Dab2IP Regulates Laminar Organization in the Mammalian Brain

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Shuhong Qiao and Ramin Homayouni
Department of Biology, University of Memphis, Memphis, TN 38152

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Introduction

Reelin signaling pathway controls cell migration and cortical histogenesis during development (Rice and Curran, 2001; D'Arcangelo, 2006). Reelin functions by binding to lipoprotein receptors ApoER2 and VLDLR, and inducing tyrosine phosphorylation of intracellular adapter protein disabled-1 (Dab1) via Src family tyrosine kinases.

Previously, we found that Dab1 interacts with the NPXY motif in DOC2/Dab2 Interacting Protein (Dab2IP), a new member of Ras GTPase activating proteins (RasGAP). Several isoforms of Dab2IP have been identified thus far (Wang et al., 2002; Homayouni et al., 2003). Recently, we identified a novel mouse Dab2IP transcript variant, (Dab2IP-L) that contains a considerably longer pleckstrin homology (PH) domain than previously reported isoforms of Dab2IP.

In order to investigate the role of Dab2IP during brain development, we generated a mouse model in which the *Dab2IP* gene was disrupted by a retroviral promoter trap strategy. This targeting construct contained a LacZ reporter gene which allows for in situ analysis of gene expression in various tissues, such as the brain. Western blot and q-RT-PCR analysis showed that the expression of all except one isoform of Dab2IP was abolished in Dab2IP mutant mice.

In this study, we used the Dab2IP mutant mice to characterize the expression of Dab2IP during brain development and examine its role in neuronal migration and layer formation in the developing cortex. Since Dab2IP interacts with Dab1, it may play a role in Reelin signaling. Thus, we also investigated if Dab2IP protein levels are altered in *reeler* mice.

Dab2IP gene structure and knock-out strategy

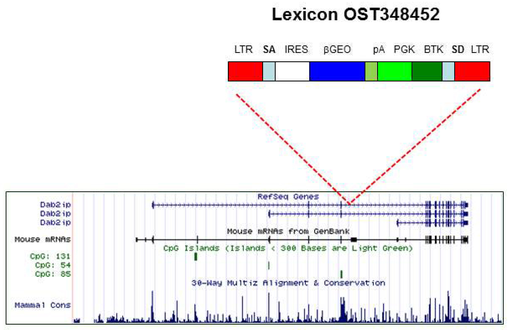

Fig. 1. Insertion of the retroviral gene trap cassette into the Dab2IP gene locus.

The mouse *Dab2IP* gene structure was examined using the UCSC Genome Browser (July 2007 build). Dab2IP contains 18 exons spanning over 175Kb. At least 4 different variants of Exon 18 have been isolated from EST libraries.

To generate Dab2IP KO mice, blastocysts were injected with Omnibank® OST348452 ES cells (Lexicon Genetics, Inc.), which contained a retroviral gene-trap inserted immediately after Exon 4 of the *Dab2IP* gene. The promoter-trap cassette encodes the β-galactosidase-neomycin (β-neo) gene flanked by 5'-splice acceptor site, which results in the β-geo gene under the control of the native Dab2IP promoter. The presence of BTK under the control of PGK promoter upstream of a splice donor site confers a selectable marker to identify targeted ES cells in culture.

Dab2IP expression is developmentally regulated

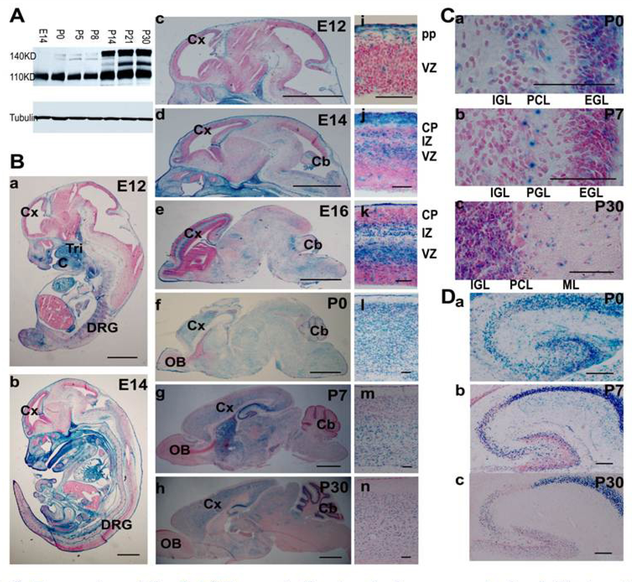

Fig 2. Expression of the Dab2IP gene in the developing mouse brain. *A*, Western blot analysis of Dab2IP expression in cortex at various developmental ages. ***B,*** Sagittal sections of E12 and E14 embryos (***a, b***) or of brains from E12 to P30. (***a-h***) were processed for β-gal and counterstained with nuclear fast red. Scale bars: 1.0 mm. (***i-n***) Higher-magnification images of *c-h* . Scale bars, 50 um ***C***, Expression of Dab2IP in the cerebellum. Scale bars: 50 um ***D***, Expression of Dab2IP in the hippocampus. Scale bar: 10 um.

Defective lamination in Dab2IP KO Mice

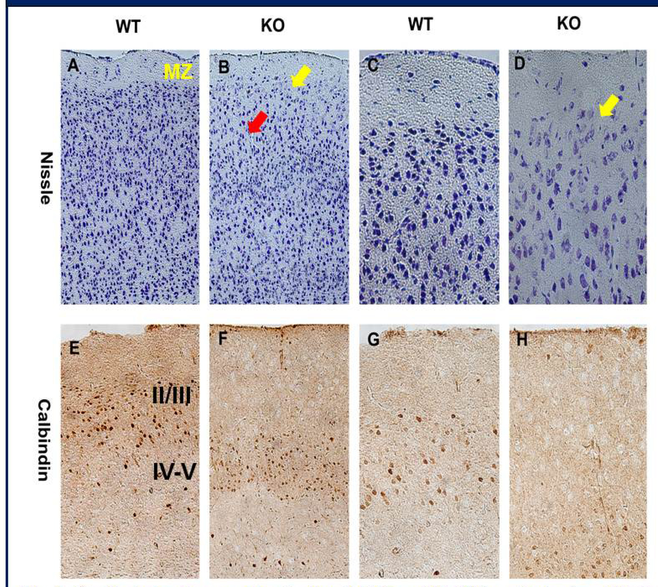

Fig. 3. Cortical structure and lamination in WT and Dab2IP mutant mice. Histological analysis of WT and KO cerebral cortex from 4-week-old-mice. Low magnification ***(A,B)*** and high magnification ***(C,D)*** Nissl staining of P30 WT and KO cortex. Defects were observed in marginal zone boundries (yellow arrow) and layer II/III (red arrow). Low ***(E,F)*** and high magnification ***(G,H)*** images of calbindin immunostaining shows layer II/III neurons are misplaced in Dab2IP mutant mice.

Dab2IP regulates neuronal migration in late-generated neurons

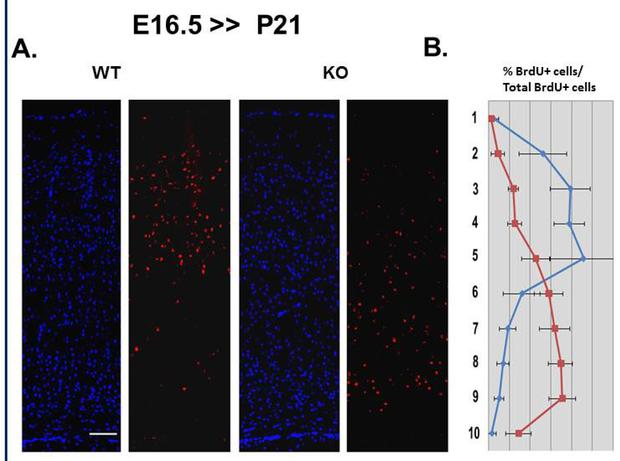

Fig. 4. Birthdating studies in the developing cortex. *A,* BrdU was injected into pregnant females at E16.5, and the brains were collected at post-natal day 21 (P21) and immunostained with anti-BrdU antibody. ***B,*** Quantitation of of BrdU-positive cells as a percentage in each of 10 bins relative to the total number of BrdU-positive cells in WT (**blue line**) and Dab2IP mutant (**red line**) brains. Data represent mean ± SD, n=3. Neurons which are born at E16.5 normally occupy layers II/III in the cortex. However, in Dab2IP mutant mice, E16.5 born neurons occupy deeper layers.

Preplate and subplate are normal in Dab2IP mutant cortex

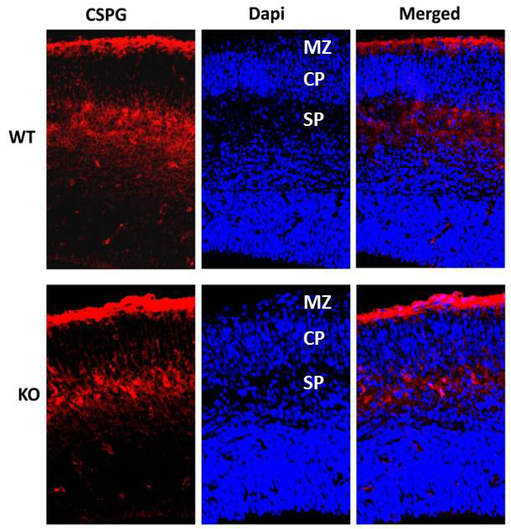

Fig. 5. CSPG immunostaining of WT and Dab2IP mutant cortex at E16.5. Coronal sections from E16.5 WT or Dab2IP mutant embryos were immunostained with CSPG (**red**) along with a DAPI nuclear stain (**blue**). The marginal zone (MZ), cortical plate (CP) and subplate region (SP) are indicated. These results indicate that the preplate splits properly at E11.5 and that the cortical plate is forming correctly until E16.5

Dab2IP levels accumulate in *reeler* cortex

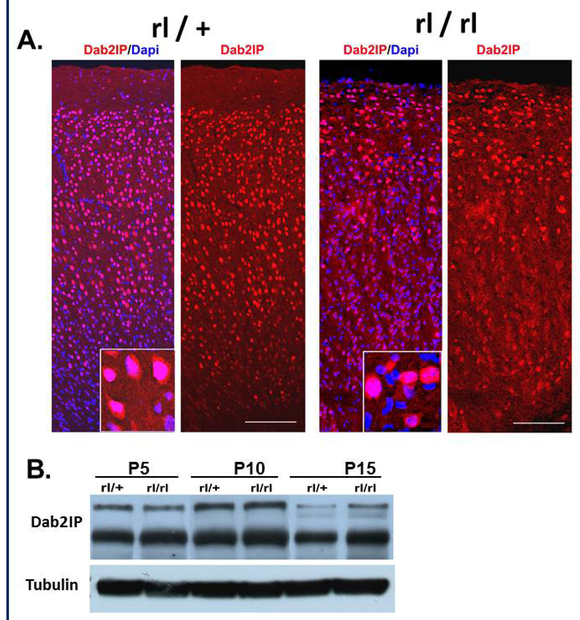

Fig. 6. Dab2IP protein levels in *reeler* cortex. *A,* Dab2IP (red) and DAPI (blue) immunostaining in the cortex of wild-type and *reeler* (rl/rl) mice at post-natal day 30. Higher-magnifications are shown in the insets. Scale bar=100 μm. ***B,*** Immunoblots of Dab2IP and beta-tubulin of pooled cortical lysates from two rl/+ and two rl/rl at various developmental ages. Dab2IP levels are higher in *reeler* brains, particularly at later developmental ages.

Summary and Conclusions

1. Dab2IP is a Ras and Rap1 GTPase activating protein which interacts with Dab1, an adapter molecule critical in Reelin signaling pathway (Homayouni et al., 2003; Kim and Homayouni, 2010).
2. Using the promoter trap lacZ marker, we show that Dab2IP is widly expressed during embryonic development. Unlike *reeler* mice, the Dab2IP mutant mice have normal gross cortical and cerebellar morphology.
3. Interestingly, Dab2IP mutants have defective lamination in the somatosensory cortex.
4. It appears that early events during cortical development are normal in Dab2IP mutant animals, whereas migration of later-born neurons that occupy layers II/III of the cortex are disrupted by deletion of Dab2IP. Also, the marginal zone boundry is disrupted in Dab2IP mutants.
5. The levels of Dab2IP are higher in a subset of cortical neurons in *reeler* mice.
6. Our data suggest that Dab2IP may play an important role in some aspects of Reelin signaling during brain development.

Acknowledgement

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