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Reelin Signaling and Cdk5 in the Control of Neuronal Positioning

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Abstract

Neuronal positioning is important for higher brain function because it is the architectural basis of the formation of precise synaptic circuits. Analysis of neurological mutant mice has led to dramatic progress in the identification and characterization of molecules important for neuronal positioning in the developing mammalian brain. Among these molecules, identification of signal pathways mediated by Reelin and Cdk5 kinase has provided a conceptual framework for exploring the molecular mechanisms underlying proper neuronal positioning in the developing mammalian brain. Recent evidence has implicated synergism between Reelin signaling and Cdk5 in contributing to the proper positioning of selective neuronal populations.

Index Entries: Reelin; Dab1; CR-50; VLDLR; ApoER2; CNR; Cajal-Retzius cell; Cdk5; p35; p39.

Introduction

Most neurons migrate from the site of their birth near the ventricle toward the outer surface of the central nervous system (CNS), where they are integrated into specific brain circuits. Proper positioning of neurons is particularly critical for the formation of cyto-architecturally distinct brain regions, such as the cerebral cortex, hippocampus, and cerebellum. During the development of laminated brain structures, a series of coordinated migrations takes neurons from their site of origin to their final destinations, where they adopt definitive morphological phenotypes by elaborating dendritic and axonal processes *(1)*. In the mammalian brain, neurons settle into six neuronal

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laminae within the cerebral cortex in an inside-out manner, with the earliest-generated neurons positioned in the deepest layers, and later-generated neurons occupying the more superficial layers *(2).*

Remarkable progress in the identification and characterization of genes important to neuronal positioning in the developing brain has been made by analysis of neurological mutant mice *(3).* In addition to this, genetic studies of human brain malformations have also proven a valuable means of identifying the molecules that regulate neuronal migration *(4).* Such studies also serve to relate the significance of genes initally identified in mutant mice, such as *reelin,* to more complex human brain development *(5,6).* The mutations of *reelin* were first identified in several known alleles of *reeler (5),* and mutations have also been recently reported in humans with autosomal recessive lissencephaly associated with cerebellar hypoplasia *(6).* Lissencephaly is a condition caused by several neuronal migration disorders that result in a smooth brain. Although mice are normally lissencephalic, neuronal migration defects result in similar misplacements of neurons in laminated brain structures in both humans and mice.

Reelin Signaling

The mouse mutant *reeler* exhibits an ataxic gait and has served as a prototype for the investigation of neuronal migration *(7).* The *reeler* mouse is characterized by abnormalities of neuronal alignment in several neural systems, including the cerebral cortex, cerebellar cortex, hippocampus, and neuronal ectopia in several brainstem nuclei and the spinal cord *(8–14).* The reason for the intense interest in a single ataxic mouse is that the *reeler* phenotype highlights most of the major events in normal cortical development. Neocortical development begins with the appearance of the preplate above the ventricular zone; the preplate is composed of subcortical projections, Cajal–Retzius cells, and subplate neurons *(15).* In the next phase, the neurons destined to form the future projection neurons of the neocortex leave the ventricular zone, migrate into preplate, and split it in two layers: 1) a superficial layer, known as the marginal zone, and 2) a deeper layer, known as the subplate *(15).* Ogawa et al. generated CR-50 monoclonal antibody by immunizing homogenates of wild-type embryonic brain to *reeler* mutant mouse *(16),* and it recognized Cajal–Retzius neurons in the marginal zone of the cerebral cortex and external granular layer of the cerebellum. The cerebral cortex of *reeler* did not react with this antibody, even though Cajal–Retzius cells are present *(16,17),* and CR-50 antigen was therefore considered a candidate for the *reeler*-gene product. Independently, the *reeler*-gene named *reelin (Reln)* has been cloned by insertional mutation of a transgene, and molecular cloning has revealed that Reelin is a large (approx 385 kDa) secreted protein *(5).* Subsequent studies have shown that CR-50 antibody recognizes a tertiary structure of a region near the N-terminal of Reelin, which is defective in Jackson-type *reeler (18).* In the Orleans *reeler,* Reelin is truncated by a mutation near the C-terminal, and the truncated Reelin protein is not secreted *(19).* It is important to note that several experimental approaches have revealed that CR-50 functions as a blocking antibody both in vivo and in vitro. CR-50 antibody disrupts the normal alignment of neurons and converts to *reeler* phenotype in reaggregation-culture of cerebral cortex *(16),* and in hippocampal structure when injected in the embryonic stage *(20).* Miyata et al. showed that normal alignment of Purkinje cells is blocked by CR-50 antibody, and that abnormal positioning of Purkinje cells in *reeler* cerebellum is rescued by transplanted granule cells isolated from normal cerebellum *(21).* Biochemistry and structural biology methods have shown that Reelin forms a homomultimer driven by the CR-50 epitope through electrostatic interaction *(22).* Mutant Reelin that lacks the CR-50 epitope region does not form homomultimers, and Reelin

homomultimers are disrupted in the presence of CR-50, suggesting that multimerization of Reelin is required for it to function *(22).* Another biochemical study demonstrated a potential role for Reelin as a serine protease of extracellular matrix *(23).*

Shortly after Reelin was identified, Davisson et al. at Jackson Laboratory described a *reeler* phenotype in a new spontaneous mutant named *scrambler (24).* Another *reeler* phenotype, spontaneous mutant mouse named *yotari,* was found in a colony of IP_3R1 mutant mice, and was shown to have normal Reelin protein expression *(25).* The *scrambler* locus was mapped to near the *disabled-1 (Dab1)* gene on chromosome 4 *(24),* and mice with targeted disruption of Dab1 were found to exhibit behavioral and anatomical abnormalities indistinguishable from those of *reeler* and *scrambler/yotari (26).* It was then shown that *scrambler/yotari* mice express a mutated form of Dab1 mRNA; a drastic decrease in Dab1 protein was found in *scrambler* brain, while an absence of Dab1 protein was found in *yotari* brain *(27).* Later, Kojima et al. found that the *yotari* mutation in Dab1 is caused by a replacement of gene sequence with a long interspersed nuclear element (L1) fragment *(28).* Characterization of the gene mutation of Dab1 in *yotari* made it easy to identify *yotari* embryos by PCR-based genotyping *(28).* Dab1 is an intracellular adaptor protein containing a motif known as a protein interaction/phosphotyrosine binding (PI/PTB) domain *(29).* Tyrosine phosphorylation of Dab1 promotes its interaction with several nonreceptor tyrosine kinases, including Src, Fyn, and Ab1 through their SH2 domains, implicating Dab1 function in kinase-signaling cascades during brain development *(29).*

In 1999, behavioral and neuroanatomical defects identical to those in *reeler* were reported in mice lacking both very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor-2 (ApoER2) *(30).* These members of the low-density lipoprotein receptor (LDLR) family have been shown to bind to the PI/PTB domain of Dab1 *(31).* VLDLR and ApoER2 were originally identified as the receptors for lipoproteins, such as ApoE, and both receptors were enriched in brain. The patterns of expression of their mRNA considerably overlap that of Dab1 *(30),* indicating that VLDLR and ApoER2 act in concert as components of the Reelin receptors. Two groups using different methods have independently demonstrated their ability to bind Reelin *(32,33).* Several lines of evidence have now placed Reelin, VLDLR/ ApoER2, and Dab1 in a common signal pathway that controls neuronal positioning in the developing CNS.

1. Mutations in these genes result in identical neuroanatomical defects of neuronal positioning *(5,25,26,27,30).*
2. Reelin binds extracellularly to the ectodomain of VLDLR/ApoER2, and Dab1 interacts intracellularly with the cytoplasmic tails of these lipoprotein receptors *(31–33).*
3. The levels of Dab1 protein increase several-fold and tyrosine-phosphorylation of Dab1 protein decreases in the brains of *reeler* and VLDLR/ ApoER2-deficient mice *(34).*
4. Reelin is expressed in the cells located adjacent to the migrating neurons that express Dab1 and VLDLR/ApoER2 *(26,27,30).*
5. Addition of Reelin to neuronal culture induces tyrosine-phosphorylation of Dab1 *(35).*

Tyr^{198} and Tyr^{220} are considered to represent the major sites of Reelin-induced Dab1-phosphorylation in embryonic neurons *(36).* Mutation of five tyrosines including these two residues in Dab1 results in *reeler* phenotype, suggesting that tyrosine-phosphorylation of Dab1 is essential for Reelin signaling *(37).*

So far, the kinases responsible for in vivo tyrosyl-phosphorylation of Dab1 are not known. Reelin has also been shown to bind to cadherin-related neuronal receptors (CNRs) *(38)* and $\alpha 3\beta 1$ integrins *(39).* A number of genes have been suggested to play a role in the Reelin signaling (*see* Table 1), and while many of them have been directly implicated in this signaling, others *(40–46),* including the genes coding neurotrophic factors *(41,42)* and transcription factors, appear to be involved in the generation or survival of Cajal–Retzius cells *(43–46).*

Table 1
Genes Implicated in Reelin Signaling and Cdk5 for the Control of Neuronal Positioning

Gene	Name	Protein type	Chromosome location		Refs.
			Human	Mouse	
Bdnf	Brain-derived neurotrophic factor	Neurotrophin	11p13	1	*(41)*
NGFβ	Nerve growth factor β subunit	Neurotrophin	1p13	3	*(101)*
Ntf-4	Neurotrophin 4	Neurotrophin	19	7	*(42)*
Reln	Reelin	Extracellular protein	7q22	5	*(5)*
ApoER2	Apolipoprotein E receptor 2	Transmembrane	1p34	ND[a]	*(30)*
CNR	Cadherin-related neuronal receptor	Transmembrane	5q31	18	*(38)*
itga-3	α3 integrin	Integrin receptor subunit	17	11	*(39)*
PS-1	Presenilin-1	Transmembrane	14q24	ND	*(40)*
Vldlr	Very low density lipoprotein receptor	Transmembrane	9p24	19	*(30)*
Abl	Abelson proto-oncogene	Nonreceptor type tyrosine kinase	9q34	2	*(102)*
CASK	Membrane-associated guanylate kinase	Cytoplasmic	Xp11	X	*(43)*
Cdk5	Cyclin-dependent kinase 5	Serine/threonine kinase	7q36	5	*(47)*
Cdk5r	p35	Cdk5 regulatory sununit	17q11	11	*(48)*
Cdk5r2	p39	Cdk5 regulatory sununit	2q35	ND	*(55)*
Dab1	Disabled 1	Cytoplasmic adoptor protein	1q	4	*(26,27)*
Src	Src proto-oncogene	Non-receptor type tyrosine kinase	20q13	2	*(29)*
Brn-1	Brn-1	POU domain transcription factor	3p14	1	*(103)*
Brn-2	Brn-2	POU domain transcription factor	6q16	4	*(103)*
Emx2	Emx2	Transcription factor related empty spiracles	10q26	19	*(45)*
p73	p73 (Transformation-related protein)	p53 related protein	1p36	ND	*(44)*
Tbr-1	T-brain 1	Transcription factor related Brachyury gene	2q23–27	2	*(46)*

[a] ND, Not determined.

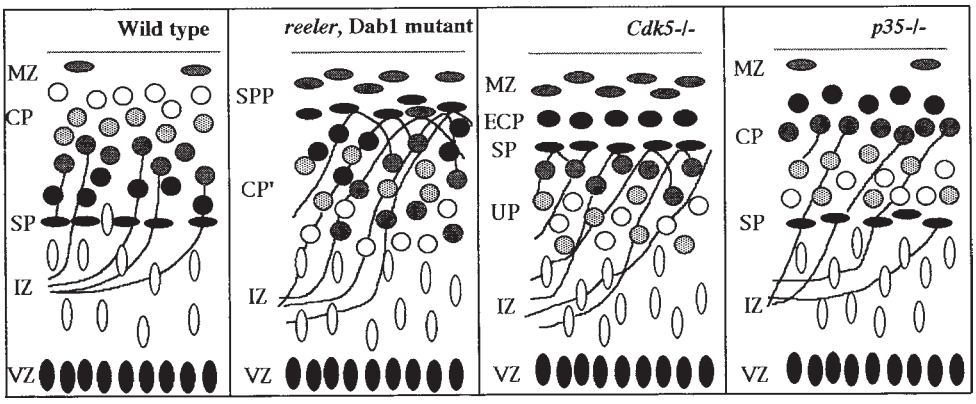

Fig. 1. Schematic representation of the development of the cerebral cortex in wild-type and mutant mice. In wild-type mice, the initial wave of migrating neurons splits the preplate into a marginal zone (MZ) and subplate (SP), and successive waves of migrating neurons move past their predecessors and form the cortical plate (CP) in an inside-out fashion. Marginal zone contains Cajal-Retzius and other cells. The migrating neurons are represented as dark ovals. At the sites where they stopped to form the cortical plate, the neurons are indicated by circles in gray-scale with respect to their final cell division. In *reeler* and Dab1 mutant mice, migrating cortical plate neurons are incapable of splitting the preplate, which remains as a superplate (SPP) with mutant neurons stacked up in inverted order. In *Cdk5–/–* mice, the initial wave of migrating neurons, destined to become layer VI (indicated by black circles), from the ventricular zone (VZ) cross the intermediate zone (IZ) and split the preplate forming a narrow CP. However, later-born cortical plate neurons (light-gray and white circles) stack up *reeler*-like under the subplate in an inverted fashion termed the 'underplate' (UP). In *p35–/–* mice, initial defects result in an inverted CP similar to that seen in *Cdk5–/–* mice, but later compensatory effects by Cdk5/p39 result in the normal positioning of the subplate neurons. In the adult of *p35–/–* mice there is distinguishable layer I, while *in reeler* no cell-free layer I can be observed in the cerebral cortex.

Cdk5 Kinase

Based on the phenotypes of knockout mice, Cdk5 and its activating subunit p35 are considered to be other critical molecules for neuronal migration *(47,48).* Cdk5 is a serine/threonine kinase with close homology to other Cdks *(49,50)* and is a unique Cdk because its kinase activity can be detected mainly in postmitotic neurons *(51).* Association of Cdk5 with a neuron-specific regulatory subunit, either p35 or its isoform p39, is critical for kinase activity *(52,53). Cdk5–/–* mice exhibit embryonic lethality associated with disruption of the cortical laminar structures in the cerebral cortex, olfactory bulb, hippocampus, and cerebellar cortex *(47),* whereas *p35–/–* mice showed milder phenotypes than *Cdk5–/–* mice owing to the redundancy of Cdk5 activator isoforms *(48,54).* Moreover, *p35–/–p39–/–* mice display a phenotype identical to *Cdk5–/–* mice, confirming redundancy in these subunits *(55).* Neuronal birthdate labeling by BrdU revealed an inverted pattern of cell layers in the cerebral cortex in *Cdk5–/–* mice *(56).* While *Cdk5–/–* and *p35–/–* mice demonstrate some similarities with *reeler* and *scrambler/yotari* mice, the development of the embryonic cerebral cortex in *Cdk5–/–* and *p35–/–* mice also shows significant differences including the splitting of the preplate *(56,57)* (*see* Fig. 1). In *reeler* and *scrambler/yotari* mutants, the migrating cortical neurons appear incapable of splitting the preplate, with cortical plate neurons stacking up in an inverted order beneath the preplate *(8,16).* In *Cdk5–/–* and *p35–/–* mice, earlier-born neurons split the preplate successfully, and later-born neurons stack up in an inverted

layer under the subplate *(56,57).* The observations of Morest and others, along with the observations in the analysis of *Cdk5–/–* mice, suggest that the earliest cortical plate neurons (destined for layer VI) may translocate their nucleus within their leading process *(58,59),* and this migration mode is Cdk5-independent *(56).* Recent analyses using in vitro slice cultures have supported the view that nuclear (somal) translocation is prominent within the early precursor, while migration guided by radial glia is used by the later-born cortical neurons *(59,60).* Because the migration of later-born neurons is selectively impaired in *Cdk5–/–* mice *(56),* it has been suggested that Cdk5 may be involved in the locomotion of migrating neurons along radial fibers *(59).* The precise role of Cdk5 kinase in neuronal positioning, however, remains to be expounded upon.

Similarities and Differences Between *Reeler* Phenotype and Cdk5-Deficient Phenotype

In addition to the cerebral cortex, *reeler* phenotype and Cdk5/p35 deficiency share similarities and differences in abnormalities of neuronal positioning in other areas of the CNS. With the exception of a few cell types, Reelin signaling and Cdk5 seem to be involved in the neuronal positioning of the same neuronal types, including Purkinje cells in the cerebellum. The cerebellum is another typical region of the CNS where both Reelin/Dab1 deficiency and Cdk5/p35 deficiency result in a similar phenotype at first glance, but detailed analysis has revealed significant differences. *Cdk5–/–* mice exhibit a cerebellar abnormality that resembles *reeler* and Dab1 mutants *(47,61). Cdk5–/–* ↔ *Cdk5*+/+ chimeric mice were used to analyze the role of Cdk5 in the neuronal positioning in the cerebellum because most cerebellar morphogenesis occurs after birth, and the *Cdk5–/–* mice die in the perinatal period *(61).* Analysis of the cerebellum of these chimeric mice demonstrated that Cdk5 deficiency arrests the migration of Purkinje cells in a cell-autonomous manner *(61).* This is in contrast to the case of normal ↔ *reeler* chimeric mice in which migration of reeler-derived Purkinje cells are rescued *(62).* Cdk5 deficiency also results in a failure of granule cells to complete migration from the external to the internal granule cell layer of the cerebellum *(61).* A small but significant number of mutant Purkinje cells successfully complete migration to the Purkinje cell layer in *reeler* and *scrambler/yotari (10,54,63),* whereas none of the *Cdk5–/–* Purkinje cells in the chimeras migrate successfully to the cerebellar cortex *(61).* This finding suggests that the Cdk5 mutation causes a more severe arrest of Purkinje cell migration than the *reeler* or Dab1 mutation. Cdk5-deficiency also results in significant defects in the migration of granule cells *(61),* whereas defective positioning of granule cells is considered to be a secondary phenomenon in *reeler* and Dab1 mutants.

Relation Between Reelin Signaling and Cdk5/p35

Because of the phenotypic similarities and differences between Cdk5/p35 and Reelin/Dab1 mutants, several models have been proposed regarding the relation between Reelin/Dab1 signaling and Cdk5/p35 *(64,65).* There is however, no evidence that Cdk5/p35 is a downstream effector of Reelin/Dab1 signaling. The close chromosomal proximity of loci for *cdk5 (66)* and *reeler* on chromosome 5 *(67)* raises concerns that the *reelin* gene might be down-regulated owing to an insertion-induced alteration in a long-distance enhancer located in the vicinity of *cdk5.* However, no differences in the levels of expression of *reelin* and Dab1 mRNA were found between wild-type and *Cdk5–/–* mouse brains at E16.5 *(56),* confirming that the migration defect of *Cdk5–/–* neurons is not mediated by changes in the expression of either Reelin or Dab1. To investigate the relation between Reelin signal-

ing and Cdk5/p35, p35 and Reelin/Dab1 double-mutant mouse lines were established *(54).* The deteriorations of the migration defects in these double mutants are typically seen in the Purkinje cells in the cerebellum and the pyramidal neurons in the hippocampus *(54);* these synergistic effects between two pathways have also been observed in neuronal positioning in the hindbrain. Migration arrest of facial branchiomotor (fbm) neurons and lack of typical alignment of the inferior olive (IO) have been observed in *Cdk5–/–* mice *(68).* Interestingly, abnormalities in the positioning of fbm and IO neurons have been observed in the *reeler* and Dab1 mutant, and are more severe in the double-null mice for p35 and Dab1 *(68).* These findings indicate that Cdk5/p35 is not a downstream effector of Reelin signaling, but that Cdk5/p35 and Reelin/Dab1 synergistically contribute to the positioning of the neurons in the developing brain. However, we also find that the addition of heterozygosity of the Dab1 mutation to the *p35–/–* genotype results in extensive migration defects of the granule and Purkinje cells in the cerebellum *(54).* Therefore, Reelin signaling can influence Cdk5 kinase activity even though Reelin signaling and Cdk5 do not lie in a single cascade. Alternatively, this can also be explained by a regulatory function of Cdk5 in Reelin signaling. Interestingly, Cdk5 phosphorylates serine/threonine sites in the C-terminal region of Dab1 in vitro (cited in ref. *33* as unpublished data). Most recently, mutant mice that express truncated Dab1 lacking the C-terminal region of Dab1 have been generated by a knock-in strategy *(69),* and analysis of these mutant mice has revealed that a single copy of the truncated gene does not support normal development of the neocortex and hippocampus—suggesting that the C-terminal region of Dab1 p80 is involved in signaling to downstream effector molecules *(69).* Thus, it will be important to investigate the significance of serine/threonine phosphorylation of Dab1 by Cdk5 in Reelin signaling in vivo.

Possible Function of Reelin Signaling in the Control of Neuronal Positioning

Despite the remarkable progress in the identification of the components of Reelin signaling, the exact function of Reelin in the control of neuronal positioning still remains to be determined. Several hypotheses have been proposed to explain the *reeler* phenotype and Reelin function in neuronal positioning. There is no doubt that Reelin plays an important role in the final phases of neuronal positioning. Reelin is expressed by Cajal–Retzius cells in the cerebral cortex, and is secreted at sites where migrating neurons terminate their migration and detach from radial fibers. Since granule cells in the external granule cell layer of the cerebellum secrete Reelin, and migrating Purkinje cells respond to it, it has been suggested that Reelin functions as a stop signal (or a repellent signal) for migrating neurons in the cerebral cortex *(39,70),* and similarly as a stop signal or chemoattractant for migrating Purkinje cells in the cerebellum *(71,72).* A recent attempt to resolve this issue has been made by using transgenic mice, in which Reelin is ectopically expressed in the ventricular zone *(73).* No effect on neuronal positioning in the cerebrum and cerebellum was observed in wild-type background, but ectopic Reelin has actually been shown to rescue some parts of the neuroanatomical abnormalities in *reeler* background *(73).* Based on this observation, the authors suspect that Reelin might participate in multiple events critical for neuronal positioning, instead of being a simple positional signal as previously thought *(73).* However, the fact that the transgene produces only small amounts of Reelin (estimated to be 10–20% of endogenous Reelin protein levels) it is difficult to interpret the effects of ectopic-Reelin from the transgene. Another possible function of Reelin has been proposed based on the study of autonomic neurons in the spinal cord *(14).* It was shown to regulate the migration of sympathetic preganglionic neurons in

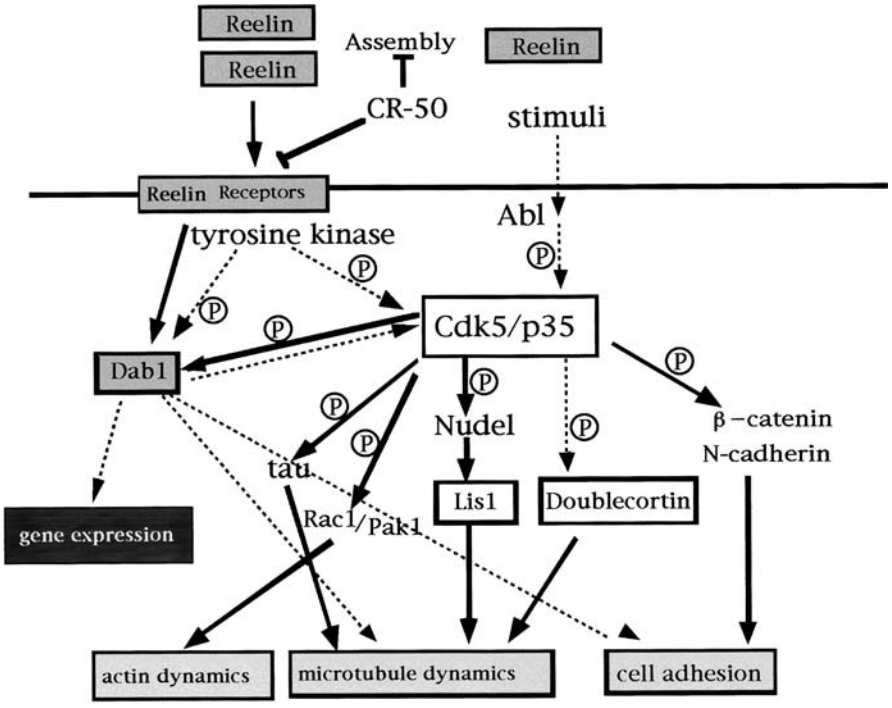

Fig. 2. Molecular model of the signaling pathway of Reelin and Cdk5 in the control of neuronal positioning. Reelin forms homomultimers and binds to its receptors, VLDLR/ApoERs and others. CR-50—a functional blocking antibody to Reelin—is thought to inhibit multimerization of Reelin and binding to receptors. Binding of Reelin to its receptors induces Dab1 tyrosine-phosphorylation, and tyrosine-phosphorylated Dab1 may act as an adapter for the recruitment of various SH2-domain containing proteins and activate downstream signaling cascades (broken lines). Cdk5 phosphorylates Dab1 on serine/threonine residues and may modulate Reelin-induced tyrosine-phosphorylation, or affect Dab1-localization within neurons. Cdk5 phosphorylates cytoskeletal proteins and their associating proteins, which affects the organization of the cytoskeleton. Interaction and phosphorylation of Pak1 by Cdk5 may modulate actin cytoskeleton dynamics. Cdk5/p35 also interacts with β-catenin, resulting in decreased association between β-catenin and N-cadherin, and loss of cell adhesion.

the spinal cord, and based on the observation of a relationship between Reelin and migrating preganglionic neurons, it has been proposed that Reelin may function as a barrier to migrating preganglionic neurons *(14).*

Possible Function of Cdk5/p35 in the Control of Neuronal Positioning

The results of several studies have suggested that Cdk5/p35 kinase regulates actin dynamics and/or microtubules. The localization of Cdk5 and p35 in the growth cone and interactions with Rac1 and Pak1 suggest that Cdk5/p35 kinase may be involved in the modulation of actin cytoskeletal dynamics *(74).* Cdk5/p35 may also regulate microtubules, because microtubule-associated protein tau and MAP1b are good substrates of Cdk5/p35 kinase *(75,76).* Interestingly, the causative genes of human lissencephaly, *Lis1 (77)* and *doublecortin (78,79)* are implicated in microtubule reorganization. Possible involvement of Cdk5 in Lis1 function in the neuronal migration has been discussed, because *Nudel* is one of the *Aspergillus* NUDE gene homologs and interacts with Lis1, and is also an in vivo sub-

strate of Cdk5/p35 *(80,81).* Another possible role of Cdk5/p35 kinase is regulation of cell adhesion mediated by N-cadherin during cortical development. Cdk5 has been shown to negatively regulate N-cadherin-mediated adhesion via an association between p35 and β-catenin *(82).* This has led to the proposal that Cdk5 kinase activity functions to suppress N-cadherin-mediated adhesion in migrating neurons during corticogenesis. An influence of Reelin signaling on Cdk5 kinase activity, resulting in abrogation of N-cadherin-mediated adhesion, has also been proposed as a possible function of Reelin *(65,82).* This hypothesis is consistent with the observation that early generated cohorts of neurons in *reeler* mice are more adhesive than their normal counterparts *(83,84).*

Other Functions of Reelin Signaling and Cdk5 Besides Neuronal Positioning in Developing CNS

Soriano and colleagues have shown that Reelin is important for correct formation of synaptic circuits in the hippocampus *(85,86). Reeler* mice exhibit several alterations in entorhinohippocampal projections, including reduced axonal branching, a decreased number of synapses, and abnormal topography of synapses *(85,86);* exposure of entorhinohippocampal slices to CR-50 leads to the formation of a *reeler*-type abnormality. Rice et al. demonstrated involvement of Reelin signaling in the organization of synpatic connections in the retina, where Reelin and Dab1 are expressed in adjacent cell layers in the adult *(87).* After postnatal day 6, Dab1 was found to accumulate in type AII amacrine cells, and defective synaptic layerings of type AII amacrine cells within the inner plexiform layer was observed in *reeler* and *scrambler* retina *(87).* These findings suggest that, in addition to its essential role during neuronal migration, Reelin signaling contributes to the proper patterning of synpatic connectivity.

Cdk5–/– and *p35–/–* mice have defects in the fasciculation of several axonal tracts including callosal and thalamocortical axons *(56,57)* (T. Ohshima, unpublished observation). In *Drosophila,* an increase or decrease in Cdk5 activity results in errors in axon pathfinding and target recognition of motor nerves *(88).* These observations suggest a role of Cdk5 in axon guidance and targeting. This is another example for the comparison with the *reeler* phenotype. In *reeler,* afferent projections to the visual, olfactory, somatosensory, and motor cortices find their correct targets despite being located in ectopic position *(89–91).*

Reelin and Cdk5 in Neuronal Degeneration

A recent study detected increased levels of tau phosphorylation in *reeler* and VLDLR/ApoER2 null mice in adulthood *(32).* Hyperphosphorylation of tau, which leads to microtubule dissocation, has been associated with Alzheimer's disease (AD) *(92);* and we have found an increased level of phosphorylated-tau in *yotari* brain (T. Ohshima et al., unpublished observation), suggesting that Reelin signaling participates in regulating cytoskeletal protein dynamics in the adult brain. Since it is well-known that the inheritance of the ApoE4 allele is a risk factor in the late onset of AD *(93),* and binding of Reelin to VLDLR/ApoER2 is inhibited by ApoE3 and ApoE4, but not by ApoE2 *(32),* it can be hypothesized that Reelin signaling acts antagonistically to a pathway involving ApoE, ApoE receptors, and tau phosphorylation *(3).* Dab1 has been shown to be capable of binding to their NPxY motif of the cytoplasmic domains of the APP family proteins: APP, APLP1, and APLP2 via the PTB domain *(31,94,95).* It will be interesting to see whether Reelin signaling affects the generation of amyloid β-protein, which is a proteolytic product of APP.

In addition to its possible physiological function in adult brains as well as in synaptic plas-

ticity, dopamine signaling, and drug addiction [reviewed in ref. *96*]), Cdk5 is also considered to be involved in the pathology of neurodegenerative disorders, such as AD *(96,97)* and amyotrophic lateral sclerosis (ALS) *(98).* Accumulation of p25—which is a cleavage product of p35 and a stable protein—and elevated Cdk5 activity have been reported in the brains of AD patients *(97).* Although these observations have not been consistent among investigators *(99,100),* a proposed model, in which elevated Cdk5 activity causes hyperphosphorylation of tau and neuronal apoptosis, is an appealing explanation of the neuropathology of neurodegenerative disorders with hyperphosphorylation of neurocytoskeletal proteins, such as tau and neurofilament proteins, and neuronal loss.

Conclusion

Further studies are necessary to understand the molecular mechanism of Reelin signaling and Cdk5 in neuronal positioning by analysis of mutant mice, and investigating the signal cascades and molecular interactions within migrating neurons in vivo and in vitro. Identification of the downstream cascade of Reelin signaling and in vivo substrates of Cdk5 involved in neuronal migration will provide valuable insights into the molecular and cellular mechanism of the neuronal migration underlying the precise positioning of the neurons in brain development.

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References

1. Ram n y Cajal S. (1955 [1911]) *Histology of the Nervous System of Man and Vertebrates.* (L. Swanson, N. Swanson, Transl.) Oxford Univ. Press, Oxford, UK.
2. Angevine J. B. and Sidman R. L. (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192,** 766–768.
3. Rice D. S. and Curran T. (2001) Role of the reelin signaling pathway in central nervous system development. *Annu. Rev. Neurosci.* **24,** 1005–1039.
4. Ross M. E. and Walsh C. A. (2001) Human brain malformation and their lesson for neuronal migration. *Annu. Rev. Neurosci.* **24,** 1041–1070.
5. 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.
6. 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.
7. Falconer D. S. (1951) Two new mutants trembler and reeler, with neurological actions in the house mouse. *J. Genet.* **50,** 192–201.
8. Caviness V. S., Jr. Crandall J. E., and Edwards M. A. (1988) The reeler malformation: implications for neocortical histogenesis, in *Cerebral Cortex.* (A Peters and EG Jones ed.) Vol. 17, Plenum, New York, pp. 59–89.
9. Caviness V. S. Jr. and Sidman R. L. (1973) Retrohippocampal, hippocampal and related structures of the forebrain in the reeler mutant mouse. *J. Comp. Neurol.* **147,** 235–254.
10. Mariani J., Crepel F., Mikoshiba K., Changeux J. P., and Sotelo C. (1977) Anatomical, physiological and biochemical studies of the cerebellum from Reeler mutant mouse. *Philos. Trans. R. Soc. London* **281,** 1–28.
11. Mikoshiba K., Nagaike K., Kohsaka S., Takamatsu K., Aoki E., and Tsukada Y. (1980) Developmental study on the cerebellum from reeler mutant mouse in vivo and in vitro. *Dev. Biol.* **79,** 64–80.
12. Goffinet A. M. (1983) The embryonic development of the inferior olivary complex in normal and reeler mutant mice. *J. Comp. Neurol.* **219,** 10–24.
13. Goffinet A. M. (1984) Abnormal development of the facial nerve nucleus in reeler mutant mice. *J. Anat.* **138,** 207–215.
14. Yip J. W., Yip Y. P. L., Nakajima K., and Capriotti C. (2000) Reelin controls position of auto-

nomic neurons in the spinal cord. *Proc. Natl. Acad. Sci. USA* **97,** 8612–8616.
15. Marin-Padilla M. (1998) Cajal-Retzius cells and the development of the neocortex. *Trend. Neurosci.* **21,** 64–71.
16. Ogawa M., Miyata T., Nakajima K., Yagyu K., Seike M., Ikenaka K., Yamamoto H., and Mikoshiba K. (1995) The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14,** 899–912.
17. Allendoerfer K. L. and Shatz C. J. (1994) The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annu. Rev. Neurosci.* **17,** 185–218.
18. D'Arcangelo G., Nakajima K., Miyata T., Ogawa M., Mikoshiba K., and Curran T. (1997) Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. *J. Neurosci.* **17,** 23–31.
19. de Bergeyck V., Nakajima K., Lambert de Rouvroit C., Naerhuyzen B., et al. (1997) A truncated Reelin protein is produced but not secreted in the Orleans reeler mutation (Reln[rl-Orl]). *Brain Res. Mol. Brain Res.* **50,** 85–90.
20. 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.
21. 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.
22. 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.
23. Quattrocchi C. C., Wannenes F., Persico A. M., et al. (2002) Reelin is a serine protease of the extracellular matrix. *J. Biol. Chem.* **277,** 303–309.
24. Sweet H. O., Bronson R. T., Johnson K. R., Cook S. A., and Davisson M. T. (1996) Scrambler, a new neurological mutation of the mouse with abnormalities of neuronal migration. *Mamm. Genome* **7,** 798–802.
25. 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.
26. 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.
27. 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.
28. Kojima T., Nakajima K., and Mikoshiba K. (2000) The disabled 1 gene is disrupted bya replacement with L1 fragment in yotari mice. *Mol. Brain Res.* **75,** 121–127.
29. 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.
30. 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.
31. 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.
32. Hiesberger T., Trommsdorff M., Howell B. W., Goffinet A., Mumby M. C., and Cooper J. A., Herz J. (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.
33. 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.
34. Rice D. S., Sheldon M., D'Arcangelo G., Nakajima K., Goldowitz D., and Curran T. (1998) Disabled-1 acts downstream of Reelin in a signaling pathway that controls laminar organization in the mammalian brain. *Development* **125,** 3719–3729.
35. 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.
36. Keshvara L., Benhayon D., Magdaleno S., and Curran T. (2001) Identification of Reelin-induced sites of tyrosyl-phosphorylation of disabled 1. *J. Biol. Chem.* **276,** 16,008–16,014.
37. Howell B. W., Herrick T. M., Hildebrand J. D., Zhang Y. N., and Cooper J. A. (2000) Dab1

tyrosine-phosphorylation sites relay positional signals during mouse brain development. *Curr. Biol.* **10,** 877–885.
38. Senzaki K., Ogawa M., and Yagi T. (1999) Proteins of the CNR family are multiple receptors for reelin. *Cell* **99,** 635–647.
39. Dulabon L., Olson E. C., Taglienti M. G., et al. (2000) Reelin binds alpha-3-beta-1 integrin and inhibits neuronal migration. *Neuron* **27,** 33–44.
40. 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.
41. 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.
42. Brunstrom J. E., Gray-Swain M. R., Osborne P. A., and Pearlman A. L. (1997) Neuronal heterotopias in the developing cerebral cortex produced by neurotrophin-4. *Neuron* **18,** 505–517.
43. Hsueh Y. P., Wang T. F., Yang F. C., and Sheng M. (2000) Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2. *Nature* **404,** 298–302.
44. Yang A., Walker N., Bronson R., et al. (2000) p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. *Nature* **404,** 99–103.
45. Mallamaci A., Mercurio S., Muzio L., Cecchi C., Pardini C. L., Gruss P., and Boncinelli E. (2000) The lack of Emx2 causes impairment of Reelin signaling and defects of neuronal migration in the developing cerebral cortex. *J. Neurosci.* **20,** 1109–1118.
46. Bulfone A., Smiga S. M., Shimamura K., Peterson A., Puelles L., and Rubenstein J. L. (1995) T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* **15,** 63–78.
47. 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.
48. Chae T., Kwon Y. T., Bronson R., Dikkes P., Li E., and Tsai L. H. (1997) Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* **18,** 29–42.
49. Hellmich M. R., Pant H. C., Wada E., and Betty J. F. (1992) Neuronal cdc2like kinase: A cdc2-related protein kinase with predominantly neuronal expression. *Proc. Natl. Acad. Sci. USA* **89,** 10,867–10,871.
50. Meyerson M., Enders G. H., Wu C. L., et al. (1992) A family of human cdc2-related protein kinases. *EMBO J.* **11,** 2909–2917.
51. Tsai L-H., Takahashi T., Caviness Jr. V. S., and Harlow E. (1993) Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic mouse central nervous system. *Development* **119,** 1029–1040.
52. Tsai L-H., Delalle I., Caviness Jr. V. S., Chae T., and Harlow E. (1994) p35 is a neuronal-specific regulatory subunit of cyclin-dependent kinase 5. *Nature* **371,** 419–423.
53. Tang D., Yeung J., Lee K-Y., et al. (1995) An isoform of the neuronal cyclin-dependent kinase 5 (cdk5) activator. *J. Biol. Chem.* **270,** 26,897–26,903.
54. Ohshima T., Ogawa M., Veeranna, et al. (2001) Synergistic contribution of Cdk5/p35 and Reelin/Dab1 to the positioning of cortical neurons in the developing mouse brain. *Proc. Natl. Acad. Sci. USA* **98,** 2764–2769.
55. Ko J., Humbert S., Bronson R. T., Takahashi S., Kulkarni A. B., Li E., and Tsai L-H. (2001) p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *J. Neurosci.* **21,** 6758–6771.
56. Gilmore E. C., Ohshima T., Goffinet A. M., Kulkarni A. B., and Herrup K. (1998) Cyclin-dependent kinase 5-deficient mice demonstrate novel developmental arrest in cerebral cortex. *J. Neurosci.* **18,** 6370–6377.
57. 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.
58. Morest D. K. (1970) A study of neurogenesis in the forebrain of opossum pouch young. *Z. Anat. Entwicklungsgesch.* **130,** 265–305.
59. Nadarajah B., Brunstrom J. E., Grutzendler J., Wong R. O. L., and Pearlman, A. L. (2001) Two modes of radial migration in early development of the cerebral cortex. *Nat. Neurosci.* **4,** 143–150.
60. Miyata T., Kawaguchi A., Okano H., and Ogawa M. (2001) Asymmetric inheritance of radial fibers by cortical neurons. *Neuron* **31,** 727–741.

61. Ohshima T., Gilmore E. C., Longenecker G., et al. (1999) Migration defects of cdk5$^{(-/-)}$ neurons in the developing cerebellum is cell autonomous. *J. Neurosci.* **19,** 6017–6026.
62. Terashima T., Inoue K., Inoue Y., Yokoyama M., and Mokoshiba K. (1986) Observations on the cerebellum of normal-reeler mutant mouse chimera. *J. Comp. Neurol.* **252,** 264–278.
63. Goldowitz D., Cushing R. C., Laywell E., et al. (1997) Cerebellar disorganization characteristic of reeler in scrambler mutant mice despite presence of reelin. *J. Neurosci.* **17,** 8767–8777.
64. Goffinet A. M. (1997) Unscrambling a disabled brain. *Nature* **389,** 668–669.
65. Homayouni R. and Curran T. (2000) Cortical development: Cdk5 gets into sticky situations. *Curr. Biol.* **10,** R331–R334.
66. Ohshima T., Nagle J. W., Pant H. C., et al. (1995) Molecular cloning and chromosomal mapping of the mouse cyclin-dependent kinase 5 gene. *Genomics* **28,** 585–588.
67. Dernoncourt C., Ruelle D., and Goffinet A. M. (1991) Estimation of genetic distances between reeler and nearby loci on mouse chromosome 5. *Genomics* **11,** 1167–1169.
68. Ohshima T., Ogawa M., Takeuchi K., Takahashi S., Kulkarni A. B., and Mikoshiba K. (2002) Cdk5/p35 contributes synergistically with Reelin/Dab1 to the positioning of facial branchiomotor and inferior olive neurons in the developing mouse hindbrain. *J. Neurosci.* **22,** 4036–4044.
69. 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.
70. Frotscher M. (1997) Dual role of Cajal-Retzius cells and reelin in cortical development. *Cell Tissue Res.* **290,** 315–322.
71. Miyata T., Nakajima K., Aruga J., et al. (1996) Distribution of a reeler gene-related antigen in the developing cerebellum: an immunohistochemical study with an allogeneic antibody CR-50 on normal and reeler mice. *J. Comp. Neurol.* **372,** 215–228.
72. Gilmore E. C. and Herrup K. (2000) Cortical development: receiving reelin. *Curr. Biol.* **10,** R162–R166.
73. 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.
74. 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.
75. Ishiguro K., Takamatsu M., Tomizawa K., et al. (1992) Tau protein kinase I converts normal Tau protein into A68-like component of paired helical filaments. *J. Biol. Chem.* **267,** 10,897–10,901.
76. Pigino G., Paglini G., Ulloa L., Avila J., and Caceres A. (1997) Analysis of the expression, distribution and function of cyclin dependent kinase 5 (Cdk5) in developing cerebellar macroneurons. *J. Cell Sci.* **110,** 257–270.
77. Liu Z., Steward R., and Luo L. (2000) *Drosophila* Lis1 is required for neuroblast proliferation, dendritic elaboration and axonal transport. *Nature Cell Biol.* **2,** 776–783.
78. 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.
79. Gleeson J. G., Lin P. T., Flanagan L. A., and Walsh C. A. (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron* **23,** 257–271.
80. Sasaki S., Shionoya A., Ishida M., Gambello M. J., Yingling J., Wynshaw-Boris A., and Hirotsune S. (2000) A LIS1/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. *Neuron* **28,** 681–696.
81. Niethammer M., Smith D. S., Ayala R., Peng J., Ko J., Lee M-S., Morabito M., and Tsai L-H. (2000) NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* **28,** 697–711.
82. Kwon Y. T., Gupta A., Zhou Y., Nikolic M., and Tsai L. H. (2000) Regulation of N-cadherin-mediated adhesion by the p35-Cdk5 kinase. *Curr. Biol.* **10,** 363–372.
83. De Long G. R. and Sidman R. L. (1970) Alignment deficit of reaggregating cells in culture of developing brains of reeler mutant mice. *Dev. Biol.* **22,** 584–600.
84. Hoffarth R. M., Johnston J. G., Krushel L. A., and van der Kooy D. (1995) The mouse mutation reeler causes increased adhesion within a subpopulation of early postmitotic cortical neurons. *J. Neurosci.* **15,** 4838–4850.
85. 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.
86. 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.
87. 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 synpatic circuitry. *Neuron* **31,** 929–941.
88. Connell-Crowley L., Le Gall M., Vo D. J., and Giniger E. (2000) The cyclin-dependent kinase Cdk5 controls multiple aspects of axon patterning *in vivo. Curr. Biol.* **10,** 599–602.
89. Caviness V. S., Jr. (1976) Patterns of cell and fiber distribution in the neocortex of the reeler mutant mouse. *J. Comp. Neurol.* **170,** 435–474.
90. Simmons P. A., Lemmon V., and Pearlman A. L. (1982) Afferent and efferent connections of the striate and extrastriate visual cortex of the normal and reeler mouse. *J. Comp. Neurol.* **211,** 295–308.
91. Terashima T., Inoue K., Inoue Y., Mikoshiba K., and Tsukada Y. (1983) Distribution and morphology of corticospinal tract neurons in reeler mouse cortex by the retrograde HRP method. *J. Comp. Neurol.* **218,** 314–326.
92. Hardy J., Duff K., Hardy K. G., Perez-Tur J., and Hutton M. (1998) Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nat. Neurosci.* **1,** 355–358.
93. Rubinsztein D. C. (1997) The genetics of Alzheimer's disease. *Prog. Neurobiol.* **52,** 447–454.
94. 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.
95. 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.
96. Dhavan R. and Tsai L. H. (2001) A decade of Cdk5. *Nat. Rew. Mol. Cell Biol.* **2,** 749–759.
97. Patrick G. N., Zukenberg L., Nikolic M., et al. (1999) Conversion of p35 to p25 de-regulates Cdk5 activity and promotes neurodegeneration. *Nature* **402,** 615–622.
98. Nguyen M. D., Lariviere R. C., and Julien J. P. (2001) Deregulation of Cdk5 in a mouse model of ALS: toxicity alleviated by perikaryal neurofilament inclusions. *Neuron* **30,** 135–147.
99. Yoo B. C. and Lubec G. (2001) p25 protein in neurodegeneration. *Nature* **411,** 763–764.
100. Takashima A., Murayama M., Yasutake K., Takahashi H., Yokoyama M., and Ishiguro K. (2001) Involvement of cyclin dependent kinase5 activator p25 on tau phosphorylation in mouse brain. *Neurosci. Lett.* **306,** 37–40.
101. Harada T., Morooka T., Ogawa S., and Nishida E. (2001) ERK induces p35, a neuron-specific activator of Cdk5, through induction of Egr 1. *Nature Cell Bio.* **3,** 453–459.
102. Zukerberg L. R., Patrick G. N., Nikolic M., et al. (2000) Cables links Cdk5 and c-Ab1 and facilitates cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. *Neuron* **26,** 633–646.
103. McEvilly R. J., de Diaz M. O., Schonemann M. D., Hooshmand F., and Rosenfeld M. G. (2002) Transcriptional regulation of cortical neuron migration by POU domain factors. *Science* **295,** 1528–1532.