Brief Communications

Collapsin Response Mediator Protein 1 Mediates Reelin Signaling in Cortical Neuronal Migration

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Collapsin response mediator protein 1 (CRMP1) is one of the CRMP family members that mediates signal transduction of axon guidance molecules. Here, we show evidence that CRMP1 is involved in Reelin (Reln) signaling to regulate neuronal migration in the cerebral cortex. In *crmp1*$^{-/-}$ mice, radial migration of cortical neurons was retarded. This phenotype was not observed in the *sema3A*$^{-/-}$ and *crmp1*$^{+/-}$;*sema3A*$^{+/-}$ cortices. However, CRMP1 was colocalized with disabled-1 (Dab1), an adaptor protein in Reln signaling. In the *Reln*$^{rl/rl}$ cortex, CRMP1 and Dab1 were expressed at a higher level, yet tyrosine phosphorylated at a lower level. Loss of *crmp1* in a *dab1* heterozygous background led to the disruption of hippocampal lamination, a Reeler-like phenotype. In addition to axon guidance, CRMP1 regulates neuronal migration by mediating Reln signaling.

Key words: **CRMP; Reln; Dab1; tyrosine phosphorylation; neuronal migration; cerebral cortex**

Introduction

The development of the neocortex has been of keen interest, because the cortex is uniquely mammalian and forms the basis for higher function. The neocortex consists of six layers of neurons that have distinct morphological and functional identities. The development of these neuronal layers involves the migration of neurons to their final laminar positions (Bielas et al., 2004).

Reelin (Reln) is a secreted protein that is involved in neuronal migration (Lambert de Rouvroit and Goffinet, 1998). During Reln binding to ApoER2/VLDLR receptors, the cytoplasmic adaptor disabled-1 (Dab1) becomes phosphorylated on tyrosine residues by Src-type tyrosine kinases (Hiesberger et al., 1999; Howell et al., 1999a; Bock and Herz, 2003). Perinatal lethality caused by widespread defects in neuronal migration during CNS development is evident in mice deficient for cyclin-dependent kinase 5 (Ohshima et al., 1996). Although how these molecules regulate neuronal migration remains obscure, several intracellular molecules, including Lis 1 (PAFAH1b1), doublecortin, Nudel, microtubule associated protein (MAP)-1B, MAP-2, and the small GTPase Rac1, have been implicated as mediators of these molecules and are involved in cytoskeletal organization (Gupta et al., 2002).

Collapsin response mediator protein (CRMP) was identified as a signaling molecule of Sema3A (Goshima et al., 1995). CRMPs are now known to be composed of five homologous cytosolic proteins; all of the family proteins are phosphorylated and are highly expressed in developing and adult nervous systems (Wang and Strittmatter, 1996; Fukada et al., 2000; Inatome et al., 2000; Yuasa-Kawada et al., 2003). CRMPs also mediate other signaling, such as NT3, and are involved in many aspects of neuronal cell development by regulating cytoskeletal organization (Goshima et al., 1995, 2002; Quach et al., 2004; Arimura and Kaibuchi, 2005; Uchida et al., 2005; Yoshimura et al., 2005). However, *in vivo* roles of CRMPs are mostly unknown.

To elucidate *in vivo* roles of CRMP1 in the development of the CNS, we have generated *crmp1*$^{-/-}$ mice (Charrier et al., 2006). We demonstrated that CRMP1 mediates Sema3A signaling *in vivo*, which is involved in spine maturation (N. Yamashita, A. Morita, Y. Uchida, F. Nakamura, H. Usui, M. Taniguchi, J. Honnorat, P. Kolattukudy, N. Thomasset, K. Takei, T. Takahashi, and Y. Goshima, unpublished observations). Intense *crmp1* expression is observed in the cerebral cortex at the embryonic day 16.5 (E16.5), the period of neuronal migration (Bielas et al., 2004). In

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the present study, we investigated whether CRMP1 plays a role in neuronal migration in the cerebral cortex. We found that neuronal migration was retarded in the *crmp1*$^{-/-}$ cerebral cortex. In embryonic *Reln*$^{rl/rl}$ cortex, CRMP1 was tyrosine phosphorylated at a lower level and expressed at a higher level when compared with *Reln*$^{rl/+}$. Furthermore, loss of *crmp1* in a *dab1* heterozygous background led to the disruption of hippocampal lamination, a Reeler-like phenotype. We propose that CRMP1 is involved in radial neuronal migration through Reln signaling.

Materials and Methods

Materials. 5-Bromo-2′-deoxyuridine (BrdU) was purchased from GE Healthcare (Piscataway, NJ). Anti-Dab1 (B3; rabbit polyclonal) antibody was a kind gift from B. Howell (National Institute of Neurological Disorders and Stroke, Bethesda, MD). Monoclonal antibodies against CRMP1 (2C6G and 2E7G) were raised as described previously (N. Yamashita, A. Morita, Y. Uchida, F. Nakamura, H. Usui, M. Taniguchi, J. Honnorat, P. Kolattukudy, N. Thomasset, K. Takei, T. Takahashi, and Y. Goshima, unpublished observations). Other antibodies used were anti-BrdU (mouse monoclonal; MBL, Nagoya, Japan), anti-β-actin (mouse monoclonal; Sigma, St. Louis, MO), anti-Myc (9E10; mouse monoclonal; Sigma), anti-MAP-2 (2a + 2b) (mouse monoclonal; Sigma), anti-Nestin (mouse monoclonal; Chemicon, Temecula, CA), anti-p-Tyr (PY99; mouse monoclonal; Santa Cruz Biotechnology, Santa Cruz, CA), Cy3-labeled goat anti-Armenian hamster (Jackson ImmunoResearch, West Grove, PA), and Alexa 488-labeled goat anti-rabbit (Invitrogen, Cergy Pontoise, France).

Mutant mice. reeler B6C3Fe mice were from The Jackson Laboratory (Bar Harbor, ME). *yotari* mice were spontaneous mutants at the *dab1* allele (Yoneshima et al., 1997). *sema3A* and *crmp1* mutant mice were generated as described previously (Taniguchi et al., 1997; Charrier et al., 2006). Genotypes of the offspring of all mutant mice were assessed using PCR, as described previously (D'Arcangelo et al., 1997; Kojima et al., 2000; Sasaki et al., 2002; Charrier et al., 2006). Mice were housed in the standard mouse facility and fed with an autoclaved diet and water. All procedures were performed according to the guidelines outlined by the institutional Animal Care and Use Committee of the Yokohama City University Graduate School of Medicine. Throughout the experimental procedures, all efforts were made to minimize the number of animals used and their suffering.

Immunoblot analysis and immunoprecipitation. Brain samples were homogenized in immunoprecipitation (IP) buffer [20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 10 mM NaF, 1 mM Na_3VO_4, 1% Nonidet P-40, 50 μM ρ-APMSF (ρ-amidinophenylmethanesulfonyl fluoride), and 10 μg/ml of aprotinin]. The lysates were centrifuged at 1200 rpm for 15 min at 4°C, and supernatants were normalized for total protein concentrations. The samples were then used for immunoblot analysis of anti-CRMP1 (2E7G; 1:2500), anti-β-actin (1:5000), anti-Dab1 (1:500), anti-MAP-2 (2a + 2b) (1:2000), and anti-Nestin (1:1000) antibodies. To detect the tyrosine phosphorylation of CRMP1, the samples were incubated with 1 μg of anti-CRMP1 (2E7G) antibody overnight at 4°C, followed by additional incubation with protein G–Sepharose (GE Healthcare) for 2 h at 4°C. After washing three times with IP buffer, the samples were used for immunoblot analysis of anti-CRMP1 (2E7G) and anti-p-Tyr (1:5000) antibodies.

In vivo *phosphorylation assay.* HEK293T cells were seeded at 2.0×10^5 cells/well in a six-well plate. One day later, the cells were transfected with CRMP1-Myc with or without the wild-type, constitutive-active or dominant-negative form of Fyn expression vector using Fugene6 transfection reagent (Roche, Meylan, France). After 24 h, cells were lysed by IP buffer and then immunoprecipitated with 1 μg of anti-Myc antibody. The samples were used for immunoblot analysis of anti-Myc (1:2500) and anti-p-Tyr antibodies.

Immunohistochemistry. Cryostat brain sections (15 μm thick) were treated with 0.1% Triton X-100 in TBS with Tween 20 (TBST) for 10 min at room temperature. Immunostaining was performed according to standard protocols using anti-CRMP1 (2C6G; 1:1000) and anti-Dab1 (1:100) antibodies. Hydrolyzed, paraffin wax-embedded sections (6 μm thick) were used for immunohistochemistry of anti-MAP-2 (2a + 2b) (1:200) and anti-Nestin (1:100) antibodies. Slides were analyzed using a laser-scanning microscope (LSM510) with a water-immersed objective at 40× (C-Apochromat/1.2W corr) equipped with an Axioplan 2 imaging microscope (Carl Zeiss, Jena, Germany).

BrdU-labeling analysis. For birth-dating analysis, cells were labeled with BrdU (30 mg/kg) in the E14.5 or E16.5 neocortical wall. Pups were killed at E18.5, postnatal day 3 (P3) or P10, and brains were fixed in 4% paraformaldehyde for paraffin wax-embedded sectioning. Hydrolyzed, paraffin wax-embedded sections (6 μm thick) were heat-treated at 120°C for 20 min in 10 mM sodium citrate buffer, pH 6.0. Sections were then treated with 0.1% Triton X-100 in TBST for 10 min and with 1.5N HCl for 30 min at room temperature. Immunostaining was performed according to standard protocols using anti-BrdU antibody (1:1000). Slides were analyzed using Olympus (Tokyo, Japan) IX-71 microscopy using a 10× objective. For quantitative measurements, comparable sections were chosen at the somatosensory cortex, which was divided into 10 horizontal bins from the superficial to the deep, and labeled nuclei in each bin were counted (Teng et al., 2001).

Statistical significance. Data are shown as mean ± SEM. The statistical significance of the results was analyzed using a Student's *t* test.

Results

Neuronal migration in *crmp1*$^{-/-}$ cortex

To examine neuronal migration, we performed BrdU birth-dating analysis in the *crmp1*$^{-/-}$ cerebral cortex. In the brains of P10 injected at E14.5 (P10–BrdU–E14.5) *crmp1*$^{+/-}$ cortex, most labeled neurons were positioned in the deep layers of the cerebral cortex destined to form layers IV and V. In *crmp1*$^{-/-}$ mice, the majority of labeled cells were positioned at layers IV and V. The percentage of labeled cells positioned in those regions, however, was decreased, and the percentage of other regions was increased (Fig. 1*A*). A similar phenotype was also observed in the P10–BrdU–E16.5 *crmp1*$^{-/-}$ cortex. Most labeled neurons were positioned in the upper layers of the cortex, which were destined to form layers II and III in *crmp1*$^{+/-}$ cortex. However, the percentage of correctly positioned neurons was decreased, whereas the percentage of the cells in the other regions tended to be increased in *crmp1*$^{-/-}$ mice (Fig. 1*B*). We also investigated E18.5–BrdU–E14.5 and P3–BrdU–E16.5 *crmp1*$^{+/-}$ and *crmp1*$^{-/-}$ mouse cortices to observe the radial migration. In both brains, the retarded phenotype of neuronal migration was observed in the *crmp1*$^{-/-}$ cortex (data not shown). We next investigated neuronal differentiation and survival in the *crmp1*$^{-/-}$ cortex. At E16.5, apoptotic cells were rarely observed by terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling staining. There was no difference in the proliferation of cortical progenitor cells between *crmp1*$^{-/-}$ and *crmp1*$^{+/-}$ cortices by BrdU proliferative analysis (data not shown). Furthermore, immunoreactivity and protein expression levels of Nestin and MAP-2 (2a + 2b), markers of dividing precursors and of matured neurons, respectively, in E16.5 *crmp1*$^{-/-}$ mice, were not different from those in *crmp1*$^{+/-}$ mice (Fig. 1*C*,*D*). These results indicate that CRMP1 plays a major role in neuronal cells at the postmitotic phase.

We next investigated whether a Sema3A-CRMP1 signaling cascade was also involved in neuronal migration in the cerebral cortex. Because most of the *sema3A*$^{-/-}$ pups died after birth, we performed only E18.5–BrdU–E14.5 analysis in the *sema3A*$^{-/-}$ cortex. We did not observe any differences between *wt*, *sema3A*$^{+/-}$, and *sema3A*$^{-/-}$ in the E18.5–BrdU–E14.5 cortices (Fig. 2*A*). In addition, double heterozygous *crmp1* and *sema3A* mice did not show any differences compared with *crmp1*$^{+/-}$ mice in the E18.5–BrdU–E14.5, P10–BrdU–E14.5, P3–BrdU–E16.5, and P10–BrdU–E16.5 cortices (Fig. 2*B*,*C*) (data not

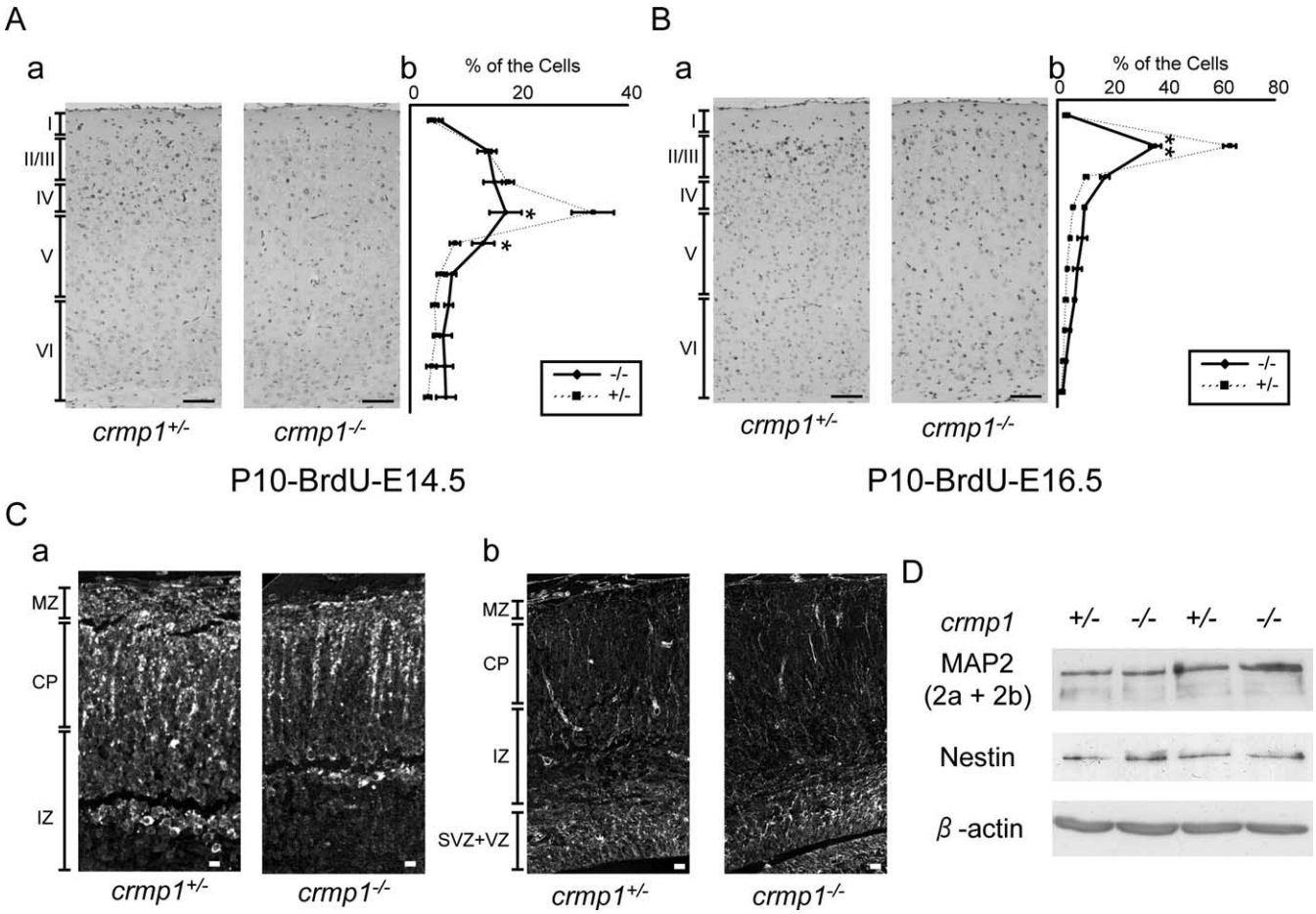

Figure 1. Abnormally positioned neurons in the *crmp1*$^{-/-}$ cerebral cortex. ***A***, Distribution of cells in the P10–BrdU–E14.5 *crmp1*$^{+/-}$ or *crmp1*$^{-/-}$ coronal cortical sections. The rate of the BrdU-labeled nuclei of each bin (see Materials and Methods) is shown in ***b***. ***B***, Distribution of cells in the P10–BrdU–E16.5 *crmp1*$^{+/-}$ and *crmp1*$^{-/-}$ coronal cortical sections. The rate of the BrdU-labeled nuclei of each bin is shown in ***b***. In all cases, three littermates of each genotype were used for quantification. ***C***, E16.5 *crmp1*$^{+/-}$ or *crmp1*$^{-/-}$ coronal cortical sections immunostained with anti-MAP-2 (2a + 2b) (***a***) and anti-Nestin (***b***) antibodies. ***D***, Expression levels of MAP-2 (2a + 2b) and Nestin at E16.5 *crmp1*$^{+/-}$ or *crmp1*$^{-/-}$ mouse cortex lysates from two individual embryos of each genotype. Equal amounts of protein were analyzed, as indicated by the loading control (β-actin). Cortical layers are shown on the left. Scale bars: ***A***, ***B***, 100 μm; ***C***, 10 μm. MZ, Marginal zone; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. *$p < 0.05$, **$p < 0.01$ compared with the corresponding value in *crmp1*$^{+/-}$.

shown). These results suggest that CRMP1 mediates some signaling molecules other than Sema3A in cortical layer formation.

CRMP1 is involved in Reln signaling

In radial migration in the cerebral cortex, tyrosine phosphorylation of Dab1 by a Reln signaling cascade has been shown to be essential. Biochemical analysis has revealed a decreased tyrosine phosphorylation of Dab1 in the *Reln*$^{rl/rl}$ cortex (Howell et al., 1999a, 2000). One of the tyrosine kinases that is involved in phosphorylation of Dab1 is Fyn, an Src-type tyrosine kinase (Bock and Herz, 2003). Because we found that CRMP1 was tyrosine phosphorylated by Fyn in HEK293T cells (Fig. 3*A*), we assumed that CRMP1 was one of the substrates phosphorylated by tyrosine kinases through a Reln signaling cascade. To examine this hypothesis, we immunoprecipitated *Reln*$^{rl/+}$ and *Reln*$^{rl/rl}$ brain lysates with anti-CRMP1 antibody and analyzed tyrosine phosphorylation levels of CRMP1 by anti-phospho-tyrosine antibody. The phosphorylation of CRMP1 at tyrosine residue(s) was detected in the brain lysate from *Reln*$^{rl/+}$ mice. The level of tyrosine phosphorylation of CRMP1 was decreased in the *Reln*$^{rl/rl}$ cortex (Fig. 3*B*). The relative level of tyrosine phosphorylation of CRMP1 in *Reln*$^{rl/rl}$ normalized by the amount of immunoprecipitated CRMP1 in *Reln*$^{rl/+}$ (100.0 ± 25.0) was 38.8 ± 5.2% ($N = 3$; $p < 0.05$). In addition, the expression of CRMP1 showed a higher level in *Reln*$^{rl/rl}$ than in *Reln*$^{rl/+}$ mice. The CRMP1 expression was increased to a lesser extent than was the Dab1 expression in the brain lysates from *Reln*$^{rl/rl}$ mice (Fig. 3*C*). The relative amount of CRMP1 normalized by β-actin in *Reln*$^{rl/+}$ and *Reln*$^{rl/rl}$ was 100.0 ± 11.8 and 155.8 ± 17.3%, respectively ($N = 3$; $p < 0.05$).

In *Reln*$^{rl/rl}$ cortex, reduced Dab1 expression is observed at superplate, whereas its increased expression is observed at cortical plate (Rice et al., 1998). We examined the protein expression pattern of CRMP1 in *Reln*$^{rl/+}$ and *Reln*$^{rl/rl}$ cortices. Intense anti-CRMP1 and Dab1 immunoreactivities were observed at the marginal zone in *Reln*$^{rl/+}$ cortex, whereas reduced immunoreactivities were observed at superplate in *Reln*$^{rl/rl}$ cortex. In addition, increased immunoreactivities of anti-CRMP1 and Dab1 at the cortical plate were also observed in *Reln*$^{rl/rl}$ cortex. Increased CRMP1 expression was observed in some neurons, whereas increased Dab1 expression was in almost all of the neurons of the cortical plate in *Reln*$^{rl/rl}$ mice (Fig. 3*D*).

We further attempted to analyze the genetic interaction with *crmp1* and *dab1*; we used *yotari* mice, one of the spontaneous mutants of *dab1*. Nissl staining of the brain at P10 revealed a disrupted hippocampal lamination in the loss of *crmp1* in a *dab1* heterozygous background (*crmp1*$^{-/-}$;*dab1*$^{yot/+}$), one of the

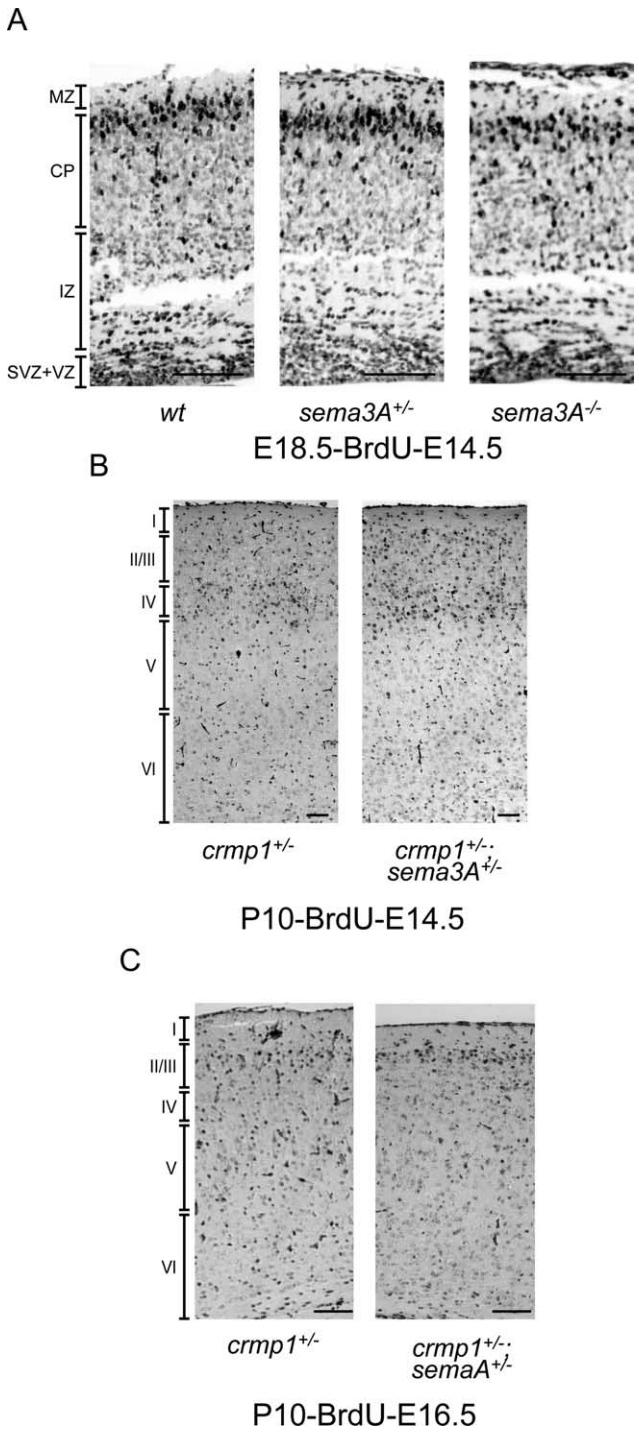

Figure 2. Observation of BrdU birth-dating analysis in *sema3A*$^{-/-}$ and *sema3A*$^{+/-}$; *crmp1*$^{+/-}$ cortices. ***A***, Distribution of cells in the E18.5–BrdU–E14.5 *wt*, *sema3A*$^{+/-}$, and *sema3A*$^{-/-}$ coronal cortical sections. MZ, Marginal zone; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. ***B***, Distribution of cells in the P10–BrdU–E14.5 *crmp1*$^{+/-}$ and *crmp1*$^{+/-}$;*sema3A*$^{+/-}$ coronal cortical sections. ***C***, Distribution of cells in the P10–BrdU–E16.5 *crmp1*$^{+/-}$ and *crmp1*$^{+/-}$;*sema3A*$^{+/-}$ coronal cortical sections. Cortical layers are shown on the left. Scale bar, 100 μm.

Reeler-like phenotypes (Fig. 3*E*). This phenotype was not observed in *crmp1*$^{-/-}$ and *dab1*$^{yot/+}$ hippocampus (Fig. 3*E*) (Assadi et al., 2003), implying that CRMP1 and Dab1 were synergistically involved in Reln signaling for neuronal migration.

Discussion

We provide, for the first time, evidence that CRMP1 is involved in radial neuronal migration through Reln signaling in the developing cerebral cortex. In *Reln*$^{rl/rl}$ cortex, CRMP1 as well as Dab1, an adaptor molecule in the transduction of the Reln signal, were expressed at a higher level, yet these were tyrosine phosphorylated at a lower level. Loss of *crmp1* in a *dab1* heterozygous background led to the disruption of hippocampal lamination, a typical Reeler-like phenotype.

In the *crmp1*$^{-/-}$ mouse cortex, retardation in neuronal migration was observed by BrdU birth-dating analysis when compared with the *crmp1*$^{+/-}$ cerebral cortex (Fig. 1) (data not shown). It is possible that Sema3A regulates neuronal migration in the developing cerebral cortex. Indeed, Sema3A regulates the tangential migration of GABAergic neurons from ganglionic eminence to the cerebral cortex (Marin et al., 2001; Tamamaki et al., 2003). Expression of *nrp1* and *sema3A* were also observed in the cerebral cortex at E16.5 (Kawakami et al., 1996; Skaliora et al., 1998). However, it appears that Sema3A signaling is not involved in radial migration during the period of cortical layer formation, because the retarded phenotype in radial neuronal migration was not seen in either the *sema3A*$^{-/-}$ or double heterozygotes of *crmp1* and *sema3A* mouse cortices (Fig. 2). Consistently, the overall expression pattern of *nrp1* was different from that of *crmp1* in the cerebral cortex at E16.5 (N. Yamashita, A. Morita, Y. Uchida, F. Nakamura, H. Usui, M. Taniguchi, J. Honnorat, P. Kolattukudy, N. Thomasset, K. Takei, T. Takahashi, and Y. Goshima, unpublished observations).

In contrast, the expression pattern of *crmp1* was similar to that of *dab1*. We clearly showed that the expression of CRMP1 was increased in the *Reln*$^{rl/rl}$ cortex and reduced tyrosine phosphorylation of CRMP1 (Fig. 3*B*,*C*). Furthermore, the protein expression pattern of CRMP1 was also similar to that of Dab1 at the marginal zone and the cortical plate. The expression level of CRMP1 was decreased at superplate and increased at the cortical plate in the *Reln*$^{rl/rl}$ cortex, respectively (Fig. 3*D*). In adult *Reln*$^{rl/rl}$ cerebellum, the number of granule cells was reduced, and transient reduction of granule cells was observed in *crmp1*$^{-/-}$ cerebellum (Rice et al., 1998; Charrier et al., 2006). We found that loss of *crmp1* in a *dab1* heterozygous background led to the disruption of hippocampal lamination, a Reeler-like phenotype (Fig. 3*E*). Because the mutations in *Reln* pathway genes cause distinctive abnormalities in many laminated brain regions, known as the Reeler phenotype (D'Arcangelo et al., 1999; Hiesberger et al., 1999; Howell et al., 1999b), these findings strongly suggest that CRMP1 mediates Reln signaling to regulate neuronal migration.

We assumed that Reln regulates neuronal migration through tyrosine phosphorylation of CRMP1 because decreased tyrosine phosphorylation of Dab1 is observed in the *Reln*$^{rl/rl}$ cortex (Howell et al., 1999a, 2000), and animals expressing the nonphosphorylated Dab1 protein have a phenotype similar to the *dab1* null mutant (Howell et al., 2000). Fyn and Fes tyrosine kinases have also been proposed to be signaling components of Sema3A (Mitsui et al., 2002; Sasaki et al., 2002). We found that when CRMP1 and Fyn were introduced into HEK293T cells, CRMP1 was phosphorylated at tyrosine residue(s) (Fig. 3*A*). We tried to detect phosphorylation of CRMP1 at tyrosine residue(s) in the cultured cortical neurons treated with Reln. Up to now, we have not yet obtained clear evidence for tyrosine phosphorylation of CRMP1 after Reln stimulation. The important finding is, however, that the level of tyrosine phosphorylation of CRMP1 was decreased in the *Reln*$^{rl/rl}$ cortex (Fig. 3*B*). Additional studies are required to

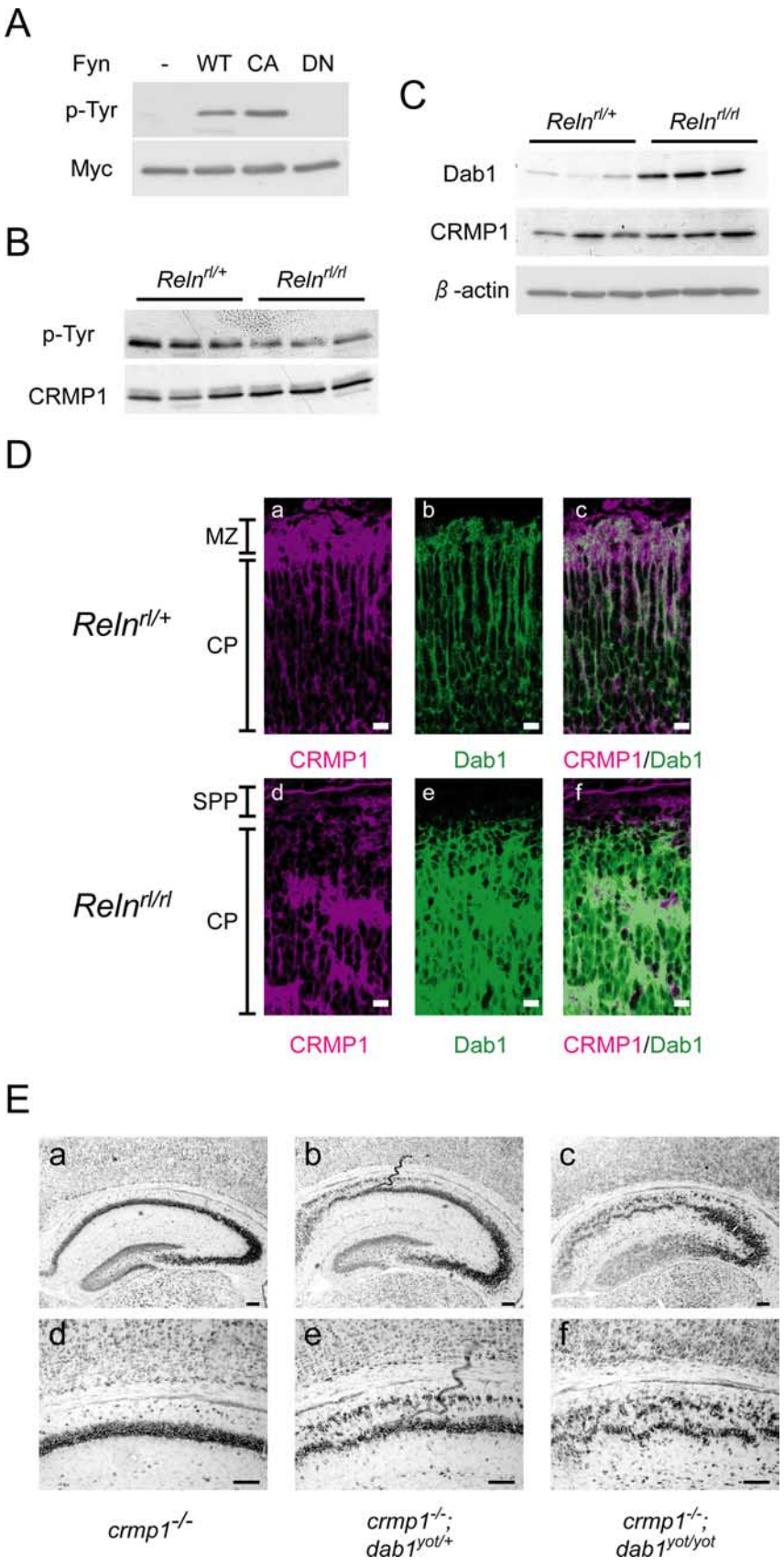

Figure 3. Expression and tyrosine phosphorylation of CRMP1 in *Reln*$^{rl/rl}$ cortex and the genetic interaction between *crmp1* and *dab1* in the hippocampus. ***A***, Tyrosine phosphorylation of CRMP1 by Fyn. CRMP1-Myc was introduced with or without the wild-type (WT), constitutive-active (CA), or dominant-negative (DN) form of Fyn in HEK293T cells. Immunoprecipitation with anti-Myc antibody was performed and was thereafter immunoblotted with anti-p-Tyr and anti-Myc antibodies. ***B***, Tyrosine phosphorylation of CRMP1 in *Reln*$^{rl/+}$ and *Reln*$^{rl/rl}$ lysates from mouse cortex at E16.5. Immunoprecipitation with anti-CRMP1 antibody was performed and was thereafter immunoblotted with anti-p-Tyr and anti-CRMP1 antibodies. ***C***, Expression levels of CRMP1 at E16.5 *Reln*$^{rl/+}$ and *Reln*$^{rl/rl}$ brain lysates. Immunoblot analysis of anti-CRMP1 and anti-Dab1 antibodies of three individual embryos of each genotype was performed. Equal amounts of protein were analyzed, as indicated by the loading control (β-actin). ***D***, Immunohistochemistry with anti-CRMP1 (magenta) and anti-Dab1 (green) antibodies in E16.5 *Reln*$^{rl/+}$ (***a–c***) and *Reln*$^{rl/rl}$ (***d–f***) coronal cortical sections. Cortical layers are shown on the left. MZ, Marginal zone; CP, cortical plate; SPP, superplate. ***E***, Nissl staining of the brains at P10 of *crmp1*$^{-/-}$ (***a***, ***d***), *crmp1*$^{-/-}$;*dab1*$^{yot/+}$ (***b***, ***e***), and *crmp1*$^{-/-}$;*dab1*$^{yot/yot}$ (***c***, ***f***) mice. Coronal sections of the hippocampus region are presented. Magnified images of the CA1 region in ***a***, ***b***, and ***c*** are shown in ***d***, ***e***, and ***f***, respectively. Scale bars: ***D***, 10 μm; ***E***, 100 μm.

delineate phosphorylation site(s) of CRMP1 and its functional significance in Reln signaling.

The presence of Reln correlates with reduced Dab1 protein levels during embryonic development (Rice et al., 1998; Howell et al., 1999a). Our study showed that the expression of CRMP1 was higher in *Reln*$^{rl/rl}$ cortex compared with *Reln*$^{rl/+}$ mice (Fig. 3*C*), suggesting that Reln regulates CRMP1 protein levels in the cerebral cortex. This further indicates that CRMP1 possesses a biochemical property similar to Dab1. Interestingly, some neurons exhibited an increased immunoreactivity of CRMP1 in the *Reln*$^{rl/rl}$ cortical plate at E16.5, whereas Dab1 accumulation was observed in almost all neurons in the cortical plate (Fig. 3*D*). This result suggests that CRMP1 may regulate migration of a subset of the cortical neurons. The neurons exhibiting increased immunoreactivity of CRMP1 in the *Reln*$^{rl/rl}$ cortex were distributed throughout the cerebral cortex (Fig. 3*D*). Consistent with this observation, *crmp1*$^{-/-}$ showed a retarded migration phenotype in both the upper and deep layers of the cerebral cortex (Fig. 1*A*,*B*). These findings suggest that neurons in which CRMP1 may regulate their migration are composed of a heterogeneous subpopulation, being unrelated to a specific cortical layer. The characterization of the CRMP1-positive neurons remains to be determined. The expression pattern of another CRMP family member, *crmp2*, was almost similar to that of *crmp1* (Wang and Strittmatter, 1996). Both CRMP1 and CRMP2 are involved in mediating Sema3A signaling (Uchida et al., 2005). These findings are consistent with a mild defect in neuronal migration in the *crmp1*$^{-/-}$ cortex, rather than in the *Reln*$^{rl/rl}$ cerebral cortex. Indeed, disruption of a single gene, having overlapping func-

tions, sometimes does not result in any phenotypic defects in the mutant animals (Kuo et al., 2005). The combined disruption with other CRMP family genes will therefore tell us the exact roles of CRMP1 in the developing cerebral cortex.

This is the first evidence for an important role of CRMP1 in Reln signaling. It appears that the CRMP family is involved in cytoskeletal organization by receiving various extracellular cues that contribute to neuronal development and maturation.

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