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Identification of Src, Fyn, and Lyn SH3-binding proteins: implications for a function of SH3 domains

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Src homology 3 (SH3) domains mediate protein-protein interactions necessary for the coupling of cellular proteins involved in intracellular signal transduction. We previously established solution- binding conditions that allow affinity isolation of Src SH3-binding proteins from cellular extracts (Z. Weng, J. A. Taylor, C. E. Turner, J. S. Brugge, and C. Seidel-Dugan, J. Biol. Chem. 268:14956-14963, 1993). In this report, we identified three of these proteins: Shc, a signaling protein that couples membrane tyrosine kinases with Ras; p62, a protein which can bind to p21rasGAP; and heterogeneous nuclear ribonucleoprotein K, a pre-mRNA-binding protein. All of these proteins contain proline-rich peptide motifs that could serve as SH3 domain ligands, and the binding of these proteins to the Src SH3 domain was inhibited with a proline-rich Src SH3 peptide ligand. These three proteins, as well as most of the other Src SH3 ligands, also bound to the SH3 domains of the closely related protein tyrosine kinases Fyn and Lyn. However, Src- and Lyn-specific SH3-binding proteins were also detected, suggesting subtle differences in the binding specificity of the SH3 domains from these related proteins. Several Src SH3-binding proteins were phosphorylated in Src-transformed cells. The phosphorylation of these proteins was not detected in cells transformed by a mutant variant of Src lacking the SH3 domain, while there was little change in tyrosine phosphorylation of other Src-induced phosphoproteins. In addition, the coprecipitation of v-Src with two tyrosyl-phosphorylated proteins with M(r)s of 62,000 and 130,000 was inhibited by incubation with a Src SH3 peptide ligand, suggesting that the binding of these substrate proteins is dependent on interactions with the SH3 domain. These results strongly suggest a role for the Src SH3 domain in the recruitment of substrates to this protein tyrosine kinase, either through direct interaction with the SH3 domain or indirectly through interactions with proteins that bind to the SH3 domain.