Molecular Neurobiology

ISSN0893-7648/04/30(3): 225–251/$25.00

Neuronal Migration and the Role of Reelin During Early Development of the Cerebral Cortex

Yves Jossin*

Developmental Neurobiology Unit, University of Louvain Medical School, Brussels, Belgium. E-mail: Yves.Jossin@dene.ucl.ac.be

Abstract

During development, neurons migrate to the cortex radially from periventricular germinative zones as well as tangentially from ganglionic eminences. The vast majority of cortical neurons settle radially in the cortical plate. Neuronal migration requires an exquisite regulation of leading edge extension, nuclear translocation (nucleokinesis), and retraction of trailing processes. During the past few years, several genes and proteins have been identified that are implicated in neuronal migration. Many have been characterized by reference to known mechanisms of neuronal and non-neuronal cell migration in culture; however, probably the most interesting have been identified by gene inactivation or modification in mice and by positional cloning of brain malformation genes in humans and mice. Although it is impossible to provide a fully integrated view, some patterns clearly emerge and are the subject of this article. Specific emphasis is placed on three aspects: first, the role of the actin treadmill, with cyclic formation of filopodial and lamellipodial extensions, in relation to surface events that occur at the leading edge of radially migrating neurons; second, the regulation of microtubule dynamics, which seems to play a key role in nucleokinesis; and third, the mechanisms by which the extracellular protein Reelin regulates neuronal positioning at the end of migration.

Index Entries: Reeler; Dab1; VLDLR; ApoER2; cortical plate; actin; microtubule; Lissencephaly.

Introduction

During the past few years, new data and observations have been reported on the molecular and cellular mechanisms of cerebral cortex development. Although it is still impossible to integrate them into a coherent view, a global picture is progressively emerging. This article focuses on the mechnisms that control neuronal migration and positioning, with particular emphasis on Reelin signaling.

Received 3/15/04; Accepted 4/29/04

The Development of the Cerebral Cortex

The formation of the cortex requires widespread cell migration from germinal zones to the cortical anlage. In the mouse, founder proliferating cells complete 11 cell cycles over 6 d, from 10 d postcoitus (E10) to E16 for the lateral cortical zone, and from E11 through E17 for the dorsomedial cortical zone *(1).* During the cell cycle, cells undergo an interkinetic nuclear migration: nuclei move away from the apical, ventricular surface during G1 to occupy the basal half of the ventricular zone during *S*-phase and return apically in G2 so that mitosis occurs at the ventricular surface. Movement of the nucleus could allow regionally different cytoplasmic factors to enter the nucleus and promote differential gene activity. A fraction of postmitotic cells leave the ventricular zone with each cycle and migrate toward the surface of the hemispheres.

Multipotent progenitors are present in the ventricular zone, some of which are believed to be self-renewing stem cells *(2).* They could be generated by progenitors dividing symmetrically in a plane roughly perpendicular to the ventricular surface (vertical cleavage) to produce two identical daughter cells that remain within the ventricular zone, thus expending the proliferative population. As cortical neurogenesis progresses, asymmetrical division, which occurs in a plane parallel to the ventricular surface (horizontal cleavage), starts to predominate. It has been proposed that asymmetrical division produces two different daughter cells—one that remains within the ventricular zone as a progenitor and one that migrates away to differentiate *(3,4).* Although these hypotheses are attractive, no direct evidence proves that the symmetry of division predicts cell fate. The mechanisms that regulate the cell cycle of cortical progenitors and the transition from predominantly symmetrical to predominantly asymmetrical divisions in the ventricular zone remain poorly understood. Recently, evidence was presented that the transcription factor Pax6 is involved in the regulation of this progression *(5).* From E12.5 to E15.5, the proportion of asymmetrical divisions increased more rapidly in Pax6 mutants than in wild-type embryos.

The first neurons that reach the cortical anlage form a horizontal, loose network known as preplate. These neurons include pioneer neurons in the future marginal zone, such as Cajal-Retzius cells (Reelin and p73 positive cells), and future subplate cells. Some preplate neurons migrate radially from the ventricular zone, whereas others (including some Cajal-Retzius cells) may follow a tangential route *(6).* Recent work has indicated that radially migrating preplate neurons may not need to extend a leading edge and could migrate by simple somal translocation (also called nucleokinesis) *(7).* They are attached to the pial surface by a radially oriented process that shortens in parallel to translocation of the nucleus followed by detachment of the apical process from the ventricle.

After preplate formation, two distinct modes of radial and tangential migration occur in the cortex. Tangentially migrating neurons move parallel to the pial surface, along axons or other neurons. They are principally γ-aminobutyric acid (GABA)ergic interneurons and originate in the medial ganglionic eminences in the basal forebrain *(8,9).* During radial migration, neurons generated in the proliferative ventricular zone move perpendicularly to the surface (along radial glial fibers that span the whole thickness of the tissue) from the ventricular to the marginal zone where they form endfeet. Thus, radial migration is also called gliophilic migration. Radially migrating neurons (the majority of cortical neurons) form principally glutamatergic cells. During radial migration, the leading process is not in contact with the pial surface, and it is shorter than the one that leads somal translocation to the preplate *(7).* The cell moves intermittently (saltatory migration) and has resting periods, and each movement is preceded by an increase of intracellular calcium. A similar sequence is observed in tangentially migrating cells from the medial ganglionic eminences *(10).* At the end of migration, when their leading processes reach the vicinity of the pial surface, neurons stop gliophilic

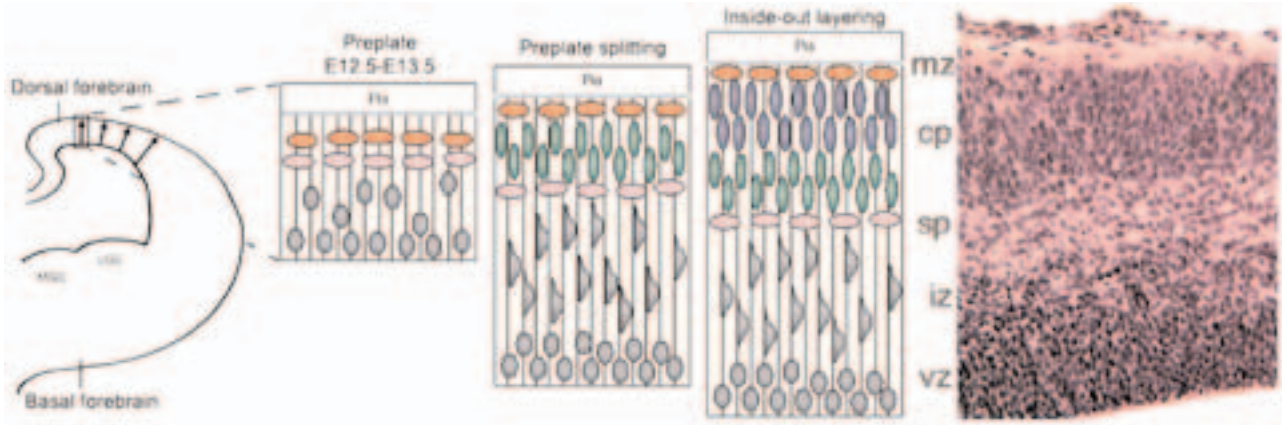

Fig. 1. Preplate splitting and inside-out layering during the formation of the cortical plate. At the preplate stage (between 12.5 and 13.5 d of embryonic age in mice). neurons form a horizontal loose network that will be splitted into marginal zone (mz; orange) cells and subplate (sp; pink) cells by insertion of first neurons (green) of the future cortical plate (cp). During the formation of the cp, youger neurons (blue) migrate past the sp and layers of neurons already installed and set off just beneath the mz. This phenomenon is called "inside-out layering."

The sagittal view of an embryonic mouse brain cortex at 16.5 d exhibits a cell-poor mz; the tightly packed cells of the cp; the sp; the intermediate zone (iz), which contains migrating cells; and the ventricular zone (vz) of precursor cell proliferation.

migration and terminate their journey by a nucleokinetic movement.

During the formation of the cortical plate, neurons invade the preplate, which is split into two parts. Some preplate neurons particularly (but not exclusively) Cajal-Retzius cells, are displaced outward, in the marginal zone, and become pioneer neurons. Others are displaced inward to form the subplate and probably will become future polymorphic neurons of cortical layer VIb. Within the cortical plate, younger neurons migrate beyond the older, previously established contingent and settle beneath the marginal zone. This results in an "inside-to-outside" layering, with younger neurons in the outermost field of the cortical plate and older neurons more toward the inside *(11)* (Fig. 1).

Mechanisms of Cell Migration

Similarly to other motile cells, neurons migrate by extending a leading process (a growth cone or a dendritic tip) that attaches to a substratum. This is followed by a nuclear translocation (nucleokinesis) within this extended furrow and then by retraction of the trailing process *(12).* The actin and microtubule cytoskeletons are involved and interact with each other to regulate these events.

Leading Edge Extension

Leading edge extension is characterized by a high dynamic instability of actin-based complexes, with forming and disassembling of filopodia and lamellipodia, which sense attractive or repulsive molecules in the environment *(13).*

Modifications of the actin cytoskeleton localized at the migration tip are controlled by small Rho GTPases *(14).* This family is divided into three groups: Rho (RhoA, RhoB, RhoC, RhoD, RhoE, TTF), Rac (Rac1, Rac2, Rac3, RhoG), and Cdc42 (Cdc42 and TC10) *(15);* the latter two families are particularly important at this level.

In fibroblasts, formation of filopodia at the cell periphery is followed by formation of lamellipodia, membrane ruffles, and focal complexes and

then by formation of focal adhesions and stress fibers in the cell body *(16,17).* The formation of filopodia is regulated by the binding of Cdc42 and phosphatydylinositol biphosphate to neural Wiscott-Aldrich Syndrome protein (N-WASP), which stabilizes its active conformation. N-WASP binds the actin-related protein 2 (Arp2) and (Arp3) complex, thereby stimulating actin nucleation and polymerization to form filopodia *(18,19).* The formation of lamellipodia and membrane ruffles is regulated by Rac proteins that activate p21-activated kinases (PAKs) and Arp2 and -3 *(20–22).* PAKs inhibit actomyosin contractility and actin depolymerization *(23–26).* In the cell body, Rho proteins stimulate actin–myosin contraction and the formation of stress fibers and focal adhesion via the activation of Rho-associated serine-threonine kinases (ROCKs) and the mammalian homolog of Diaphanous *(24,27–30).* Rac and Cdc42 also promote the formation of small adhesive structures called focal complexes, which resemble the focal adhesions induced by Rho but are smaller and appear only at the cell periphery *(31).*

Similar mechanisms have been demonstrated during growth cone and neurite extension in neurons, which are blocked by dominant negative Rac or Cdc42 in neuroblastoma cells *(32).* By contrast, Rho activation, which induces stress fibers and focal adhesion in fibroblast, triggers neurite retraction in neural cells *(32–34).* GTP-bound Rac and Cdc42 may induce the GDP-bound state of Rho that is permissive for axonal growth. Thus, GTP-loaded Cdc42 and Rac may allow axonal growth by controlling the nucleotide content of Rho *(35).*

Chemoattractants or repellents may act on the growth cone by locally activating Rac and/or Cdc42 or Rho to guide axonal growth and cell migration. In hippocampal neurons, activation of plexin-B1 by semaphorin 4D regulates the activity of the Rho guanine nucleotide exchange factors PDZ-RhoGEF and LARG, leading to RhoA activation and growth cone collapse *(36).* Similarly, Ephrin-A5 induces growth cone collapse by activating RhoA and ROCK and inhibiting Rac1 and Cdc42 *(37,38).* The RhoGEF ephexin, which acts downstream of the Ephrin receptor EphA, is required for Ephrin-A-induced growth cone retraction *(38).* The Netrin-1 receptor deleted in colorectal cancer (DCC) promotes filopodia formation and cell spreading in embryonic rat spinal commissural neurons *(39)* and induces neurite outgrowth in neuroblastoma cells *(40)* by activating Cdc42 and Rac1.

It is tempting to infer a similar role of RhoGTPases in neuronal migration. For example, Robo activation by Slit activates the GTPase-activating protein (GAP) that inactivates CDc42 srGAP1. This inhibits neurite growth and induces repulsion of migratory cells from the anterior subventricular zone of the forebrain *(41).* Haploinsufficiency of *LIS1*—a gene that is implicated in lissencephaly discussed later—is associated with upregulation of RhoA and downregulation of Rac1 and Cdc42 activity at the leading edge of migrating neurons, with a reduction of filamentous actin *(42).*

Studies of mammalian enabled (Mena)/vasodilator-stimulated phosphoprotein (VASP) proteins extended our understanding of the relationship between leading edge extension and cell migration. In fibroblasts, IRSp53 serves as an adapter between Cdc42 and Mena and stimulates filopodial extension *(43).* Unexpectedly, this inhibited motility, because Mena/VASP-deficient fibroblasts exhibited increased motility *(44).* Moreover, constitutive membrane targeting of Mena/VASP increased lamellipodia dynamics and ruffling and reduced motility, whereas cells with sequestered Mena displayed more stable lamellipodia and increased motility *(45).* A mechanism has been suggested by which Mena/VASPs bind to the barbed ends of actin filaments and compete with capping proteins, allowing formation of longer filaments that are presumably too flexible to counteract the forces of membrane tension *(45).* Whole cell speed probably correlates with the persistence of lamellipodial protrusion rather than the velocity of extension of individual protrusions. Mena/VASP proteins have a profound influence on neuronal motility. They are concentrated in filopodial tips and at the edge of lamellipodial

veils in neurons *(46).* Inhibition of Mena/VASP function through retroviral injections *in utero* led to aberrant placement of early-born pyramidal neurons in the superficial layers in a cell-autonomous fashion *(47).* This aberrant migration does not result from a delayed onset of neuronal migration; the abnormally placed pyramidal neurons exhibit grossly normal morphology and polarity, and glial cells are not affected. A role is also established for Ena/ VASP proteins for filopodia formation in neurons *(48).* It would be interesting to determine whether the Reelin pathway influences Mena/ VASP proteins during corticogenesis. However, genetic studies are complicated because mutant mice with double inactivation of the two Mena/VASP genes die during embryonic development.

As discussed previously, cell migration requires polarization, characterized by oriented protrusions containing actin that is organized into lamellipodia and filopodia. It also requires a reorientation of the microtubule organizing center (MTOC), the microtubule cytoskeleton, and the Golgi to face the direction of migration. Cdc42, in synergy with a Par6-atypical protein kinase C(aPKC) complex, is required to establish cellular asymmetry during directed migration, as shown in astrocyte migration assays *(49)* and in cultured hippocampal neurons *(50).* This complex leads to the phosphorylation and inactivation of GSK3-β (a "τ" kinase) and to the spatially restricted association of adenomatous polyposis coli protein, with the "plus" end of microtubules at the leading edge, which is essential for establishing polarity *(51).* Cdc42 also regulates MTOC reorientation in a dynein–dynactin-dependent manner *(52).* Golgi reorientation in a scratch-wound assay is actin-dependent in National Institutes of Health (NIH)-3T3 cells but not in astrocytes *(53).* In NIH-3T3 cells, MTOC and Golgi polarization are separately controlled, because the relocation of the MTOC is independent of actin but inhibited by disruption of microtubules, whereas Golgi polarity is dependent on actin and independent of microtubules *(53).*

Nucleokinesis

The control of nucleokinesis differs from that of leading edge extension. An obvious example includes the migration of inferior olivary neurons that first emit a leading process in which the nucleus translocates until it reaches the floorplate. At this level, the leading process crosses the midline, whereas the nucleus remains homolateral *(54–56).* In comparison to leading edge extension, nucleokinesis relies more on the microtubule cytoskeleton *(57).* As discussed later, mutation in the *LIS1* gene leads to a defect in neuronal migration. The *LIS1* protein is homologous to NudF and interacts with orthologs of other Nud proteins that are involved in nuclear translocation in *Aspergillus nidulens.* Like other *Nud* genes, *LIS1* interacts directly with microtubules.

Within the soma, a cagelike distribution of microtubules surrounds the nucleus. During the translocation of the nucleus of migrating cerebellar granules, "plus" ends of microtubules assemble at the leading edge, and the depolymerizing "minus" ends face the nucleus. Microtubule polymerization in the direction of the leading edge could help in the extension of the leading process, whereas depolymerization in front of the nucleus may assist nuclear movement toward the front of migration. By contrast, microtubules in the trailing process are of mixed polarity and cannot induce any moving force. This model is consistent with the saltatory movement of migrating neurons, with a slow microtubule association phase reflecting the slow leading process extension and the rapid nuclear translocation reflecting the rapid dissociation of tubulin subunits. The influx of cytosolic Ca^{++} that is seen during movement may reflect calcium's need for tubulin polymerization *(58).*

Interactions Between Actin and Microtubules

There is ample evidence of interactions between the actin and microtubule networks during migration. For example, cytochalasin B,

which inhibits assembly of actin subunits, reversibly inhibits gliophilic migration *(59)* and disturbs microtubules *(60).* Reciprocally, the depolymerization of microtubules in fibroblasts perturbs cell shape polarization and increases the size of focal adhesions via activation of RhoA *(61,62),* whereas repolymerization of microtubules following their disassembly is associated with the activation of Rac1 *(63).* Therefore, the microtubule network can modulate the activation of Rho GTPases, which in turn influence the actin cytoskeleton.

In fibroblasts, microtubule growth—and not the sole presence of microtubule polymers—at the leading edge promotes lamellipodia protrusion through activation of Rac1 *(63–65).* Moreover, as focal complexes or focal adhesions polymerize toward the cell periphery microtubules specifically target them and promote their disassembly *(66).* There are several interactions between microtubules and proteins that regulate the actin network: the Rac1-GTP binds to tubulin *(67);* the guanine nucleotide exchange factor GEF-H1, an activator of Rac1 and RhoA, is localized to microtubules *(68);* and RhoG (which is closely related to Rac1) activates Rac1 and Cdc42 in a microtubule-dependent manner *(69).* Therefore, microtubules may act, at least partly, by modulating adhesion site turnover through the local delivery of signaling molecules that inhibit Rho activity and activate Rac1.

Retraction of the Trailing Process and Actomyosin Tension

In some slowly moving cells, tail detachment depends on the action of the protease calpain that degrades focal adhesions at the rear *(70).* In migrating leukocytes, ROCK, through myosin light-chain phosphorylation, is essential for detachment *(71).* The intracellular tension induced by RhoA-mediated acto-myosin contraction is correlated with an increase of large and elongated focal contacts localized to the ends of actin stress fibers and with increased integrin density and turnover. In focal adhesion sites, integrins are anchored within the actin cytoskeleton. Contraction of the actin filament backbone induced by myosin II may lead to increased integrin density and mobility and is blocked by an inhibitor of myosin contraction *(72,73).* At the rear of the cell, focal adhesions are more dense and mobile; this mobility may be important for retraction and migration *(72).* Many studies have pointed to physical tension as a key factor in the development and dissolution of adhesion complexes; however, other signals, such as cellular homologue of the chicken oncogen of Rous Sarcoma Virus (Src), focal adhesion kinase (FAK), and PAK, can also induce focal adhesion disassembly and contribute to tail detachment *(74).*

The role of microtubules in trail retraction is not clear and is cell type-dependent. Contrary to fibroblasts and epithelial cells, the microtubule polymerization inhibitor nocodazole does not affect rapidly migrating cells such as neutrophils (which do not have focal adhesions) and keratinocytes *(74).* However, in slow moving cells, tension in the actin cytoskeleton is necessary for the formation of focal adhesions and for the retraction of the trailing process. Microtubules target focal adhesions and induce their relaxation and dissolution, perhaps by activating Rac or Cdc42 *(66);* this may explain the reason that microtubules are required for tail retraction in some cells *(75).* The local application of inhibitors of contractility mimics the microtubule-mediated cell edge retraction and induces the depolymerization of microtubules *(66).* Tension may induce the polymerization of microtubules that target focal adhesion where they deliver signals that inhibit tension to reverse focal adhesion development.

Intracellular Molecules That Influence Cortical Plate Formation

Studies of human and mouse cortical malformations led to the discovery of genes that are involved in the radial migration of neurons into the cortex. Many of the corresponding proteins have a tight relationship with the microtubule or actin cytoskeletons, and defects in leading edge extention or in nucleokinesis

may explain some of the phenotypes that result from their deficiency.

Type I Lissencephaly

LIS1, the gene mutated in the Miller-Dieker Syndrome and type I lissencephaly, encodes the noncatalytic β-subunit of platelet-activating factor (PAF) acetylhydrolase AH1-β1, an enzyme that inactivates PAF; however, there is no apparent relationship between this function and lissencephaly. *LIS1* binds to dynein motor proteins and to microtubules and may stabilize the microtubule network during cell migration *(57,76).* In both humans and mice, homozygous *LIS1* mutations are lethal, and heterozygotes are characterized by a migration disorder in the neocortex, hippocampus, olfactory bulb, and cerebellum. Using knock-in strategies, mice were generated that express reduced levels of *LIS1* in the brain. Compared to control animals, null/heterozygotes express 45% *LIS1* protein and compound null/hypomorphics express 35% *(77,78).* The development of the preplate, Cajal-Retzius cells and the radial glial scaffold are unaffected by *LIS1* levels. Contrary to the reeler cortex, the preplate is split correctly and the layering of the cortex proceeds from inside to outside. However, there is a dose-dependent disorganization of the subplate and a cell autonomous slowing of migration in vivo and in vitro *(77–79).* Additionally, *LIS1* influences the interkinetic nuclear migration and neuroblast proliferation. *LIS1* is similar to nuclear distribution (Nud) protein F *(80)* and interacts physically and biochemically with the mammalian ortholog of NudC and with mNudE and mNudeL during neuronal migration in vivo *(81).* Nud proteins are required for nuclear translocation in *Aspergillus nidulans,* a process that is controlled by dynein, dynactin, and other components of microtubules *(57).* This suggests that the *LIS1* gene product may have a function similar to that of NudF and supports the hypothesis that nuclear migration (nucleokinesis) plays a key role in neuronal migration.

In a model for the cooperation of NudC, *LIS1*, and dynein in mediating nuclear movement in migrating neurons, NudC and *LIS1* may be involved in targeting and regulating the function of dynein at the leading pole of migrating neurons. The minus end-directed activity of dynein could pull the MTOC and the associated nucleus in the direction of migration. NudC, *LIS1*, and dynein also may be involved in tethering the nucleus to the MTOC and transporting it along microtubules *(81).* Moreover, neurons from *LIS1* haploinsufficient mice exhibit a reduced F-actin content associated with upregulation of RhoA activity at the leading edge and shorter filopodia. The use of an inhibitor of p 160 ROCK rescues their motility defect *(82),* although the effect of *LIS1* on Rho GTPases does not result from a direct interaction. *LIS1* may act on actin through its effect on microtubules. Alternatively, migrating neurons may use dynein to transport signaling molecules from the leading process to the nucleus to convey cortical positioning information *(57).*

Female patients with mutations of the X-linked doublecortin *(DCX)* gene have a "double cortex" syndrome in which a band of gray matter is embedded within the cortical white matter, whereas male patients with *DCX* demonstrate lissencephaly. The difference in phenotypes is attributed to mosaicism in females that reflect X-inactivation. Surprisingly, the mouse *Dcx* mutant has no defect in cortical development *(83),* showing that this gene is less important in rodents than in man. This difference may result from the presence of proteins that are homologous to Dcx and are able to support neuronal migration in mice but not in the human brain, in which migration is more complex.

Doublecortin binds to tubulin and has a microtubule stabilizing activity in vitro *(84,85).* Doublecortin and *LIS1* interact physically (coprecipitation assays) and functionally (microtubule polymerization assays) and probably function in the same pathway *(86).* Similarly to other microtubule interacting proteins, function of Dcx is regulated by phosphorylation. Its association with microtubules is inhibited through phosphorylation by PKA, microtubule affinity

regulating kinase (MARK) (orthologs of which in *Caenorhabiditis elegans* [PAR-1] and *Drosophila* [dPAR-1] are involved in cell polarity), and Cyclin-dependent kinase 5 (Cdk5) *(87,88).*

Cdk5 is a member of the cyclin-dependent serine/threonine kinase family. However, unlike other Cdks, it does not associate with cyclins and does not regulate the cell cycle. In mammals, Cdk5 messenger RNA (mRNA) and protein are expressed in the kidney, testes, and ovary, with the highest expression in postmitotic neurons in the developing and adult nervous system *(89,90).* The Cdk5 activity is detected only in the brain, because it depends on the presence of the regulatory subunit p35 or p39 *(91).* Mutant mice that are defective in Cdk5 or, to a lesser extent, its activator p35 have defects in radial migration that exhibit some similarity to the reeler phenotype, with inversion of cortical layering but a correct preplate splitting *(92).* Whereas inactivation of p39 (another activator of Cdk5) causes subtle abnormalities, a combined defect of p35 and p39 induces the same malformation as Cdk5 inactivation *(93),* suggesting functional redundancy of these two proteins in the activation of Cdk5.

Cdk5 associates directly with Pak1 and Rac. It phosphorylates and downregulates the activity of Pak1, an effector of the Rac GTPase involved in actin reorganization and in the progression of the leading process, as mentioned previously *(94).*

In addition to its role on the actin cytoskeleton, Cdk5/p35 may be involved in the control of microtubule dynamics and may affect neuronal migration though interference with nucleokinesis. The *LIS1* interacting protein Nudel and the microtubule-associated proteins τ, MAP2, and doublecortin all are substrates of Cdk5/p35 *(88,95,96).* Cdk5 phosphorylates FAK on Ser 732, which may be important for nuclear movement during neuronal migration *(97).* However, the role of FAK in neuronal migration has not been confirmed with the analysis of conditional Fak mutant mice. Inactivation of FAK in radial glia precursors that express the Emx1 promoter resulted in disruption of the external limiting membrane, with excessive migration of neurons in the marginal zone and even in the meninges. This phenotype, which we call "overmigration" for short, is reminiscent of type II cobblestone lissencephaly and different from defective or slow migration. However, no anomaly was observed when FAK was inactivated in cells that expressed the neuron-specific Nex1 promoter *(98).*

X-Linked Periventricular Heterotopia

Studies of human X-linked periventricular heterotopias confirmed the involvement of actin binding proteins in radial migration. In affected female subjects, this disorder is characterized by the failure of a subset of neurons to migrate to cerebral cortex. Migrational arrest results in the formation of nodules of ectopic neurons in the periventricular zone. The heterotopias lie beneath a normal-appearing cerebral cortex. Some periventricular heterotopias result from mutations in the X-linked gene *filamin A (FLNA),* a member of a family of three (*FLNA, -B,* and *-C*) *(99).* Similarly to DCX, the presence of two populations of normal and ectopic neurons is believed to reflect mosaicism resulting from X-inactivation. *FLNA* encodes an actin-binding protein and may be involved in the extension of filopodia along glial fibers *(100).* Filamin cooperates with other actin-binding proteins, including the Arp2–Arp3 complex, to promote orthogonal actin polymerization *(101)* and is able to bind membrane proteins such as β-1 integrin *(102).* It also binds to Rho, Rac, Cdc42, RalA (a Ras-related small GTPase) *(103),* PAK *(104),* and Trio (a Rho guanine nucleotide-exchange factor that controls the GTPases RhoG, Rac1, and RhoA) *(105).* Therefore, filamin may serve as a scaffolding protein for Rho GTPases-mediated signaling and may regulate the assembly of integrin-actin structures. Filamin also may serve to anchor actin filaments to mitogen inducible gene-2, thus recruiting Migfilin to cell–extracellular matrix adhesions *(106).* Another FLNA interacting protein, FILIP, binds to and induces the degradation of filamin in cos-7 cells. Its overexpression in cells of the

ventricular zones in vitro inhibits neuronal migration *(107).* Periventricular heterotopias are also induced by mutations in the adenosine-diphosphate-ribosylation factor guanine nucleotide exchange factor, an autosomal gene that encodes the BIG2 protein involved in vesicle and membrane trafficking from the *trans*-Golgi network. Inhibition of BIG2 by brefeldin A decreases cell proliferation and prevents transport of E-Cadherin and β-catenin from the Golgi to the surface *(108).*

"Overmigration" in the Meninges and Type 2 Lissencephaly

Several disorders in man and mice are associated with migration of cortical neurons in the marginal zone and even in the meninges, an anomaly that is occasionally referred to as "overmigration." In man, when overmigration is profuse, it results in a lissencephaly called cobblestone or lissencephaly type 2. In mice, deficiency in the myristoylated alanine-rich C kinase substrate (MARCKS) induces overmigration with invasion of the marginal zone and formation of neuronal streams through the basal lamina and limiting membrane *(109).* MARCKS is a prominent substrate for PKCs that might bridge filamentous actin and the plasma membrane *(110).* A similar phenotype observed in Dreher mutant mice is an autosomal recessive mutation in the LIM homeobox transcription factor-α1, Lmx1a *(111).* Presenilin-1 (PS1)-deficient mice develop a cortical dysplasia with leptomeningeal fibrosis and migration of cortical-plate neurons beyond their normal position into the marginal zone and subarachnoid space *(112).* PS1 is a transmembrane protein located in the endoplasmic reticulum and the *cis* Golgi apparatus. A depletion of the ECM proteins Reelin and chondroitin sulfate proteoglycans in the marginal zone could contribute to neuronal overmigration.

In man, lissencephaly type 2 is part of several syndromes, such as Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB) and Fukuyama muscular dystrophy (FCMD), all of which are associated with mutations in genes that encode known or putative glycosylation enzymes. WWS is caused by mutation in the *POMT1* gene in 20% of patients. POMT2, a closely related protein, may account for some of the other cases. POMT1 is a putative *O*-mannosyltransferase that catalyses the transfer of mannose to a Ser or Thr residues *(113).* Attempts to detect the *O*-mannosyltransferase activity of POMT1 or POMT2 alone have not been successful, but coexpression of both enzymes shows activity in vitro *(114).* POMGnT1 is mutated and inactivated in MEB. *POMGnT1* encodes the protein *O*-linked mannose β1,2-*N*-acetylglucosaminyltransferase 1, which catalyses the transfer of *N*-acetylglucosamine from uridyl di-phosphate (UDP)-GlcNAc to *O*-mannosyl glycoproteins *(115).* FCMD is caused by mutation of the protein Fukutin. Its sequence predicts a glycosylation activity that has not yet been demonstrated *(116).* Interestingly, a loss-of-function mutation of the protein Large (a putative glycosyltransferase) in mice is responsible for myodystrophy (*myd* mouse) and neuronal migration abnormalities that resemble lissencephaly type 2.

In patients with WWS, MEB, and FCMD and in the *myd* mouse, α-dystroglycan is deficient and/or hypoglycosylated. The brain-selective deletion of dystroglycan in mice produces a phenotype with neuronal overmigration *(117).* Dystroglycan is a component of the dystrophin–glycoprotein complex (DGC) in the sarcolemmal muscle membrane, and some DGC components are associated with dystroglycan and dystrophin isoforms in other tissues, including the brain *(118,119).* In the mouse, dystroglycan, fukutin, and POMGnT1 are coexpressed in late embryonic and early postnatal cerebellar neurons and glial cells *(120).* DGC is involved in linking cytoskeletal actin (via dystrophin) to components of the ECM (via α-dystroglycan). α-dystroglycan binds to laminin-α1 and -α2, agrin, perlecan, and neurexin in a calcium-dependent manner *(121).* The importance of this interaction is underlined by the fact that lissencephaly type 2 is also associated with merosin (laminin-α2)

deficiency *(122)* or perlecan deficiency *(123, 124).* Disruption of the ECM protein-binding activity of α-dystroglycan through aberrant glycosylation seems a plausible mechanism of muscle cell degeneration and abnormal neuronal migration in WWS, MEB, and FCMD in humans and in *myd* mice.

Laminin is a ligand for α-dystroglycan and α6β1 and α6β4 integrins, and defects similar to lissencephaly type 2 are observed in mice that are deficient in β-1 *(125)* and α-6 integrins *(126).* The signaling pathways triggered by laminin binding appear to be essential for basement membrane integrity. The FAK is activated following integrin binding to the ECM, and the deficiency of FAK in meningeal cells of the dorsal telencephalon resulted in cobblestone lissencephaly, whereas removal of FAK in neurons had no effect *(98).* DGC, integrins, and FAK can associate, and there is an integrin-dependent tyrosine phosphorylation of the DGC components α- and γ-sarcoglycan, perhaps through FAK activity *(127).* Altogether, these data point to the importance of the structure of the basal lamina, limiting membrane, and local ECM to limit neuron migration. However, more data are required—particularly to define the cell autonomy of the different gene products concerned.

Guidance Molecules That Influence the Formation of the Cortical Plate

Neurons that move tangentially extend a growth cone that is similar to axonal growth cones. This migration proceeds in apposition to other neurons or axons and is sometimes called "neuronophilic." Radially migrating neurons extend a dendritic tip that becomes the dendritic pole and is closely apposed to and follows glial fibers; this mode of migration is referred to as "gliophilic" *(128).*

Many molecules that influence tangential migration are the same as those that influence axonal guidance. Although many molecules that modulate axonal guidance also regulate dendritic growth *(129),* most have no effect on radial migration. The two known proteins that regulate axonal and dendritic growth and influence radial migration do so indirectly. The Notch receptor regulates axon and dendritic outgrowth *(129)* and influences radial migration via the formation of radial glia *(130),* and the neurotrophin brain-derived neurotrophic factor (BDNF) *(129)* influences radial migration by regulating the expression of Reelin by Cajal-Retzius cells *(131).*

Four main classes of attractive or repulsive guidance molecules regulate tangential migration: Ephrins, Semaphorins, Slit, and Netrins *(132,133).* For example, Slit expressed in the ventricular zone of the ganglionic eminences (GEs) repels subventricular zone neurons that leave the GE to settle in the striatum or migrate to the cortex *(134).* The striatum expresses Semaphorins, which repel neurons of the GE that express the neuropilin receptor, thus allowing these neurons to avoid the striatum and migrate into the cortex *(135).*

Although tangential migration is considered independent of glia, the Rostral Migratory Stream (RMS) interacts closely with glial cells. In the RMS, inhibitory interneurons migrate tangentially from the subventricular zone of the cortex to the olfactory bulb inside "tubes" of astrocytic cells that appear to surround migrating neurons *(136).* Moreover, migration-inducing activity (MIA), a soluble peptide produced by astrocytes, modulates the migration of RMS cells by converting the inhibitory effect of Slit (expressed in the septum) into a repulsive action *(137).*

Unlike tangential migration, attractive or repulsive guidance cues have not been identified in radial migration, which appears to be primarily guided by radial glia. Accordingly, the molecules involved in radial migration act on the interactions between neurons and glial cells and include astrotactin, neuregulins (NRGs), epidermal growth factor (EGF) receptors, integrins, and Reelin. Although Reelin also has been proposed to be an attractive cue *(138),* its mechanism of action remains largely debated. To date, very little is known of molecular action of their molecules on migration.

Astrotactin is expressed in early postmitotic neurons in the cerebellum, hippocampus, cerebrum, and olfactory bulb. In migrating cerebellar granule cells, it mediates interactions between neurons and Bergmann fibers *(139).* In astrotactin null mutant mice, the cerebellum is diminutive and granule cells migration is slow because of a decreased neuron–glia interaction *(140).* Cerebellar granule cells express NRG, the ligand of the ErbB4 receptor that is present on the surface of radial glial cells. Blocking radial glial differentiation by anti-NRG antibodies reduces the rate of granule cell migration *(141).* NRG may regulate the differentiation of the radial glia in the cerebellum, and the elongation of radial glial cells is essential for radial migration. This effect also occurs in the cortex *(142).* Radial glial cell generation is significantly impaired in the cerebral cortex of NRG mutants and can be rescued by exogenous NRG *(143).* The NRG receptor ErbB2 is required to promote the radial glial phenotype.

The EGF receptor (EGFR) also seems to be important because *Egfr –/–* mutant mice exhibit accumulation of precursor cells in the ventricular zone *(144).* Moreover, Caric et al. *(145)* showed that cells overexpressing EGFR displayed increased radial migration in the cortex and olfactory bulb.

Integrin receptors consist of membrane-spanning heterodimers of α- and β-subunits. More than 20 integrin receptors can be formed by combinations of 18 α- and 8 β-subunits. Mice that are deficient in most integrin subunits have been produced. Some of them, such as α2, α5, and β1, die during early embryonic development, precluding analysis of cortical development *(146).* Others (α1, -4, -7, -8, and -9 and β2, -3, -5, -6, and -7) have a normal cortex. Knockout mice for α3, α6, and αv and a brain-specific mutant for β1 exhibit cortical malformations similar to lissencephaly type 2. Anton et al. *(147)* suggested that integrin α3β1 and αv integrins are implicated in interaction between radially migrating cortical neurons and radial glial fibers. The involvement of the integrins in the Reelin pathway is discussed in the following section.

The Reeler Mutant Mouse and the Reelin Signaling Pathway

Reeler is an autosomal recessive mutation in a gene that encodes Reelin *(148).* In man, defective Reelin is the cause of the Norman-Roberts-type lissencephaly *(149)* Online Mendelian Inheritance in Man (OMIM 257320). Reelin mutant mice exhibit neuronal ectopia in laminated brain structures such as cerebral and cerebellar cortices and the hippocampus. Additionally, there are subtle anomalies in the facial nerve nucleus, inferior olivary complex, olfactory bulb, cochlear nuclei, thalamus, tectum, retina, spinal cord, and throughout the brain *(150–152).* Here, we focus on the cortical phenotype.

In Reeler mutant mice, neurons are generated in normal numbers and at the normal time. The preplate is also formed normally. However, the neurons destined for the cortical plate settle beneath the preplate, which is not split into the marginal zone and the subplate but is displaced *en bloc* externally to form a "superplate." At the end of development, the marginal zone is absent, and cells are closely apposed to the basal lamina (Fig. 2).

Young neurons are unable to migrate past their predecessors, and maturation of the cortex proceeds from outside to inside. Thus, the Reeler defect is a failure in cell positioning in the terminal stage of radial migration rather than a disorder of cell migration *per se (150,153,154).* Besides neurons, radial cells and their radial fibers ramify abnormally.

In addition to Reelin, a Reeler-like phenotype results from mutations in the Disabled1 (Dab1) gene *(155–157)* and from a double inactivation of the lipoproteins receptor genes very low-density lipoprotein receptor (VLDLR) and Apolipoprotein E Receptor type 2 (ApoER2) *(158).*

Reelin

Reelin is a secreted glycoprotein detected by 11 to 12 d of embryonic age in mice. Its concentration increases until birth and decreases

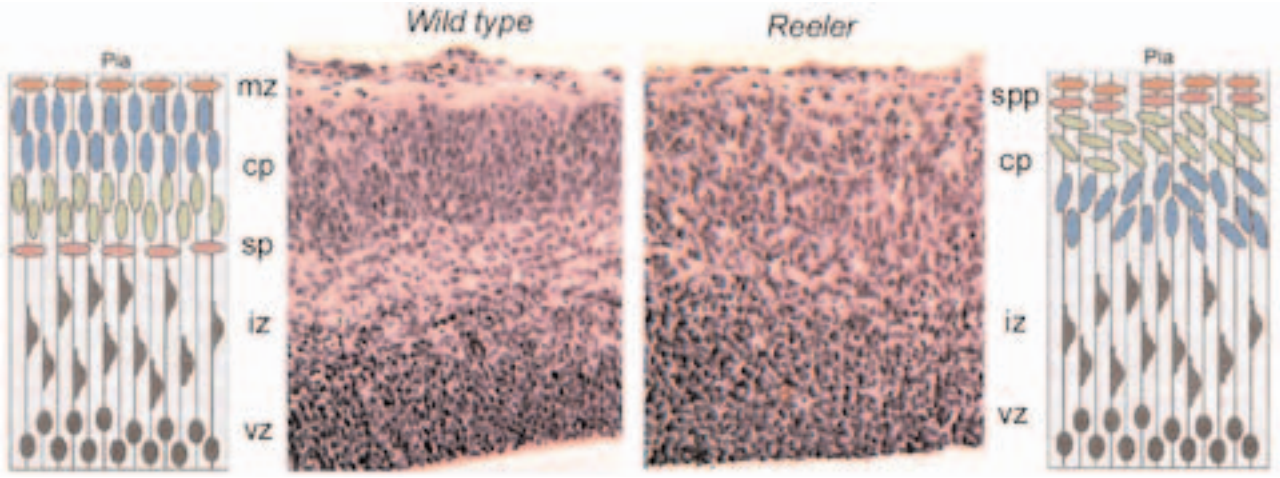

Fig. 2. The Reeler embryonic cortex.

with maturation. It is secreted by several neurons, such as Cajal-Retzius cells in the cortex and hippocampus, granule cells in the cerebellum, mitral cells in the olfactory bulb, ganglion cells in the retina, and neurons of the spinal cord *(152,159,160).* After birth, Reelin is expressed by a subset of cortical GABAergic interneurons in the cortex and hippocampus and by glutamatergic granule cells in the cerebellum *(161).* It is also expressed in peripheral organs such as liver and kidney and is present in the plasma and cerebrospinal fluid *(162,163),* but its function in these sites is unknown.

Reelin is a large extracellular protein of 420 to 450 kDa. Its structure is described in ref. *148* and is shown in Fig. 3.

A signal peptide (25–27 residues) is followed by an *F*-spondin homology domain (163 residues) and a unique region (309 residues). Its main body consists of eight repeats of 350 to 390 amino acids. Each repeat consists of two related subdomains (A and B) flanking a pattern of conserved cystein residues that form EGF-like motifs that are very similar to each other and are most closely related to those of the ECM proteins tenascin C, tenascin X, restrictin, and the integrin β-chain family. The protein ends with a basic stretch of 33 amino acids.

Reelin is cleaved in vivo *(164).* The use of a Reelin C-terminal antibody in Western blot reveals three bands (approx 450, 370, and 180 kDa), whereas the use of Reelin N-terminal antibodies also reveals three fragments (450, 270, and 80 kDa). Comparison of the sizes of these immunoreactive products and of recombinant Reelin proteins suggests that two cleavage sites are located between repeats 2 and 3 and between repeats 6 and 7 (Fig. 3). Recently, we raised an antibody against the central fragment and this antibody revealed four fragments (450, 370, 270, and 190 kDa; Gui et al., unpublished data 2004) in Western blots, thus confirming cleavage at two sites.

Disabled 1

Dab1 is mostly expressed in the nervous system, with traces of mRNA in the kidney and a few other organs. It is expressed by cortical plate neurons in the neocortex and hippocampus, Purkinje cells in the cerebellum, inferior olivary cells, cranial nerve nuclei, and several others, all of which are target cells of Reelin *(165).*

The *Dab1* gene encodes an intracellular protein with a protein interaction/phosphotyrosine binding domain (PI/PTB) and tyrosine residues

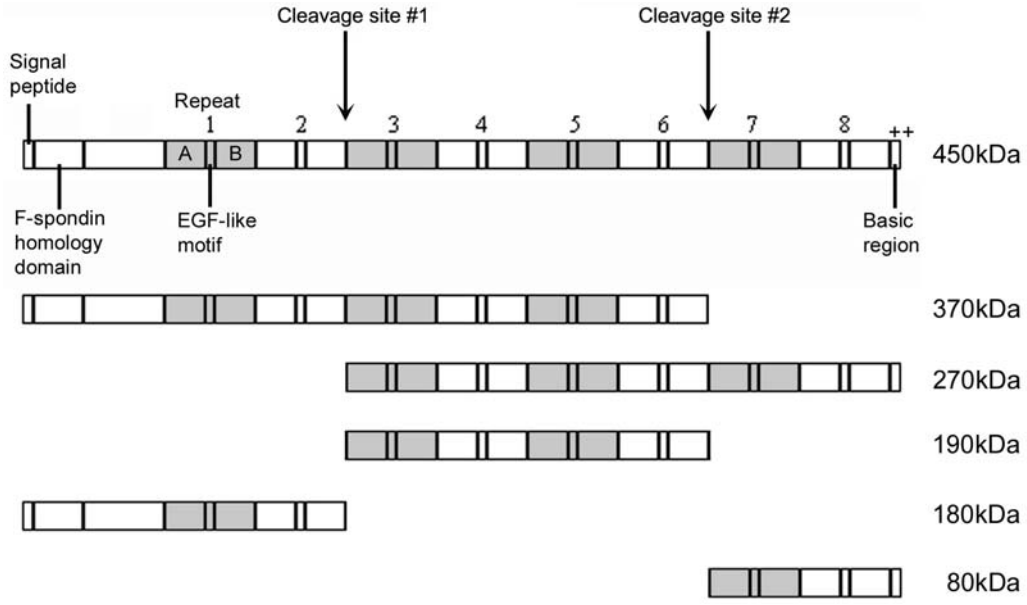

Fig. 3. Organization of Reelin and its cleavage fragments.

in the right sequence to bind to SH2 domains, suggesting that it is an adaptor protein.

The NPxY (Asp-Pro-any amino acid-Tyr) sequences in the cytoplasmic tail of transmembrane receptors of the LDLR family (LDLR, VLDLR, ApoER2, LRP, megalin) and amyloid precursor proteins (APP; APLP1, APLP2) bind Dab1 through the PI/PTB domain *(158,166–169).* However, unlike other PI/PTB domains (i.e., Shc, IRS1), the Dab1 PTB motif binds preferentially to unphosphorylated NPxY sites *(170).*

Some phosphatidyl inositols, especially phosphatidylinositol-4,5-bisphosphate ($PI4,5P_2$) can bind the PTB domain of Dab1 without affecting its interaction with lipoprotein receptors *(167, 171,172).*

Dab 1 mutations, either induced *(157)* or spontaneous (in *scrambler* and *yotari* mutant mice), generate a Reeler-like phenotype *(155, 156,173).* There is no additional cortical defect in mice that lack both Reelin and Dab1, suggesting that the two proteins function in a linear pathway *(170).* Dab1 is phosphorylated on five tyrosine residues by Src in vitro *(174);* this allows binding to the SH2 domain of Src, Fyn, Abl, and, probably, of the p85 regulatory subunit of PI3K *(175)* and Nckβ *(176).* Two of these five tyrosine residues, Y198 and Y220, are phosphorylated in vivo in response to the binding of Reelin to lipoprotein receptors VLDLR and ApoER2 *(177).* Knock-in mice that express a complementary DNA (cDNA) in which the five tyrosine residues are replaced by phenylalanines have a Reeler-like phenotype *(174),* and the replacement of the *Dab1* gene by a partial cDNA that encodes its PTB domain and the stretch of the five tyrosine residues is able to rescue most, but not all, features of the Dab1 mutant phenotype *(178).* This suggests that tyrosine phosphorylation is essential, but not sufficient, for all Dab1 functions and that the C-terminal part of Dab1 may serve unidentified roles. Interestingly, it contains consensus S/T phosphorylation sites, some of which can be phosphorylated by Cdk5-p35 independently of Reelin *(179,180).* The *Dab1* gene is highly complex. It extends over more than 1 MBp of genomic DNA and possesses at least five alternative promoters. Additionally, many internal alternative splicing events generate several

isoforms of the protein. The respective roles of the different promoters and protein isoforms are unknown *(181).*

VLDLR and ApoER2

Mice with double inactivation of the lipoprotein receptor genes coding for VLDLR and ApoER2 have a Reeler-like phenotype, whereas single receptor gene mutations generate subtle phenotypes. The analysis of single mutants suggests that VLDLR is most important in the cerebellum and ApoER2 is most important in the cortex and hippocampus *(158).* Reelin binds directly to the ectodomains of these receptors *(182,183),* and Dab1 docks to their cytoplasmic NPxY motif. Both receptors are expressed by neurons of the cortex and other areas that also express Dab1, and both are Reelin target cells *(158).* The other major sites of expression of VLDLR are the heart, skeletal muscle, and endothelial cells of major blood vessels. ApoER2 expression is almost exclusively limited to the brain, testes, and placenta *(184–186).*

VLDLR and ApoER2 are similar to the LDL receptor. They contain eight complement-type repeats implicated in ligand binding for VLDLR or four, five, seven, or eight for ApoER2. These repeats are 40 amino acids long and are rich in cystein and negatively charged amino acids. The repeats are followed by EGF-precursor homology domains essential for the pH-dependent release of ligands in endosomes *(185,187).* Their cytoplasmic tails contain one NPxY motif involved in the internalization of receptors via clathrin-coated pits *(188)* and in docking PTB domain-containing cytoplasmic adaptors *(189,190).*

Alternative splicing generates a variant of ApoER2, with an insert of 59 amino acids in the cytoplasmic tail. This insert, which is rich in proline, contains two potential SH3 domain binding sites and is responsible for the binding to JIP1 and JIP2 (JNK interacting protein 1 and 2), which cannot bind to VLDLR *(191).* The expression of this variant coincides with neuronal differentiation. JIPs are kinase scaffolding proteins involved in the JNK pathway by binding to mixed lineage kinase 3 (MLK3), mitogen-activated protein kinase 7 (MKK7), and JNK. They also interact with RhoGEF and a subunit of the kinesin anterograde microtubule motor that is involved in transport of signaling molecules *(192,193).* JIPs are localized to the neurite tips *(192),* where neurons sense their environment, making them candidates in the Reelin pathway. However, the cortex and cerebellum of *Jip1–/–* are normally laminated *(193),* although it may reflect the compensatory effect of JIP2. Another argument against the involvement of JIPs in Reelin signaling is the subtle phenotype observed in ApoER2–/– mice, indicating that VLDLR (which cannot bind to JIPs) can transduce the Reelin signal by itself.

Other functions of ApoER2 and VLDLR still are not fully defined, despite their broad panel of potential ligands, such as ApoE, the activated form of α2-macroglobulin, thrombospondin 1, lipoprotein lipase, urokinase-type plasminogen activator (uPA), vitellogenin *(187).* For instance, VLDLR is involved in the internalization and clearance from the membrane of the complex formed by uPA, the uPA-plasminogen activator inhibitor-1 and the uPA receptor *(195,196).* This receptor is also involved in the endocytosis of vitellogenin and VLDL from the yolk by the endodermal endothelial cells and subsequently delivered to the embryos during oogenesis in the chicken *(197,198).* In mice, VLDLR has been shown to play a role in cholesterol homeostasis in heart and skeletal muscle when LDLR is absent *(199).*

The Reelin Signaling Pathway

The components described in the previous section define a new signaling pathway that is identified genetically but still poorly characterized at the cellular and biochemical levels *(200–202).* The role of the Reelin pathway during cortical development can be summarized as follows: Reelin, secreted by Cajal-Retzius cells in the marginal zone of the cortex, is cleaved in vivo after secretion, probably by a metalloproteinase *(164).* Reelin, or a processing fragment, binds to VLDLR and ApoER2 that

are present on migrating neurons. This induces tyrosine phosphorylation of Dab1, which is attached to the NPxY cytoplasmic motif of receptors, thus triggering an intracellular cascade that instructs neurons to assume their proper shape and location. The NPxY motif is involved in internalization of lipoprotein receptors, but it remains unclear whether receptor internalization plays a role in Reelin signaling.

The importance of proteolytic processing for Reelin function is also unknown. It has been reported that the N-terminal fragment of Reelin binds to the protocadherin cadherin-related neuronal receptor 1 (CNR1), which could act as a coreceptor for Reelin *(203),* but this finding is controversial *(204).* There is evidence that CR-50, an antibody against the N-terminal region of Reelin, could block its function in vivo and in vitro *(205–207).* This antibody interferes with the aggregation of Reelin, which may affect its function *(208,209).* However, an N-terminal Reelin protein containing the first 437 amino acids, including the F-spondin homology domain and the CR-50 epitope, does not bind to VLDLR and ApoER2 *(183).* The observation that the central cleavage fragment is sufficient to bind to the VLDLR and ApoER2 receptors, to induce Dab1 phosphorylation, and to rescue the Reeler phenotype in a slice culture assay (whereas N- and C-terminal fragments that lack this central portion are not) demonstrated that the proteolytic processing of Reelin does not inhibit its function *(204).* Contrary to a previous report, the binding of CNR1 to Reelin was not confirmed in a pull-down assay *(204),* and this protocadherin is probably not a Reelin receptor.

The tyrosine kinases that phosphorylate Dab1 were recently identified. Yeast-two-hybrid experiments showed that Dab1 interacts with Abl, Src, and Fyn *(166),* and studies in mutant mice indicated that Fyn and, to a lesser extent, Src are involved in Dab1 phosphorylation, whereas Yes is not *(210,211).* As in Reelin- and receptor-deficient mice, the concentration of the Dab1 protein is increased in Fyn-deficient tissue and even more so in Fyn and Src compound mutant mice but not in Src and Yes compound mutant animals, suggesting that phosphorylation by Fyn and Src kinases controls Dab1 levels. Following tyrosine phosphorylation, Dab1 is polyubiquitinated and degraded by the proteasome; this may be important to ensure a transient response to Reelin *(212).*

The addition of Reelin to neurons from Fyn–/–Src+/–, Fyn+/–Src–/–, or Fyn–/–Src–/– mice still induces normal levels of Dab1 tyrosine phosphorylation, and no mutant mice exhibit a Reeler-like phenotype. To our knowledge, Fyn–/–Src–/– mutants die perinatally and have not been studied. Among the eight members of Src kinases, Src, Fyn, and Yes are highly expressed in postmitotic developing neurons, whereas expression of Blk, Fgr, Hck, Lck, and Lyn is restricted to hematopoietic cell lineages *(213).* However, weak expression of Lck and Lyn has been described in neurons *(214–216).* Using the slice culture assay, we demonstrated that the use of PP2, a well-validated cell permeant inhibitor of the Src family and Abl kinases induced a Reeler-like phenotype, with poor definition of the marginal zone, disorganization of the cortical plate, inversion of the inside-out layering, absence of preplate splitting, inhibition of Dab1 phosphorylation, and increase of Dab1 protein levels *(217).* Gleevec, which inhibits Abl but not Src kinases, had no effect on radial migration and cortical plate formation. These results provide strong evidence that Src family kinases, perhaps including Abl kinases, are responsible for Reelin-induced Dab1 phosphorylation.

Because, lipoprotein receptors have no intrinsic kinase activity and are not directly associated with tyrosine kinases or phosphatases, a coreceptor that is able to dock Src family kinases might be involved in Reelin signaling. Our observation that the induction of Dab1 phosphorylation in Reeler slices by addition of antireceptor antibodies is not sufficient to correct their phenotype suggests the presence of a coreceptor that is not involved in the recruitment of Src kinases *(204).* Other results using a divalent RAP-Fc fusion protein

support this observation *(218).* However, this negative result could result from other causes, and the presence of a Reelin coreceptor remains to be demonstrated.

α3β1 integrins apparently are able to bind Reelin; this could inhibit neuronal migration by stimulating the detachment of neurons from radial glia *(147,219).* However, mice with a brain-specific inactivation of β1 integrins induced a phenotype reminiscent of cobblestone lissencephaly and very different from Reeler, suggesting that these integrins regulate the anchorage of glial endfeet to the basal lamina but are not essential for neuronal migration *per se (125).* Mutation in the α3-subunit induces neuronal heterotopias in the intermediate zone, with premature transformation of radial glial cells into astrocytes and impaired neuron–glia interaction *(147).* In an in vitro assay, α3β1 promoted association of neurons with radial glia. In the absence of α3 integrin, the preference of neurons for glia changed to an increased association with other neurons *(147),* providing an explanation for the neuronal heterotopias in mice that lack α3 integrin. The relatively high expression of the 180-kDa N-terminal Reelin fragment and the reduction of Dab1 protein levels in cerebral cortices of α3 integrin-deficient mice also suggest an interaction with the Reelin pathway *(219).* Although no single integrin defect generates a Reeler-like phenotype, redundancy may hamper the evaluation of interactions with the Reelin pathway. For example, αv-containing integrins can bind Reelin and may compensate for the absence of α3 integrins *(147).* Therefore, integrins remain interesting candidates as partners of Reelin signaling.

At least two models have been proposed to explain the effect of Reelin on migrating neurons. Reelin, which is secreted in the marginal zone, might serve as an attractant for radially migrating neurons, enabling them to move beyond their predecessors that are already installed in the cortical plate. Alternatively, Reelin may provide a stop signal to migrating neurons, helping to detach them from the radial glia, which would then be free to guide the migration of the next wave of neurons.

Ectopic expression of Reelin in the ventricular zone was induced in transgenic mice that expressed the Reelin cDNA under control of the nestin promoter. Interestingly, Reelin expression in the ventricular zone did not stop premature neuronal migration but partially rescued the Reeler phenotype—namely, the restoration of preplate splitting but not of the normal, inside-to-outside gradient of cortical plate formation *(220).* Similarly, addition of Reelin to a Reeler slice in culture partially rescued the Reeler phenotype, with induction of preplate splitting and development of a better defined cortical plate, suggesting that the tissue localization of Reelin was not essential to its activity *(204).* These data argue against a direct attractant or stop signal effect of Reelin. However, Reelin may allow neurons to respond to another signal present that is in the marginal zone, or reception of the Reelin signal may require another cue present in the marginal zone; for example, Reelin may concentrate locally by binding to a component of the ECM.

Finally, recent observations point to a possible role of Reelin not only on end-migrating neurons but also on radial precursor cells. ApoER2, VLDLR, and Dab1 are expressed in radial glia precursors *(221),* and recombinant Reelin induce branching of glial processes in vitro *(222).* Therefore, Reelin may affect the differentiation of the glia via a Dab1-dependent mechanism. In the Reeler mouse, the radial glial scaffold fails to form normally and this may also impact on the radial migration of cortical neurons. Reelin, Dab1, and Reelin receptors are expressed in neural stem cells and may be important for their migration and differentiation during embryogenesis and in the adult brain *(223).*

Downstream Elements of the Reelin/Dab1 Pathway

Increased phosphorylation of the microtubule-associated protein τ is present in mice that are deficient in Reelin, Dab1, or both VLDLR and ApoER2 *(183,224).* Strain back-

ground has a major influence of this phenomenon, suggesting the presence of modifier genes that need to be further identified *(225).* Reelin stimulation of cultured neurons triggers the interaction of Y-phosphorylated Dab1 with the regulatory p85α-subunit of PI3K and increases Akt phosphorylation at S473 and GSK-3β phosphorylation at S9 in a VLDLR-, ApoER2-, and Dab1-dependent manner *(175,224,226).* GSK-3β acts on many downstream targets that include τ and the microtubule motor kinesin *(227).* In an astrocyte scratch wound assay, this is regulated by the Par6–aPKC complex, which promotes the polarization of the centrosome and controls the direction of cell protrusions *(51).* Unfortunately, disruption of the murine *GSK3β* gene results in embryonic lethality at 13.5 and 14.5 d *(228),* precluding analysis of its role in cortical development.

The aPKC (ζ and λ/ι) also phosphorylate GSK3β at S9, and we recently showed that inhibition of aPKC in a Slice culture induces a Reeler-like phenotype. However, additional data are needed to more accurately assess whether aPKCs interact directly with, or in parallel to, the Reelin pathway *(217).*

As summarized earlier, Cdk5/p35, another main τ kinase, is implicated in neuronal migration. Cdk5 null mice die shortly after birth, with defective migration in the cerebral cortex, cerebellum, and hippocampus *(229).* The activity of Cdk5 is not affected by Reelin stimulation of primary neuronal culture and is unchanged in mice with mutations of Reelin, Dab1, or VLDLR and ApoER2 *(226).* The phosphorylation of Dab1 by Cdk5 is independent of Reelin *(179).* Mice lacking both Cdk5/p35 and Reelin/Dab1 exhibit some synergistic phenotypes, but most defects in neuron migration are additive, suggesting that Reelin and Cdk5s do function in parallel rather than serially *(180).* Both pathways affect τ phosphorylation, but τ knockout animals display normal brain organization and neuronal migration *(230).* However, functional redundancies between τ and other microtubule-associated proteins such as mitogen activated protein-1B must be taken into account *(231,232).*

Reelin and Axonal Guidance

In addition to its role in architectonic development, Reelin has been suggested to influence axonal guidance *(233).* Hippocampal CR cells are able to guide entorhinal axons to the stratum lacunosum moleculare. However, hippocampal afferents successfully reach their target in Reeler mice, despite a significant delay *(234,235),* and we showed that Reelin is neither attractive nor repulsive for embryonic cortical axons *(236).* In this regard, Teillon et al. *(237)* showed that Reelin expressed in the accessory olfactory system does not provide a guidance cue for vomeronasal axons. Thus, the effect of the Reelin deficiency on axons may be secondary to the profuse architectonic malformation resulting from the absence of Reelin.

Concluding Remarks

Neuronal migration is a complex biological mechanism in which the Reelin signal plays a key part in instructing neurons to achieve a normal differentiation when they reach their final destination at the end of radial migration. Reelin signaling seems very different from canonical signal transduction pathways, making it quite refractory to mechanistic dissection. The involvement of a coreceptor and the putative role of Reelin processing remain open issues, and events downstream of Dab1 phosphorylation have only started to yield to investigation. We hope that improvements of in vitro techniques to study radial migration will enable identification of the different components of the signal and, most of all, better understanding of the overall control that Reelin exerts on end migrating neurons.

Acknowledgments

YJ is a research fellow with the Fonds National de la Recherche Scientifique, Belgium. I thank André Goffinet for critical reading of the manuscript.

References

1 Takahashi T., Goto T., Miyama S., Nowakowski R. S., and Caviness V. S. Jr. (1999) Sequence of neuron origin and neocortical laminar fate: relation to cell cycle of origin in the developing murine cerebral wall. *J. Neurosci.* **19,** 10,357–10,371.

2 Reid C. B., Liang I., and Walsh C. (1995) Systematic widespread clonal organization in cerebral cortex. *Neuron* **15,** 299–310.

3 Kornack D. R. and Rakic P. (1995) Radial and horizontal deployment of clonally related cells in the primate neocortex: relationship to distinct mitotic lineages. *Neuron* **15,** 311–321.

4 Chenn A. and McConnell S. K. (1995) Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. *Cell* **82,** 631–641.

5 Estivill-Torrus G., Pearson H., van Heyningen V., Price D. J., Rashbass P. (2002) Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. *Development* **129,** 455–466.

6 Meyer G., Soria J. M., Martinez-Galan J. R., Martin-Clemente B., and Fairen A. (1998) Different origins and developmental histories of transient neurons in the marginal zone of the fetal and neonatal rat cortex. *J. Comp. Neurol.* **397,** 493–518.

7 Nadarajah B. and Parnavelas J. G. (2002) Modes of neuronal migration in the developing cerebral cortex. *Nat. Rev. Neurosci.* **3,** 423–432.

8 Anderson S. A., Eisenstat D. D., Shi L., and Rubenstein J. L. (1997) Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes. *Science* **278,** 474–476.

9 Lavdas A. A., Grigoriou M., Pachnis V., and Parnavelas J. G. (1999) The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* **19,** 7881–7888.

10 Bellion A., Wassef M., and Metin C. (2003) Early differences in axonal outgrowth, cell migration and GABAergic differentiation properties between the dorsal and lateral cortex. *Cereb. Cortex* **13,** 203–214.

11 Caviness V. S. Jr. and Rakic P. (1978) Mechanisms of cortical development: a view from mutations in mice. *Annu. Rev. Neurosci.* **1,** 297–326.

12 Lambert de Rouvroit C. and Goffinet A. M. (2001) Neuronal migration. *Mech. Develop.* **105,** 47–56.

13 Bradke F. and Dotti C. G. (1999) The role of local actin instability in axon formation. *Science* **283,** 1931–1934.

14 Luo L. (2000) Rho GTPases in neuronal morphogenesis. *Nat. Rev. Neurosci.* **1,** 173–180.

15 Prieto-Sanchez R. M. and Bustelo X. R. (2003) Structural basis for the signaling specificity of RhoG and Rac1 GTPases. *J. Biol. Chem.* **278,** 37,916–37,925.

16 Takai Y., Sasaki T., and Matozaki T. (2001) Small GTP-binding proteins. *Physiol. Rev.* **81,** 153–208.

17 Hall A. (1998) Rho GTPases and the actin cytoskeleton. *Science* **279,** 509–514.

18 Miki H., Sasaki T., Takai Y., and Takenawa T. (1998) Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. *Nature* **391,** 93–96.

19 Rohatgi R., Ma L., Miki H., et al. (1999) The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97,** 221–231.

20 Miki H., Yamaguchi H., Suetsugu S., and Takenawa T. (2000) IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. *Nature* **408,** 732–735.

21 Du Y., Weed S. A., Xiong W. C., Marshall T. D., and Parsons J. T. (1998) Identification of a novel cortactin SH3 domain-binding protein and its localization to growth cones of cultured neurons. *Mol. Cell Biol.* **18,** 5838–5851.

22 Weed S. A., Du Y., and Parsons J. T. (1998) Translocation of cortactin to the cell periphery is mediated by the small GTPase Rac1. *J. Cell Sci.* **111,** 2433–2443.

23 Horwitz A. R. and Parsons J. T. (1999) Cell migration—movin' on. *Science* **286,** 1102,1103.

24 Bagrodia S. and Cerione R. A. (1999) Pak to the future. *Trends Cell Biol.* **9,** 350–355.

25 Edwards D. C., Sanders L. C., Bokoch G. M., and Gill G. N. (1999) Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat. Cell Biol.* **1,** 253–259.

26 Ohashi K., Nagata K., Maekawa M., Ishizaki T., Narumiya S., and Mizuno K. (2000) Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J. Biol. Chem.* **275,** 3577–3582.

27 Totsukawa G., Yamakita Y., Yamashiro S., Hartshorne D. J., Sasaki Y., and Matsumura F.

(2000) Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J. Cell Biol.* **150,** 797–806.

28 Maekawa M., Ishizaki T., Boku S., et al. (1999) Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* **285,** 895–898.

29 Watanabe N., Madaule P., Reid T., et al. (1997) p140mDia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO J.* **16,** 3044–3056.

30 Riveline D., Zamir E., Balaban N. Q., et al. (2001) Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J. Cell Biol.* **153,** 1175–1186.

31 Nobes C. D. and Hall A. (1999) Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J. Cell Biol.* **144,** 1235–1244.

32 Kozma R., Sarner S., Ahmed S., and Lim L. (1997) Rho family GTPases and neuronal growth cone remodelling: relationship between increased complexity induced by Cdc42Hs, Rac1, and acetylcholine and collapse induced by RhoA and lysophosphatidic acid. *Mol. Cell Biol.* **17,** 1201–1211.

33 Katoh H., Negishi M., and Ichikawa A. (1996) Prostaglandin E receptor EP3 subtype induces neurite retraction via small GTPase Rho. *J. Biol. Chem.* **271,** 29,780–29,784.

34 Postma F. R., Jalink K., Hengeveld T., and Moolenaar W. H. (1996) Sphingosine-1-phosphate rapidly induces Rho-dependent neurite retraction: action through a specific cell surface receptor. *EMBO J.* **15,** 2388–2392.

35 Sander E. E., ten Klooster J. P., van Delft S., van der Kammen R. A., and Collard J. G. (1999) Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J. Cell Biol.* **147,** 1009–1022.

36 Swiercz J. M., Kuner R., Behrens J., and Offermanns S. (2002) Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. *Neuron* **35,** 51–63.

37 Shamah S. M., Lin M. Z., Goldberg J. L., et al. (2001) EphA receptors regulate growth cone dynamics through the novel guanine nucleotide exchange factor ephexin. *Cell* **105,** 233–244.

38 Wahl S., Barth H., Ciossek T., Aktories K., and Mueller B. K. (2000) Ephrin-A5 induces collapse of growth cones by activating Rho and Rho kinase. *J. Cell Biol.* **149,** 263–270.

39 Shekarabi M. and Kennedy T. E. (2002) The netrin-1 receptor DCC promotes filopodia formation and cell spreading by activating Cdc42 and Rac1. *Mol. Cell Neurosci.* **19,** 1–17.

40 Li X., Saint-Cyr-Proulx E., Aktories K., and Lamarche-Vane N. (2002) Rac1 and Cdc42 but not RhoA or Rho kinase activities are required for neurite outgrowth induced by the Netrin-1 receptor DCC (deleted in colorectal cancer) in N1E-115 neuroblastoma cells. *J. Biol. Chem.* **277,** 15,207–15,214.

41 Wong K., Ren X. R., Huang Y. Z., et al. (2001) Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit–Robo pathway. *Cell* **107,** 209–221.

42 Kholmanskikh S. S., Dobrin J. S., Wynshaw-Boris A., Letourneau P. C., and Ross M. E. (2003) Disregulated RhoGTPases and actin cytoskeleton contribute to the migration defect in Lis1-deficient neurons. *J. Neurosci.* **23,** 8673–8681.

43 Krugmann S., Jordens I., Gevaert K., Driessens M., Vandekerckhove J., and Hall A. (2001) Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. *Curr. Biol.* **11,** 1645–1655.

44 Bear J. E., Loureiro J. J., Libova I., Fassler R., Wehland J., and Gertler F. B. (2000) Negative regulation of fibroblast motility by Ena/VASP proteins. *Cell* **101,** 717–728.

45 Bear J. E., Svitkina T. M., Krause M., et al. (2002) Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. *Cell* **109,** 509–521.

46 Lanier L. M., Gates M. A., Witke W., et al. (1999) Mena is required for neurulation and commissure formation. *Neuron* **22,** 313–325.

47 Goh K. L., Cai L., Cepko C. L., and Gertler F. B. (2002) Ena/VASP proteins regulate cortical neuronal positioning. *Curr. Biol.* **12,** 565–569.

48 Lebrand C., Dent E. W., Strasser G. A., et al. (2004) Critical role of Ena/VASP proteins for filopodia formation in neurons and in function downstream of netrin-1. *Neuron* **42,** 37–49.

49 Etienne-Manneville S. and Hall A. (2001) Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* **106,** 489–498.

50 Shi S. H., Jan L. Y., and Jan Y. N. (2003) Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* **112,** 63–75.
51 Etienne-Manneville S. and Hall A. (2003) Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity. *Nature* **421,** 753–756.
52 Palazzo A. F., Joseph H. L., Chen Y. J., et al. (2001) Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr. Biol.* **11,** 1536–1541.
53 Magdalena J., Millard T. H., and Machesky L. M. (2003) Microtubule involvement in NIH 3T3 Golgi and MTOC polarity establishment. *J. Cell Sci.* **116,** 743–756.
54 Bourrat F. and Sotelo C. (1990) Migratory pathways and selective aggregation of the lateral reticular neurons in the rat embryo: a horseradish peroxidase in vitro study, with special reference to migration patterns of the precerebellar nuclei. *J. Comp. Neurol.* **294,** 1–13.
55 Bloch-Gallego E., Ezan F., Tessier-Lavigne M., and Sotelo C. (1999) Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons. *J. Neurosci.* **19,** 4407–4420.
56 Causeret F., Danne F., Ezan F., Sotelo C., and Bloch-Gallego E. (2002) Slit antagonizes netrin-1 attractive effects during the migration of inferior olivary neurons. *Dev. Biol.* **246,** 429–440.
57 Morris N. R., Efimov V. P., and Xiang X. (1998) Nuclear migration, nucleokinesis and lissencephaly. *Trends Cell Biol.* **8,** 467–470.
58 Rakic P., Knyihar-Csillik E., and Csillik B. (1996) Polarity of microtubule assemblies during neuronal cell migration. *Proc. Natl. Acad. Sci. USA* **93,** 9218–9222.
59 Rivas R. J. and Hatten M. E. (1995) Motility and cytoskeletal organization of migrating cerebellar granule neurons. *J. Neurosci.* **15,** 981–989.
60 Forscher P. and Smith S. J. (1988) Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. *J. Cell Biol.* **107,** 1505–1516.
61 Danowski B. A. (1989) Fibroblast contractility and actin organization are stimulated by microtubule inhibitors. *J. Cell Sci.* **93,** 255–266.
62 Kaverina I., Krylyshkina O., and Small J. V. (2002) Regulation of substrate adhesion dynamics during cell motility. *Int. J. Biochem. Cell Biol.* **34,** 746–761.
63 Waterman-Storer C. M., Worthylake R. A., Liu B. P., Burridge K., and Salmon E. D. (1999) Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nat. Cell Biol.* **1,** 45–50.
64 Rodionov V. I., Gyoeva F. K., Tanaka E., Bershadsky A. D., Vasiliev J. M., and Gelfand V. I. (1993) Microtubule-dependent control of cell shape and pseudopodial activity is inhibited by the antibody to kinesin motor domain. *J. Cell Biol.* **123,** 1811–1820.
65 Mikhailov A. and Gundersen G. G. (1998) Relationship between microtubule dynamics and lamellipodium formation revealed by direct imaging of microtubules in cells treated with nocodazole or taxol. *Cell Motil. Cytoskeleton* **41,** 325–340.
66 Kaverina I., Krylyshkina O., and Small J. V. (1999) Microtubule targeting of substrate contacts promotes their relaxation and dissociation. *J. Cell Biol.* **146,** 1033–1044.
67 Best A., Ahmed S., Kozma R., and Lim L. (1996) The Ras-related GTPase Rac1 binds tubulin. *J. Biol. Chem.* **271,** 3756–3762.
68 Ren Y., Li R., Zheng Y., and Busch H. (1998) Cloning and characterization of GEF-H1, a microtubule-associated guanine nucleotide exchange factor for Rac and Rho GTPases. *J. Biol. Chem.* **273,** 34,954–34,960.
69 Gauthier-Rouviere C., Vignal E., Meriane M., Roux P., Montcourier P., and Fort P. (1998) RhoG GTPase controls a pathway that independently activates Rac1 and Cdc42Hs. *Mol. Biol. Cell* **9,** 1379–1394.
70 Huttenlocher A., Palecek S. P., Lu Q., et al. (1997) Regulation of cell migration by the calcium-dependent protease calpain. *J. Biol. Chem.* **272,** 32,719–32,722.
71 Alblas J., Ulfman L., Hordijk P., and Koenderman L. (2001) Activation of Rhoa and ROCK are essential for detachment of migrating leukocytes. *Mol. Biol. Cell* **12,** 2137–2145.
72 Smilenov L. B., Mikhailov A., Pelham R. J., Marcantonio E. E., and Gundersen G. G. (1999) Focal adhesion motility revealed in stationary fibroblasts. *Science* **286,** 1172–1174.
73 Ballestrem C., Hinz B., Imhof B. A., and Wehrle-Haller B. (2001) Marching at the front and dragging behind: differential alphaV-beta3-integrin turnover regulates focal adhesion behavior. *J. Cell Biol.* **155,** 1319–1332.
74 Ridley A. J. (2001) Rho GTPases and cell migration. *J. Cell Sci.* **114,** 2713–2722.

75 Ballestrem C., Wehrle-Haller B., Hinz B., and Imhof B. A. (2000) Actin-dependent lamellipodia formation and microtubule-dependent tail retraction control-directed cell migration. *Mol. Biol. Cell* **11,** 2999–3012.

76 Wynshaw-Boris A. and Gambello M. J. (2001) LIS1 and dynein motor function in neuronal migration and development. *Genes Dev.* **15,** 639–651.

77 Hirotsune S., Fleck M. W., Gambello M. J., et al. (1998) Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. *Nat. Genet.* **19,** 333–339.

78 Gambello M. J., Darling D. L., Yingling J., Tanaka T., Gleeson J. G., and Wynshaw-Boris A. (2003) Multiple dose-dependent effects of Lis1 on cerebral cortical development. *J. Neurosci.* **23,** 1719–1729.

79 Cahana A., Escamez T., Nowakowski R. S., et al. (2001) Targeted mutagenesis of Lis1 disrupts cortical development and LIS1 homodimerization. *Proc. Natl. Acad. Sci. USA* **98,** 6429–6434.

80 Xiang X., Osmani A. H., Osmani S. A., Xin M., and Morris N. R. (1995) NudF, a nuclear migration gene in Aspergillus nidulans, is similar to the human LIS-1 gene required for neuronal migration. *Mol. Biol. Cell* **6,** 297–310.

81 Aumais J. P., Tunstead J. R., McNeil R. S., et al. (2001) NudC associates with Lis1 and the dynein motor at the leading pole of neurons. *J. Neurosci.* **21,** RC187.

82 Kholmanskikh S. S., Dobrin J. S., Wynshaw-Boris A., Letourneau P. C., and Ross M. E. (2003) Disregulated RhoGTPases and actin cytoskeleton contribute to the migration defect in Lis1-deficient neurons. *J. Neurosci.* **23,** 8673–8681.

83 Corbo J. C., Deuel T. A., Long J. M., et al. (2002) Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. *J. Neurosci.* **22,** 7548–7557.

84 Francis F., Koulakoff A., Boucher D., et al. (1999) Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron* **23,** 247–256.

85 Olson E. C. and Walsh C. A. (2002) Smooth, rough and upside-down neocortical development. *Curr. Opin. Genet. Dev.* **12,** 320–327.

86 Caspi M., Atlas R., Kantor A., Sapir T., and Reiner O. (2000) Interaction between LIS1 and doublecortin, two lissencephaly gene products. *Hum. Mol. Genet.* **9,** 2205–2213.

87 Schaar B. T., Kinoshita K., and McConnell S. K. (2004) Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. *Neuron* **41,** 203–213.

88 Tanaka T., Serneo F. F., Tseng H. C., Kulkarni A. B., Tsai L. H., and Gleeson J. G. (2004) Cdk5 phosphorylation of doublecortin ser297 regulates its effect on neuronal migration. *Neuron* **41,** 215–227.

89 Tsai L. H., Takahashi T., Caviness V. S. Jr., and Harlow E. (1993) Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic mouse nervous system. *Development* **119,** 1029–1040.

90 Ino H., Ishizuka T., Chiba T., and Tatibana M. (1994) Expression of CDK5 (PSSALRE kinase), a neural cdc2-related protein kinase, in the mature and developing mouse central and peripheral nervous systems. *Brain Res.* **661,** 196–206.

91 Lew J., Qi Z., Huang Q. Q., Paudel H., Matsuura I., Matsushita M., Zhu X., and Wang J. H. (1995) Structure, function, and regulation of neuronal Cdc2-like protein kinase. *Neurobiol. Aging* **16,** 263–268.

92 Kwon Y. T. and Tsai L. H. (1998) A novel disruption of cortical development in p35(−/−) mice distinct from reeler. *J. Comp. Neurol.* **395,** 510–522.

93 Ko J., Humbert S., Bronson R. T., et al. (2001) p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *J. Neurosci.* **21,** 6758–6771.

94 Nikolic M., Chou M. M., Lu W., Mayer B. J., and Tsai L. H. (1998) The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. *Nature* **395,** 194–198.

95 Niethammer M., Smith D. S., Ayala R., et al. (2000) NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* **28,** 697–711.

96 Patrick G. N., Zukerberg L., Nikolic M., de la Monte S., Dikkes P., and Tsai L. H. (1999) Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* **402,** 615–622.

97 Xie Z., Sanada K., Samuels B. A., Shih H., and Tsai L. H. (2003) Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration. *Cell* **114,** 469–482.

98 Beggs H. E., Schahin-Reed D., Zang K., et al. (2003) FAK deficiency in cells contributing to the

basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. *Neuron* **40,** 501–514.
99 Sheen V. L., Feng Y., Graham D., Takafuta T., Shapiro S. S., and Walsh C. A. (2002) Filamin A and Filamin B are co-expressed within neurons during periods of neuronal migration and can physically interact. *Hum. Mol. Genet.* **11,** 2845–2854.
100 Hatten M. E. (2002) New directions in neuronal migration. *Science* **297,** 1660–1663.
101 Flanagan L. A., Chou J., Falet H., Neujahr R., Hartwig J. H., and Stossel T. P. (2001) Filamin A, the Arp2/3 complex, and the morphology and function of cortical actin filaments in human melanoma cells. *J. Cell Biol.* **155,** 511–517.
102 Loo D. T., Kanner S. B., and Aruffo A. (1998) Filamin binds to the cytoplasmic domain of the beta1-integrin. Identification of amino acids responsible for this interaction. *J. Biol. Chem.* **273,** 23,304–23,312.
103 Ohta Y., Suzuki N., Nakamura S., Hartwig J. H., Stossel T. P. (1999) The small GTPase RalA targets filamin to induce filopodia. *Proc. Natl. Acad. Sci. USA* **96,** 2122–2128.
104 Vadlamudi R. K., Li F., Adam L., et al. (2002) Filamin is essential in actin cytoskeletal assembly mediated by p21-activated kinase 1. *Nat. Cell Biol.* **4,** 681–690.
105 Bellanger J. M., Astier C., Sardet C., Ohta Y., Stossel T. P., and Debant A. (2000) The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. *Nat. Cell Biol.* **2,** 888–892.
106 Tu Y., Wu S., Shi X., Chen K., and Wu C. (2003) Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* **113,** 37–47.
107 Nagano T., Yoneda T., Hatanaka Y., Kubota C., Murakami F., and Sato M. (2002) Filamin A-interacting protein (FILIP) regulates cortical cell migration out of the ventricular zone. *Nat. Cell Biol.* **4,** 495–501.
108 Sheen V. L., Ganesh V. S., Topcu M., et al. (2004) Mutations in ARFGEF2 implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. *Nat. Genet.* **36,** 69–76.
109 Stumpo D. J., Bock C. B., Tuttle J. S., and Blackshear PJ. (1995) MARCKS deficiency in mice leads to abnormal brain development and perinatal death. *Proc. Natl. Acad. Sci. USA* **92,** 944–948.
110 Hartwig J. H., Thelen M., Rosen A., Janmey P. A., Nairn A. C., and Aderem A. (1992) MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium–calmodulin. *Nature* **356,** 618–622.
111 Costa C., Harding B., and Copp A. J. (2001) Neuronal migration defects in the Dreher (Lmx1a) mutant mouse: role of disorders of the glial limiting membrane. *Cereb. Cortex.* **11,** 498–505.
112 Hartmann D., De Strooper B., and Saftig P. (1999) Presenilin-1 deficiency leads to loss of Cajal-Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly. *Curr. Biol.* **9,** 719–727.
113. Beltran-Valero de Bernabe D., Currier S., Steinbrecher A., et al. (2002) Mutations in the *O*-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg Syndrome. *Am. J. Hum. Genet.* **71,** 1033–1043.
114 Manya H., Chiba A., Yoshida A., et al. (2004) Demonstration of mammalian protein *O*-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. *Proc. Natl. Acad. Sci. USA* **101,** 500–550.
115 Yoshida A., Kobayashi K., Manya H., et al. (2001) Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev. Cell* **1,** 717–724.
116 Aravind L. and Koonin E. V. (1999) The fukutin protein family—predicted enzymes modifying cell-surface molecules. *Curr. Biol.* **9,** R836–R837.
117 Moore S. A., Saito F., Chen J., et al. (2002) Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. *Nature* **418,** 422–425.
118 Grewal P. K. and Hewitt J. E. (2003) Glycosylation defects: a new mechanism for muscular dystrophy? *Hum. Mol. Genet.* **12,** R259–R264.
119 Endo T. and Toda T. (2003) Glycosylation in congenital muscular dystrophies. *Biol. Pharm. Bull.* **26,** 1641–1647.
120 Henion T. R., Qu Q., and Smith F. I. (2003) Expression of dystroglycan, fukutin and POMGnT1 during mouse cerebellar development. *Brain Res. Mol. Brain Res.* **112,** 177–181.
121 Winder S. J. (2001) The complexities of dystroglycan. *Trends Biochem. Sci.* **26,** 118–124.

122 Jones K. J., Morgan G., Johnston H., et al. (2001) The expanding phenotype of laminin alpha2 chain (merosin) abnormalities: case series and review. *J. Med. Genet.* **38,** 649–657.

123 Arikawa-Hirasawa E., Watanabe H., Takami H., Hassell J. R., and Yamada Y. (1999) Perlecan is essential for cartilage and cephalic development. *Nat. Genet.* **23,** 354–358.

124 Costell M., Gustafsson E., Aszodi A., et al. (1999) Perlecan maintains the integrity of cartilage and some basement membranes. *J. Cell Biol.* **147,** 1109–1122.

125 Graus-Porta D., Blaess S., Senften M., et al. (2001) Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron* **31,** 367–379.

126 Georges-Labouesse E., Mark M., Messaddeq N., and Gansmuller A. (1998) Essential role of alpha 6 integrins in cortical and retinal lamination. *Curr. Biol.* **8,** 983–986.

127 Yoshida T., Pan Y., Hanada H., Iwata Y., and Shigekawa M. (1998) Bidirectional signaling between sarcoglycans and the integrin adhesion system in cultured L6 myocytes. *J. Biol. Chem.* **273,** 1583–1590.

128 Rakic P. (1990) Principles of neural cell migration. *Experientia* **46,** 882–891.

129 McAllister A. K. (2000) Cellular and molecular mechanisms of dendrite growth. *Cereb. Cortex* **10,** 963–973.

130 Sobeih M. M. and Corfas G. (2002) Extracellular factors that regulate neuronal migration in the central nervous system. *Int. J. Dev. Neurosci.* **20,** 349–357.

131 Ringstedt T., Linnarsson S., Wagner J., et al. (1998) BDNF regulates reelin expression and Cajal-Retzius cell development in the cerebral cortex. *Neuron* **21,** 305–315.

132 Flanagan J. G. and Vanderhaeghen P. (1998) The ephrins and Eph receptors in neural development. *Annu. Rev. Neurosci.* **21,** 309–345.

133 Hamasaki T., Goto S., Nishikawa S., and Ushio Y. (2003) Neuronal cell migration for the developmental formation of the mammalian striatum. *Brain Res. Brain Res. Rev.* **41,** 1–12.

134 Zhu Y., Li H., Zhou L., Wu J. Y., and Rao Y. (1999) Cellular and molecular guidance of GABAergic neuronal migration from an extracortical origin to the neocortex. *Neuron* **23,** 473–485.

135 Marin O. and Rubenstein J. L. (2001) A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev. Neurosci.* **2,** 780–790.

136 Doetsch F. and Alvarez-Buylla A. (1996) Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc. Natl. Acad. Sci. USA* **93,** 14,895–14,900.

137 Mason H. A., Ito S., and Corfas G. (2001) Extracellular signals that regulate the tangential migration of olfactory bulb neuronal precursors: inducers, inhibitors, and repellents. *J. Neurosci.* **21,** 7654–7663.

138 Curran T. and D'Arcangelo G. (1998) Role of reelin in the control of brain development. *Brain Res. Brain Res. Rev.* **26,** 285–294.

139 Edmondson J. C., Liem R. K., Kuster J. E., and Hatten M. E. (1988) Astrotactin: a novel neuronal cell surface antigen that mediates neuron-astroglial interactions in cerebellar microcultures. *J. Cell Biol.* **106,** 505–517.

140 Adams N. C., Tomoda T., Cooper M., Dietz G., and Hatten M. E. (2002) Mice that lack astrotactin have slowed neuronal migration. *Development* **129,** 965–972.

141 Rio C., Rieff H. I., Qi P., Khurana T. S., and Corfas G. (1997) Neuregulin and erbB receptors play a critical role in neuronal migration. *Neuron* **19,** 39–50.

142 Anton E. S., Marchionni M. A., Lee K. F., and Rakic P. (1997) Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. *Development* **124,** 3501–3510.

143 Schmid R. S., McGrath B., Berechid B. E., Boyles B., Marchionni M., and Sestan N., and Anton E. S. (2003) Neuregulin 1-erbB2 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex. *Proc. Natl. Acad. Sci. USA* **100,** 4251–4256.

144 Threadgill D. W., Dlugosz A. A., Hansen L. A., et al. (1995) Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* **269,** 230–234.

145 Caric D., Raphael H., Viti J., Feathers A., Wancio D., and Lillien L. (2001) EGFRs mediate chemotactic migration in the developing telencephalon. *Development* **128,** 4203–4216.

146 Schmid R. S. and Anton E. S. (2003) Role of integrins in the development of the cerebral cortex. *Cereb. Cortex* **13,** 219–224.

147 Anton E. S., Kreidberg J. A., and Rakic P. (1999) Distinct functions of alpha3 and alpha(v) integrin receptors in neuronal migration and laminar organisation of the cerebral cortex. *Neuron* **22,** 277–289.

148. D'Arcangelo G., Miao G. G., Chen S. C., Soares H. D., Morgan J. I., and Curran T. (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374,** 719–723.
149 Hong S. E., Shugart Y. Y., Huang D. T., et al. (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat. Genet.* **26,** 93–96.
150. Lambert de Rouvroit C. and Goffinet A. M. (1998) The Reeler mouse as a model of brain development. *Adv. Anat. Embryol. Cell Biol.* **150,** 1–106.
151 Rice D. S., Nusinowitz S., Azimi A. M., Martinez A., Soriano E., and Curran T. (2001) The Reelin pathway modulates the structure and function of retinal synaptic circuitry. *Neuron* **31,** 929–941.
152 Yip J. W., Yip Y. P., Nakajima K., and Capriotti C. (2000) Reelin controls position of autonomic neurons in the spinal cord. *Proc. Natl. Acad. Sci. USA* **97,** 8612–8616.
153 Goffinet A. M. (1984) Events governing organization of postmigratory neurons: studies on brain development in normal and reeler mice. *Brain Res.* **319,** 261–296.
154 Rice D. S. and Curran T. (2001) Role of the reelin signaling pathway in central nervous system development. *Annu. Rev. Neurosci.* **24,** 1005–1039.
155 Sheldon M., Rice D. S., D'Arcangelo G., et al. (1997) Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* **389,** 730–733.
156 Ware M. L., Fox J. W., Gonzalez J. L., et al. (1997) Aberrant splicing of a mouse disabled homolog, mdab1, in the scrambler mouse. *Neuron* **19,** 239–249.
157 Howell B. W., Hawkes R., Soriano P., and Cooper J. A. (1997) Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* **389,** 733–737.
158 Trommsdorff M., Gotthardt M., Hiesberger T., et al. (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* **97,** 689–701.
159 Schiffmann S. N., Bernier B., and Goffinet A. M. (1997) Reelin mRNA expression during mouse brain development. *Eur. J. Neurosci.* **9,** 1055–1071.
160 Tissir F. and Goffinet A. M. (2003) Reelin and brain development. *Nat. Rev. Neurosci.* **4,** 496–505.
161 Pesold C., Impagnatiello F., Pisu M. G., et al. (1998) Reelin is preferentially expressed in neurons synthesizing gamma-aminobutyric acid in cortex and hippocampus of adult rats. *Proc. Natl. Acad. Sci. USA* **95,** 3221–3226.
162 Ikeda Y. and Terashima T. (1997) Expression of reelin, the gene responsible for the reeler mutation, in embryonic development and adulthood in the mouse. *Dev. Dyn.* **210,** 157–172.
163 Smalheiser N. R., Costa E., Guidotti A., et al. (2000) Expression of reelin in adult mammalian blood, liver, pituitary pars intermedia, and adrenal chromaffin cells. *Proc. Natl. Acad. Sci. USA* **97,** 1281–1286.
164. Lambert de Rouvroit C., de Bergeyck V., Cortvrindt C., Bar I., Eeckout Y., and Goffinet A. M. (1999) Reelin, the extracellular matrix protein deficient in reeler mutant mice, is processed by a metalloproteinase. *Exp. Neurol.* **156,** 214–217.
165 Walsh C. A. and Goffinet A. M. (2000) Potential mechanisms of mutations that affect neuronal migration in man and mouse. *Curr. Opin. Genet. Dev.* **10,** 270–274.
166 Howell B. W., Gertler F. B., and Cooper J. A. (1997) Mouse disabled (mDab1): a Src binding protein implicated in neuronal development. *EMBO J.* **16,** 121–132.
167 Howell B. W., Lanier L. M., Frank R., Gertler F. B., and Cooper J. A. (1999) The disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. *Mol. Cell Biol.* **19,** 5179–5188.
168 Homayouni R., Rice D. S., Sheldon M., and Curran T. (1999) Disabled-1 binds to the cytoplasmic domain of amyloid precursor-like protein 1. *J. Neurosci.* **19,** 7507–7515.
169 Trommsdorff M., Borg J. P., Margolis B., and Herz J. (1998) Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J. Biol. Chem.* **273,** 33,556–33,560.
170 Howell B. W., Herrick T. M., and Cooper J. A. (1999) Reelin-induced tryosine phosphorylation of disabled 1 during neuronal positioning. *Genes. Dev.* **13,** 643–648.
171. Stolt P. C., Jeon H., Song H. K., Herz J., Eck M. J., and Blacklow S. C. (2003) Origins of peptide selectivity and phosphoinositide binding revealed by structures of Disabled-1 PTB domain complexes. *Structure (Cambridge).* **11,** 569–579.

172 Yun M., Keshvara L., Park C. G., et al. (2003) Crystal structures of the Dab homology domains of mouse disabled 1 and 2. *J. Biol. Chem.* **278,** 36,572–36,581.
173 Yoneshima H., Nagata E., Matsumoto M., et al. (1997) A novel neurological mutant mouse, yotari, which exhibits Reeler-like phenotype but expresses CR-50 antigen/Reelin. *Neurosci. Res.* **29,** 217–223.
174 Howell B. W., Herrick T. M., Hildebrand J. D., Zhang Y., and Cooper J. A. (2000) Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. *Curr. Biol.* **10,** 877–885.
175 Bock H. H., Jossin Y., Liu P., et al. (2003) Phosphatidylinositol 3-kinase interacts with the adaptor protein Dab1 in response to Reelin signaling and is required for normal cortical lamination. *J. Biol. Chem.* **278,** 38,772–38,779.
176 Pramatarova A., Ochalski P. G., Chen K., et al. (2003) Nck beta interacts with tyrosine-phosphorylated disabled 1 and redistributes in Reelin-stimulated neurons. *Mol. Cell Biol.* **23,** 7210–7221.
177 Keshvara L., Benhayon D., Magdaleno S., and Curran T. (2001) Identification of Reelin-induced sites of tyrosyl phosphorylation on Disabled 1. *J. Biol. Chem.* **276,** 16,008–16,014.
178 Herrick T. M. and Cooper J. A. (2002) A hypomorphic allele of Dab1 reveals regional differences in Reelin-Dab1 signaling during brain development. *Development* **129,** 787–796.
179 Keshvara L., Magdaleno S., Benhayon D., and Curran T. (2002) Cyclin-dependent kinase 5 phosphorylates disabled 1 independently of Reelin signaling. *J. Neurosci.* **22,** 4869–4877.
180 Ohshima T., Ogawa M., Veeranna, et al. (2001) Synergistic contributions of cyclin-dependant kinase 5/p35 and Reelin/Dab1 to the positioning of cortical neurons in the developing mouse brain. *Proc. Natl. Acad. Sci. USA* **98,** 2764–2769.
181 Bar I., Tissir F., Lambert de Rouvroit C., De Backer O., and Goffinet A. M. (2003) The gene encoding Disabled-1 (Dab1), the intracellular adaptor of the Reelin pathway, reveals unusual complexity in human and mouse. *J. Biol. Chem.* **278,** 5802–5812.
182. D'Arcangelo G., Homayouni R., Keshvara L., Rice D. S., Sheldon M., and Curran T. (1999) Reelin is a ligand for lipoprotein receptors. *Neuron* **24,** 471–479.
183 Hiesberger T., Trommsdorff M., Howell B. W., et al. (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of Disabled-1 and modulates tau phosphorylation. *Neuron* **24,** 481–489.
184 Herz J. and Bock H. H. (2002) Lipoprotein receptors in the nervous system. *Annu. Rev. Biochem.* **71,** 405–434.
185 Brandes C., Kahr L., Stockinger W., Hiesberger T., Schneider W. J., and Nimpf J. (2001) Alternative splicing in the ligand binding domain of mouse ApoE receptor-2 produces receptor variants binding Reelin but not alpha 2-macroglobulin. *J. Biol. Chem.* **276,** 22,160–22,169.
186 Kim D. H., Iijima H., Goto K., et al. (1996) Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. *J. Biol. Chem.* **271,** 8373–8380.
187 Nykjaer A. and Willnow T. E. (2002) The low-density lipoprotein receptor gene family: a cellular Swiss army knife? *Trends Cell Biol.* **12,** 273–280.
188 Willnow T. E., Nykjaer A., and Herz J. (1999) Lipoprotein receptors: new roles for ancient proteins. *Nat. Cell Biol.* **1,** E157–E162.
189 Batzer A. G., Blaikie P., Nelson K., Schlessinger J., and Margolis B. (1995) The phosphotyrosine interaction domain of Shc binds an LXNPXY motif on the epidermal growth factor receptor. *Mol. Cell Biol.* **15,** 4403–4409.
190 Gustafson T. A., He W., Craparo A., Schaub C. D., and O'Neill T. J. (1995) Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. *Mol. Cell Biol.* **15,** 2500–2508.
191 Stockinger W., Brandes C., Fasching D., et al. (2000) The Reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. *J. Biol. Chem.* **275,** 25,625–25,632.
192 Meyer D., Liu A., and Margolis B. (1999) Interaction of c-Jun amino-terminal kinase interacting protein-1 with p190 rhoGEF and its localization in differentiated neurons. *J. Biol. Chem.* **274,** 35,113–35,118.
193 Verhey K. J., Meyer D., Deehan R., et al. (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell Biol.* **152,** 959–970.
194 Whitmarsh A. J., Kuan C. Y., Kennedy N. J., et al. (2001) Requirement of the JIP1 scaffold protein for stress-induced JNK activation. *Genes Dev.* **15,** 2421–2432.
195 Heegaard C. W., Simonsen A. C., Oka K., et al. (1995) Very low density lipoprotein receptor

binds and mediates endocytosis of urokinase-type plasminogen activator-type-1 plasminogen activator inhibitor complex. *J. Biol. Chem.* **270,** 20,855–20,861.
196 Webb D. J., Nguyen D. H., Sankovic M., and Gonias S. L. (1999) The very low density lipoprotein receptor regulates urokinase receptor catabolism and breast cancer cell motility in vitro. *J. Biol. Chem.* **274,** 7412–7420.
197 Nimpf J. and Schneider W. J. (1998) The VLDL receptor: an LDL receptor relative with eight ligand binding repeats, LR8. *Atherosclerosis* **141,** 191–202.
198 Hermann M., Mahon M. G., Lindstedt K. A., Nimpf J., and Schneider W. J. (2000) Lipoprotein receptors in extraembryonic tissues of the chicken. *J. Biol. Chem.* **275,** 16,837–16,844.
199 Tiebel O., Oka K., Robinson K., et al. (1999) Mouse very low-density lipoprotein receptor (VLDLR).: gene structure, tissue-specific expression and dietary and developmental regulation. *Atherosclerosis* **145,** 239–251.
200. Lambert de Rouvroit C. and Goffinet A. M. (1998) A new view of early cortical development. *Biochem. Pharmacol.* **56,** 1403–1409.
201 Bar I. and Goffinet A. M. (1999) Developmental neurobiology. Decoding the Reelin signal. *Nature* **399,** 645, 646.
202 Jossin Y., Bar I., Ignatova N., Tissir F., De Rouvroit C. L., and Goffinet A. M. (2003) The Reelin signaling pathway: some recent developments. *Cereb. Cortex* **13,** 627–633.
203 Senzaki K., Ogawa M., and Yagi T. (1999) Proteins of the CNR family are multiple receptors for Reelin. *Cell* **99,** 635–647.
204 Jossin Y., Ignatova N., Hiesberger T., Herz J., Lambert de Rouvroit C., and Goffinet A. M. (2004) The functional activity of Reelin is critically dependent on the central repeat region, but not on the N-terminus, nor on interaction with CNR1. *J. Neurosci.* **24,** 514–521.
205 Miyata T., Nakajima K., Mikoshiba K., and Ogawa M. (1997) Regulation of Purkinje cell alignment by Reelin as revealed with CR-50 antibody. *J. Neurosci.* **17,** 3599–3609.
206 Nakajima K., Mikoshiba K., Miyata T., Kudo C., and Ogawa M. (1997) Disruption of hippocampal development in vivo by CR-50 mAb against Reelin. *Proc. Natl. Acad. Sci. USA* **94,** 8196–8201.
207 Ogawa M., Miyata T., Nakajima K., et al. (1995) The Reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14,** 899–912.
208 Utsunomiya-Tate N., Kubo K., Tate S., et al. (2000) Reelin molecules assemble together to form a large protein complex, which is inhibited by the function-blocking CR-50 antibody. *Proc. Natl. Acad. Sci. USA* **97,** 9729–9734.
209 Kubo K., Mikoshiba K., and Nakajima K. (2002) Secreted Reelin molecules form homodimers. *Neurosci. Res.* **43,** 381–388.
210 Arnaud L., Ballif B. A., Forster E., and Cooper J. A. (2003) Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. *Curr. Biol.* **13,** 9–17.
211 Bock H. H. and Herz J. (2003) Reelin activates SRC family tyrosine kinases in neurons. *Curr. Biol.* **13,** 18–26.
212 Arnaud L., Ballif B. A., and Cooper J. A. (2003) Regulation of protein tyrosine kinase signaling by substrate degradation during brain development. *Mol. Cell Biol.* **23,** 9293–9302.
213 Kefalas P., Brown T. R., and Brickell P. M. (1995) Signalling by the p60c-src family of protein-tyrosine kinases. *Int. J. Biochem. Cell Biol.* **27,** 551–563.
214 Umemori H., Wanaka A., Kato H., Takeuchi M., Tohyama M., and Yamamoto T. (1992) Specific expressions of Fyn and Lyn, lymphocyte antigen receptor-associated tyrosine kinases, in the central nervous system. *Brain Res. Mol. Brain Res.* **16,** 303–310.
215 Chen S., Ren Y. Q., and Hillman D. E. (1996) Transient expression of lyn gene in Purkinje cells during cerebellar development. *Brain Res. Dev. Brain Res.* **92,** 140–146.
216 Omri B., Crisanti P., Marty M. C., et al. (1996) The Lck tyrosine kinase is expressed in brain neurons. *J. Neurochem.* **67,** 1360–1364.
217 Jossin Y., Ogawa M., Metin C., Tissir F., and Goffinet A. M. (2003) Inhibition of SRC family kinases and non-classical protein kinases C induce a Reeler-like malformation of cortical plate development. *J. Neurosci.* **23,** 9953–9959.
218 Strasser V., Fasching D., Hauser C., et al. (2004) Receptor clustering is involved in Reelin signaling. *Mol. Cell Biol.* **24,** 1378–1386.
219 Dulabon L., Olson E. C., Taglienti M. G., et al. (2000) Reelin binds alpha3beta1 integrin and inhibits neuronal migration. *Neuron* **27,** 33–44.
220 Magdaleno S., Keshvara L., and Curran T. (2002) Rescue of ataxia and preplate splitting by ectopic expression of Reelin in Reeler mice. *Neuron* **33,** 573–586.

221 Luque J. M., Morante-Oria J., and Fairen A. (2003) Localization of ApoER2, VLDLR and Dab1 in radial glia: groundwork for a new model of Reelin action during cortical development. *Brain Res. Dev. Brain Res.* **140,** 195–203.
222 Forster E., Tielsch A., Saum B., et al. (2002) Reelin, Disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proc. Natl. Acad. Sci. USA* **99,** 13,178–13,183.
223 Kim H. M., Qu T., Kriho V., et al. (2002) Reelin function in neural stem cell biology. *Proc. Natl. Acad. Sci. USA* **99,** 4020–4025.
224 Ohkubo N., Lee Y. D., Morishima A., et al. (2003) Apolipoprotein E and Reelin ligands modulate tau phosphorylation through an apolipoprotein E receptor/disabled-1/glycogen synthase kinase-3beta cascade. *FASEB J.* **17,** 295–297.
225 Brich J., Shie F. S., Howell B. W., et al. (2003) Genetic modulation of tau phosphorylation in the mouse. *J. Neurosci.* **23,** 187–192.
226 Beffert U., Morfini G., Bock H. H., Reyna H., Brady S. T., and Herz J. (2002) Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta. *J. Biol. Chem.* **277,** 49,958–49,964.
227 Morfini G., Pigino G., Beffert U., Busciglio J., and Brady S. T. (2002) Fast axonal transport misregulation and Alzheimer's disease. *Neuromolecular Med.* **2,** 89–99.
228 Hoeflich K. P., Luo J., Rubie E. A., Tsao M. S., Jin O., and Woodgett J. R. (2000) Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature* **406,** 86–90.
229 Ohshima T., Ward J. M., Huh C. G., et al. (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc. Natl. Acad. Sci. USA* **93,** 11,173–11,178.
230 Harada A., Oguchi K., Okabe S., et al. (1994) Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature* **369,** 488–491.
231 Takei Y., Teng J., Harada A., and Hirokawa N. (2000) Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. *J. Cell Biol.* **150,** 989–1000.
232 Teng J., Takei Y., Harada A., Nakata T., Chen J., and Hirokawa N. (2001) Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic outgrowth, and microtubule organization. *J. Cell Biol.* **155,** 65–76.
233 Del Rio J. A., Heimrich B., Borrell V., et al. (1997) A role for Cajal-Retzius cells and Reelin in the development of hippocampal connections. *Nature* **385,** 70–74.
234 Borrell V., Del Rio J. A., Alcantara S., et al. (1999) Reelin regulates the development and synaptogenesis of the layer-specific entorhinohippocampal connections. *J. Neurosci.* **19,** 1345–1358.
235 Borrell V., Ruiz M., Del Rio J. A., and Soriano E. (1999) Development of commissural connections in the hippocampus of Reeler mice: evidence of an inhibitory influence of Cajal-Retzius cells. *Exp. Neurol.* **156,** 268–282.
236 Jossin Y. and Goffinet A. M. (2001) Reelin does not directly influence axonal growth. *J. Neurosci.* **21,** RC183.
237. Teillon S. M., Yiu G., and Walsh C. A. (2003) Reelin is expressed in the accessory olfactory system, but is not a guidance cue for vomeronasal axons. *Brain Res. Dev. Brain Res.* **140,** 303–307.