Proteomic Identification of CrkL-SH3 Binding Proteins from Embryonic Murine Brain: Implications for Multiprotein Complexes in Reelin Signaling

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Cells respond to a vast array of extracellular cues that regulate their intracellular activities and thereby alter their functions and fates. Many extracellular cues transduce their signal inside of the cell by initiating signaling cascades involving reversible tyrosine phosphorylation. Tyrosine phosphorylation may alter a protein's activity directly or it may serve as a docking site to which proteins with phosphotyrosine-binding domains are recruited. The Crk and CrkL (Crk/L) family of adaptor proteins have an amino-terminal Src Homology 2 (SH2) domain which can bind to phosphotyrosine. At their carboxyl-termini Crk/L have SH3 domains which are known to bind proline-rich regions in a number of proteins. Thus extracellular signals that induce tyrosine phosphorylation enable the recruitment of Crk/L proximal to the site of stimulation. This concomitantly can recruit cargo proteins bound to the Crk/L-SH3 domains which may be effector proteins of the signaling cascade. The signaling cue Reelin is essential for proper positioning of neurons during embryonic brain development in vertebrates. Reelin binds to its receptors and clusters them into a macromolecular signaling complex. This leads to the activation of Src Family Kinases (SFKs) and induces tyrosine phosphorylation of Disabled-1 (Dab1). Crk/L are then recruited to phosphorylated Dab1. Recent work has shown that mice conditionally deficient in Crk and CrkL exhibit some of the neuronal mis-positioning phenotypes observed in mice deficient in Reelin, Reelin receptors, SFKs, Dab1 or knock-in mice expressing a form of Dab1 that cannot be phosphorylated on tyrosine. This leads to the hypothesis that Crk and CrkL-SH3 binding proteins are recruited to the Reelin signaling complex where they become locally activated to effectuate the Reelin signal. Here we describe biochemical and proteomic approaches leading to the identification of a number of CrkL-SH3 binding proteins from embryonic murine brain. We discuss these protein complexes and their implication for Reelin signaling.