The extracellular matrix protein Reelin, secreted by Cajal-Retzius (CR) cells in the marginal zone (MZ) of the cerebral cortex, is important for neuronal migration during development. Two lipoprotein receptors for Reelin have been identified, apolipoprotein E receptor 2 (ApoER2) and the very low-density lipoprotein receptor (VLDLR). The binding of Reelin to these receptors induces tyrosine phosphorylation of an adapter protein, Dab1. T ogether these studies indicate that Reelin, its lipoprotein receptors, Dab1 and PI3K are all members of the same signaling pathway.

Mutations in the genes encoding for Reelin, its receptors, Dab1 or SFKs such as fyn and src, cause a common reeler-like phenotype. In cultures of the dentate gyrus, the reeler phenotype could be rescued by wild-type co-culture, and the rescue was found to be mediated by lipoprotein receptors for Reelin and Dab1. Together these studies indicate that Reelin, its lipoprotein receptors, Dab1 and PI3K are all members of the same signaling pathway.

How to explain the function of Reelin in the control of neuronal migration? How does Reelin control the actin cytoskeleton? To address these questions, it may be useful to have a look at the modes of neuronal migration during development. Two different modes of radial migration have been described: somal translocation and glia-guided migration. Somal translocation predominates during the early phases of cortical development with the leading process attaching the marginal zone; thereafter, when an inside-out patterning with early generated neurons occupying the deep layers and late-born neurons bypassing their predecessors to reach more superficial layers. In contrast, in reeler mutant mice lacking Reelin, cortical lamination is inverted. Neurons are unable to bypass their predecessors and pile up underneath. In wild-type animals the marginal zone is cell-sparse, whereas it is densely populated in reeler mutants. Thus, Reelin has been suggested to act as a stop signal or positional signal for radially migrating neurons. However, the mechanism of Reelin’s action on the migration process is still poorly understood.

Many studies have shown that Reelin binds to ApoER2 and VLDLR in the membranes of the migrating neurons. Binding of Reelin to ApoER2 and VLDLR induces tyrosine phosphorylation of an adapter protein, Dab1, which interacts with the intracellular domains of these receptors. It has been shown that non-receptor tyrosine kinases, SFKs, phosphorylate Dab1 upon Reelin binding. Another kinase in the Reelin signaling pathway is PI3K. Blocking of PI3K by LY294002 was found to induce inverted cortical layering reminiscent of the reeler phenotype in embryonic slice cultures.

Mutations in the genes encoding for Reelin, its receptors, Dab1 or SFKs such as fyn and src, cause a common reeler-like phenotype. In cultures of the dentate gyrus, the reeler phenotype could be rescued by wild-type co-culture, and the rescue was found to be mediated by lipoprotein receptors for Reelin and Dab1. Together these studies indicate that Reelin, its lipoprotein receptors, Dab1 and PI3K are all members of the same signaling pathway.
the migratory route to the cortical plate has increased, postmitotic neurons born in the ventricular zone migrate towards the pial surface with the guidance of radial glial fibers extending from the subventricular zone to the pial surface. As soon as the leading processes of late generated neurons approach the Reelin-containing marginal zone, the neurons switch from locomotion to somal translocation to terminate the migrational process.6,18

Essentially, nuclear translocation consists of three steps: extension of a lamellipodium, forward movement of the cell body, and retraction of the trailing process. These dynamic cytoskeletal changes involve the actin cytoskeleton of migrating cells. The actin cytoskeleton (F-actin) at the leading edge is subjected to an organized process of assembly/disassembly to allow for the changes in cell shape required for the cell to move forward.20 These cytoskeletal changes are regulated by actin-binding proteins such as cofilin, an essential regulator of actin dynamics.21 Cofilin acts as an actin-depolymerizing protein that has severing activity; it binds to F-actin and promotes its disassembly. Cofilin is located at the membrane of the leading edge of migrating cells, promotes lamellipodia formation by providing actin monomers,22 and is required for directional migration.23,24 In response to extracellular stimuli, cofilin is phosphorylated at serine 3 by LIMK1.25 Phosphorylation makes cofilin unable to disassemble F-actin. As a result, actin dynamics and subsequent process extension are inhibited26,27 and the cytoskeleton is stabilized. Such a scenario has to be assumed for a variety of cells including migrating neurons of the cerebral cortex.28

In our recent studies, we asked whether Reelin acts on cofilin phosphorylation. We performed a western blot analysis for p-cofilin and found that phosphorylated n-cofilin is severely reduced in E18 reeler cortices when compared to wild-type or heterozygous mice. Exogenous Reelin increased phosphorylated cofilin in reeler mutant cortices, and this increase in p-cofilin was accompanied by an increase of p-LIMK1, the cofilin kinase. In addition, in the presence of SFK inhibitor PP2, Reelin-induced p-cofilin was downregulated. In the presence of inhibitors of PI3K, LY294002 and Wortmannin, Reelin-induced p-cofilin was abolished. We also found that in Dab1 and ApoER2 mutant mice Reelin application could not induce the phosphorylation of cofilin. Together these results suggested that Reelin induces phosphorylation of cofilin in the cortex, and that this process involves ApoER2, Dab1, SFKs and PI3K.

Our next question was: Where would we find phospho-cofilin? To address this question, we immunostained cortical slices from E17.5 wild-type or reeler mice and found that p-cofilin is located in the marginal zone, i.e., the zone of Reelin-synthesizing CR cells. High-power magnification revealed that p-cofilin is present in the leading processes of radially migrating neurons as they approach the Reelin-containing marginal zone (Fig. 1).

While p-cofilin was found strongly reduced in ApoER2 knockout mice, it appeared almost normal in VLDLR mutants, comparable with the level of phosphorylation of Dab1 in these mutants (Fig. 2).29 We concluded that ApoER2 and VLDLR play divergent roles in the migration of cortical neurons.30 In ApoER2 mutants late generated neurons are unable to bypass early-generated cells and remain close to the subventricular zone. In contrast, in VLDLR mutants, many neurons invade the marginal zone. We hypothesize that Reelin binds to ApoER2 receptors on the surface of migrating neurons and in turn induces phosphorylation of cofilin to stabilize the leading process. Reelin binding to VLDLR receptors may be involved in the arrest of the nucleus in the terminal phase of migration by nuclear translocation.
Reelin stabilizes the actin cytoskeleton of neurons

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