

ROLE OF MEMBRANE PHOSPHOINOSITIDES IN REGULATION OF REELIN SIGNALING

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Development of the mammalian brain is a complex process involving proper migration and positioning of billions of neurons that ultimately form distinct layers in various brain structures. Studies of mice with neurological defects have led to identification of numerous signaling pathways that are required for normal mammalian brain development. One such pathway is called the Reelin pathway. Reelin is a secreted glycoprotein that serves as a ligand for very low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) in brain. Effect of reelin is mediated through an intracellular protein named Disabled 1 (Dab1), which associates with intracellular tails of the lipoprotein receptors upon reelin binding. Mice that lack reelin, Dab1 or both VLDLR and ApoER2 display similar neuroanatomical defects, indicating the linear nature of this pathway. The hallmark feature of this pathway is phosphorylation of Dab1 on multiple tyrosine residues induced by Reelin, which is mediated by Src family kinases. This tyrosine phosphorylation is thought to be critical as it is able to transmit the reelin signaling to downstream by recruiting Src homology 2 (SH2) domain containing proteins.

While tyrosine phosphorylation of Dab1 is critical to its function, little is known about the mechanisms that regulate Dab1 phosphorylation. A phosphotyrosine binding domain (PTB) within the amino terminal region of Dab1 binds to the cytoplasmic tails of the lipoprotein receptors. In addition to a binding pocket that recognizes the NPXY sequence motifs within the receptor tails, Dab1 PTB domain contains a Pleckstrin Homology (PH) superfold that binds to the phospholipid phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P₂). Since PtdIns-4,5-P₂ is enriched in the cell membrane, we investigated the role of the PH superfold in regulation of Dab1 membrane localization and phosphorylation. Here, we show that disruption of Dab1-phospholipid interaction abrogates both membrane localization of Dab1 as well as its basal and Reelin-induced phosphorylation. Our results suggest that phospholipid-binding is the first critical step in recruitment of Dab1 to the plasma membrane followed by its activation in response to Reelin stimulation. The data also raises the possibility that this pathway may be modulated through regulation of PtdIns-4,5-P₂ concentration in certain membrane compartments, such as lipid rafts, that may harbor components of the Reelin pathway. Furthermore, accumulation of PtdIns-4,5-P₂ within the leading edge of a neuron may establish the directionality of its migration in response other positional cues.