Lipoprotein Receptors

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Lipoprotein Receptors: Signaling Functions in the Brain?

Minireview

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While some nutrients enter cells through transporters, others, including iron and cholesterol, are actively imported into cells by specialized receptors. These import receptors continuously recycle between the cell surface and intracellular vesicles. Cargo is carried from the cell surface via clathrin-coated pits to endosomes, acidified compartments where the cargo is released. The receptors then return to the cell surface for another cycle of import. One import receptor, the low density lipoprotein receptor (LDLR), has been intensively studied because its malfunction is a cause of atherosclerosis. The LDLR is essential to transport LDL (complexes of cholesterol, triglycerides, and specific apolipoproteins) out of the plasma, as first shown by the hallmark analysis of familial hypercholesterolemia by Brown and Goldstein. In subsequent years, other import receptors related to the LDLR have been discovered, and the LDLR superfamily now includes at least five mammalian and several invertebrate proteins. It has become clear that most of the new members of the family do not have a primary function in LDL import, and instead bind and import multiple ligands (see Figure 1). Indeed, the ability to transport LDL is a recent evolutionary adaptation of an ancient receptor family. Even so, there have been few reasons to suspect that these proteins regulate cellular events during development. Thus, it is remarkable to discover that the ablation of two LDLR family members (VLDLR and ApoER2) causes very specific alterations in the development of the nervous system (Trommsdorff et al., 1999 [this issue of *Cell*]). Moreover, the characteristic changes in the mutant brains indicate that neurons interpret or respond to positional information with the help of VLDLR and ApoER2, which may therefore be signaling receptors. These findings herald a new opportunity for interactions between researchers in atherosclerosis and neurobiology.

The Phenotype of VLDLR$^-$ apoER2$^-$ *Mice*

Brain development is markedly altered in mice lacking both VLDLR and ApoER2 (Trommsdorff et al., 1999). The double mutants have obvious and highly characteristic defects in neuronal layering in cerebral cortex, hippocampus, and cerebellum. Detailed analysis shows that the *VLDLR$^-$ apoER2$^-$* phenotype is virtually identical to that seen when either of two other genes, *reln* (reelin) or *dab1* (mammalian disabled-1), is mutated. Extensive neuroanatomical and cell marking analysis of *reln* mutant mice, and more recent analysis of *dab1* mutant mice, has shown that the defective layering is due to misplacement of committed neurons (Goldowitz et al., 1997; Gonzalez et al., 1997; Howell et al., 1997; Lambert de Rouvroit and Goffinet, 1998). That is, each neuron adopts its correct fate but in the wrong place. This occurs most conspicuously in the cerebral cortex, hippocampus, and cerebellum. Each of these parts of the brain has a characteristic architecture of packed layers of cells with specific functional identities. In *VLDLR$^-$ apoER2$^-$*, *reln$^-$*, and *dab1$^-$* brains, these layers are disorganized.

For example, the Purkinje cells of the cerebellum normally migrate outward from an origin near the fourth ventricle, to form a thin layer beneath cerebellar granule cell precursors. In the mutants, the Purkinje cells initiate migration but remain clustered deeper in the cerebellar primordium (Lambert de Rouvroit and Goffinet, 1998; Trommsdorff et al., 1999). Since the proliferation of granule cells requires mitogens, such as Sonic hedgehog, made by the Purkinje cells, the number of granule cells is markedly reduced in the mutants, and the mutant cerebella are about five times smaller than usual.

The development of the cortex is orchestrated differently (Pearlman et al., 1998). Early neurons form two layers, an inner subplate and an outer layer of Cajal-Retzius (CR) neurons. Later neurons, which will form the cortical plate of the embryonic cortex, move through the subplate layer and come to rest below the CR cells. As each subsequent cortical plate neuron migrates, it bypasses its predecessors, coming to rest immediately below the CR neurons. In the mutants, the migrating cells neither penetrate between subplate neurons nor overtake their brethren (Pearlman et al., 1998; Rice et al., 1998). The CR, subplate, and early cortical plate neurons remain superficial to subsequent generations of cortical plate neurons, which pile-up underneath them

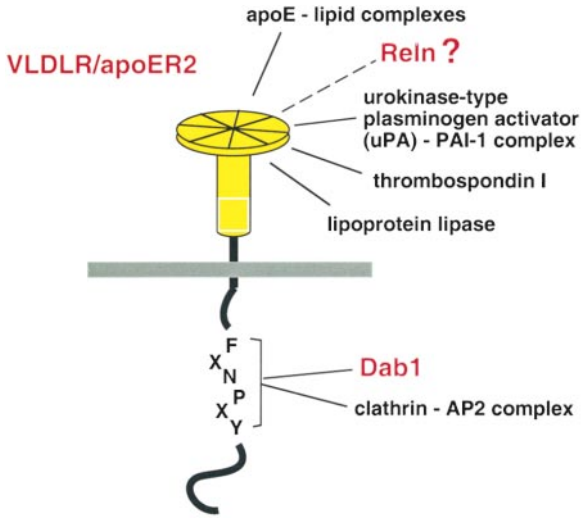

Figure 1. Binding Interactions of the Extracellular and Intracellular Domains of VLDLR and ApoER2

Additional ligands for the extracellular domain that are not shown here include other serine protease–protease inhibitor complexes and a protein named RAP that is normally retained in the endoplasmic reticulum and probably assists extracellular domain folding. The direct or indirect binding of Reln to the extracellular domain is currently speculation. Reln may instead bind to other cell surface signaling molecules, or may bind to both VLDLR/ApoER2 and unknown coreceptors.

as the brain expands. It is clear that neurons in the affected regions of the brain can and do migrate, but they seem to lack the ability to sense their position and respond appropriately.

A Reln–Dab1 Signaling Pathway in Brain Development

The characterization of the *reln* and *dab1* genes has made it possible to interpret the complex neuroanatomical defects of the mutants in terms of a signaling pathway. The *reln* gene encodes a large glycoprotein (Reln) that is secreted by specialized neurons within restricted regions of the brain (D'Arcangelo et al., 1995; Ogawa et al., 1995). In the cerebellar primordium, Reln is made by the external layer of granule cells, and by the deep nuclei, below the Purkinje cells, but not by the Purkinje cells themselves. This suggests that Purkinje cell position is influenced by external signals, in the form of Reln.

In the cortex, Reln is made by the CR neurons but not by the migratory cells (D'Arcangelo et al., 1995; Ogawa et al., 1995). Early in development, it induces a change that allows cortical neurons through the subplate. Later, as the CR neurons are displaced outward, the zone of Reln production marks the stopping point for each wave of migrating cortical neurons. Reln may instruct these neurons to stop migrating and to allow subsequent arrivals to pass by. Thus, in *reln*$^-$ animals, cortical plate neurons can migrate but cannot pass through the subplate or overtake other cortical plate neurons. There is also evidence that Reln influences the targeting of specific axons, suggesting that Reln may also mark the stopping points for axonal growth cones (Del Río et al., 1997).

The cellular responses induced by Reln, and how they contribute to the anatomical defects, are still mysterious. In principle, changes in motility, adhesion, or gene expression (e.g., of chemotaxis receptors) could be important. One clue is that neurons from *reln*$^-$ brains have increased homotypic adhesion in vitro (Hoffarth et al., 1995; Ogawa et al., 1995). In vitro assays for Reln-regulated cell migration are currently unavailable but will be crucial to understanding the chain of events.

The *dab1* gene encodes a cytoplasmic, tyrosine-phosphorylated protein that is found in the neurons whose migrations are altered in each part of the brain, including Purkinje cells and cortical plate neurons (Howell et al., 1997; Rice et al., 1998). Therefore, Dab1 seems likely to be part of the response to a signal from Reln. Indeed, Dab1 tyrosine phosphorylation is stimulated directly by Reln in neuronal cultures, and Dab1 tyrosine phosphorylation is reduced in the brains of embryos that lack Reln (Howell et al., 1999). Thus, Reln is likely to act, in whole or in part, by stimulating tyrosine phosphorylation of Dab1. Consistent with this implied order of events, Dab1 is not required for Reln expression, and Reln has no detectable Dab1-independent functions. In addition, Reln regulates Dab1 protein levels, such that Dab1 protein levels increase conspicuously in brains that lack Reln (Rice et al., 1998; Howell et al., 1999).

Relative Roles of VLDLR and ApoER2 in Brain Development

Although *VLDLR*$^-$ *apoER2*$^-$ mice phenocopy *reln*$^-$ and *dab1*$^-$ mutants, analysis of single mutants suggests that the VLDLR is more important in the cerebellum while ApoER2 is more important in the cortex (Trommsdorff et al., 1999). This is consistent with higher expression of VLDLR, relative to ApoER2, in the cerebellum than in the cortex. Therefore, VLDLR and ApoER2 may be functionally redundant in a mechanistic sense, with each receptor working identically and its relative importance determined solely by its abundance. However, the simple hypothesis of a common molecular mechanism of action raises the question of why closely related proteins that are also expressed in the developing cortex and cerebellum, such as LRP and the LDLR, do not compensate for lack of VLDLR and ApoER2 in the mutants. On the other hand, it is also possible that the VLDLR and ApoER2 have distinct molecular mechanisms of action in the cerebellum and cortex, respectively. For example, they may interact with cerebellum-specific and cortex-specific effectors, respectively. "Knocking-in" one receptor gene into the other's genomic locus may be needed to resolve the question of redundant versus specialized functions.

VLDLR and ApoER2 Are Likely to Be Involved in the Reln Response

In support of the functional evidence that Reln, Dab1, VLDLR, and ApoER2 may be on the same pathway, biochemical studies suggest that there may be a direct physical link between VLDLR or ApoER2 and Dab1 (see Figure 1). The Dab1 protein contains a domain that binds specifically to an FXNPXY signal present in all LDLR family proteins (Trommsdorff et al., 1998). This sequence is also required for LDLR internalization, since it associates with clathrin and adaptor proteins (Kibbey et al., 1998). Although a complex between VLDLR or ApoER2 and Dab1 has not yet been demonstrated in neurons, such complexes do form in vitro and in heterologous cells, and would provide a molecular mechanism for signals to be transmitted between the receptors and Dab1. It would also suggest that the receptors would work in the cells that express Dab1. Indeed, VLDLR and ApoER2 are expressed by the neurons that express Dab1, as well as other cells in the cerebellum and cortex, and are not expressed in the cells that make Reln (Trommsdorff et al., 1999). As in the *dab1*$^-$ brain, *VLDLR*$^-$ *apoER2*$^-$ neurons localize as though they have not sensed the Reln signal. In addition, Dab1 protein levels are increased, as in a *reln*$^-$ mutant. Coupled with the direct binding of Dab1 to the receptors, these results suggest that VLDLR and ApoER2 are part of the Reln response pathway, although a parallel pathway cannot be rigorously excluded at this time.

Molecular Models for Signal Relay

Assuming a single signaling cascade raises the question: Do the receptors regulate Dab1, or conversely, does Dab1 regulate the receptors?

An intriguing possibility is that VLDLR and ApoER2 may be cell surface receptors or coreceptors for Reln (Figure 2, left). If Reln does bind to the extracellular domain of either VLDLR or ApoER2, it may allow the receptor to bring its associated Dab1 close to a tyrosine kinase for phosphorylation. Such a kinase may be contained in the cellular compartment through which the receptors cycle, such as coated pits, or may be supplied by a Reln coreceptor that has associated tyrosine kinase activity. It remains to be seen whether Reln actually binds to VLDLR and ApoER2, and whether the receptors

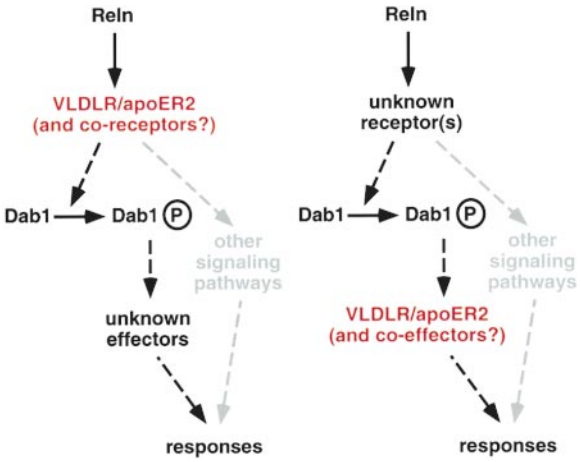

Figure 2. Alternative Schemes for the Reln Signaling Pathway

VLDLR/ApoER2 may serve as receptors (or coreceptors) for Reln, thereby inducing the tyrosine phosphorylation of Dab1 and cellular changes that cause neurons to position correctly in the brain (left panel). Alternatively, VLDLR/ApoER2 may be effectors (or coeffectors) for tyrosine-phosphorylated Dab1 (right panel). In either scheme, there may be additional signals induced by Reln that may cooperate with Dab1 to induce the response. A third scheme, not shown, would place VLDLR/ApoER2 on a parallel pathway that is required for the Reln response through Dab1 but is not necessarily regulated by Reln.

are needed for Reln to induce Dab1 tyrosine phosphorylation in vivo and in cultured neurons.

VLDLR and ApoER2 also have the potential to be effectors for the response (Figure 2, right). For example, they may regulate migration by controlling exocytosis of new membrane at the leading edge of the cell. Dab1 could regulate membrane trafficking by binding to the internalization signals of VLDLR and ApoER2 and interacting with the vesicle transport machinery. In addition, VLDLR has been shown to be a receptor for proteins that regulate cell motility in other systems. The motility of cultured epithelial cells is increased when the urokinase-type plasminogen activator (uPA) binds to the cell surface uPA receptor (uPAR) (Webb et al., 1999). The uPAR lacks internalization signals, but the VLDLR acts as a coreceptor for internalization. VLDLR-mediated clearance of uPA and uPAR from the surface inhibits motility. If these cell culture experiments are relevant to neuronal migrations in vivo, these observations suggest that VLDLR and ApoER2 could play an effector role in a Reln–Dab1 signaling pathway by altering cell surface levels of proteases required for motility.

Involvement of Lipoproteins

Whether the receptors are upstream, downstream, or parallel to the Reln–Dab1 pathway, it is fairly certain that lipoproteins will not play an essential regulatory role in the phenotype. The only lipoproteins that bind to both VLDLR and ApoER2 have apolipoprotein E (ApoE) as their protein component. An *apoE*$^{-}$ knockout mouse has been made, and does not show any obvious abnormalities in brain development, although neurodegenerative symptoms are detected in later life (Masliah et al., 1995). This suggests that ApoE lipoproteins will not play an instructive role in neuronal migrations. On the other hand, lipoproteins may regulate the levels of VLDLR and ApoER2 available for interaction with other ligands, and thus modify the response to Reln. As a corollary, Dab1 and similar proteins that bind the internalization signal of LDLR relatives could regulate receptor trafficking, and thus modify lipoprotein internalization kinetics. These possibilities remain to be explored.

Implications for Development and Aging

Mutations in some other genes cause phenotypes that are reminiscent of some aspects of the *reln*$^{-}$, *dab1*$^{-}$ and *VLDLR*$^{-}$ *apoER2*$^{-}$ phenotype, suggesting they may be involved in the same signaling events. These genes encode a serine/threonine kinase, Cdk5, its stimulatory subunit, p35, and the integrin α3 (Ohshima et al., 1996; Chae et al., 1997; Anton et al., 1999). This suggests that serine/threonine phosphorylation and integrin function could be regulated by Dab1. However, these mutations do not give perfect phenocopies, suggesting that they may act on parallel pathways or on branches in the Reln–Dab1 pathway that are important in some cell types but not others. The existence of parallel or branched pathways may become apparent if compound homozygotes show synthetic phenotypes, but these crosses have not yet been reported. In addition, a number of human genetic disorders cause defects in neuronal migration, but none of the mapped genes has been associated with the Reln signaling pathway. Defects in the Reln pathway may have more serious consequences in human development and lead to embryonic death.

In addition to a role in development, there is reason to believe that pathways regulated by VLDLR and ApoER2 may also be involved in neurodegenerative diseases. Individuals with specific alleles of *apoE*, *app*, *ps1*, or *ps2* are predisposed to Alzheimer's disease (Tanzi et al., 1996). The *apoE* gene encodes ApoE, which can bind to VLDLR or ApoER2. The *app* gene encodes the amyloid precursor protein (APP), a transmembrane protein that contains a YXNPXY internalization signal that binds Dab1. The *ps1* and *ps2* genes encode presenilins; apparent proteases or protease regulators involved in cleaving APP. The roles for Reln, Dab1, and the ApoE receptors in adult animals is presently unknown but now seems ripe for exploring.

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