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Review

Reelin mouse mutants as models of cortical development disorders

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Abstract

Developmental defects in neuronal positioning and synaptic connectivity are commonly found in neurological diseases, and they are believed to underlie many cognitive and affective disorders. Several mouse mutants are currently available that model at least some aspects of human developmental brain disorders. With the identification of the genes mutated in these animals and the study of the cellular basis of the phenotypes, we have taken significant strides toward an understanding of the mechanisms controlling proper brain development and the consequences of their dysfunction. In particular, mouse mutants deficient in the *Reelin* gene have provided valuable insights into the mechanisms of cortical development. Absence of *Reelin* expression in the spontaneous mutant mouse *reeler* leads to extensive defects in neuronal position and dendrite development. In humans, loss of Reelin results in a type of lissencephaly with severe cortical and cerebellar malformation. Genetic and biochemical studies using mouse mutants suggest that the Lis1 protein may participate in the Reelin signaling pathway controlling cortical development. Reduced levels of Reelin are also present in postmortem brains of patients with schizophrenia, suggesting a possible link with this cognitive disorder. The regulation of the *Reelin* gene may thus provide insights into the mechanisms of this disease.

Keywords: Reeler; Disabled-1; Very low density lipoprotein receptor; Apolipoprotein E receptor 2; Platelet-activating factor acetylhydrolase 1b1; Hydrocephalus; Epilepsy; Cerebral cortex; Schizophrenia

1. Introduction

Homozygous mutations in the *Reelin* (*Reln*) gene result in a severe disruption of brain development in humans and in rodents. The gene encodes a large extracellular glycoprotein that bears little similarity to other known proteins, reflecting its unique properties in the control of brain development. The mouse mutant *reeler* has been studied extensively as a model for understanding the molecular and cellular mechanisms governing the orderly development of cortical structures (see [1–3] for comprehensive reviews). In this mutant, cellular layer formation in the cerebral cortex, hippocampus, and cerebellum goes awry and ectopic neurons can be found in most anatomical structures of the central nervous system. The loss of cellular organization in the cerebellum of *reeler* mice results in severe hypoplasia, which in turn causes an ataxic phenotype characterized by tremors, dystonia, and a reeling gate. Similar behavioral and anatomical traits are present in mutant mice in which essential components of the Reelin signaling pathway are disrupted. This pathway includes two high-affinity Reelin receptors, the very low density lipoprotein receptor (VLDLR) and the apolipoprotein E receptor 2 (ApoER2) [4,5], and an adapter molecule called Disabled-1 (Dab1) (Fig. 1). Binding of Reelin to its receptors causes clustering [6] and promotes the phosphorylation of Dab1 on specific tyrosine residues through the activation of src-family kinases [7–11]. Additional components of the signaling cascade have recently been identified, but their role in mediating Reelin function in cortical layer

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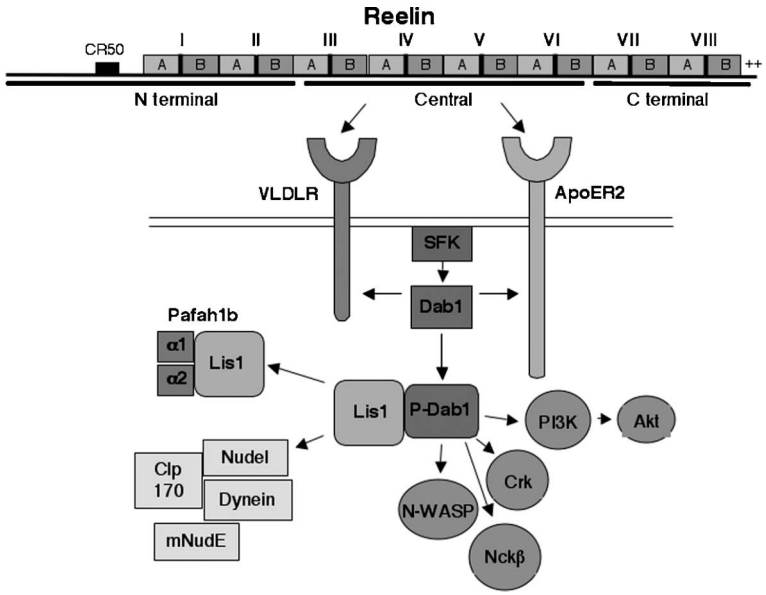

Fig. 1. Diagram of the Reelin protein and its signaling molecules. Reelin is synthesized as a large protein containing eight consecutive repeats (I–VIII). Each repeat contains two subrepeats (A and B) separated by an EGF-like cysteine pattern (thick black vertical line). The N-terminal CR-50 epitope and the C-terminal stretch of positively charged amino acids (++) are indicated. Full-length Reelin is cleaved to produce three major fragments: the N-terminal, central, and C-terminal fragments (thick black horizontal lines). Full-length Reelin and the central fragment bind to VLDLR and ApoER2 and activate an SFK, src family kinase; PI3K, phosphatidylinositol 3 kinase; (other abbreviations are standard and not defined in the literature) that phosphorylates Dab1. PhosphoDab1 then binds Lis1 and several other signaling proteins (gray circles). Lis1 also binds dynein-associated molecules and the catalytic subunits of the Pafah1b complex (α1 and α2). Coordinated Dab1- and Lis1-dependent activities mediate cortical layer formation in the developing brain.

formation has not been fully elucidated. One Dab1-binding protein that is particularly relevant to cortical development is Lis1, the product of the *PAFAH1b1* gene. The interaction between Dab1 and Lis1 allows cross-talk between Reelin signaling and Lis1-dependent molecular events that may be important for cellular layer formation in the neocortex [12]. This interaction is discussed in detail as it relates to the appearance of lissencephaly and hydrocephalus in humans. The expression level of the *REELIN* gene is regulated by methylation and it is reduced in the brain of subjects with schizophrenia [13,14]. The significance of these findings is also discussed. As a first step in considering mutant mice as models for human developmental brain disorders, I review expression studies of *Reelin* in rodents and primates.

2. *Reelin* gene identification and regulation

The *Reelin* gene was first identified in the mouse as the coding sequence that is disrupted in two distinct strains of *reeler* mutants [15]. In both strains, one generated by random transgene insertion (rl^{tg}) and the other by spontaneous mutation (rl^{Ed}, now renamed $Reln^{rl}$), large deletions of the *Reelin* gene on the distal region of chromosome 5 result in the loss of mRNA and protein expression. In a third strain of *reeler* (rl^{Orl}, now renamed $Reln^{rl\text{-}Orl}$) insertion of an L1 element causes a frameshift that alters the C terminus of the Reelin protein [16]. Since its identification, several additional alleles of *reeler* have been identified in mouse [17,18] and rat [19,20], all carrying loss-of-function mutations in the *Reelin* gene. The complete structure of the mouse *Reelin* gene has been determined and found to contain 65 exons, spanning approximately 450 kb of genomic DNA [21]. Alternative splicing of a microexon and alternative polyadenylation were also observed [22]. The murine cDNA encodes a large core protein containing 3461 amino acids forming a distinct N-terminal region, followed by eight highly similar repeats and a positively charged C-terminal region [15] (Fig. 1). The protein is glycosylated, increasing its molecular weight from the predicted 388 kDa to approximately 400 kDa, and it is secreted in the extracellular environment [23]. Secretion occurs through a constitutive pathway [24], and requires the C-terminal region [23]. Disruption of this region in the $Reln^{Orl}$ strain results in the complete loss of extracellular Reelin and the appearance of a phenotype indistinguishable from that of *Reelin* null mutants [16,25]. Reelin is secreted as a full-length protein, but it is then subjected to proteolytic cleavage, which produces three major fragments: an N-terminal fragment of approximately 180 kDa (N terminus to repeats 1 and 2), a central fragment of approximately 120 kDa (repeats 3 to 6), and a C-terminal fragment of approximately 100 kDa (repeat 7 and 8 to C terminus) [26]. An intermediate cleavage product of approximately 300 kDa, corresponding to the N-terminal fragment plus the central fragment, is also commonly detected using antibodies directed against the N terminus of Reelin. The protease that cleaves Reelin has not been identified, but pharmacological studies suggest it may be a metalloproteinase [27]. Functional studies have demonstrated that