Development/Plasticity/Repair

Absence of Fyn and Src Causes a Reeler-Like Phenotype

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Nonreceptor protein tyrosine kinases of the Src family regulate the survival, proliferation, differentiation, and motility of many cell types, but their roles in brain development are unclear. Biochemical and in vitro experiments implicate Src and Fyn in the Reelin-dependent tyrosine phosphorylation of Dab1, which controls the positioning of radially migrating neurons in many brain regions. However, genetic evidence that either Src or Fyn mediates Reelin-dependent migrations in vivo has been lacking. Here, we report that, although Src is dispensable and although the absence of Fyn causes an intermediate phenotype, the combined absence of Src and Fyn almost abolishes tyrosine phosphorylation of Dab1 and causes defects in the fetal cortex and cerebellum very similar to those of dab1 mutants of the same age. Neurogenesis is not detectably affected, but the layering of neurons in the cortex is inverted, and the formation of the Purkinje plate is impaired. This implies that Src and Fyn are needed for Reelin-dependent events during brain development.

Key words: cerebellum; cortex; reelin; Fyn; Dab1; Src; tyrosine phosphorylation

Introduction

The Src family of nonreceptor protein tyrosine kinases has been highly conserved over metazoan evolution and plays key roles in relaying signals that regulate cell proliferation, differentiation, and motility (Brown and Cooper, 1996). At least three Src-family kinases (SFKs)—Src, Fyn, and Yes—have been detected in the developing mammalian brain (Cotton and Brugge, 1983; Martinez et al., 1987; Sudol et al., 1988; Cooke and Perlmutter, 1989; Zhao et al., 1991; Umemori et al., 1992). Targeted knock-outs of src and yes in the mouse do not lead to obvious defects in brain development (Soriano et al., 1991; Stein et al., 1994; Lowell and Soriano, 1996), although src is required for cultured cerebellar neurons to extend neurites on certain surfaces (Igelnzli et al., 1994). In contrast, fyn gene disruption causes obvious phenotypes in the developing and adult brain. There are increased numbers of granule cells in the dentate gyrus and pyramidal neurons in the CA3 region of the temporal hippocampus (Grant et al., 1995), the dendrites of pyramidal neurons of layer V of the medial cortex are misoriented (Sasaki et al., 2002), and late-generated cortical neurons are found in deeper layers than normal (Yuasa et al., 2004). These defects likely reflect a requirement for Fyn in neurons or their progenitors during development. In addition, Fyn is important for other aspects of brain function. Cultured neurons require fyn for neurite extension on specific extracellular ligands (Beggs et al., 1994), whereas oligodendrocytes require Fyn for myelination (Umemori et al., 1994; Sperber et al., 2001). In mature neurons, Fyn is involved in the regulation of NMDA and AMPA receptors (Grant, 1996; Kojima et al., 1997; Miyakawa et al., 1997; Narisawa-Saito et al., 1999), and Fyn mutant mice exhibit defects in learning and memory (Grant et al., 1992; Kojima et al., 1997).

Src-family kinases share a common mechanism of activation and can phosphorylate many of the same substrate proteins in cells, thus there is the potential for redundancy. Indeed, although individual knock-outs of src, fyn, or yes do not impact the survival of embryos or pups, 85–90% of src fyn double mutants die perinatally, and all src fyn yes triple mutants die early in gestation, suggesting that these Src-family kinases can partially compensate for each other in vivo (Stein et al., 1994; Klinghoffer et al., 1999). Compensation is also suggested by the observation that Src and Fyn kinase activities are slightly increased in fyn and src mutant neonatal brains, respectively (Grant et al., 1995). Specific developmental phenotypes caused by combined mutations of fyn and src or yes in the nervous system have not been described previously.

The mammalian neocortex has characteristic laminations, containing different neuron types arranged in stereotypical patterns. Layer formation requires proper migrations of projection neurons from their origins in the neuroepithelium that lines the ventricles. Layering is disrupted by mutations that either affect the migratory ability of the neurons or their ability to sense the position at which they should cease migration (Walsh and Goffinet, 2000; Rice and Curran, 2001; Gupta et al., 2002). In particular, mutations in Reelin pathway genes cause distinctive abnormalities in many laminated brain regions, known as the Reeler phenotype. Reelin is a secreted protein, made by Cajal-Retzius neurons of the marginal zones of the neocortex and hippocampus and in the nuclear transitory pathway and external granule layer of the cerebellum (D’Arcangelo et al., 1995; Rice et al., 1998). In the cortex, reln mutations prevent splitting of the preplate and cause inversion of the cortical plate. In addition, the radial glia, which serve both as neuronal precursors and as guides for migration, show abnormal end feet and branching, and the
projection neurons in the cortical plate are misoriented. In the cerebellum, rehl mutations prevent proper migrations of the Purkinje cells, and an inner granule cell layer fails to form.

The combined mutation of vldlr and apoER2, or the single mutation of dab1, also causes a Reeler phenotype. The gene products Vldlr, ApoER2, and Dab1 are expressed by radial glia and migrating cortical plate cells of the neocortex and hippocampus and by Purkinje cells of the cerebellum. Vldlr and ApoER2 are receptors that bind to Reelin through their extracellular domains and to Dab1 through their cytoplasmic domains (D’Arcangelo et al., 1999; Hiesberger et al., 1999; Howell et al., 1999b). Dab1 is an intracellular protein that becomes tyrosine phosphorylated, and then degraded, in response to Reelin (Rice et al., 1998; Hiesberger et al., 1999; Howell et al., 1999a; Arnaud et al., 2003b; Bock et al., 2004). An allele of Dab1 that lacks the phosphorylated tyrosine residues behaves as a null allele (Howell et al., 2000).

Despite the importance of tyrosine phosphorylation in Reelin signaling, there have been no reports of a Reeler phenotype resulting from mutations in tyrosine kinase genes. However, biochemical and genetic evidence implicates Src-family kinases in Reelin-induced Dab1 phosphorylation. Inhibitors of Src-family kinases reduce Dab1 tyrosine phosphorylation in vitro (Arnaud et al., 2003a; Bock and Herz, 2003) and inhibit preplate splitting and formation of a normal cortical plate in cortical slice cultures, although layer inversion was not documented (Jossin et al., 2003). In addition, fyn mutant neurons show reduced Dab1 tyrosine phosphorylation and degradation in response to Reelin (Arnaud et al., 2003a; Bock and Herz, 2003). Additional reductions in Dab1 tyrosine phosphorylation and degradation occur if src gene dosage is reduced in fyn homozygotes, suggesting partial redundancy (Arnaud et al., 2003a).

Here, we provide genetic evidence suggesting that fyn and src are partly redundant members of the Reelin pathway. Although postnatal src fyn double homozygous mutant mice cannot be analyzed, because of perinatal lethality (Stein et al., 1994), the development of the src fyn double-mutant fetal brain strongly resembles that of dab1 mutants. Combined with previous results showing that Src-family kinases are required for biochemical events in Reelin signaling, the new data provide genetic evidence implicating Fyn and Src in the Reelin signaling pathway.

Materials and Methods

Animals and tissue preparation. Animals were derived from an src fyn yes breeding colony in the mixed C57BL/6 × 129Sv strain background and genotyped as described previously (Arnaud et al., 2003a). Embryos were removed from timed pregnant dams and cerebral hemispheres were dissected for protein assays and neuron cultures. For protein analysis, embryonic day 16.5 (E16.5) embryo cerebrosomes were frozen on dry ice and stored at −80°C until use. Neuron cultures were prepared essentially as described previously (Herrick and Cooper, 2002; Arnaud et al., 2003a). For histology, embryo heads were dissected out and soaked overnight in 20% acrylamide gels, as described previously (Arnaud et al., 2003a). Blots were probed with mouse anti-phosphotyrosine 4G10 (Upstate Biotechnology), rabbit anti-Src-family kinases (recognizing Src, Fyn, and Yes; SRC-2; Santa Cruz Biotechnology), mouse anti-beta tubulin (TuJ1; Covance, Princeton, NJ), rabbit anti-FAK pY397 (catalog #44-624; Biosource International, Camarillo, CA), or the same antibodies used for immunoprecipitation. Dab1 tyrosine phosphorylation stoichiometry was determined using ImageJ to quantify Western blot signals from blots that probed first for phosphotyrosine and then stripped and reprobed for Dab1 protein. Phosphorylation level was divided by Dab1 protein level to result in Western blot signals from blots that probed first for phosphotyrosine and then stripped and reprobed for Dab1 protein. Phosphorylation level was divided by Dab1 protein level to calculate phosphorylation stoichiometry, relative to wild type. Means, SEs, and numbers of determinations are shown.

Immunohistochemistry. Slides were rehydrated with PBS for 5 min and blocked with 5% goat serum in PBS and 0.1% Tween 20 at room temperature for 1 h. A 20 min boil with 0.01 M sodium citrate, pH 6.0, was used for antigen retrieval. Slides were incubated with primary antibodies overnight at 4°C, washed three times with PBS, and then incubated with secondary antibodies for 2 h at room temperature, washed, incubated with 4',6-diamidino-2-phenylindole dihydrochloride (2.5 μg/ml; Sigma, St. Louis, MO) in PBS for 5–10 min, washed, and mounted with ProLong Gold (Invitrogen, Carlsbad, CA).

The following primary antibodies were used in 5% goat serum in PBS plus 0.1% Tween 20: mouse anti-chondroitin sulfate proteoglycan (CSPG; diluted 1:100; Sigma), rabbit anti-calretinin (1:1000; Chemicon, Temecula, CA), rabbit anti-calcibindin (1:400; Chemicon), guinea pig anti-Bnr1 (1:500; kind gift from Robert J. McEwilly, University of California, San Diego, CA), rabbit anti-Tbr1 (1:1000; kind gift from Robert Hevner, University of Washington, Seattle, WA), rabbit anti-Cux1 (1:400; kind gift from Chris Walsh, Harvard Medical School, Boston, MA), mouse anti-reelin (1:400; kind gift from André M. Goffinet, University of Louvain Medical School, Brussels, Belgium), and rabbit anti-Dab1 B3 (1:400).

The following secondary antibodies were used at 1:400 in 20% goat serum in PBS plus 0.1% Tween 20: Alexa Fluor 488 anti-mouse (Invitrogen), Alexa Fluor 568 anti-rabbit (Invitrogen), and FITC anti-guinea pig (Jackson ImmunoResearch, West Grove, PA). Images were collected by epifluorescence with MetaMorph software (Universal Imaging, Downingtown, PA) and levels adjusted in Photoshop (Adobe Systems, San Jose, CA). The distribution of neurons was quantified by dividing the thickness of the cortex, excluding the ventricular zone, into nine bins of equal thickness, and counting the labeled cells in each bin. For each genotype, the mean and SE of counts from replicate sections from each embryo from different litters were calculated, except for Cux1, in which data are based on sections from a single embryo.

Results

Fyn- and Src-dependent protein phosphorylation in the developing cortex

To investigate the role of Src and Fyn in brain development, we generated a breeding colony of src−/− fyn−/− mice and set up timed matings. Because most, but not all, src−/− fyn−/− pups die perinatally (Stein et al., 1994), we were concerned that surviving pups might represent a subpopulation with less severe phenotype. Therefore, we recovered embryos at E16.5 or E18.5 for analysis.

We first studied the levels of Dab1 protein and tyrosine phosphorylation in embryonic cortex and in cultured cortical neurons (Fig. 1). Protein extracts were prepared from cortices dissected from littermate E16.5 embryos from a src−/−fyn−/− intercross and from two wild-type E16.5 embryos from a different litter, and analyzed by Western blotting (Fig. 1a). Tyrosine phosphorylation of some proteins (Fig. 1a, asterisks) was reduced by homozygous mutation of fyn (lane 2) relative to controls (lanes 1 and 7). Phosphorylation was further reduced by heterozygous

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Kuo et al. • Reelin Signaling Requires Fyn and Src

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Expression levels and tyrosine phosphorylation of Dab1 in mutant embryonic brain.

**Figure 1.** Expression levels and tyrosine phosphorylation of Dab1 in mutant embryonic brain and neuron cultures. **a, b,** Protein extracts were prepared from E16.5 embryonic brains. *fyn*−/− littermate embryos with various src genotypes were obtained from a src+/−*fyn*−/− intercross. Wild-type embryo brains were from another litter. **a,** Protein samples were analyzed directly by immunoblotting for phosphotyrosine, neuron-specific βIII-tubulin, Src-family kinases, and Dab1. The asterisks indicate proteins whose phosphorylation was reduced by src *fyn* mutation. Note the reduction in Src-family kinase expression and the increase in Dab1 protein levels (lanes 5–6), and an additional decrease in Fyn alone or *fyn* and *src* were mutated (Fig. 1c). These results show that primarily Fyn, and secondarily Src, is required for high-level phosphorylation of p190, FAK, and Dab1 in developing cortex.

Effects of double mutation of *fyn* and *src* on cortical development: expression of Reelin and Dab1

We examined the structure of the developing cortex in embryos derived from src+/−*fyn*−/− intercrosses at E16.5 and E18.5. For comparison, we used embryos of the same ages derived from *dab1*+/+ intercrosses. To control for regional variation, we compared regions of dorsomedial cortex near the hippocampus.

**Fyn** mutation, or **src** *fyn* double mutation, did not significantly affect Reelin expression, which in all cases was restricted to scattered neurons (presumably Cajal-Retzius neurons) in the marginal zone (Fig. 2). However, as expected from Western blots (Fig. 1), Dab1 levels were increased throughout the developing cortical plate, subplate, and ventricular zone in *fyn* and *src* *fyn* mutants (Fig. 2). The Dab1 antibody was specific, because staining of *dab1* mutant cortex was strongly reduced (Fig. 2).

Nissl staining revealed no significant differences in the brain size, cortex thickness, or total number of nuclei in control, *fyn*−/−, *src*−/−, or src+/*fyn*−/− E18.5 embryos (supplemen-
Figure 2. Dab1 staining is increased in the src−/−fyn−/− neocortex. Coronal sections of E18.5 neocortices were double stained with antibodies against Reelin and Dab1. The separate channels are shown. All images were captured using the same exposure time, and levels were adjusted equally. a–d, Reelin was expressed equally by scattered neurons in the marginal zone across all genotypes. However, Dab1 staining was absent in dab1−/− (b′) and elevated in fyn−/− (c′) and src−/−fyn−/− (d′) neocortices relative to wild-type (a′) neocortex, ps, Pial surface; MZ, marginal zone; II and V, layers II/III and V/VI of the cortical plate, respectively; SP, subplate; IZ, intermediate zone; v, ventricle.

Preplate splitting in src fyn mutant embryos

One of the earliest defects detected in the Reeler neocortex is abnormal splitting of the preplate (Angervin and Sidman, 1961; Sheppard and Pearlman, 1997). The preplate is composed of Cajal–Retzius neurons, which migrate into the marginal zone from either local or distant origins (Hevner et al., 2003; Takiguchi-Hayashi et al., 2004), and subplate neurons, which migrate radially from the neocortical ventricular zone to lie below Cajal–Retzius neurons. Subsequent divisions in the neocortical ventricular zone give rise to cortical plate neuroblasts, which enter the subventricular zone and may go through one or two divisions before migrating outward along or between radial glia guides. Each neuron migrates almost to the pial surface, passing between the subplate neurons and stopping short of the Cajal–Retzius neurons in the marginal zone. The preplate is thus split into subplate and Cajal–Retzius neurons. In the Reeler mouse, subplate neurons remain close to the Cajal–Retzius neurons and are displaced outward above the developing cortical plate to form a “superplate.”

We used antibodies to CSPG, which is secreted by subplate neurons (Sheppard et al., 1991; Sheppard and Pearlman, 1997), to visualize the subplate. As shown in Figure 3, the subplate was readily detected in E18.5 control embryos and was absent in dab1−/− embryos (Fig. 3a,b). CSPG staining was more dispersed in fyn−/− cortex (Fig. 3c) and was weak in src−/−fyn−/− cortex (Fig. 3d). Similar results were obtained at E16.5 (Fig. 3e,f) and at E18.5 with antibodies to calretinin, which marks cells and axons in the subplate and marginal zone (Fonseca et al., 1995) (Fig. 3g,h). The defects in preplate splitting likely contribute to the hypercellularity of the marginal zone in mutant embryos (supplemental Figs. S1, S4, available at www.jneurosci.org as supplemental material).

Cortical inversion in src fyn mutant embryos

Because each cortical plate neuron stops migrating when it reaches the edge of the marginal zone, the outer, shallower layers of wild-type cortex contain younger neurons than the inner, deeper layers (Rakic, 1972; Caviness and Sidman, 1973; Caviness, 1982). In the Reeler mutant mouse, this inside-out layering order is inverted (Caviness and Sidman, 1973; Caviness, 1982). The later-born neurons seem to have difficulty passing their earlier siblings, which are in abnormally close contact with radial glia fibers (Pinto-Lord et al., 1982). The cortex is also inverted in dab1 mutants, and dab1 mutant neurons remain closely associated with radial glia fibers (Gonzalez et al., 1997; Howell et al., 1997; Sheldon et al., 1997; Sanada et al., 2004).

Layering of the cortical plate in fyn src mutant cortex was assessed using layer-specific markers at E18.5. Tbr1 is expressed in the preplate and cortical plate, most abundantly in the subplate and layer VI cortical plate neurons (Hevner et al., 2001) (Fig. 4a). In dab1 mutants, as shown before (Herrick and Cooper, 2002), Tbr1+ neurons migrate excessively and are found in the marginal zone and upper cortical plate (Fig. 4b). In src fyn double-mutant cortex, Tbr1+ neurons were present in superficial layers of the cortex and marginal zone (Fig. 4d). This suggests that src fyn mutation, like dab1 mutation, causes early-born cortical plate neurons to migrate excessively into the Reelin-containing marginal zone.

We used Brn1 and Cux1 as markers for late cortical plate neurons. Brn1 is expressed throughout the cortical plate but at highest levels in layers II–IV (McEvilly et al., 2002). Similarly, Cux1 is most strongly expressed by a subset of layer II–IV neurons (Feng and Walsh, 2004; Nieto et al., 2004). It is not clear whether Cux1 and Brn1 are always coexpressed. By E18.5 in wild-type cortex, most Brn1+ and Cux1+ neurons have settled in the upper cortical plate (Fig. 4e). In dab1 mutant cortex, Brn1 and Cux1 were expressed at high levels in the lower cortical plate, as well as in the intermediate zone (Fig. 4f), although some neurons in the intermediate zone were Brn1 and Cux1 positive and were presumably in transit (Fig. 4e). This is consistent with cortical plate inversion. In src fyn double-mutant cortex, Cux1+ and
Brn1+ neurons were excluded from the marginal zone and upper cortical plate and were positioned in the lower cortical plate, as in the dab1 mutant (Fig. 4h). The cortical phenotypes were quantified and are shown in Figure 4i–k. The results show that src fyn mutation causes layer inversion similar to, but not as pronounced as, dab1 mutation. Although only five E18.5 and four E16.5 src fyn double-mutant embryos were analyzed using Tbr1 and Brn1, layer inversion was found in all cases. This is statistically significant (p < 0.005; χ² test).

The fyn mutant phenotype was more subtle. Tbr1+ neurons were positioned above the residual subplate in a broader region than in controls (Fig. 4c). Fyn−/− Tbr1+ neurons were not detected in the upper layers or marginal zone. This suggests that, although most Tbr1+ neurons respond to Reelin and positioned appropriately, some may have migrated excessively before settling. Cux1+ neurons were found in a broad region, including the marginal zone and entire cortical plate down to the top of the residual subplate (Fig. 4g). Thus, some late-born Cux1+ neurons have overmigrated into the marginal zone, some are positioned correctly in the upper cortical plate, some are mispositioned in the lower cortical plate, and yet others are in transit in the intermediate zone. Brn1+ neurons were also broadly scattered, but a majority were positioned, as in dab1 mutant cortex, at the bottom of the cortical plate (Fig. 4g). This suggests a partial Reeler phenotype, with most early cortical plate neurons positioned correctly. Some late cortical plate neurons may be trapped below excessively adherent early cortical plate neurons, whereas others successfully migrated into the upper cortical plate but failed to stop at the Reelin-containing marginal zone.

In contrast to fyn mutant cortex, the positions of Tbr1+ and Brn1+ neurons in src−/− cortex were normal (supplemental Fig. S2, available at www.jneurosci.org as supplemental material). Thus, Src is only needed for cortical lamination when Fyn is absent.

Cerebellar Purkinje cell migrations require Src and Fyn

Cerebellar Purkinje cells arise between E11 and E13 and migrate outwards along radial glia to lie below the external granule layer. In Reeler mutants, the Purkinje cells move away from the ventricular zone but do not reach the Purkinje plate (Rice and Curran, 2001). Although the primary defect is unknown, Purkinje cells appear to be obstructed by disorganized radial glia in the intermediate zone (Yuasa et al., 1993). Purkinje cells can be detected with antibodies to calbindin (Jande et al., 1981) or Dab1 (Gallagher et al., 1998; Rice et al., 1998). At E18.5, calbindin staining revealed a Purkinje layer in wild-type embryos, with characteristic gaps corresponding to future parasagittal stripes (Fig. 5a). In both dab1 and src fyn mutants, most Purkinje cells were misplaced in clusters between the ventricular zone and the external granule layer, although a partial Purkinje layer was detected in src fyn cerebellum (Fig. 5bc). Similar results were obtained with Dab1 staining (supplemental Fig. S5, available at www.jneurosci.org as supplemental material). Thus, the dab1 and src fyn double mutation cause similar defects in Purkinje cell migration in the cerebellum.

Discussion

Although mutational activation of Src-related kinases can have dramatic effects on cell biology, ranging from malignant transformation to induction of differentiation, loss-of-function mutations cause a phenotype in only a subset of the cells in which the genes are normally expressed. Here, we report that src−/− embryonic brains seem normal, whereas fyn−/− embryonic brains have a complex phenotype, with scattering of both early and late cortical neurons into inappropriate layers. However, the phenotype is clearly distinct from dab1 and, by implication, from Reeler. In contrast, mutation of both src and fyn reveals a phenotype that is similar to, but less severe than, the dab1 null and Reeler phenotypes. The marginal zone is hypercellular, and the cortical plate is inverted (Fig. 4). This phenotype correlates with a virtual absence of Reelin-induced Dab1 tyrosine phosphorylation and Dab1 degradation in cultured src−/− fyn−/− neurons and in fetal cortex (Fig. 1). These results provide genetic evidence that src/fyn, together with reelin, vldlr/apoer2, and dab1, are involved in the Reelin signaling pathway.

Src fyn double-mutant fetal brains are subtly different from those of dab1 mutants. There is a residual subplate, the cortical plate is not as clearly inverted, and a rudimentary Purkinje plate is detected. The incomplete phenotype is consistent with the slight residual response of cultured src−/− fyn−/− neurons to Reelin stimulation and is likely attributable to other Src-family kinases. Yes and lyn are also expressed at low levels in developing forebrain (Sudol et al., 1988; Zhao et al., 1991; Umemori et al., 1992) and are candidates for phosphorylation of Dab1 when Fyn and Src are absent. However, brain development cannot be studied in src−/− fyn−/− yes−/− mutant embryos because they die too
Figure 4. Cortical lamination is inverted in the src−/− fyn−/− mutant. a–d, E18.5 cortices stained with antibodies to Tbr1 (red) and Brn1 (green). e–h, Staining with antibodies to Cux1 (red) and Brn1 (green). Tbr1 marks subplate and early-born cortical plate neurons, whereas Cux1 and Brn1 mark overlapping populations of late-born cortical plate neurons. Note approximate layer inversion in dab1−/− and src−/−/fyn−/− relative to wild type. i–k, Quantification of phenotypes. The percentage of Tbr1+ (i), Brn1+ (j), and Cux1+ (k) in different layers from marginal zone (bin 1) to the top of the ventricular zone (bin 9) was calculated based on replicate sections from one (Cux1) or two (Tbr1 and Brn1) embryos of each genotype. Tbr1 and Brn1 staining of a total of five E18.5 and four E16.5 src−−, dab1−−, fyn−−, src−−/fyn−− cortices showed that all had an inverted cortical plate. pS, Pial surface; v, ventricle; WT, wild type.
both important for integrin signaling and cell movement (Ilic et al., 1995; Parsons and Parsons, 1997; Schlaepfer et al., 1999; Arthur et al., 2000; Kulkarni et al., 2000). Serine phosphorylation of FAK is important for radial migrations (Xie et al., 2003), and FAK is required for normal cortical lamination (Beggs et al., 2003), but it is not known whether FAK tyrosine phosphorylation is required. In addition, FAK and Src-family kinases are involved in axon guidance and fasciculation (Beggs et al., 2003). Together, these results suggest that neurons migrate independently of Src-family-catalyzed phosphorylation events.

To date, the Reeler phenotype results whenever mutations are made in components acting in the signaling cascade. Thus, mutations in Reelin, its receptors Vildr and ApoER2, the substrate Dab1, and, as shown here, the tyrosine kinases Fyn and Src, all cause a Reeler-like phenotype. When homozygous mutations at different levels of the cascade have been combined, there are no additional phenotypes, implying that the pathway is linear (Howell et al., 1999a). However, genetic interactions have not been apparent when transheterozygotes of reelin and dab1 are prepared (B. Howell and Cooper, unpublished results). The pathway seems to be robustly buffered from variation, presumably by negative feedback loops such as the regulation of Dab1 protein levels after Reelin stimulation. It will be interesting to see whether other components in the signaling cascade, working downstream of activated Src-family kinases and Dab1, are also required for normal neuron positioning.

References


Sheppard AM, Hamilton SK, Pearlman AL (1991) Changes in the distribution of extracellular matrix components accompany early morpho-