PERSPECTIVE

A missed exit: Reelin sets in motion Dab1 polyubiquitination to put the break on neuronal migration

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In 1951, Douglas Scott Falconer first described the reeler spontaneous mutant mouse (Falconer 1951). In those mice, cortical neurons are generated normally but migrate abnormally, resulting in an inversion of the cortical laminar organization, with later-born neurons remaining in the deeper layers of the cortex. Forty-four years later, D’Arcangelo et al. (1995) identified the causative gene \( Rln \) and the encoded protein Reelin. Reelin pathway mutants, and particularly mice with mutations of its intracellular effector Dab1 (Disabled-1), probably represent the most-studied phenotype of altered neuronal migration. The observations that mutations of \( Dab1 \) phenocopy the \( Rln \) mutation, and that \( Dab1 \) is up-regulated in reeler mice suggested the existence of a negative feedback loop in Reelin signaling via the regulation of Dab1 levels. In the previous issue of Genes & Development, Feng et al. (2007) presented the first coherent model to explain the mechanism of Reelin-mediated Dab1 down-regulation. They proposed that Reelin both activates and down-regulates its effector Dab1, first by inducing its phosphorylation, which then causes its targeting by an E3 ubiquitin ligase complex (EBC) containing SOCS family proteins and Cullin5 (Cul5). The impairment of this down-regulation mechanism in vivo leads to a unique phenotype in the cortex where neurons migrate past their target layer.

The known side of Reelin/Dab1 signaling

Reelin is a large matrix-associated transmembrane glycoprotein expressed by specific neuronal populations throughout the developing CNS. Reelin has been shown to homodimerize, at least in vitro, and to bind to and cluster two receptors from the LDL [low-density lipoprotein] family: VLDLR [very low-density lipoprotein receptor] and ApoER2 [apolipoprotein E receptor type2] (D’Arcangelo et al. 1999, Hiesberger et al. 1999, Trommsdorff et al. 1999, Kubo et al. 2002, Strasser et al. 2004). The binding of Reelin to its receptors induces the tyrosine phosphorylation of the cytoplasmic adaptor protein Dab1, which interacts with the conserved motif NPxY in the cytoplasmic tails of VLDLR and ApoER2 (Trommsdorff et al. 1998; Howell et al. 1999).

In addition, Dab1 binds to and is phosphorylated by nonreceptor tyrosine kinases from the Src family (SFK): Src and Fyn [Arnaud et al. 2003a; Kuo et al. 2005]. Noticeably, the mouse Dab1 was originally identified in a yeast two-hybrid screen for Src-interacting proteins [Howell et al. 1997]. Src and Fyn are activated [phosphorylated] through the Reelin pathway, and thus together, Dab1 tyrosine phosphorylation and SFK phosphorylation are two interdependent mechanisms that are part of a positive feedback loop [Arnaud et al. 2003b; Bock and Herz 2003].

In the developing cortex, Reelin is specifically expressed by horizontally oriented Cajal-Retzius neurons located in the preplate before its splitting and then in the outermost layer of the cortex [the marginal zone of the developing brain, becoming layer I of the mature brain] [Fig. 1; D’Arcangelo et al. 1995; Ogawa et al. 1995]. On the other hand, ApoER2, VLDLR, and Dab1 are expressed by both cortical neurons and radial glia cells supporting their migration [Forster et al. 2002, Frotscher et al. 2003; Hartfuss et al. 2003; Luque et al. 2003]. Strikingly, mutations of both VLDLR and ApoER2, of Dab1 and particularly point mutations at five Dab1 tyrosine phosphorylation sites [\( Dab1^{5F} \) mutation of Tyr185, Tyr198, Tyr200, Tyr220, and Tyr232], or mutation of both SFK Src and Fyn result in a phenocopy of the reeler phenotype, with a failure to split the preplate as well as inverting cortical layering [Howell et al. 1997, 2000; Sheldon et al. 1997; Benhayon et al. 2003; Kuo et al. 2005], validating that they participate in a common signaling pathway regulating cortical neuronal migration.

Down-regulating Dab1

Desensitization is a key feature of most signaling pathways as it allows a system to reset and thus regain sensitivity to repeated stimulation. Many studies demonstrated that the Reelin pathway displays such a negative...
feedback mechanism. It has been shown that the disruption of Reelin signaling [by genetic disruption of 
Reelin, VLDLR, ApoER2, or Fyn and Src] leads to the accumulation of Dab1 protein in vivo, suggesting that Reelin limits its action in responsive neurons [i.e., earlier-born] neurons [lighter green], thus organizing the cortex in an “inside-out” manner. Reelin is secreted by Cajal-Retzius cells (red) located at early developmental stages [i] within the preplate (PP) before it splits into the subplate (SP) and the marginal zone (MZ), and at later stages (ii) within the marginal zone. Both neurons and radial glia cells express Reelin receptors VLDLR and ApoER2 (orange) and are responsive to Reelin. Reelin signaling displays a negative feedback regulation by which Reelin limits its own action by down-regulating the level of its effector Dab1. Feng et al. [2007] demonstrated that reelin-induced phosphorylation of Dab1 on Tyr185 and Tyr198 cause the polyubiquitination of Dab1 (Ub) by E3 ubiquitin ligases containing SOCS proteins and Cul5. Polyubiquitinated Dab1 is then degraded by the proteasome. Correct cortical development requires a strictly regulated balance of Reelin signaling and its negative feedback. During neuronal migration through the deeper layers of the cortex [a], Dab1 level is normally low [light yellow], potentially due to its distance to the Reelin source at this time, or to its down-regulation induced by Reelin encountered at an early stage of development [i]. [b] The uninhibited Dab1 signaling [bright yellow] may be required for the neuron to progress through previously deposited neuronal layers. [c] In turn, Dab1 down-regulation may be necessary to stop neuron movement and avoid “overmigration.” [SVZ] Subventricular zone; [IZ] intermediate zone.
Feng et al. (2007) identified another type of EBC potentially regulating Dab1 in vivo. They also used non-neuronal cell lines such as COS7, in which Dab1, when coexpressed with Fyn and Src, is known to be constitutively tyrosine phosphorylated but not degraded. This suggests that a component of the degradation machinery targeting phospho-tyrosine Dab1 is absent or limiting in this in vitro system, which thus makes it particularly adapted for a candidate approach. Feng et al. (2007) tested several E3 ubiquitin ligases in this nonneuronal cell system and show that the SOCS1–3 ligases can bind to Dab1, and induce Tyr185 or Tyr198 phosphorylated Dab1 degradation, in a Fyn-dependent manner. Interestingly, they also tested Cbl in this assay and show that it fails to induce Dab1 degradation, further invalidating the Cbl implication hypothesis. Because many SOCS proteins are expressed during brain development (among them SOCS1–3) and might be functionally redundant, Feng et al. (2007) chose to study the consequence of the inactivation of another component of the SOCS-containing EBC on Reelin-induced degradation of Dab1: the cullin Cul5 (Petroski and Deshaies 2005). Cul5 recently has been shown and confirmed in the study by Feng et al. (2007) to be expressed in mouse cortical neurons (Lein et al. 2007), but its role was so far completely unknown. Feng et al. (2007) showed that in cultured cortical neurons, Cul5 binds to Dab1, and that Cul5 knockdown specifically protects Dab1 from Reelin-induced degradation. Noticeably, Dab1 interaction with ubiquitin ligases may also have other biological significance. Dab1 has been shown to interact with the E3 ubiquitin ligase Siah-1A in yeast two-hybrid assays and coimmunoprecipitation experiments in 293T cells. But in that case, Dab1–Siah1 binding induces the inhibition of Siah-1A ubiquitinating activity (Park et al. 2003). Thus, one might wonder if Dab1 could additionally regulate EBC containing SOCS and Cul5 as well, adding a step to the complexity of this Reelin feedback loop.

Overmigration: when Dab1 down-regulation fails to occur

Feng et al. (2007) showed for the first time the in vivo consequences of a failure of Dab1 down-regulation on neuronal migration. Bock et al. (2004) showed that blocking proteasome activity disturbs the proper organization of the cortical plate may be difficult as the depth and the layered organization of the cortical plate are continuously changing. A transcriptional mode of regulation implies latency of protein synthesis, which is slow in the case of Dab1, and of the protein half-life (12 h for Dab1) (Arnaud et al. 2003a), and thus is probably not an appropriate way to control a timely switch mechanism. Controlling expression level by regulating the degradation of a constantly synthesized protein, in a continuously responding neuron can constitute a very efficient method, in contrast. In migrating cortical neurons, Dab1 is constantly down-regulated [as implied by the fact that in reeler mice, the Dab1 level is increased throughout the depth of the cortex] (Arnaud et al. 2003a) via the Cul5/ SOCS-containing E3 ligase complex. Dab1 regulation

endfeet [Hu et al. 2007]. Interestingly, Cul5 knockdown neurons remain at the surface of the cortex, instead of being bypassed by younger neurons. This defect is partially rescued in vivo by the coelectroporation of Dab1 short hairpin RNA (shRNA). Furthermore Cul5 shRNA stops Dab1 degradation in vitro, validating that the overmigration is due to the inhibition of Dab1 degradation.

In the developing cortex, neurons migrate along the processes of their parental radial glia from the ventricular zone to the more superficial cortical layer and stop when they reach the marginal zone. Later-born neurons migrate through previously deposited neurons in cortical plate, leading to an inside-out lamination of the cortex [Fig. 1]. The reeler phenotype displayed by Rhl-deficient mice as well as mice deficient for VLDLR and ApoER2, Dab1, and Src and Fyn mutant show a rough inversion of the normal inside-out pattern of cortical migration and an excess of neurons in the normally cell-sparse marginal zone (Caviness and Sidman 1973; Howell et al. 1997, 2000; Sheldon et al. 1997; Benhayon et al. 2003; Kuo et al. 2005). The commonly accepted explanation for this phenotype is that the newly arrived neurons fail to penetrate the preplate and split it appropriately into the marginal zone and subplate, and cannot bypass previously deposited neurons and accumulate in an outside-in manner. Dab1 seems necessary to achieve the bypassing step in mosaic Dab1 wt; Dab1<sup>-/-</sup> embryos, mutant neurons lie below wild-type neurons (Hammond et al. 2001), and BrdU pulse-labeling experiments showed that Dab1 knockdown neurons accumulate in deep cortical plate and fail to pass their earlier-born siblings. Furthermore, neurons showing an abnormally high level of Dab1 migrate presumably faster through the cortical plate and stick to the marginal zone, preventing the passage of their siblings [Feng et al. 2007]. Overall, those results strongly suggest that Dab1 is necessary for neurons to cross previously deposited neuronal layers, and in turn, Dab1 should be down-regulated in neurons to properly terminate their migration and allow their siblings to bypass them. According to this hypothesis, a precise regulation of Dab1 levels should be required to control the precise location of the migration arrest. A threshold response to a gradient of a signaling molecule may be an efficient way to control a timely stop. However, maintaining a Reelin gradient within the cortical plate may be difficult as the depth and the layered organization of the cortical plate are continuously changing. A transcriptional mode of regulation implies latency of protein synthesis, which is slow in the case of Dab1, and of the protein half-life (12 h for Dab1) [Arnaud et al. 2003a], and thus is probably not an appropriate way to control a timely switch mechanism. Controlling expression level by regulating the degradation of a constantly synthesized protein, in a continuously responding neuron can constitute a very efficient method, in contrast. In migrating cortical neurons, Dab1 is constantly down-regulated [as implied by the fact that in reeler mice, the Dab1 level is increased throughout the depth of the cortex] (Arnaud et al. 2003a) via the Cul5/ SOCS-containing E3 ligase complex. Dab1 regulation
thus relies on the EBC activity, which may be rapidly controlled by protein complex assembly, to rapidly decrease Dab1 levels and definitively terminate neuronal migration.

Although knockout results are very informative, essential complementary data would come from the analysis of the brain phenotype of Cul5-deficient mice. The inhibition of ubiquitination in cortical slices by Bock and Herz (2003) may provide some clues. Those slices showed massive disorganization of the layering, however, complementary cell tracing experiments, necessary to fully understand this phenotype, were not performed. In humans, cortical migration disorders caused by Reelin signaling deficiency lead to classical lissencephaly (Hong et al. 2000; Boycott et al. 2005; Chang et al. 2007; Zaki et al. 2007), a condition characterized by a paucity of cortical gyration, leading to severe epilepsy and mental retardation. Impairment of the down-regulation component of Reelin signaling is thus predicted to cause strong cortical developmental defects as well. A precise description of the phenotype of the Cul5-deficient mice cortex may pinpoint human brain abnormalities linked to this mechanism.

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References

Arnaud, L., Ballif, B.A., and Cooper, J.A. 2003a. Regulation of protein tyrosine kinase signaling by substrate degradation during brain development. Mol. Cell. Biol. 23: 9293–9302.

Arnaud, L., Ballif, B.A., Forster, E., and Cooper, J.A. 2003b. Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. Curr. Biol. 13: 9–17.

Benhayon, D., Magdaleno, S., and Curran, T. 2003. Binding of purified Reelin to ApoER2 and VLDLR mediates tyrosine phosphorylation of Disabled-1. Brain Res. Mol. Brain Res. 112: 33–45.

Bock, H.H. and Herz, J. 2003. Reelin activates SRC family tyrosine kinases in neurons. Curr. Biol. 13: 18–26.

Bock, H.H., Jossin, Y., May, P., Bergner, O., and Herz, J. 2004. Apolipoprotein E receptors are required for reelin-induced proteosomal degradation of the neuronal adaptor protein Disabled-1. J. Biol. Chem. 279: 33471–33479.

Boycott, K.M., Flavelle, S., Bureau, A., Glass, H.C., Fujiwara, T.M., Howell, B.W., Godinho, L., and Tan, S.S. 2001. Disabled-1 functions cell autonomously during radial migration and cortical layering of pyramidal neurons. J. Neurosci. 21: 8798–8808.

Hartfuss, E., Forster, E., Bock, H.H., Hack, M.A., Leprince, P., Luque, J.M., Herz, J., Frotscher, M., and Gotz, M. 2003. Reelin signaling directly affects radial glia morphology and biochemical maturation. Development 130: 4597–4609.

Hiesberger, T., Strommdorff, M., Howell, B.W., Goffinet, A., Mumby, M.C., Cooper, J.A., and Herz, J. 1999. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and mediates tau phosphorylation. Neuron 24: 481–489.

Hong, S.E., Shugart, Y.Y., Huang, D.T., Shahwan, S.A., Grant, P.E., Hourihane, J.O., Martin, N.D., and Walsh, C.A. 2000. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat. Genet. 26: 93–96.

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.

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.

Howell, B.W., Herrick, T.M., Hildebrand, J.D., Zhang, Y., and Cooper, J.A. 2000. Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. Curr. Biol. 10: 877–885.

Hu, H., Yang, Y., Eade, A., Xiong, Y., and Qiu, Y. 2007. Breaches of the pial basement membrane and disappearance of the glia limitans during development underlie the cortical laminarization defect in the mouse model of muscle-eye-brain disease. J. Comp. Neurol. 501: 168–183.

Kubo, K., Mikoshiba, K., and Nakajima, K. 2002. Secreted Reelin molecules form homodimers. Neurosci. Res. 43: 381–388.

Kuo, G., Arnaud, L., Kronstad-O’Brien, P., and Cooper, J.A. 2005. Absence of Fyn and Src causes a reeler-like phenotype. J. Neurosci. 25: 8578–8586.

Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boc, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., et al. 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 166–176.

Levковitz, G., Waterman, H., Ettenger, S.A., Katz, M., Tsyganov, A.Y., Alroy, I., Lavi, S., Iwai, K., Reiss, Y., Cicchenero,
A., et al. 1999. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell* 4: 1029–1040.

Lupher Jr., M.L., Songyang, Z., Shoelson, S.E., Cantley, L.C., and Band, H. 1997. The Cbl phosphotyrosine-binding domain selects a D(N/D)XpY motif and binds to the Tyr292 negative regulatory phosphorylation site of ZAP-70. *J. Biol. Chem.* 272: 33140–33144.

Luque, J.M., Morante-Oria, J., and Fairen, A. 2003. Localization of ApoER2, VLDLR and Dab1 in radial glia: Groundwork for a new model of reelin action during cortical development. *Brain Res.* 140: 195–203.

Meng, W., Sawasdikosol, S., Burakoff, S.J., and Eck, M.J. 1999. Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. *Nature* 398: 84–90.

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.

Park, T.J., Hamanaka, H., Ohshima, T., Watanabe, N., Mikoshiba, K., and Nukina, N. 2003. Inhibition of ubiquitin ligase Siah-1A by disabled-1. *Biochem. Biophys. Res. Commun.* 302: 671–678.

Petroski, M.D. and Deshaies, R.J. 2005. Function and regulation of cullin–RING ubiquitin ligases. *Nat. Rev.* 6: 9–20.

Rice, D.S., Sheldon, M., D’Arcangelo, G., Nakajima, K., Goldwitz, 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.

Sheldon, M., Rice, D.S., D’Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., Howell, B.W., Cooper, J.A., Goldwitz, D., and Curran, T. 1997. Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* 389: 730–733.

Strasser, V., Fasching, D., Hauser, C., Mayer, H., Bock, H.H., Hiesberger, T., Herz, J., Weeber, E.J., Sweatt, J.D., Pramatarova, A., et al. 2004. Receptor clustering is involved in Reelin signaling. *Mol. Cell. Biol.* 24: 1378–1386.

Suetugu, S., Tezuka, T., Morimura, T., Hattori, M., Mikoshiba, K., Yamamoto, T., and Takenawa, T. 2004. Regulation of actin cytoskeleton by mDab1 through N-WASP and ubiquitination of mDab1. *Biochem. J.* 384: 1–8.

Thien, C.B. and Langdon, W.Y. 2001. Cbl: Many adaptations to regulate protein tyrosine kinases. *Nat. Rev.* 2: 294–307.

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: 33556–33560.

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R.E., Richardson, J.A., and Herz, J. 1999. Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97: 689–701.

Zaki, M., Shehab, M., El-Aleem, A.A., Abdel-Salam, G., Koeller, H.B., Ilkin, Y., Ross, M.E., Dobyns, W.B., and Gleeson, J.G. 2007. Identification of a novel recessive RELN mutation using a homozygous balanced reciprocal translocation. *Am. J. Med. Genet.* 143: 939–944.
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