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Apoer2: A Reelin Receptor to Remember

The extracellular protein Reelin is crucial for neuronal positioning during brain development, but its expression persists long after cell migration is completed. In this issue of *Neuron*, Beffert et al. demonstrate that Reelin exerts an additional function in the mature brain, to modulate synaptic plasticity and to favor memory formation. This activity is carried out exquisitely by the Apoer2 receptor and critically requires the presence of an alternatively spliced exon. This exon encodes an intracellular domain that interacts with postsynaptic proteins and promotes binding and phosphorylation of NMDA receptors.

Our ability to acquire new information and to remember is dependent upon synaptic plasticity in brain regions, particularly the hippocampus, where neurons are known to modulate the strength of specific connections by regulating neurotransmitter release and/or postsynaptic responsiveness. Experience is envisioned to initiate molecular events that lead to potentiation or depression of synapses, thereby enabling learning and shaping our memories. A key molecular complex in this process is the NMDA receptor, which mediates calcium influx in response to glutamate release at excitatory synapses. The activity of the NMDA receptor is regulated at many levels, including subunit composition, which in turn is controlled by gene expression, splicing and protein trafficking, and phosphorylation. While the movement of calcium ions is elicited by glutamate binding to the NMDA receptor, postsynaptic responsiveness is also subject to tonic modulation by other factors. In this issue, investigators identify Reelin as a novel factor that modulates NMDA receptor activity and synaptic plasticity in the hippocampus (Beffert et al., 2005). They further elucidate the molecular mechanism of this previously unrecognized activity of Reelin and demonstrate the crucial involvement of a particular splice variant of Apoer2, one of two known high-affinity Reelin receptors.

Reelin is an extracellular protein essential for the development of laminated cortical brain structures in vertebrates (D'Arcangelo, 2005; Rice and Curran, 2001). Loss of Reelin in *reeler* mutant mice results in widespread neuronal ectopia and ataxic behavior. Reelin is secreted during embryonic brain development by cells that are critically positioned near the pial surface of the cortical structures where neuronal radial migration terminates and cellular layers are formed. Forward and reverse genetics in combination with biochemical studies led to the discovery of some components of Reelin signal transduction that are essential for cortical layer formation. These include two high-affinity Reelin receptors belonging to the lipoprotein receptor superfamily, the apolipoprotein E receptor 2 (Apoer2) and the very low density lipoprotein receptor (Vldlr) (D'Arcangelo et al., 1999; Hiesberger et al., 1999). These receptors are partially redundant, as double disruption of both corresponding genes is required to recapitulate the *reeler* phenotype, but disruption of the Apoer2 gene alone results in some detectable layering defects in the cortex and hippocampus (Trommsdorff et al., 1999). Thus, Apoer2 appears to be the dominant Reelin receptor, at least in the forebrain. Another essential component of the Reelin pathway identified by multiple genetic studies is Disabled-1 (Dab1). This adaptor protein becomes phosphorylated on tyrosine residues in response to Reelin by src family kinases (SRKs). Phospho-Dab1 then interacts with a variety of intracellular proteins that presumably carry out the molecular program necessary for the final stages of neuronal migration and the assembly of cellular layers (D'Arcangelo, 2005).

Apoer2 and Vldlr have very similar biochemical properties in the extracellular and transmembrane compartments (Beffert et al., 2004) and bind Reelin with similar affinities. On the intracellular side, they also share an internalization motif that is also required for Dab1 binding. The intracellular region of Apoer2, however, is subject to alternative splicing of exon 19 that generates receptor molecules containing or lacking a domain capable of binding additional adaptor proteins such as PSD-95 and JIP1/2. The significance of these interactions has been elusive…until now. In a series of elegant experiments, Beffert et al. show that exon 19 plays an essential role in the transmission of the Reelin signal that underlies learning and memory in the adult brain. The authors used a knockin strategy to generate strains of mice that always express or always lack the exon 19-encoded intracellular domain of Apoer2. Anatomically, these mice look normal. However, exon 19-deficient mice performed poorly in a series of behavioral tests designed to assay hippocampal-dependent associative learning. Building on the previous observation that recombinant Reelin can induce long-term potentiation (LTP) in hippocampal slices (Weeber et al., 2002), Beffert et al. further show that exon 19 of Apoer2 is required for this activity, consistent with behavioral studies. Field potential and whole-cell EPSC recordings further demonstrated that Reelin-induced LTP in the presence of the long form of Apoer2 is dependent on NMDA receptor activity (Figure 1).

How does Reelin affect NMDA receptor activity? The study by Beffert et al. points to at least two potentially related modalities. First, Apoer2 physically interacts with the NMDA receptor subunits NR2A and NR2B and may thus alter their conformation. This interaction could be solidified by PSD-95 at the synapse, since this protein can bind Apoer2 and the NMDA receptor subunits simultaneously. Furthermore, the authors show that Reelin induces tyrosine phosphorylation of NR2A and NR2B in hippocampal slices derived from mice expressing Apoer2 with the exon 19-encoded domain and not from mice lacking this region of the receptor. Even though the molecular details of this phosphorylation

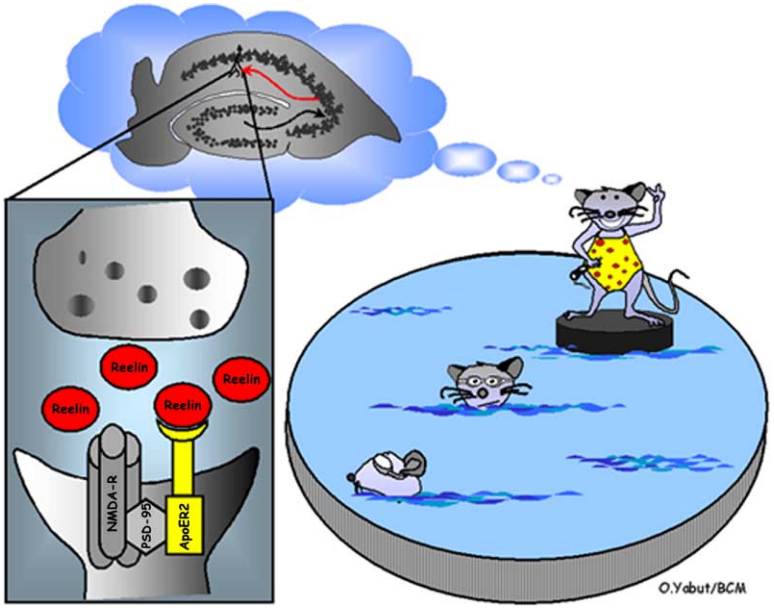

Figure 1. Interaction of Apoer2 with Reelin and with the NMDA Receptor at the Synapse Is Required for Hippocampal LTP and for Good Performance in Learning and Memory Tests

event have not yet been determined, the ability of Reelin to modify the NMDA receptor in a manner strictly dependent on the long form of Apoer2 represents a striking and truly exciting finding. The role of the exon 19-encoded domain does not appear to be in receptor trafficking, since both Apoer2 variants were detected at hippocampal postsynaptic densities, although it is not clear whether or not they are enriched in this compartment. Most likely, the intracellular domain mediates physical interaction with synaptic proteins such as PSD-95 and coupling of the Reelin signal with the NMDA receptor function.

Another exciting observation in the Beffert et al. study is that activity regulates Apoer2 splicing so that exon 19-containing receptors are produced preferentially when the animal is awake and feeding. This correlates with the need to learn and remember during periods of activity. How this splicing event is regulated is a complete mystery at this point, but a fascinating subject for further investigation.

An important aspect of the Beffert et al. study is the unequivocal demonstration of a role of Reelin in adult brain function. This function has long been suspected but never before directly shown in vivo. The observation that GABAergic interneurons express Reelin in the adult forebrain without regard to layer specificity initially raised suspicion that Reelin may play roles beyond the regulation of neuronal migration. Then came the observation that Reelin accumulates at postsynaptic densities in the hippocampus and neocortex. Third, Reelin was shown to induce LTP in hippocampal slices. A recent study further showed that Reelin induces changes in the composition of the NMDA receptor in hippocampal slices (Sinagra et al., 2005). The absolute requirement for Reelin signaling in brain development prevented an earlier discovery of its role in adult synaptic function, since homozygous mutant mice presented with gross disruptions of cellular organization. The sophisticated knockin approach of Beffert et al., however, circumvented this obstacle, since removal of the exon 19-encoded domain of Apoer2 did not interfere with Dab1 phosphorylation and layer formation and thus enabled the authors to study synaptic function and animal behavior without confounding factors such as abnormal anatomy or ataxia. It will be important in the future to confirm the present findings with direct demonstrations of the requirement of Reelin in learning and memory in vivo. This could be accomplished by a conditional knockout approach that removes Reelin expression in the postnatal brain. A similar approach could be used to explore the role of Dab1 in this adult brain function. Given the known requirement of Reelin in neuritic outgrowth in the developing postnatal hippocampus, future studies should also elucidate whether Reelin regulates synaptogenesis or synapse stability and whether Apoer2 is important for synapse development.

Postnatal expression of Reelin is particularly elevated in the primate and human brain, where the protein has been detected in several axonal tracts, in the neuropil as well as selected neuronal populations in addition to GABAergic neurons (Martinez-Cerdeno et al., 2002). These reports and the present findings may be relevant to human neurological disorders and mental illnesses that have a cognitive component, such as schizophrenia. In fact, several studies have shown that in postmortem brains of schizophrenic patients *REELIN* mRNA expression is reduced as a result of promoter hypermethylation (Grayson et al., 2005). This epigenetic mechanism may thus modulate Reelin-dependent synaptic function in the hippocampus and perhaps in other brain regions, leading to cognitive impairment. In light of the findings of Beffert et al., it will be interesting to examine Apoer2 splicing in the brain of schizophrenic patients as well as in other patient populations affected by learning and memory defects, such as Alzheimer's disease.

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Selected Reading

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