2–Par3–Tiam1–Rac1) could function in axon specification and/or elongation induced by reelin (Figure 4a).

Other reelin-related proteins involved in axonal growth are the adaptor protein Nck (non-catalytic region of tyrosine kinase adaptor protein 1) and GTPases of the Ral family (Figure 4). Nck binds to pDab1 in response to reelin [197]. This adaptor has been associated with both axon repulsion and attraction mechanisms. Depending on the upstream regulators and the timing of engagement in attraction and growth, Nck downstream partners include Rac1 and Cdc42 acting on the remodeling of the actin cytoskeleton at the growth cone [182]. Ral GTPases have also been associated with axonal growth [198]. Reelin signaling could affect on these GTPases considering the described role of Rap1 as an activator of RalA and RalB GTPases in the context of neuronal migration during development [101]. As mentioned, Ral GTPases activate the exocyst complex [107,108], which also regulates cortical neuronal migration [109]. In hippocampal neurons, the exocyst subunit Exo70 interacts with the small GTPase TC10 (related to Cdc42), and this interaction promotes the translocation of the exocyst complex to the distal axon positively regulating axonal specification and expansion [199]. Axonal branching is part of the neuronal developmental process. *Reeler* mice exhibit reduced branching [173], and the *in vitro* stimulation of cortical neurons with reelin in the presence of the VIP (vasoactive intestinal polypeptide)/PACAP38 (pituitary adenylyl cyclase activating polypeptide 38) peptidergic system increases axonal branching [189]. Reelin signaling could also be part of the regulation of membrane trafficking required for axon outgrowth and branching, including dendritic outgrowth (see below). This is reinforced by the observation that *in vitro* activation of hippocampal neurons with reelin results in an increase in intracellular vesicle trafficking [189] associated with the growth of neuronal processes. As mentioned, reelin/ApoER2 activates LIMK activity. The presence of LIMK in the Golgi apparatus has been linked to axonal growth and to the presence of Par3–Par6, IGFR (insulin-like growth factor receptor) and NCAM (neural cell adhesion molecule) in the growth cone [126]. In this regard, it could be interesting to investigate if reelin is able to trigger local (Golgi apparatus) activation of LIMK, required for the formation of axonal cargoes exiting from the Golgi, as has been demonstrated for apical proteins in epithelial cells [200].

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Axonal growth is also under the control of the small GTPases of the Rab family [201,202], especially Rab5, Rab8, Rab11 and Rab35 as well as Arf6 [203]. In this regard, and as was described for neuronal migration, there is no information about the role of reelin in the activation of these GTPases. While the early endosomal protein Rab5 has controversial roles in axonal growth, at least in hippocampal neurons, this GTPase is activated by its GEF Rabex5 in the axon; and the reduction in either Rab5 or Rabex5 is associated with a substantial reduction in total axonal length [204]. In the case of Rab11, associated with the formation of vesicular cargoes from recycling endosomes, it has a positive role in axonal growth *in vitro* [205] and can be indirectly activated by Fyn kinase [201], one of the targets of reelin signaling [38]. Rab8 interacts with Rab11 in a GRAB (guanine nucleotide exchange factor for Rab3A)-dependent manner. GRAB, a Rab8 GEF, promotes axonal outgrowth [206]. Interestingly, GRAB is phosphorylated by Cdk5, which has been also associated with reelin signaling [130,207]. Another GTPase connected with axonal growth is Rab35, a negative regulator of Arf6 [208]. While active Arf6 inhibits axonal growth [209], the activation of Rab35 is involved in hippocampal axonal growth [201].

## Dendritic development

Dendritogenesis underlies the formation of layers during neocortex development [92]. In *reeler* mice, this process is severely impaired [18,19,31,91,210–216], including dendritogenesis of the radial glial cells [217]. However, reduced neuronal arborization is not necessarily caused by defects in cell migration; in fact, neurons from heterozygous *reeler* mice have reduced neuronal arborization [18], but do not necessarily show severe alterations in neuronal positioning. The effects of reelin in dendritic growth and branching require its binding to ApoER2 (and VLDLR) [18,35,44,54] as well as the presence of pDab1 [44,141].

As previously mentioned, reelin signaling promotes Golgi orientation and deployment, an event that occurs after axonal specification [218] and before the determination of the leading process (future apical dendrite). Neurons of cortical layer VI from *reeler* and Dab1-KO mice have altered dendrite length and orientation, and present a compact Golgi apparatus. These features correlate with the lack of a defined apical dendrite and the presence of several primary dendrites emerging from the soma [219]. The addition of reelin to brain slices from *reeler* mice induces a significant polarization of layer VI neurons, with reorientation of the nucleus and Golgi towards the pia and an evident dendritogenesis process [92]. In the hippocampus, reelin determines Golgi translocation and deployment into the primary dendrite via the activation of Cdc42 GEF αPix/cool2 [105]. Therefore, reelin regulates Golgi position and deployment as part of the mechanism that determines the formation of the dendritic tree (Figure 6).

Upon arriving at their final destination, migratory neurons anchor to the MZ, detach from the RG and develop the dendritic arbor in a reelin-dependent manner (Figure 6) [217]. As discussed previously, the activity of LIMK1 is required for leading process stabilization and attachment to the MZ. Dendritic branching depends on controlled cytoskeleton dynamics and may also require activation of LIMK1. The expression of the GEF — αPix/cool2 — has been associated with the phosphorylation of *n*-cofilin [220], supporting a Cdc42-dependent activation of LIMK1. In this way, reelin could activate a signaling axis including Dab1–αPix/cool2–Cdc42/Rac–PAK1–LIMK1 to regulate dendritic development (Figure 6) [105,122,220,221]. Dendritic outgrowth and Golgi assembly/disassembly also relay on the microtubular cytoskeleton; Rho GTPases also control microtubule dynamics [81,222,223] and reelin promotes microtubule stabilization in growing dendrites through plus-end tracking proteins EB3 [161] and CLASP2 [53].

In the hippocampus, the conditional postnatal elimination of Dab1 from progenitors of the dentate gyrus cells induces anomalous grow of basal dendrites in the hilus and decrease in the length and branching of the correctly oriented dendrites [224]. Dendritogenesis induced by reelin depends on the Crk/CrkL pathway but also on the activation of mTOR complexes, downstream from PI3K and Akt [54]. mTOR activation allows phosphorylation of its substrate p70S6K, a kinase involved in the control of protein translation [51]. Interestingly, during early postnatal stages in the hippocampus, reelin-induced dendritogenesis of CA1 neurons is absolutely dependent on Crk/CrkL and therefore probably less dependent on mTOR, suggesting that the machinery required would rely on cellular context and/or developmental stage. Moreover, Crk/CrkL is specifically needed for reelin effects but not for the outgrowth induced by other trophic factors such as BDNF (brain-derived neurotrophic factor) [44].

The extent of reelin-induced signaling is also highly dependent on the machinery regulating reelin receptors trafficking and availability. Hence, the regulation of ApoER2 trafficking has a direct impact on dendritic growth [117]. SNX17 is an endosomal protein that binds to the NPxY motif in the cytoplasmic tail of ApoER2, regulating its exit from Rab5 early endosomes into the Rab11 recycling endosome and to the plasma membrane

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(Figure 5). Disruption of this pathway in cortical and hippocampal neurons leads to a significant reduction in reelin signaling and dendritic outgrowth (Figure 6) [117]. SNX17 also binds to the cytoplasmic domain of other reelin receptors such as VLDLR [225] and APP [226] and of β1 integrins [227]. It can be predicted that the effects of SNX17 reduction on reelin signaling probably include other targets and could alter neuronal migration during development, an aspect that has not been yet tested. Another way to control ApoER2 availability is through its proteolytic processing induced not only by reelin [228] but also by neurotrofin-induced signaling of Trk receptors [228]. Specifically, the activation of hippocampal neurons with BDNF induces, via its receptor TrkB, the shedding of the ApoER2 ectodomain [229].

In *Drosophila* and *Caenorhabditis elegans*, dendritic outgrowth requires the participation of the exocyst complex [230–232]. As it was already discussed, the exocyst has a role in mammalian cortical neuronal migration [109] and axonal branching [198]. The function and localization of this complex is regulated by its association with microtubules [233] and the activation of several small GTPases [234], including Rab11 [235], Arf6 [236], TC10 [199] and Ral (Figure 4) [107,108]. Therefore, reelin-induced dendritic outgrowth could also include the activation of the exocyst at least for the direct activation of Rap1 that acts upstream of Ral GTPases. In another context, in primary hippocampal neurons, reelin activates CaMKIIβ [58]. Interestingly, this kinase has been connected with BDNF-induced activation of LIMK1 in the process of neuritogenesis of cortical neurons *in vitro* [237], opening the possibility that reelin-induced dendritogenesis would also include CaMKIIβ/LIMK1. In this regard, and in contrast with what happens at the MZ in the neocortex, hippocampal reelin expression continues postnatally and in the adult, specifically in the MZ of the dentate gyrus (outer molecular layer), allowing the migration and polarization of granular neurons.

As for its role on neuronal migration [115,116], the function of the small GTPase Arf6 is also puzzling in terms of neuronal differentiation/maturation. The inactivation of Arf6 in hippocampal neurons *in vitro* results in the enhancement of dendritic and axonal outgrowth and branching [110,209]. Accordingly, *in vivo* electroporation of the Arf6 inhibitor TCBID24 increases dendritic outgrowth similarly to what happens when the GDP form of Arf6 is expressed [115]. Overall, these data suggest that Arf6 is a negative regulator of dendrite and axon formation/extension. As possible mechanisms for these actions, it has been shown that Arf6 counteracts the activation of Rab35. Arf6 and Rab35 mutually affect their activation state; specifically, the Arf6 effector EPI64B is a Rab35 GAP [238] and Rab35 effector ACAP2 (centaurin-β2) is an Arf6 GAP [208]. Rab35 activation is also associated with the formation and trafficking of vesicles from recycling endosomes to the neurite tip during outgrowth [239,240] and, as already mentioned, to axonal growth in hippocampal neurons [241]. In contrast, another set of evidence shows that Arf6 activity is required for neurite outgrowth. For example, Arf6 is activated by the adaptor protein Fe65, which binds to APP and to several members of the low-density lipoprotein family including ApoER2 [242,243]. This activation of Arf6 is followed by Rac1 activation at the growth cone [244]. The predominant view is that Arf6 could contribute to Rac1 activation by inducing its trafficking from endosomes to specific domains of the plasma membrane where it is activated and required [245,246]. It is difficult at present to reconcile the inhibitory actions of Arf6 in neurite extension with its Rac1-promoting activity, since the latter is a well-recognized factor crucial for axon/dendrite formation and neuronal polarization [223,247]. Not surprisingly, there is also evidence that Arf6 could inactivate Rac1 [110,248]. This exemplifies the complexity of Arf6 function and highlights the importance of linking the analysis of GTPases with precise knowledge about their spatio-temporal patterns of activation. Arf6 also regulates endosomal recycling, and this aspect could be associated with the activation of Rab11 in the recycling of N-cadherin during migration of neurons at the upper IZ [116].

## Dendritic refinement

After development, reelin keeps its functions related to neuronal polarization and remodeling, including the less characterized process of dendritic pruning. In mice, reelin is still secreted by Cajal–Retzius cells in layer I 2 weeks after birth. These cells respond to serotonin via the 5-HT3A [5-hydroxytryptamine (serotonin) receptor 3A, ionotropic] receptors. Interestingly, this secretion of reelin is required for the refinement of the apical dendritic arbor of later II/III pyramidal neurons and is not dependent on the canonical-signaling pathway that requires ApoER2 and/or VLDLR [249]. In contrast, in mature hippocampal neurons, the refinement of the dendritic tree is dependent on reelin and the receptors, and interference with reelin signaling increases dendritogenesis both *in vitro* and *in vivo* [250]. This evidence indicates that, depending on the maturation stage of the neuron, reelin could have opposite effects on dendritic arborization. The pruning induced by reelin could be, as suggested by the work of Ampuero et al. [251], dependent on increased insertion of the NMDAR NR2B

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(NMDAR subunit 2B) subunit, which is normally negatively regulated by reelin during the maturation of neurons *in vitro* [252]. However, and in contrast with the wealth of information regarding the cellular and molecular elements involved in the process of dendritic outgrowth, there is no information on how reelin could induce refinement. In other systems, including *Drosophila*, the flavoprotein monooxygenase of the MICAL-1 (microtubule-associated monooxygenase, calponin and LIM domain-containing 1) family is involved in dendritic pruning [253]. This enzyme induces the disassembly of F-actin by generating reactive oxygen species [254,255]. Rab5 and ESCRT (endosomal sorting complexes required for transport)-mediated endocytic pathways are also critical for dendrite pruning in *Drosophila*, decreasing the cell surface levels of Neuroglian [256]. Another possible mechanism that could be responsible for this reelin-mediated pruning role, independent of the MICAL-dependent pathway, could involve the inactivation of mTOR activity [257]. If this last pathway is operating in mature neurons in the presence of reelin, the mTOR pathway that is activated by reelin during neuronal development should be turned off.

## Role of reelin in synaptogenesis

Learning and memory require the modification of synaptic strength. The underlying crucial events are dendritic spine formation and remodeling. Dendritic spines are micron-sized membrane protrusions of neuronal dendrites that constitute the major sites of contact for presynaptic excitatory inputs in the CNS. Reelin and its receptors, especially ApoER2, have a direct role in these processes [35,51,258]. For instance, heterozygous *reeler* mice have fewer spines and reduced levels of the scaffold postsynaptic protein PSD95 (postsynaptic density protein 95) and of the NMDAR [51]. The same is observed *in vitro* in hippocampal neurons from *reeler* animals, which upon the addition of reelin are able to restore the number of spines [35]. Heterozygous *reeler* mice have more immature spines and filopodia, and show a concomitant decrease in size and density of mature spines [259]. Moreover, the addition of reelin on mature hippocampal neurons increases the number of postsynaptic compartments characterized by the presence of PSD95, in a mechanism that is dependent on CaMKII expression [58]. Interestingly, despite the central role of Dab1 in reelin-mediated hippocampal function in the adult, its role is not associated with the formation of dendritic spines. In fact, the conditional deletion of Dab1 in the adult forebrain only affects the size of the spines, but not their formation or number [34]. In the same direction, it was recently shown that in the hippocampus, spinogenesis of newborn granule cells from young animals is not affected by reelin overexpression or by the inactivation of Dab1. What is substantially altered in these cells is the morphology of the dendritic spines; reelin disruption is associated with a decrease in mature, mushroom type spines and with an increase in filopodial inmature spines [260]. Therefore, reelin effects on spinogenesis and synaptogenesis may depend on neuronal population and developmental stages. The proline-rich insert in the cytoplasmic domain of the ApoER2, encoded by exon 19 in mice [36] and exon 18 in humans [261], interacts with PDS95 (Figure 2). This interaction is modulated by reelin and by the activation of the NMDAReceptor, and is associated with an increase in the surface levels of ApoER2 [243]. Reelin receptors determine the number of synapses. For instance, cortical neurons from 1-month-old ApoER2 KO mice exhibit reduced size and the number of spines, but in the adults (1-year-old mice) the spines are only slightly reduced in size [258]. Other work has emphasized the role of VLDLR in spinogenesis of mature hippocampal neurons *in vitro*; both at pre- and postsynaptic sites, although the role of reelin was not tested [57]. Interestingly, the increase in the number spines requires the activation of the Ras regulator GRF1 (growth hormone releasing factor 1) as well as the downstream effectors CaMKIIα and β, even though VLDLR only interacts with CaMKIIα [57]. In a different aspect, reelin also has a role in the processes that determine synapse structure and maturation. In immature neurons, the predominant NMDAR at the excitatory synapse contains the NR1 (NMDAR subunit 1)/NR2B subunits, which is replaced by the lower conductance NR1/NR2A (NMDAR subunit 2A)-containing receptor upon maturation [258]. *Reeler* mice have lower levels of the NR2A-containing receptor and of PSD95 [35]. In addition, reelin treatment decreases NR2B from the synapse through lateral mobility [252] and also accelerates the maturation process by increasing the presence of NR2A subunits [55]. The increase in NR2A is dependent on the canonical reelin signaling pathway, including activation of Src and the presence of the receptors [55].

Small GTPases of the Rab and Rho families as well as Arf6 have been linked to the formation, stabilization and changes in the shape and size of dendritic spines, which depend significantly on cytoskeleton dynamics and membrane trafficking [112,262,263]. Again, there is no direct evidence regarding the activation of Rab proteins by reelin, but it is an interesting possibility to consider in the process of spinogenesis and synapse regulation. Among Rab proteins, a specific role of Rab17 has been described in the somatodendritic domain of

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neurons and in the generation of dendritic spines starting from filopodia [263]. The early/recycling endosome protein Rab4 functions in the constitutive delivery of membrane in the synapse [264] and, through its effector GRASP-1 [general receptor for phosphoinositides 1-associated scaffold protein (also known as Tamalin)], allows membrane delivery to the Rab 11 recycling compartment [265]. Rab11 functions are critical for cargo recycling to the spine [266]. The activity of Rac1 is required for spinogenesis, while Rho activation interferes with this process [267,268]. In this regard, Par3 is also involved in spine morphogenesis by restricting Rac activation to the dendritic spines, via its interaction with Tiam1 [269]. The interaction of ApoER2 with Par3, including the activation of Tiam1 [124] along with the presence of the receptor in the postsynaptic compartment, raises the possibility of local Tiam1-dependent Rac1 activation, regulating spine formation and morphogenesis independently of Dab1. This proposed mechanism would be similar to the one shown for the G-protein-coupled receptor BAI1 (brain-specific angiogenesis inhibitor 1) in synaptogenesis [270]. The role of Arf6 in the formation of dendritic spines has been associated with its activation by GEF EFA6A (guanine nucleotide exchange factor for Arf6), which is highly expressed in the adult brain [262,271]. Reelin signaling could act upstream of Arf6 activation in its role related to the maturation and/or stabilization of dendritic spines. Recently, spine maturation in the hippocampus was connected to Arf6 activity, which was directly regulated by NMDAR subunit composition; NR2B interacts with the Arf6 GEFS, BRAG1 (Brefeldin-resistant Arf GEFs), whereas NR2A interacts with BRAG2 [272]. Dendritic spines increase membrane expression and the number of AMPA-R as a result of long-term potentiation (LTP), a phenomenon that requires membrane recycling from endosomes [273]. Reelin has an important role in learning and memory by increasing LTP [274] that depends mostly on the presence of the ApoER2 splice variant carrying exon-19 [36]. One of the mechanisms underlying reelin-stimulated LTP involves the increase, via a PI3K-dependent mechanism, of the number of AMPA-R at the postsynaptic membrane [60]. Several endosomal proteins, such as Rab8 (localized in the *trans* Golgi network) [275], Rab11 [273,276], the retromer-associated protein SNX27 [277], the retromer protein VPS35 [278] and Arf6 [279,280], have been involved in constitutive and/or LTP-induced endosomal AMPA-R recycling. However, their activation has not been related to reelin signaling. Recently, it was shown that during LTP, the activation of NMDAR triggers the activation of Cdc42 and the recycling of AMPA-R from the Rab11 endosome to the synaptic membrane [281].

Besides its well-established postsynaptic role, reelin also regulates the presynaptic compartment. Reelin treatment of mature hippocampal neurons increases synaptophysin-positive spines [58], an effect that is also found upon overexpression of VLDLR [57]. In addition, reelin participates in the regulation of presynaptic activity by stimulating fusion of the synaptic vesicle pool that experiences spontaneous (not evoked) exocytosis. Interestingly, this fusion process is mediated by the v-SNARE (vesicle-SNAP soluble NSF attachment protein receptor) VAMP7 (vesicle-associated membrane protein 7) and requires the canonical pathway with the expression of reelin receptors [63]. Moreover, *reeler* mice have lower levels of SNAP25 (synaptosomal-associated protein 25) [62], indicating that reelin expression and function is also associated with the SNARE machinery at least at neuronal level. It is not clear yet how reelin signaling activates VAMP7-mediated synaptic vesicle fusion.

## Conclusions and perspectives

In the past decades, research into the roles of reelin, especially in the CNS, has been crucial to understand many of the processes underlying brain development and function at the cellular and molecular levels. The use of an important number of research tools, including novel *in vivo* forward and backward genetic approaches, *in utero* electroporation, *in vivo* multiphoton microscopy and the use of state-of-the-art methods in cell culture including fluorescence resonance energy transfer to follow the spatial–temporal activation of signaling molecules, have been of invaluable help in improving our understanding of the broad and complex role of reelin and its receptors during development and adult life. Altogether, these studies have clearly established that reelin is much more than an extracellular protein regulating neuronal positioning in the brain. Nevertheless, there are still many open questions waiting to be answered and therefore requiring new research approaches. The detailed knowledge of reelin function at the cellular and molecular levels discussed in this work is relevant to understand how dysfunctions in this signaling pathway are associated with pathologies of high prevalence, including neuropsychiatric diseases such as autism, schizophrenia and depression, as well as neurodegenerative diseases, such as Alzheimer's. Finally, the study of reelin in the CNS proved to be relevant and fundamental in determining and establishing the new participants and their roles in this signaling pathway not only in the CNS but also at the peripheral level (not addressed in this review). Research in this area includes the role of

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reelin in several epithelial tissues and also in the immune system. We anticipate that the regulation of the function of polarity complexes, cytoskeleton proteins and small GTPases will be part of the reelin functions in the periphery, controlling aspects of morphogenesis and cell migration. Undoubtedly, all these findings are likely to provide novel venues for therapeutic interventions.

### Abbreviations

+TIP, microtubule plus-end tracking protein; ADAMTS 4/5, A Disintegrin and Metalloproteinase with Thrombospondin motifs 4 and 5; Akt, Ak thymoma; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPA-R, AMPA receptors; ApoER2, apolipoprotein E receptor 2; Arf, ADP ribosylation factor; BAI1, brain-specific angiogenesis inhibitor 1; BDNF, brain-derived neurotrophic factor; BRAG, Brefeldin-resistant Arf GEFs; C3G, C3 glomerulopathy; CaMKII, $Ca^{2+}$/calmodulin-dependent kinase II; Cdc42, cell division control protein 42 homolog; CLASP, cytoplasmic linker protein-associated protein; CNS, central nervous system; CP, cortical plate; CrkL, Crk-like; Dab, Disabled; Dock1/Dock180, 180 kDa protein downstream from Crk; E, embryonic day; EB3, microtubule plus end-binding protein 3; ERK, extracellular signal-regulated kinases; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factors; GM130, *cis*-Golgi matrix protein; GRAB, guanine nucleotide exchange factor for Rab3A; GSK3β, glycogen synthase kinase 3β; ITSN1, intersectin 1; IZ, intermediate zone; KD, knockdown; KO, knockout; LIMK, LIM motif-containing protein kinase 1; LIS1, Lissencephaly type 1; LKB1, liver kinase B1, also known as serine/threonine kinase 11 STK11; LRP8, low-density lipoprotein receptor-related protein 8; LTP, long-term potentiation; MAP1B, microtubule-associated protein 1B; MICAL-1, microtubule-associated monooxygenase, calponin and LIM domain-containing 1; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; MTs, microtubules; MZ, marginal zone; Nck, non-catalytic region of tyrosine kinase adaptor protein 1; NICD, Notch intracellular domain; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptors; NR1, NMDAR subunit 1; NR2A, NR2B, NMDAR subunit 2A, NMDAR subunit 2B; N-WASP, neural Wiskott–Aldrich syndrome protein; p120ctn, p120 catenin; PAK1, p21-activated kinase; Par, partitioning defective; pDab1, phosphorylated Disabled-1; PI3K, phosphatidylinositol-3-kinase; PP, preplate; PSD95, postsynaptic density protein 95; Rac1, Ras-related C3 botulinum toxin substrate 1; Rap1, Ras-related protein 1; RG, radial glia; RIAM, Rap1-interacting adaptor molecule; SC, Schwann cells; SFKs, Src family of tyrosine kinases; shRNA, short hairpin RN; SNAP25, synaptosomal-associated protein 25; SNX, sorting nexins; SOCS, suppressors of cytokine signaling family; SP, subplate; Stk25, STE20 family of serine/threonine kinase; STRAD, STe20-Related ADapter; SVZ, subventricular zone; TBC1D24, Tre2–Bub2–Cdc16 1 domain, member 24; Tiam1, T-cell lymphoma invasion and metastasis-inducing protein 1; VAMP7, vesicle-associated membrane protein 7; VLDLR, very low-density lipoprotein receptor; VZ, ventricular zone; WM, white matter.

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### Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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