[Frontiers in Bioscience 7, d164-203, January 1, 2002]

THE EFFECTS OF PHOSPHORYLATION ON ADAPTOR PROTEIN FUNCTION

Lidia Cherezova, Amanda Gatesman and Daniel C. Flynn

The Mary Babb Randolph Cancer Center and the Department of Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV

TABLE OF CONTENTS

16. References

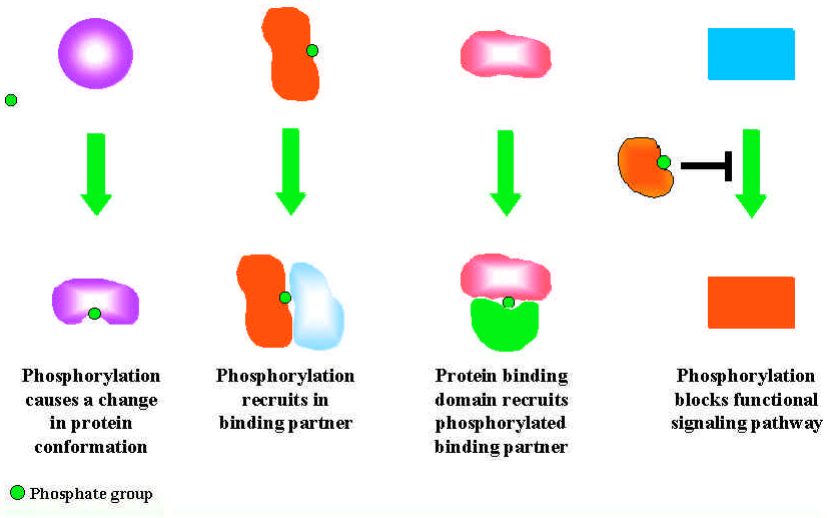

Figure 1. Four mechanisms in which phosphorylation affects protein function.

1. ABSTRACT

Adaptor proteins are specialized protein binding partners that serve to link signaling proteins to each other, as a mechanism to propagate a cellular signal. Ultimately, these signals are required for a specific biological response. Thus, it is important that the cell develop mechanisms to regulate these signaling cascades. One way these cascades can be regulated is through post translational modifications of adaptor proteins which would regulate their ability to forge protein-protein interactions. In this review, we summarize the effects of serine/threonine and tyrosine phosphorylation on adaptor protein function, with a specific focus upon those adaptor proteins in which phosphorylation has been demonstrated to regulate a signaling cascade or biological response.

2. INTRODUCTION

Adaptor proteins are defined as intracellular proteins that contain modular domains capable of recruiting additional signaling molecules, but exhibit no enzymatic activity (1). These proteins function by linking signaling molecules in such a way as to propagate a cellular signal, resulting in a biological outcome. Biological responses must be regulated and adaptor protein interactions with appropriate binding partners are also regulated, e.g., by changes in cellular localization that permit approximation to binding partners, changes in steady-state expression levels and through post translational modifications. It is the latter that is the subject of this review. Specifically, we will focus on the role of phosphorylations in modulating the activity of adaptor proteins and their ability to interact with cellular binding partners.

We consider that phosphorylation could modulate adaptor protein function in 4 significant ways (Figure 1). Here, phosphorylation could (a) alter the conformation of the adaptor protein, which in turn would alter its ability to interact with binding partners, (b) create a protein binding

motif, (c) through known protein binding modules such as SH2 or PTB, enable a protein to seek out and bind to a phosphorylated binding partner, or (d) alter the properties of an adaptor protein enabling it to impede or block the progression of signals in a signaling pathway. There are two types of phosphorylation events to cover in eukaryotes - serine/threonine and tyrosine phosphorylation. In this review, we attempt to comprehensively cover how phosphorylation events alter the function of adaptor proteins. We initiate this review with the effects of tyrosine phosphorylation on adaptor protein function, then segue into the well studied signaling cascades associated with T cell and B cell signaling that are dependent upon the function of several adaptor proteins. Finally, we discuss the role of serine/threonine phosphorylation in regulating adaptor protein function. Each of these two topics are covered within the context of the 4 ways we envision phosphorylation effecting the function of adaptor proteins. We acknowledge that phosphorylation may alter adaptor protein function in ways that we have not envisioned; however, for simplicity, we group these effects of phosphorylation into these 4 main categories.

Tyrosine phosphorylation plays an important role in the function and regulation of several adaptor proteins. Many adaptor proteins contain either a modular protein binding domain for mediating protein interactions with phosphorylated substrates, tyrosine residues that, upon phosphorylation, recruit in binding partners with modular binding domains, or both. Two modular domains that are important in modulating adaptor protein function are the SH2 domain and the PTB domain. The Src homology 2 (SH2) domain is a globular protein interaction module that directs protein-protein interactions (2,3,4,5). Proteins containing these domains are involved in the control of biochemical pathways involving phospholipids metabolism, activation of Ras-like GTPases, gene expression, protein trafficking, tyrosine phosphorylation and dephosphorylation, and cytoskeletal rearrangements (3). SH2 domains bind to relatively conserved sequences

Table1. Signaling proteins that are involved in cellular signaling cascades

Protein	Binding Motif(s)	Consensus Binding Domain	Associated Proteins	References
Abp1/SH3P7/HIP55	pY sites	pY-x-x-P	?	427,428,429
APS	SH2, pY sites	Unknown	PDGFR, Cbl, EpoR, InsR, Grb2	430,431,432
BKS	SH2-like, pY sites	Unknown	BRK	433
Cbl	PTB, pY sites	PTB - D-(N/D)-x-pY PY site – Y-D-V-P	Syk, ZAP-70, Src, p85 PI3K, Crk, Vav, 3BP2, Grb2	106,102
CIN85	pY sites	--------	BLNK	434
CMS/CD2AP	SH3	--------	PY-Cbl	435
Crk/CRKL	SH2	pY-x-x-P	STAT5, Cbl, IRS1/4, Gab, p130Cas, paxillin	8,436,6
Dos (Drosophila)	pY sites	--------	Corkscrew (drosophila)	436,210,437
Gab1, 2	SH2 binding motif	--------	Crk/CRKL, Grb2, PI3K, PLC-γ, SHP-2, Shc	438,439
Grb2/Drk (Drosophila)	SH2	pY-(Q/Y/V)-N-(Y/Q/F)	Shc, Cbl, LAT, SHP-2	210,206
Grb7	SH2	No consensus binding sequence	EGFR, SHPTP2, Shc, Rnd1, Ret, c-Kit, Raf-Mek1, FGFR1, PDGFR, erbB2/erbB3, EphB1, Tek/Tie2, InsR, FAK	422
IRS	PTB	N-x-P-pY	InsR, Grb2, SHP-2	440,441
Nck	SH2	pY-x-x-P	SLP-76, HPK1, IRS-1, Dok, Cas, HGFR, VEGFR, PDGFR, Bcr-Abl, EphB1	94,283
NSP	SH2	Unknown	EGFR	442
PECAM-1	pY sites, ITIM	--------	SHP-1/2, Src	188,191,199, 194
SH2-B	SH2, pY sites	Unknown	Ins R, JAK2, TrkA, FcεRI-gamma	443,444,445
SH2D1A/SAP/DSHP	SH2, ITSM	ITSM – T-x-Y-x-x-(V/I)	SHP-2, SHIP, SLAM, 2B4	446,447,448
Shc	PTB, SH2	SH2 – pY –(I/E/Y/L)-x-(I/L/M) PTB - x-ϕ-x-N-P-x-pY	Grb2, NGFR, EGFR, SHIP, P85 PI3K	210,67,449,79

SHPS-1/2	pY sites	PY-x-x-(L/V/I)	SHP-1/2	436,450
Vav	SH2	pY-(M/L/E)-E-P	CD19, Slp-76, BLNK, EGFR, PDGFR, Syk, Zap-70, Cbl	210,25

of a phosphorylated tyrosine residue followed by specific residues (usually at position +2 to +5) that dictate binding specificity (6), and have relatively no affinity for unphosphorylated peptides (3). Binding of phosphorylated SH2-binding motifs to the SH2 domain can affect its function in several ways, including the direct stimulation of enzymatic activity, enhanced tyrosine phosphorylation, and relocalization of the protein to a different sub-cellular site (3). The phosphotyrosine-binding domain (PTB) is similar to the SH2 domain in that both domains recognize phosphorylated tyrosine consensus sequences. However, PTB domains recognize amino acids that are located amino terminal to the phosphotyrosine-binding pocket, and these residues are essential for determining PTB-binding specificity.

Table 1 summarizes several adaptor proteins that are substrates for tyrosine phosphorylation and become binding partners for signaling proteins that contain SH2 and/or PTB domains, as well as SH2 and PTB domain-containing proteins. We summarize the effects of tyrosine phosphorylation on adaptor proteins where phosphorylation is known to affect function.

3. ADAPTOR PROTEINS THAT UNDERGO A CONFORMATIONAL CHANGE IN RESPONSE TO TYROSINE PHOSPHORYLATION

3.1. Crk/CRKL

Crk (chicken tumour virus no. 10 regulator of kinase) was first identified as a fusion gene (*v-Crk*) of the viral *gag* gene and a cellular gene that, when transfected into chicken embryo fibroblasts, leads to the activation of cellular tyrosine kinases and the expression of a transformed phenotype (7). Crk is expressed as two distinct proteins of 28 (Crk-I) and 40 kDa (Crk-II) (8). Both of the Crk proteins contain an SH3 domain and an SH2 domain mediating protein-protein interactions important in the assembly of multimeric signaling complexes (8). Crk is homologous to the CRKL (Crk-like) protein; however, they originate from different genes (6). Crk-II and CRKL contain a tyrosine residue at position Y221 (Y207 in CRKL) that is absent in Crk-I, and the sequence surrounding Y221 represents the major tyrosine phosphorylation site in Bcr-Abl expressing B cells (9). Feller et al. (10) predicted that phosphorylation of Y221 resulted in the ability of c-Crk-II to bind to its own SH2 domain, blocking access of its SH3 domain from binding other proteins. Several studies have looked into the regulation of Crk signaling by folding (11,12,13,14,15,16,17,18) and their results support the inhibitory role of pY221 in Crk signaling. However, many of these studies used cell lines expressing high levels of protein and receptors, and may not be reflective of true *in vivo* conditions (6).

Alternately, Crk and CRKL do have highly homologous SH2 domains, and appear to have similar SH2-directed binding specificities, as many of the SH2 protein-binding partners of Crk have also been shown to co-immunoprecipitate with CRKL (19). The SH2 domains of Crk/CRKL have been shown to interact with phosphorylated tyrosine residues in Cbl, STAT5, p85 PI3K, Gab2, p62dok, IRS1/4, FRS2, p130Cas, ZAP-70, and paxillin in response to a number of cellular stimuli, including T cell, B cell, cytokine, hormone, and growth factor receptor engagement (20,21,22,6,23). One attractive hypothesis is that upon receptor activation, Crk becomes tyrosine phosphorylated on Y221, resulting in a conformational change that blocks access of the SH3 domain from other adaptor proteins, effectively desensitizing the Crk signaling pathway (6). Recently, Guris and colleagues were able to develop a Crk-deficient mouse model containing a targeted disruption in the *crkl* gene (24). These mice show severe defects in neural crest development, which mimics the phenotype of mice with DiGeorge syndrome. Individuals with this phenotype rarely survive until birth. Tissues and organs that depend on neural crest cells for development (thymus, thyroid, parathyroid, and head skeletal structures) were also affected. There is no mouse model to date with a targeted disruption of the *crk* gene (6). These data position Crk and CRKL as an essential adaptor protein, important in embryonic development.

3.2. Vav

The Vav family of proteins currently consists of three members: Vav, Vav2 and Vav3. The protein domain structure of Vav is diverse, containing a calponin-homology (CH) domain, an acidic region, a Dbl- and pleckstrin-homology domain (DH and PH), as well as an SH2 domain flanked by two SH3 domains (25). The most characterized role of Vav proteins is their activity as guanosine nucleotide exchange factors for Rho/Rac proteins (26,27,28,29). Vav enzyme activity catalyzes the release of GDP from inactive GTPases, favoring the binding of GTP and the concomitant change in protein conformation that allows the active GTPases to bind their specific substrate (30). The catalytic activity of Vav is modulated by tyrosine phosphorylation, which is specific to Vav (26,27,28,29). In the unphosphorylated state, tyrosine 174 contacts residues in the Rac1 binding site of the catalytic domain, directing the formation of a closed protein conformation. In this conformation, Vav is unable to interact with substrate (31). Tyrosine phosphorylation of Y174 causes a steric clash between the phosphate group and several residues present in the DH domain of Vav, resulting in an open protein conformation that is capable of binding Rac1 (31). It is possible that other residues in the amino terminal portion of Vav also contribute to its auto-inhibitory state, and in accordance with this theory, deletions in the amino terminal CH domain result in the oncogenic activation of Vav, even when the acidic domain and the inhibitory Y174 are present (32,29).

Besides functioning as a guanosine nucleotide exchange factor, several studies discussed below have implicated Vav as an important adaptor protein in signaling

pathways in hematopoietic cells. In B and T cells, Vav is instrumental in the activation of NF-kappaB and NF-AT, a transcription factor that participates in IL-2 gene transcription. Also, upon lymphocyte activation, Vav is involved in the induction of strong calcium fluxes (33,34,35,36,37,38). In B cells, tyrosine phosphorylation of Vav is dependent upon its interaction with tyrosine residues in the cytoplasmic tail of the transmembrane adaptor protein CD19 (39,40,41). The interaction with CD19 localizes Vav with the receptor associated tyrosine kinases Syk and Lyn. Mutation of the Vav binding site in CD19 (Y^{391}EEP) results in the abrogation of both Vav binding and CD-19-mediated responses (39,40), indicating that the Vav/CD19 interaction is required for both Vav tyrosine phosphorylation and the activation of CD19 mediated downstream signaling. In T cells, Vav tyrosine phosphorylation is elevated upon the co-engagement of the CD28 receptor and the TCR, as well as the presence of LAT (42). Vav localization to CD28 and LAT occurs through SH3-mediated complex formation with Grb2, which functions as a bridge molecule (43), placing Vav in the proximity of ZAP-70 kinase (44,25).

The SH2 domain in Vav has been shown to form complexes with several other adaptor proteins in both B and T cells. In T cells, Vav can interact with SLP-76, an adaptor protein that is important in transducing signals from TCR-related tyrosine kinases to downstream effector molecules (45,46,47,48). While the exact mechanism of Vav/SLP-76 signaling remains unclear, it is evident that these proteins are involved in regulating PLCγ activity, calcium mobilization, activation of MAPK/Erk pathway, and the activation of NF-AT in response to TCR-mediated activation (34,49,50). Because BLNK is highly homologous to Slp-76, it is likely that these pathways are conserved in B cells as well (25). Vav can also complex with other SH2 domain-containing proteins including Shc and p85 PI3K; however, the role of these associations have not been elucidated (28,51,25).

4. ADAPTOR PROTEINS THAT CONTAIN MODULAR DOMAINS THAT RECRUIT BINDING PARTNERS

4.1. Shc

Shc is a prototype adaptor protein that has been useful in the understanding of adaptor protein function in cellular signaling cascades (52). To date, three *shc* genes have been identified, and their protein products, ShcA, ShcB, and ShcC, appear to have a conserved protein domain structure (53,54). In mammals, ShcA is ubiquitously expressed, while ShcB and ShcC appear to be found only in neuronal cells (54,55). ShcA can be further classified into three different isoforms of 46, 52, and 66 kDa (56,57). All of the Shc isoforms contain both an SH2 domain and a phosphotyrosine-binding (PTB) domain separated by a central linker region with several essential tyrosine phosphorylation sites (54).

The main function associated with Shc is the recruitment and binding of specific signaling molecules into larger complexes, thereby linking cell surface receptor stimulation to downstream effector molecules. Earlier studies indicated that the SH2 domain of Shc appeared to be important for the recruitment of Shc to cell surface receptors, including EGFR, PDGFR, and the TCR-ζ chain (57,58,59,60). However, the SH2 domain alone is not responsible for Shc localization to the TrkA receptor or the polyoma middle T antigen (61,62). Further investigation led to the discovery of an amino-terminal PTB domain in Shc, capable of binding phosphorylated tyrosine residues N-terminal to the pY (63,64,65,66,67,68). The PTB domain of Shc has been reported to bind to several tyrosine-phosphorylated proteins, such as EGFR, NGFR, the insulin receptor (69,68,63,70), and also acidic phospholipids like PI and PIP3 (67,71,72).

The CH1 (collagen homology 1) domain of p52Shc contains three critical tyrosine residues (Y239, Y240, and Y317) that become phosphorylated in response to a variety of signals, including a growth factor and cytokine receptor stimulation, integrin engagement, and G-protein coupled receptor engagement (73,53,52). Mutation of Y317 alone or Y239/Y240/Y317 combined results in a dominant negative effect in Shc signaling (74,75,76,77,78,79,52). Tyrosine phosphorylation of these sites in the CH1 domain creates binding sites for the SH2 domain of Grb2. Grb2, in turn, binds to the Ras nucleotide exchange factor Sos, localizing this complex to the membrane and directing the subsequent activation of the Ras/MAPK pathway (52). Shc has also been implicated in the regulation of c-Myc activation (74), neuronal cell survival (55), and focal adhesion and cytoskeletal organization (80,81). In hematopoietic cells, ShcA has been shown to bind to SHIP1 and SHIP2, potent negative-regulators of cytokine signaling (82). However, the functional implication of this interaction in cell signaling events is not clear.

Hyperphosphorylation of Shc has been observed in many different tumors (83,84,85), and a knockout of the *shcA* gene results in embryonic lethality at day 11.5 (86). These data suggest that Shc is an essential adaptor protein that functions in almost every aspect of cell growth and differentiation, including embryonic development.

4.2. Grb2

Grb2 is a ubiquitously expressed adaptor protein that functions in coupling growth factor receptor engagement with Ras activation (87,1). The protein structure of Grb2 contains an SH2 domain flanked by two SH3 domains, and these regions mediate interactions between activated receptors and downstream signaling molecules (87,88,89,90,91,58).

Several studies have examined the role of Grb2 in linking tyrosine kinase receptors to downstream effector pathways. The SH2 domain of Grb2 is recruited to activated growth factor receptors, the TCR or the BCR either directly or indirectly through complex formation with Shc (92). The SH3 domain of Grb2 interacts with several binding partners, effectively positioning signaling molecules in the proximity of activating kinases. Grb2 is also able to interact with Sos, a Ras guanosine nucleotide

exchange factor, *via* its SH3 domain. Membrane localization of the Grb2/Sos complex facilitates the interaction of Sos with its substrate Ras, leading to the activation of the Ras/MAPK pathway. In conclusion, Grb2 appears to function as an adaptor protein that couples receptor activation to downstream signaling effects in both lymphoid and non-lymphoid cells. The role of Grb2 in immune cell signaling pathways will be discussed later in this review.

4.3. Nck/Dock

The Nck/Dock is an SH2/SH3 adaptor protein family that plays an important role in signaling pathways that lead to alterations in the actin cytoskeleton (93). The Nck family is comprised of Nck-alpha/Nck/Nck1 and Nck-beta/Grb4/Nck2 (93). The protein domain structure of Nck reveals one SH2 domain and three SH3 domains important in connecting upstream signals to specific downstream pathways (94). Studies by Chou (95), Li (96), Meisenhelder and Hunter (97), and Park and Rhee (98) demonstrated that Nck is tyrosine phosphorylated in cells in response to growth factor receptor engagement (EGFR, PDGFR, and Eph1/ELK) or transformation by Src. However, the functional significance of these phosphorylations remains unclear (93). Also, these studies suggested that Nck was able to interact directly with RTKs, and this interaction was dependent upon the Nck SH2 domain. While Nck possesses the ability to bind to several RTKs, a handful other proteins have been identified as SH2 binding partners in many receptor-activated pathways. Among these are p130Cas, Dok-1, IRS-1, HPK1, SLP-76 (in T cells), and BLNK (in B cells) (93,99). Nck also mediates a large number of protein interactions *via* its three SH3 domains. The main function associated with the adaptor protein Nck is to link cell surface receptors to the actin cytoskeleton through its protein binding domains (94). The role of Nck in immune cell signaling pathways will be discussed later in this paper.

5. ADAPTOR PROTEINS THAT CONTAIN BOTH MODULAR DOMAINS AND TYROSINE RESIDUES

5.1. Cbl

The c-Cbl protooncogene was first identified as the cellular form of the transforming retroviral protein v-Cbl (100,101). c-Cbl is a 906 amino acid protein that is ubiquitously expressed in mammalian cells (101), including the thymus, spleen, testis, lung, brain, heart, and cells of hematopoietic origin. Extensive research on Cbl resulted in the discovery of additional Cbl family members Cbl-b, which is also ubiquitously expressed, and Cbl-3/Cbl-c, which is expressed primarily in the gastrointestinal system (102). Structural analysis of the Cbl family proteins reveals several protein binding domains, including a highly conserved N-terminal domain composed of a zinc-binding RING finger domain, a calcium-binding EF hand domain, and a modified SH2 domain (103). The N-terminal region of Cbl is responsible for binding phosphorylated tyrosine residues of multiple protein tyrosine kinases, and is also referred to as the tyrosine kinase-binding (TKB) domain (104,105,106). Following the N-terminal region is a proline-rich sequence and a C-terminal segment containing several tyrosine phosphorylation sites (107,108,109,110,111,112,113). Tyrosines 700, 731, and 774 have each been identified as major phosphorylation sites of c-Cbl by PTKs (113). The extreme carboxy-terminus contains a leucine zipper motif (not in Cbl-3), and this motif directs the self-association of Cbl proteins (114,110,115,116).

Cbl becomes tyrosine phosphorylated in response to a variety of extracellular signals, including signaling though protein tyrosine kinases, immune recognition receptors, cytokine receptors, integrins, hormone receptors, cell surface receptors, and growth factor receptors (102). Tyrosine phosphorylation, the presence of an SH2 domain, and the ability of Cbl to homodimerize position Cbl to function as a multivalent adaptor protein. The TKB region of Cbl is involved in mediating the binding of several PTKs, including Syk, Zap-70, and Src, while the C-terminal phosphorylation sites of Cbl provide docking sites for p85 PI3K, Crk, CRKL, Src family PTKs, and Vav (102). These interactions position Cbl as an essential component of several physiologically relevant signaling complexes important in the propagation of signals to downstream effector molecules that affect cell proliferation, survival, adhesion, migration, and morphology (102).

In addition to participating in signaling pathways that positively regulate cellular functions, Cbl has also been implicated in the negative regulation of growth factor receptors and in T cell anergy. In the role of a negative regulator, Cbl proteins have emerged as mediators of ubiquitination of certain growth factor receptors, including EGF, PDGF, and CSF-1 (117,118,119,120). Within the amino terminus of Cbl is a zinc-binding RING finger motif that is associated with E3 ubiquitin-ligase activity. An E3 ubiquitin ligase recognizes phosphorylated tyrosine substrates and recruits E2 ubiquitin-conjugating enzymes through its RING domain (121,122,123). E2 and E3 work in conjunction to transfer ubiquitin to substrate proteins, targeting these substrates for degradation by the proteosome (102). Several studies have highlighted the importance of PTB-mediated interactions with target PTKs for Cbl-dependent ubiquitination. A mutant of Cbl that lacks its C terminus is still able to bind to Syk, Zap-70, EGF, and PDGF receptors (104,124,105,106,125,126). Also, a decrease in binding between Cbl and PTKs caused by an inactivation of the SH2-like domain results in a significant inhibition in tyrosine phosphorylation of Cbl (127,126), and abrogates Cbl-mediated ubiquitination of these receptors (128,127). In another experiment, the expression of a protein fragment encompassing the PTB and RING domains of Cbl was sufficient for ubiquitination-mediated desensitization of the EGF receptor (122,128). Growth factor receptors are not the only targets of Cbl-mediated ubiquitination. Syk, Fyn, and various receptors without enzyme activity have also been identified as Cbl targets, although in some cases, the PTB domain does not facilitate the binding of Cbl to the target protein (129,130,131). Through the ability of Cbl to mediate protein degradation, a possible biological function for this protein could be the negative regulation of constitutively

active PTKs that are identified to be the cause of certain oncogenic transformations (102).

5.2. FRS2/SNT

The nerve growth factor receptor (NGFR) and the fibroblast growth factor receptor (FGFR) belong to a large group of receptor tyrosines kinases that regulate cell growth, differentiation, and survival (132,133). Both the FGF and NGF receptors contain an extra-cellular ligand binding domain, a transmembrane region, a protein tyrosine kinase domain, and several tyrosine residues that serve as recognition domains upon phosphorylation (134,135). Activation of these RPTKs has been implicated in the stimulation of the Ras/MAPK signaling cascade *via* the recruitment of Grb2/Sos (136,137,135). However, both the NGF and FGF receptors lack the SH2-consensus binding sequence of Grb2, and must use a different, indirect method to recruit the Grb2/Sos complex to the plasma membrane (138).

The FRS2 protein family (FGF receptor substrate 1, also known as SNT1 (suc1-associated neurotrophic factor target protein)) is composed of the highly homologous FRS2-alpha and FRS2-beta isoforms (136,138), and these proteins serve as essential mediators of downstream signals emanating from the FGF and NGF receptor, and possibly the Insulin receptor (139). The protein domain structure of FRS2 reveals an N-terminal myristylation signal, a PTB domain, and a C-terminal region with several tyrosine residues that serve as binding sites for SH2 domain-containing proteins upon phosphorylation (138). FRS2-alpha is found to be constitutively bound to the FGFR1 *via* the FRS2 PTB domain. However, this association is not dependent upon receptor tyrosine phosphorylation. Alternatively, the PTB domain of FRS2-alpha and FRS2-beta bind to the NGF receptor TrkA in a tyrosine phosphorylation-dependent manner (140,141). Both NGF and FGF receptor stimulation results in the phosphorylation of five tyrosine residues in FRS2 (142). Four of these phosphorylated tyrosine residues (Y196, Y306, Y349, and Y392) serve as binding sites for the recruitment of Grb2/Sos complexes, while the fifth residue (Y436) binds to molecules of SHP2 (136,142,143). FRS2 interaction with SHP2 results in its own tyrosine phosphorylation, leading to the recruitment of and complex formation with other Grb2 molecules. The ability of FRS2 to recruit Grb2/Sos complexes both directly and indirectly positions FRS2 to act as a mediator of Ras/MAPK pathways in response to growth factor receptor signaling (142,136). Evidence for the role of FRS2 in Ras/MAPK activation arises from studies that utilize mutants of FRS2 that are unable to bind SHP2 and/or Grb2. Overexpression of the SHP2-binding mutant or the Grb2-binding mutant resulted in the induction of MAPK activity, but this activity was not sustained (142). Overexpression of the FRS2/Grb2 binding mutant resulted in a very weak induction of FGF-induced MAPK activation and a loss in neurite outgrowth in PC12 cells (142,136), indicating that FRS2 binding to both of these signaling molecules is important in the FGFR1-mediated activation of the MAPK pathway.

It was also demonstrated that FRS2 plays a role in the FGF-induced recruitment and tyrosine phosphorylation of Gab1 (144). Binding of the SH2 domain of Grb2 to tyrosine phosphorylated FRS2α mediates the recruitment of Gab1 to Grb2, resulting in FRS2-alpha/Grb2/Gab1 ternary complex formation (144). Gab1 is then tyrosine phosphorylated by the FGF receptor, creating a binding site for the SH2 domain of the p85 subunit of PI3K. Recently, Hadari et al. (145) introduced a targeted mutation into the *frs2a* gene, creating FRS2-deficient mice and FRS2α-deficient mouse embryo fibroblasts. These mice showed impaired cell migration, MAPK activation, PI3K activity, and cell proliferation upon induction with FGF. These FRS2-alpha-deficient mouse fibroblasts were used to investigate the role of FRS2-alpha in FGF-mediated PI3K stimulation. Lysates from FGF-stimulated wild type and FRS2-alpha-deficient fibroblasts were examined for Gab1 tyrosine phosphorylation. Phosphorylation of Gab1 on tyrosine was

Table 2. Adaptor proteins that are involved in signaling pathways downstream of Src tyrosine kinase

Protein	Expression	Binding Motif	Consensus Sequence	Associated molecules	References
Cortactin	Ubiquitous	pY sites	--------	Src, Fer	153,149,148,158
Paxillin	Ubiquitous	pY sites	--------	Crk, CRKL, Csk, Chk, Src, Lck	169,165,164,174,175,177
FRS2/SNT1	Ubiquitous	PTB, pY sites	N-P-Q-pY and N-P-X-pY	NGFR, FGFR, Grb2, SHP2, Ret	147,138,136,142,143
AFAP-110	Ubiquitous	pY sites	--------	Src	451,377,452
Cas	Ubiquitous	pY sites	7x Y-x-x-P	Crk, Nck, SHIP2, Src	408,453
SAM68	Ubiquitous	SH2	Unknown	Src, Grb2, RasGAP	454,455
RACKS	Ubiquitous	pY sites	--------	Src	185,187,182

not detected in FRS2-alpha-deficient cells; however, phosphorylation of Gab1 was restored upon expression of ectopic FRS2-alpha cDNA in these cells. More importantly, Gab1 is a known binding partner for PI3K and participates in PI3K activation (146). To test whether FRS2α is involved in PI3K activation, Gab1 immunoprecipitates of wild type and FRS2-alpha-deficient fibroblasts were analyzed for PI3K activity in response to FGF stimulation. Immunoprecipitates from wild type fibroblasts were found to contain PI3K activity, while immunoprecipitates from FRS2-alpha-deficient fibroblasts were not. Full PI3K activity was restored to FRS2-alpha-deficient cells upon the ectopic expression of FRS2-alpha cDNA. These results indicate that FRS2 plays an important role in mediating the activation of PI3K in FGF-stimulated cells.

Recently, Melillo et al. (147) found that FRS2 is tyrosine phosphorylated by the protein tyrosine kinase Ret and its oncogenic forms. It was demonstrated that Ret is constitutively associated with FRS2, leading to FRS2 tyrosine phosphorylation, MAPK pathway stimulation, and cell proliferation. Overall, FRS2 appears to be an essential docking protein that is capable of mediating the formation of several protein complexes crucial to FGF and EGF-dependent signaling pathways.

Several adaptor proteins are involved in signaling pathways modulated by Src family non-receptor tyrosine kinases. These include Src binding partners and effector proteins. Table 2 lists several of these adaptor proteins.

6. PROTEINS THAT ARE TYROSINE PHOSPHORYLATED IN SRC SIGNALING PATHWAYS

6.1. Cortactin

Cortactin is a cortical actin binding protein that is involved in RhoGTPase and Src signaling events that regulate changes in actin dynamics (148). The protein domain structure of cortactin reveals an amino terminal acidic region, a proline-rich region with several tyrosine residues, and an SH3 domain at the carboxy terminus (149,150). Cortactin was first identified as a tyrosine phosphorylated protein in Src-transformed cells (151). Since then, several studies have shown that cortactin is a substrate for Src in response to many extracellular stimuli, including growth factors (FGF and EGF), integrin signaling, and cellular stresses (H_2O_2) (148). Coimmunoprecipitation studies indicate that Src binds to cortactin directly *via* its SH2 domain, and subsequent tyrosine phosphorylation of cortactin creates binding sites for other SH2 domain-containing proteins (152,153,154). Okamura and Resh (153) demonstrated that in quiescent cells, cortactin is not tyrosine phosphorylated and is mainly associated with the detergent-soluble fraction. However, in cells expressing v-Src, tyrosine phosphorylation of cortactin by Src leads to a significant increase in cortactin association with the Triton-X insoluble cytoskeletal fraction. These data indicate tyrosine phosphorylation of cortactin serves to localize cortactin to the specific intracellular compartments.

Using Fyn-/- Csk-/- and Src-/- Csk-/- fibroblasts, Thomas et al.(155) showed that Src is the tyrosine kinase primarily responsible for the phosphorylation of cortactin resulting from knockout of the *csk* gene. Expression of a cortactin mutant with phenylalanine substitutions at Y421, Y466, and Y482 resulted in a dramatic reduction in cortactin tyrosine phosphorylation in v-Src-transformed cells (156). Huang et al. (156) showed that tyrosine phosphorylation of cortactin by Src resulted in a decrease in the ability of cortactin to cross-link actin filaments. Over-expression of a cortactin mutant that is unable to be phosphorylated by Src showed impaired cell migration, while overexpression of wild type cortactin resulted in an increase in endothelial cell migration. However, the full significance of Src-mediated tyrosine phosphorylation of cortactin has not yet been elucidated (148).

A second non-receptor tyrosine kinase implicated in cortactin tyrosine phosphorylation in response to growth factor stimulation is Fer (157). Fer immunoprecipitates with cortactin, and this interaction is thought to be mediated by the Fer SH2 domain (158). The involvement of Fer in growth factor-mediated cortactin phosphorylation is further supported by the suppression of CSF-1 receptor mediated tyrosine phosphorylation of cortactin by the expression of a kinase dead Fer (158). Further studies by Kapus et al. (159) indicated that upstream signaling through Fyn is required for Fer mediated tyrosine phosphorylation of cortactin; however, the significance of this phosphorylation event is still unknown (148).

6.2. Paxillin

Paxillin is a 68-70 kDa adaptor protein that is thought to function as a scaffold protein in the organization of signaling complexes at focal adhesions (160,161), coordinating the transmission of signals that affect cell morphology and motility (162,163). A number of motifs have been characterized in paxillin that mediate protein-protein complex formation, including a N-terminal proline-

rich region, several tyrosine residues that are consensus sequences for SH2 domains, an LD motif, and four LIM domains (164,165). The LIM domains are thought to facilitate the localization of paxillin to focal adhesions through their interaction with β-integrin tails or possibly though an intermediate adaptor protein (160). Stimulation of cells with growth factors, neuropeptides, antigens, G-protein coupled protein receptor ligands, and cellular stresses have all been described to stimulate the tyrosine phosphorylation of paxillin (161). While paxillin can be phosphorylated in response to a large number of stimuli, only a few tyrosine kinases have been implicated in this process. Focal adhesion kinase (FAK) and the FAK-related protein CAK-beta/Pyk2/CadTK/RAFTK physically associate with paxillin, and the activation of these kinases is associated with tyrosine phosphorylation of paxillin (166,167). There is also a role for Src tyrosine kinases in paxillin phosphorylation. Fibroblasts lacking Src, Fyn, and Yes demonstrate dramatic reductions in the phosphorylation state of paxillin, while fibroblasts derived from *csk*-/- mice exhibit enhanced Src family kinases activity and an equally enhanced increase in paxillin tyrosine phosphorylation (155,168). As tyrosine phosphorylation of paxillin is increased in Abl-transformed 32D cells, paxillin may also serve as a target for the Abl tyrosine kinase as well (165).

There are four sites of tyrosine phosphorylation in paxillin (possibly five), including Y31, Y118, Y40 and Y88 (169,170,162). The only known function associated with paxillin tyrosine phosphorylation is the creation of binding sites for SH2 domain-containing proteins (161). The SH2 domain of Crk and CRKL bind to paxillin with high affinity at tyrosines pY31 and pY118 (171,169,172). This enables Crk to bind other proteins like C3G guanine nucleotide exchange factor and Dock180 *via* SH3 domain binding (173). Csk and Chk, a Csk-related kinase, have also been reported to bind to phosphorylated paxillin *via* SH2 domain binding (174,169,175,176). Likewise, Src can also associate with paxillin, *in vitro*, and Lck can associate with paxillin *in vivo* in T cells (169,177). Therefore, tyrosine phosphorylation of paxillin serves as an essential step in the recruitment of diverse cytoskeletal and signaling proteins into functional complexes in focal adhesions.

6.3. RACKs

The protein kinase C (PKC) family of serine/threonine kinases are important regulators of cellular proliferation, differentiation, and cytoskeletal rearrangements (178). There are at least ten different PKC isoforms that can be divided into three subfamilies based on their sensitivity to second messengers (179,180). It has been evidenced that the activation of PKC isoforms is dependent upon the translocation of each isoform to different sub-cellular compartments, thereby conferring specificity in substrate phosphorylation and subsequent downstream cellular responses (181). The localization of individual PKC isoforms to specific sub-cellular sites is thought to involve the RACKs family of anchoring proteins (receptor for activated C-kinase). Members of the RACK protein family have been reported to display PKC isozyme selectivity, resulting in the ability of RACK to anchor activated PKCs in close proximity to their physiological substrates (182). However, RACKs themselves are not substrates for PKC kinase activity (183,184). RACKs are composed of seven repeats of the WD40 motif, a domain that is also found in the beta subunit of the heterotrimeric G-protein (185). WD40 domains are responsible for the recruitment of several PKC substrates, including PLC-gamma, RasGAP, and Dynamin-1.

Interestingly, the induction of PKC activity upon treatment with phorbol ester (PMA) resulted in the activation of Src in Swiss 3T3 cells (186). However, the mechanism by which PKC regulates Src activation is poorly understood. Using a yeast two-hybrid system, Chang (187) demonstrated that c-Src can directly bind RACK1, and this binding is increased upon the phosphorylation of a tyrosine residue in the 6^{th} WD40 domain of RACK1 (182). The interaction between Src and RACK1 is enhanced by PKC activation, as treatment of CHO cells with PKC activator PMA resulted in an increase in RACK1/Src binding (182). While it is unclear at this time which tyrosine kinase is responsible for the phosphorylation of RACK, it is tempting to suggest that RACK is a substrate for Src kinase, and this in turn allows for enhanced binding of RACK to the Src-SH2 domain (182). Overall, RACK appears to serve as a scaffolding protein that is involved in the recruitment and assembly of signaling complexes. Also, due to its ability to both bind and localize PKC and Src, RACK may serve as a novel link between the tyrosine kinase pathway and the serine/threonine kinase pathway.

6.4. PECAM-1

PECAM-1 (platelet/endothelial cell adhesion molecule-1) is a 130-kDa cell surface glycoprotein belonging to the immunoglobulin (Ig) superfamily that is abundantly expressed on endothelial cells, platelets, myeloid cells, mast cells, monocytes, and defined T cell subsets (188). The structure of PECAM-1 is highly conserved across species, and consists of an extracellular segment with six Ig-like domains (C2 type), a transmembrane domain, and a cytoplasmic domain (189). The cytoplasmic domain contains tyrosine residues and also sequences that resemble dual ITIMs that become phosphorylated in response to a variety of stimuli, including mechanical stimulation of endothelial cells (190), platelet aggregation (191), TCR engagement (192), IgE receptor activation on basophils (192), and treatment with protein-tyrosine phosphatase (PTP) inhibitors vanadate and pervanadate (193,194,191). Three main functions are attributed to PECAM-1. They include the mediation of leukocyte-endothelial interactions, inter-endothelial cell adhesion, and the transendothelial migration of cells (195,196).

Tyrosine phosphorylation of PECAM-1 is thought to be mediated by Src family kinases, as PECAM-1 tyrosine phosphorylation is abolished in cells treated with the Src family inhibitor PP2 (197). Also, PECAM-1 tyrosine phosphorylation is observed in Cos-1 cells that are transiently co-transfected with c-Src, Csk, Lck, Fyn, and Lyn (189). These data support the potential role of Csk

Table 3. Adaptor proteins that are involved in signaling pathways in lymphoid cells

Protein	Expression	Binding Motif	Consensus Sequence	Associated Molecules	References
3BP2	Lymphocytes	SH2	PY(E/M/V)(N/V/I)x	PLCγ, Vav, Cbl	456,210
Bam32/DAPP1	Bam32 - B cells DAPP1 - ubiquitous	SH2	unknown	PLC-gamma	457
BLNK/SLP-65/BASH	B cells	SH2, PY sites	pY-(E/D/M)-E-x	PLC-gamma, Vav, Syk, Btk, Grb2, Nck	458,459,201,460
BRGD1	B, myeloid cells	PY-sites	Unknown	Tec	461
Cbp	Ubiquitous	PY-sites	--------	Csk, Fyn	461
CD19	B cells	PY sites	Unknown	Vav, PI3K	200
CD22		ITIM, ITAM-like	(I/V)-x-Y-x-x-L	SHP-1, Grb2, Shc, Lyn, Syk, PI3K, PLC-gamma	245
CD72	B cells	ITIM	(I/V)-x-Y-x-x-L	SHP-1, Grb2	462,249,463
CLNK	Cytokine-stimulated hematopoietic cells	SH2, PY-sites	Unknown	P92 (HPK1)	464
Dok	Leukocytes	PTB, PY sites	PTB - N-P-x-pY PY site – Y-E-L-P and Y-D-E-P	SHIP, Crk/CRKL	465,201
FcγRIIb/CD32	B cells, myeloid cells	ITIM	(I/V)-x-Y-x-x-L	SHIP	243,244,227
GADS	T, NK, macrophages, mast cells, platelets	SH2	pYxNx	LAT, Shc	227,228,229
Grap	Lymphocytes	SH2	pYxNx	Shc	1,202,92
LAT	Lymphocytes	SH2 binding motif	--------	Grap, GADS, PLC-gamma, Grb2, p85 PI3K, SLAP	1,205
Lnk	Lymphocytes	SH2	PY-x-x-L	TCR-zeta, {Grb2, PI3K, PLC-gamma1 in vitro}	466,467,468
Sap	T, NK cells	SH2	Unknown	SLAM, SHP-2	469,201
Shb	Ubiquitous	PTB, SH2	SH2 – pY-(T/V/I)-x-L PTB – N-N-x-pY	LAT, TCR-zeta, PDGFR	470,201,471,472
SIT	T cells, thymocytes, B cells	ITIM, PY sites	(I/V)-x-Y-x-x-L	Grb2, SHP2, Csk	217,215
SKAP55	T cells	PY-sites	--------	Fyn	215
SLAM	T cells, B cells, thymocytes, dendritic cells	PY sites	Unknown	SAP	473
SLAP	Lymphocytes	SH2	Unknown	ZAP-70, Syk, LAT, TCR-zeta	204
SLAP-130/Fyb	T, myeloid cells	PY- sites	--------	SLP-76, Fyn	206
SLP-76	Lymphocytes	SH2, PY-sites	PY site – Y-(E/V)-(N/P/V)-P	Vav, Nck, GADS, SLAP-130	220,47,221,427
SOCS/JAB	Leukocytes	SH2	Unknown	JAK kinases, Cytokine receptors	292,291,293
TRIM	T cells	PY-sites	YxxM	PI3K,	217
TSAd/Lad/RIBP	T cells, NK, lung	SH2, potential PTB	SH2- ? PTB-N-P-x-pY	Lck	474,475

tyrosine kinases as mediators of PECAM-1 tyrosine phosphorylation. Recently, Newman (198) determined the importance of Lck in PECAM-1 tyrosine phosphorylation in T lymphocytes.

Tyrosine phosphorylation of the cytoplasmic ITIMs in PECAM-1 directs its association with the protein tyrosine phosphatases SHP-1 and SHP-2 (192). Tyrosines in position 663 and 686 (Y677 and Y700 in murine PECAM-1) of the ITIM sequence were found to be essential for this protein interaction to occur (194). Mutation of either of these residues reduced PECAM-1 tyrosine phosphorylation to barely detectable levels in PECAM-1-transfected 3T3 cells. Phosphorylation of tyrosine residues also creates SH2-binding sites for the SH2 domains of Src tyrosine kinases (191,199,194). As is observed with CD22 in B cells, PECAM-1 appears to function as a negative regulator of signaling pathways involved in its own tyrosine phosphorylation.

Recently, several reviews have focused on the importance of adaptor proteins in immune cell signaling events (200,201,202,203). Several adaptor proteins are expressed only in lymphoid cells where they appear to play a role in connecting cell surface receptors to intracellular effector molecules. Table 3 contains a list of adaptor proteins, their expression patterns, protein-protein binding domains, and associated binding partners. The specific role of tyrosine phosphorylation and tyrosine-mediated protein binding will be discussed for several of these proteins below.

7. TYROSINE PHOSPHORYLATION REGULATES ADAPTOR PROTEINS THAT PLAY A ROLE IN SIGNALING PATHWAYS IN T LYMPHOCYTES

T cell receptor-mediated signaling is essential for the development and function of T cells as effectors of cell-mediated immunity (204). The T cell receptor (TCR) is composed of a heterodimer of alpha and beta polypeptide chains that are non-covalently linked to an invariant polypeptide complex consisting of CD3 gamma, delta, and epsilon chains and TCR-gamma chains (1). TCR engagement leads to the activation of Src family kinases, namely Lck and Fyn, which are able to phosphorylate tyrosine-containing immunoreceptor tyrosine-based activation motifs (ITAMs) present in the cytoplasmic tails of the TCR-gamma chain. ITAMs were originally identified in the cytoplasmic tails of the BCR co-receptor

molecules Ig-alpha and Ig-beta, but have since then been observed in accessory chains of the TCR and Fc receptors on mast cells and NK cells. ITAMs are composed of two tyrosine residues separated by 13 amino acids, and have the general sequence Y-X-X- (L/V)-X_{7-11}-Y-X-X- (L/V). Upon receptor ligation, the tyrosine residues in these sequences are phosphorylated by receptor-associated tyrosine kinases, creating docking sites for several SH2-containing proteins. In T cells, the phosphorylation of the cytoplasmic ITAM sequences by Src family kinases allows for the recruitment of a second family of non receptor protein tyrosine kinases, ZAP-70, which binds to the phosphorylated ITAM sequence by virtue of its tandem SH2 repeats (205). ZAP-70 is subsequently activated through auto/transphosphorylation events (206). The activation of Src and ZAP-70 tyrosine kinase family initiates a signaling cascade that results in the activation of several other protein tyrosine kinases (PTKs), including the Tec family. The PTK signaling cascade eventually results in the activation of downstream signaling effectors such as PLC-gamma, which directs the hydrolysis of phosphoinositides, and the activation of the Ras/MAPK pathway. These pathways ultimately result in the up-regulation of several transcription factors, including AP-1, NF-AT, and NF-kappaB, known to be important in enhanced gene transcription, cellular proliferation, and differentiation (204).

Propagation of signals emanating from the TCR involves a number of adaptor proteins expressed only in specific lymphoid cells (207). The effects of protein domain interactions and tyrosine phosphorylation on the conformation and function of these adaptor proteins will be discussed.

7.1. LAT and 3BP2

Two adaptor proteins play an important role in TCR signal transduction are linker for activated T cells (LAT) and SH2-domain containing leukocyte protein of 76 kDa (SLP-76). LAT is a type III transmembrane protein that is localized in glycolipid-enriched microdomains (GEMs) due to two palmitoylated cysteine residues (208), and localization of LAT to GEMs is required for TCR signaling. The intracellular domain of LAT contains multiple tyrosine-based residues that become phosphorylated by ZAP-70 upon receptor ligation (1). Deckert et al. were able to link the adaptor protein 3BP2 to ZAP-70-mediated activation of LAT. 3BP2 is an 80kDa protein that contains an SH2 motif and is only expressed in lymphocytes (209). 3BP2 interacts with members of the Syk kinase family and LAT *via* SH2 domain interactions, although not simultaneously. LAT contains two-YEN (Tyr-Glu-Asn) sites that, upon phosphorylation, may function as a binding site for the 3BP2 SH2 domain (210,209). As it is still unknown how LAT is recruited to the TCR upon receptor ligation, it is possible that through interactions with LAT, 3BP2 is able to recruit LAT to the activated TCR and the associated tyrosine kinases, resulting in the phosphorylation and activation of LAT. The ability of 3BP2 to associate with both of these proteins provides a mechanism for the activation of LAT by Syk kinases upon TCR activation.

The phosphorylated tyrosine residues in LAT serve as docking sites for several other proteins that contain SH2 domains, including Grb2, Grap, GADS, p85 subunit of PI3K, and PLC-gamma (1,205). LAT is also able to recruit several proteins indirectly *via* linker proteins, including SLP-76 and Vav (211). The function of LAT tyrosine phosphorylation is thought to involve the re-localization of specific signaling proteins to the plasma membrane, placing them in direct proximity of lymphocytic tyrosine kinases (211). This would facilitate tyrosine phosphorylation of these downstream molecules that are important in achieving the cellular response. For example, the recruitment of Grb2 and/or Grap in complex with Sos provides a mechanism for the stimulation of the Ras/MAPK pathway, as does the recruitment and activation of PLC-gamma (212). The Ras/MAPK signaling pathway culminates in the activation of several transcription factors, including AP-1 and NF-AT (211).

Tyrosine phosphorylation plays an essential role in LAT complex formation and the ability of LAT to activate downstream signaling pathways. Mutation of LAT tyrosines at position 171 and 191 to phenylalanines has a dominant negative effect on TCR signaling, as these mutants can block activation of NF-AT and AP-1 transcription factors (1). Studies using LAT-deficient cell lines and LAT knockout mice emphasize the importance of LAT in TCR signaling. Using the LAT-deficient J.CaM2 Jurkat cell line (42), it was determined that in the absence of LAT, Cbl was hyperphosphorylated on tyrosine, while PLC-gamma1, Vav, and SLP-76 were hypophosphorylated (211). In agreement with these findings, cell lines deficient in LAT no longer mobilize calcium, do not activate the Ras pathway in response to TCR stimulation, and are defective in NF-AT activity (42,49,212). LAT-deficient mice have a block in thymocyte development at the double negative stage (CD25+CD44-), and these mice have few mature peripheral T cells (213). The phenotype of these mice is similar to the phenotype observed in Lck, Fyn, ZAP-70, and RAG 1 (-/-) knock out mice (1,214). In conclusion, LAT acts a downstream effector molecule of TCR-related kinases, and LAT phosphorylation facilitates the phosphorylation of substrates and the construction of larger signaling complexes capable of regulating TCR-mediated cellular maturation and activation (205).

7.2. SIT and TRIM

SIT (SHP-2-interacting transmembrane adaptor protein) is a disulfide-linked homodimeric transmembrane glycoprotein that is expressed in T cells, thymocytes, and, to a smaller extent, in B cells (215). SIT contains five potential tyrosine phosphorylation sites, one of which is an immunoreceptor tyrosine-based inhibitory motif (ITIM). ITIMs are found in the cytoplasmic tails of several cell surface receptors that modulate activation signals in both B and T lymphocytes. The ITIM consists of the restricted consensus sequence (I/V)-X-Y-X-X-(L/V), and functions in the recruitment of inhibitory phosphatases, such as SHP1 and/or SHIP, to the receptor (216).

Upon TCR ligation, SIT becomes tyrosine phosphorylated by Src family kinases, most notably Fyn

(215), and Syk kinase. Marie-Cardine et al. (215) proposed a two-step activation model for SIT, requiring input from both Syk and Src kinases. Upon TCR activation, SIT is phosphorylated by a Src PTK, inducing a conformational change in SIT that exposes specific tyrosine residues that can then be phosphorylated by Syk PTKs. However, further experiments are needed to determine the accuracy of this model. Nevertheless, tyrosine phosphorylation of SIT generates binding sites for proteins with SH2 domains. The adaptor protein Grb2 was found to bind to the Y90 and Y188 sequence motifs of SIT *via* its SH2 domain (217). SHP-2 protein phosphatase also inducibly associates with Y148 of activated SIT, suggesting that SIT may play a role in the negative regulation of TCR activation (201,215). SIT activation is also reported to negatively regulate TCR-mediated NF-AT activity (215), and the ability of SIT to mediate negative regulatory effects is dependent upon its phosphorylation state (217). It is hypothesized that SIT may bind to Csk, and this interaction results in the negative regulation of NF-AT activity in TCR-mediated signaling (217). In conclusion, SIT represents a transmembrane adaptor protein that has the ability to regulate signaling downstream of the TCR due to protein-protein interactions.

TRIM (T cell receptor-interacting molecule) is a type III transmembrane adaptor protein, similar to LAT, that is exclusively expressed in T cells and NK cells (218). TRIM is found to be associated with the ζchain of the TCR complex, and overexpression of TRIM in cells results in a two-fold increase in the expression of TCR/CD3 complex on the cell surface and an increase in calcium mobilization (219). Overexpression of TRIM also inhibits the spontaneous internalization of the TCR/CD3 complex, suggesting that TRIM plays a role in the stabilization of the TCR/CD3 complex on the cell surface (219).

The cytoplasmic tail of TRIM contains eight tyrosine residues, three of which are components of a sequence motif that directs interactions with SH2 domain-containing proteins (218). Upon TCR engagement, TRIM is phosphorylated by Src family members Lck and Fyn, but not ZAP-70 or Syk (218). This is evidenced by the fact that identical levels of tyrosine phosphorylation of TRIM are detected in wild type Jurkat cells and in Jurkat cells that are deficient in both Syk and ZAP-70. Also, treatment of cells with the Src inhibitor PP1 completely inhibited tyrosine phosphorylation of TRIM (218). Tyrosine phosphorylation of TRIM creates an SH2 binding motif for the recruitment of PI3K and two unknown polypeptides of 43 and 50 kDa (217,219). However, the role of tyrosine phosphorylation of TRIM in T cell-mediated signaling remains unknown.

7.3. SLP-76 and SLAP-130

Another downstream target of ZAP-70 is SLP-76, an adaptor protein whose expression is restricted to cells of hematopoietic origin (220,47,1). SLP-76 contains an SH2 motif and several tyrosine-based residues that become phosphorylated in response to TCR engagement by Syk family PTKs (220,47). The function of SLP-76 is dependent upon its association with LAT (221), and the Gads adaptor protein mediates SLP-76-association with LAT. SLP-76 also associates with SLP-76–associated phosphoprotein of 130 kDa/Fyn binding protein (SLAP-130/Fyb) *via* its SH2 domain; however, there is no known function associated with this interaction.

TCR engagement leads to the tyrosine phosphorylation of SLP-76 and the subsequent interaction with Vav and Nck *via* SH2 binding (48,45,44). The SLP-76/Nck/Vav complex is thought to recruit and activate Rac1-GTPase and p21-activated kinase (PAK), leading to Rac and Cdc42 activation and subsequent cytoskeletal reorganization (222). Mutation of tyrosines in positions 113 and 128 to phenylalanine dramatically decreases SLP-76 tyrosine phosphorylation and the ability of SLP-76 to augment NF-AT activity after TCR ligation (1). Also, mutation of either tyrosine 113 or 128 to phenylalanine blocks the interaction of SLP-76 and Vav, indicating that these tyrosine residues are important in Vav recruitment to phosphorylated SLP-76 (223). Similar to LAT, cell lines and mouse models deficient in SLP-76 have been essential in determining the role of tyrosine phosphorylation. SLP-76 gene knockout mice display a phenotype that is identical to LAT-deficient mice, and these mice frequently succumb to severe systemic hemorrhage at an early perinatal stage (224,225). J14 is a mutant Jurkat T cell line deficient in SLP-76 that has been used in several experiments to determine the function of SLP-76 in T cell signaling (49). While tyrosine phosphorylation levels of Vav, ZAP-70, Itk, and LAT were normal in these cells, tyrosine phosphorylation of PLC-gamma1 was almost completely ablated (211). This would indicate that SLP-76 is an important upstream effector in TCR-mediated PLC-gamma1 activation. Also, TCR-dependent gene transcription mediated by NF-AT was absent in J14 cells, and the re-expression of SLP-76 in these cells resulted in normal NF-AT activity (211). Recently, Yablonski et al. (226) reported that SLP-76 interacts with PLC-gamma *via* SH3 interactions, and this interaction is required for downstream activation of Erk, PLC-gamma1, and NF-AT. Therefore, it is proposed that TCR-mediated activation of PLC-γ requires a functional complex of LAT, Gads, SLP-76, and PLC-gamma, as both LAT and SLP-76 appear to be required for PLC-gamma activation in TCR-mediated signaling. Over all, SLP-76 appears to play a role in the recruitment of Vav, Nck, and other essential signaling proteins to the plasma membrane to facilitate their activation. These effector proteins subsequently transduce signals that are required for T cell-mediated immune responses.

7.4. GADS/GrpL and Grap

Gads/GrpL (Grb2-related adaptor downstream of Shc) is a Grb2/Grap-related adaptor protein that is specifically expressed in hematopoietic cells that links LAT with SLP-76. Gads is a member of the Grb2 family of adaptor proteins, and contains an SH2 binding motif, and two SH3 binding motifs (227). Gads was originally isolated as an SH2 binding partner for phosphorylated Shc in K562 leukemic cells, but this interaction has never been demonstrated in activated Jurkat T cells (227). In T cell, Gads is found to be constitutively associated with SLP-76 *via* SH3 interactions (228), and is recruited to LAT *via* its SH2 domain binding to tyrosine phosphorylated sites in

LAT upon TCR activation (229,207,228). Unlike SLP-76 and LAT-deficient mice, mice deficient in Gads exhibit a less severe phenotype, characterized by the presence of double negative and single positive thymocytes (221). These data indicate that not all SLP-76 dependent functions require Gads. In conclusion, Gads is an essential protein that facilitates crosstalk between LAT and SLP-76 signaling complexes, and provides the link between LAT and SLP-76 required for PLC-gamma activation and subsequent Ras/MAPK activation and calcium mobilization (228,221).

Grap (Grb2-related adaptor protein) is another member of the Grb2 adaptor protein family that is specifically expressed in spleen, thymus, and peripheral blood leukocytes (202,92). Grap, like Grb2 and Gads, contains an SH2 domain and two SH3 domains that direct interactions with other signaling molecules. Upon TCR ligation in Jurkat cells, Grap interacts with tyrosine phosphorylated Shc and p36/38 *via* SH2 interactions (1). Like Gads, Grap appears to function as an adaptor protein, mediating protein-protein interactions with specific binding partners in response to TCR activation.

8. TYROSINE PHOSPHORYLATION REGULATES ADAPTOR PROTEINS THAT PLAY A ROLE IN SIGNALING PATHWAYS IN B LYMPHOCYTES

B-cell receptor-mediated signaling is essential for the development and maintenance of a functional humoral immune response. Recently, new evidence has suggested that the BCR has a role in dual signaling. In the absence of antigen, it participates in the transduction of maintenance signals required for the development and survival of mature B-lymphocytes. Upon antigen binding, the BCR also regulates the activation of B-lymphocytes either for cell death or clonal expansion (230). The BCR consists of a transmembrane immunoglobulin molecule that is associated with the invariant Ig-alpha and Ig-beta chains in a non-covalent complex. As in TCR engagement, antigen binding to the BCR results in receptor aggregation and activation of non-receptor tyrosine kinases, such as Lyn, Src, Btk, and Syk, which phosphorylate ITAM sequences on the cytoplasmic tails of the BCR co-receptors Ig-alpha and Ig-beta. These tyrosine kinases co-localize with the activated receptor and facilitate the phosphorylation of several adaptor proteins essential in BCR signal transduction. Signals emanating from the BCR ultimately initiate pathways involved in phosphoinositide metabolite regulation, activation of Ras/MAPK pathway, elevation of calcium levels, and the initiation of gene transcription (200).

8.1. CD19

Similar to TCR signaling, BCR signaling also requires adaptor proteins that link receptor associated tyrosine kinases to downstream signaling molecules. CD19 is a transmembrane linker protein that is physically associated with the BCR (200), and has been implicated as a signaling partner for several other B cell surface receptors including CD40 (231), CD38 (232), CD72 (231), and VLA-4 (233). Upon receptor engagement, the cytoplasmic tail of CD19 becomes tyrosine phosphorylated by activated Lyn. This phosphorylation event provides binding sites for SH2 domain-containing proteins like Vav and PI3K. In this manner, CD19 functions to localize Vav and PI3K to the plasma membrane in close proximity to the activated BCR. Vav is then tyrosine phosphorylated by receptor associated tyrosine kinases, activating its Rho-GTPase function (26) and initiating a signaling cascade that results in the activation of MAPK/Erk pathway, JNK pathway, and increased calcium mobilization (37,36,234,40). Evidence of the involvement of CD19 in Vav activation comes from experiments using CD19-/- B cells, which display attenuated tyrosine phosphorylation of Vav (235,200). Also, CD19-/- mice show reduced B-cell lymphopoiesis and antibody responses to T-cell dependent antigens (236,237), indicating that CD19 may play a role in B cell activation and selection (238). Alternatively, the binding of PI3K to CD19 facilitates its activation, resulting in activation of Btk, which is involved in directing calcium flux, and Akt, which promotes cellular survival (239,240,241,242).

8.2 CD22 and FcgRIIb

While BCR-mediated signaling is essential for B cell maintenance and maturation, it is essential to have a mechanism of negative regulation. CD22 and Fc-gammaRIIb are two transmembrane linker proteins that are involved in the attenuation of BCR signaling. Both of these proteins contain ITIMs in their cytoplasmic tails, which function as binding sites for SH2 domain-containing phosphatases (243,244). CD22 also contains ITAM-like sequences, positioning this adaptor protein to be involved in both the positive and negative regulation of BCR-mediated signaling (245,246,247).

In response to BCR activation, Lyn phosphorylates the ITIM of CD22, and this phosphorylation event provides a docking site for the SH2 domain-containing tyrosine phosphatase SHP1. The recruitment of SHP1 to the BCR results in phosphatase activation and the dephosphorylation of a number of proteins including Lyn and Syk. SHP1 activation has also been implicated in reduced calcium flux by preventing the release of calcium from intracellular stores (248). These data demonstrate the importance in CD22 in the negative regulation of receptor activity (249,250,251,252). Also upon receptor activation, separate tyrosine residues become phosphorylated, creating binding sites for Lyn, Syk, Shc, PI3K, PLCγ, and Grb2 (245). Poe et al. (245) predict that the recruitment of Shc and Grb2 to the phosphorylated CD22 facilitates the binding of SHIP (Src homology 2-containing inositol phosphatase) as part of a CD22/Grb2/Shc/SHIP quaternary complex. The formation of this complex would position SHIP at the plasma membrane, which is considered to be critical for SHIP functional activity (245). At the cell membrane, SHIP is able to reduce the production of IP3, ultimately resulting in a decrease of extracellular calcium influx (248,253). CD22-deficient mice display reduced proliferation responses and decreased overall tyrosine phosphorylation in response to BCR engagement (254,255,256,257,258). These mice also showed an increase in intracellular calcium concentrations (259,260,261), but no activation of the JNK

pathway. Based on these data, CD22 is positioned to both transduce signals from activated tyrosine kinases to downstream effector molecules, and regulate calcium mobilization from both intracellular and extracellular sources.

FcγRIIb also mediates its inhibitory effects on BCR activation through interaction with SHIP (243,244). SHIP is recruited to the phosphorylated ITIM in the cytoplasmic domain of FcγRIIb, where the phosphatase is able to catalyze the hydrolysis of PIP3 to PIP2. As Akt and PLC-gamma require PIP3 for proper enzymatic function, FcγRIIb is capable of attenuating BCR-mediated signaling by effectively altering the pool of available phosphoinositides (262,263,264).

8.3. BLNK

While transmembrane proteins play an important role in BCR signaling, cytoplasmic adaptor proteins are also essential for signal transduction downstream of the activated receptor. BLNK (B cell linker protein) is a cytoplasmic adaptor protein that has been implicated in integrating receptor associated PTKs with downstream calcium and MAPK responses (206). BLNK (also known as SLP-65 and BASH) is thought to be the B cell equivalent of SLP-76, sharing a 33% sequence homology and related domain structures (206). Like SLP-76, BLNK contains several phosphorylated tyrosine residues, a central proline rich region, and a carboxy-terminal SH2 domain (201). Upon BCR stimulation, BLNK is recruited to the membrane by an unknown mechanism, allowing the SH2 domain of BLNK to interact with Syk, resulting in BLNK phosphorylation. This effectively creates SH2-binding sites for Btk, Grb2, Vav, Shc, Nck, and PLC-gamma2. Both Btk and Syk are necessary for the activation of PLC-gamma and subsequent calcium flux, and BLNK appears to be a link between upstream Btk and Syk kinase signaling and downstream effects on calcium mobilization. The interaction between PLC-gamma and BLNK also facilitates the activation of transcription factor NF-kappaB (265), which in turn activates genes involved in promoting cell survival. This interaction is supported by the finding that BLNK inactivation led to a signaling defect in the NF-kappaB pathway (265).

Several studies have determined the importance of BLNK in BCR-mediated signaling. The BLNK-/- DT40 cell line cannot increase intracellular calcium concentrations or activate the Erk, JNK, or p38 signaling pathways (266). However, transfection of wild-type BLNK into this cell line restored BLNK-mediated downstream effects. Additionally, overexpression of a mutant of BLNK that can not be phosphorylated on four of its tyrosine residues, thus preventing PLCγ binding, results in the dominant inhibition of BCR-stimulated PLC-gamma activation, calcium mobilization, and NF-AT activity (267). Pappu et al. (268) created a BLNK-deficient mouse model to examine the *in vivo* role of BLNK in B cells. B cell development in these mice was blocked at an early stage (from B220+CD43+ to B220+CD43-) and only a small percentage of B cells were detected in the periphery. Overall, the phenotype of these mice resembles that of *xid* (X-linked immunodeficiency) or Btk-/- mice (269), indicating that these two proteins function in the same signaling pathways. These results indicate that BLNK, unlike SLP-76 in T cells, is not essential for B cell development. However, BLNK does appear to be a necessary adaptor protein in BCR-mediated signaling, functioning as a scaffolding protein for the establishment of signaling complexes essential in calcium regulation and activation of the MAPK pathways.

9. TYROSINE PHOSPHORYLATION REGULATES ADAPTOR PROTEINS THAT PLAY A ROLE IN SIGNALING CASCADES IN B AND T LYMPHOCYTES

There is mounting evidence that highly regulated mechanisms are involved in restricting the intensity and the duration of signaling through the BCR and TCR (270,271,272,273,274,275). Several adaptor proteins have been identified as regulators of immune cell signaling, as listed in Table 3. Among these are Dok family proteins, CBP/PAG, and SOCS/JAB.

9.1. Dok

The Dok family of proteins (downstream of tyrosine kinases) comprises p62dok (Dok-1), Dok-R/Dok-2, Dok-3, Dok-4, and Dok-5 (276,277,278). The Dok proteins contain a PH domain, PTB domain, and numerous sites for tyrosine phosphorylation (279,280,281), and these proteins are found in various hematopoietic cells, including B cells and macrophages (277). Upon immunoreceptor or growth factor receptor stimulation, Dok becomes tyrosine phosphorylated on several tyrosine residues by Abl, Tek, and Lyn tyrosine kinases, facilitating the binding of Tec kinase (in B cells), RasGAP and Nck *via* SH2 binding to the phosphorylated tyrosines in Dok (279,280,282,283). Subsequent downstream binding partners are specific to individual Dok family members. It has been suggested that the interaction of Dok-1 with Ras-GAP results in the inhibition of Ras activation (284), and Kashige et al. (285) reported that Dok-1 binding to RasGAP negatively regulated its GTPase activity *in vitro*. However, effects of Dok-1 on MAPK signaling are unknown, as several other proteins involved in the activation of MAPK are also activated upon immune cell receptor stimulation. Dok-1 has also been shown to bind to RasGAP after FcγRIIb/BCR coaggregation, and Dok-1/RasGAP complex formation is dependent upon the recruitment and tyrosine phosphorylation of SHIP (286). SHIP is thought to act as an adaptor protein, linking Dok with Fc-gammaRIIb (286). Dok-3, which is highly expressed in B cells and myeloid cells, but not T cells (277), does not interact with RasGAP as is observed with Dok-1 and Dok-2. Instead, Dok-3 was found to interact with the protein tyrosine phosphatase SHIP and the PTK Csk subsequent to tyrosine phosphorylation. The interaction of Dok-3 with inhibitory molecules positions this protein to act as a negative regulator of BCR-mediated signaling (277). In conclusion, the Dok family proteins represent a key down-regulator of the Ras signaling pathway (285).

9.2. PAG/Cbp

Cbp (Csk-binding protein)/PAG (phosphoprotein associated with GEMs) is a type III transmembrane adaptor

protein that appears to function in the regulation of Src family tyrosine kinases though its association with the Src negative regulator Csk (287,288). Cbp contains a putative palmitoylation sequence, two potential SH3 binding motifs, and ten potential tyrosine phosphorylation sites. Cbp has been found to be localized in GEMs, and this facilitates interaction between the phosphorylated tyrosine residues of Cbp and the SH2 domain of Csk (287,288). Overexpression of Cbp in COS cells results in Src kinase suppression (287), and Csk association was required for this suppression to occur. Also, Cbp overexpression in Jurkat cells resulted in impaired Fyn activity and reduced TCR-mediated NF-AT activation (288). These data suggest a model in which Cbp couples Csk to Src family members in the GEMs of resting lymphocytes (206). Receptor activation results in the dephosphorylation of Cbp and subsequent disengagement of Csk, resulting in free activation of Src family members.

9.3. SOCS/JAB

Hematopoietic cells regulate their growth, function, and differentiation through a diverse cytokine signaling network (289). As most cytokine receptors lack intrinsic kinase activity, ligand-dependent phosphorylation events are mediated by non-receptor tyrosine kinases (289). The main tyrosine kinases associated with cytokine receptors are the Janus (JAK) family. Upon cytokine binding the receptors oligomerize, inducing JAK activation. The activated JAKs can in turn phosphorylate tyrosine residues in the cytokine receptor tail, leading to the recruitment and activation of other signaling molecules like STAT (signal transducers and activators of transcription) proteins. Activated STAT molecules complex and translocate to the nucleus where they activate the transcription of a variety of cytokine responsive genes. While it is known that most cytokine responses are limited in duration and magnitude, little is known about mechanisms by which cytokine signaling is regulated (289). One family of proteins that may be involved in this process are the SOCS/JAB/CIS proteins.

SOCS (suppressors of cytokine signaling) are a newly characterized family of cytokine-inducible suppressors of cytokine signaling (also called JAB – janus kinase binding protein, SSI–STAT-inducible STAT inhibitors, and CIS–cytokine-inducible SH2 protein) (290,291,292,293,294,295,296). To date, there are eight members of the SOCS family (CIS and SOCS1-7), all containing a similar protein domain structure, including an N-terminal variable region, a central SH2 domain, and a C-terminal SOCS-box motif (290,291,292,293,294,295,296). SOCS1 (JAB/SSI-1) and SOCS3 are both involved in the inhibition of the Jak/STAT pathway of various cytokines (297), while SOCS2 appears to counteract the inhibitory actions of SOCS1 (298,299). CIS has been identified as a specific inhibitor of STAT5 signaling, while the functions associated with SOCS4-7 have yet to be identified (300,301). The expression of SOCS proteins appears to be regulated by cytokine signaling, as SOCS-1 mRNA was found to be increased upon cytokine stimulation (291,293). Additionally, IFN-gamma and, to a lesser extent, IL-6 have been shown to induce SOCS protein expression (302). This presents a fascinating negative feedback loop in which the ability of SOCS to down-regulate cytokine signaling is dependent upon signaling through that particular cytokine.

CIS was first identified by Yoshimura (290) as an immediate early gene induced in response to signaling by IL-2, IL-3 and EPO. Overexpression of CIS can partially suppress IL-3 and EPO-induced proliferation, as well as STAT5 activation (290,300). CIS1 was found to specifically bind to the phosphorylated tyrosine residue at position 401 of the EPO receptor, a major site of binding for STAT5 (303). Therefore, CIS1 may function to inhibit cytokine signaling by competing with STAT5 for binding sites on the activated receptor (289). Another possibility is that CIS1, by virtue of its binding domains, functions as an adaptor protein, linking negative signaling molecules to the activated receptor. Matsumoto (301) generated transgenic mice expressing the CIS gene under control of the beta-actin promoter. These mice developed normally, though their body size and weight was decreased with respect to wild type mice, indicating a defect in hormone signaling. Female transgenic mice showed defects in the development of their mammary glands. Also, IL-2-induced activation of STAT5 was inhibited in T cells of these transgenic mice, while leukemia inhibitory factor (LIF)-induced STAT3 activation was not affected. These phenotypes resemble the phenotype of STAT5a and STAT5b knockout mice (304,305,306,307,308). These data suggest a role for CIS1 in the negative regulation of STAT5 mediated signaling pathways.

JAB/SOCS1/SSI-1 (herein referred to as SOCS1) was first identified as an inhibitor of IL-6-induced differentiation of murine monocytic leukemic M1 cells, a JAK binding protein, and also as a protein with an SH2 domain similar to that of STAT proteins (292,293,291). In fact, SOCS1 has been shown to bind to all four JAKs proteins *via* its SH2 domain, and to inhibit JAK kinase activity *in vitro*. Overexpression of SOCS1 results in the inhibition of any signaling pathway utilizing the JAK kinases, including STAT5 activation by EPO receptor, STAT3 activation by LIF or IL-6, and c-fos induction by IL-2 (302). The mechanism by which SOCS regulates JAK activation is similar to CIS/STAT5 regulation. The SOCS1-SH2 domain binds to the Y1007 in JAK2, a critical tyrosine residue within the activation loop of the JAK2 kinase domain (309). Based on extensive mutational analysis, it was determine that the interaction with JAK2 also requires an additional N-terminal 12 amino acid region (the kinase inhibitory region, KIR) (302). Thus, SOCS1 appears to play an essential role in the down-regulation of cytokine signaling by inhibiting JAKs activation.

Recently, SOCS1-/- knockout mice were developed (310,311,312). These mice displayed severe growth retardation and died within three weeks after birth. Fatty degeneration of the liver was observed, as well as monocytic infiltration of several organs. Lymphocytes in the thymus and spleen were decreased in comparison to wild type lymphocytes, and showed accelerated apoptosis with age. These phenotypes suggest that SOCS-1 is required for the *in vivo* regulation of several cell types, and is essential for normal postnatal growth and survival (302).

10. SERINE/THREONINE PHOSPHORYLATION OF ADAPTOR PROTEINS

Phosphorylation of proteins is a reversible form of posttranslational modification that occurs rapidly in response to cellular signals to regulate protein function. Phosphorylated proteins often display conformational changes and/or altered ability to interact with binding partners. Until recently tyrosine phosphorylation regulated protein-protein interactions were the subject of thorough investigation while the sole consequence of serine/threonine phosphorylation were thought to be allosteric modifications. In the past few years it became apparent that serine/threonine phosphorylation regulates protein-protein interactions not only *via* allosteric mechanism, but also results in their ability to form complexes with phospho-serine/threonine specific binding partners. Four phospho-serine/threonine binding domains that have been identified are described below.

14-3-3 ligand binding groove was the first phospho-serine/threonine specific binding domain to be identified (313). It represents a carboxy-terminal part of the 14-3-3 molecule with highly conserved residues lining the inner surface of a concave ligand-binding groove (314). Co-crystallization studies have confirmed the phosphorylation specific nature of 14-3-3-ligand interactions (315). Most well-known consensus sequences for 14-3-3 conserved binding groove are RSXpS/TXP and RXSXpS/TXP. Arginine in –3 or –4 position relative to phosphorylated serine or threonine is required, whereas serine in –2 and proline in +2 are preferable. Serine or threonine in position 0 must be phosphorylated for 14-3-3 to bind to a target protein. Phosphorylation of serine in –2 position does not enable 14-3-3 binding (316). 14-3-3 binds to a variety of signaling and structural proteins, thus it may act as a scaffold to bring different target proteins into complexes facilitated by its ability to form saddle-shaped dimers *via* interaction between amino terminal parts of the two 14-3-3 molecules (314).

WW domains are relatively small 35-40 amino acid modules that bind proline-rich sequences PPXY or PPLP. Recently it became apparent that certain WW domains bind target proteins in a phosphorylation specific manner. WW domains of prolyl isomerase Pin1 and ubiquitin ligase Nedd4 have been shown to recognize pSer/Thr-Pro motifs on target proteins (317). Pin1 is a prolyl isomerase, a protein whose function is cis-trans isomerization of peptidyl-prolyl bond. Pin 1 differs from other prolyl isomerases as it is able to catalyze isomerization of pSer/Thr-Pro bond while it is known that phosphorylation of Ser or Thr residue in Ser/Thr-Pro sequences reduces the rate of isomerization for other prolyl isomerases (318). WW domain of Pin 1 binds mitotic phosphoproteins *via* interactions with p-Ser or p-Thr. This binding occurs in a phosphorylation dependent manner, since Pin 1 does not initiate binding to mitotic targets if they were dephosphorylated. The crystal structure shows that the Pin 1 WW domain has a hydrophobic cluster formed by different strands of beta sheets (318). Co-crystallization of the WW domain of Pin 1 with a phosphopeptide substrate shows the structural basis for WW-phosphorylated target interactions (319). WW domain of ubiquitin ligase Nedd4 has also been shown to associate with target proteins *via* phosphothreonine or phosphoserine interactions, which are responsible for presentation of proteins for ubiquitination (318,317).

Forkhead associated domains (FHA) are conserved 55-75 amino acid modules that were originally found in forkhead family transcription factors (320). They are present in both eukaryotes and prokaryotes. FHA domain contains three highly conserved stretches of amino acid separated by variable regions. Each conserved stretch contains invariable residues in the middle of it – a Gly for the first and third, His for the second position. The secondary structure of FHA has a beta strand structure. The majority of FHA proteins known today are nuclear proteins that fall into three categories – transcription factors, DNA repair proteins or cell cycle proteins. Yeast FHA containing DNA repair protein Rad53p is the best studied. It has two FHA domains, FHA1 and FHA2, both of which bind to target proteins in a phosphorylation dependent manner, specifically recognizing phospho-threonine (321,322). The FHA1 domain binds phosphothreonine-containing sequences within the consensus pTXXD, whereas FHA2 binds pTXXI (323,324). Other FHA containing proteins display different preferences for amino acid in the +3 position though it is clear that their binding to target proteins is phospho-threonine specific. Liao and co-workers have shown that the FHA2 domain of Rad53p can also bind phosphotyrosine containing targets (321). Their data suggest that FHA domains may be dual specificity binding modules.

WD 40 motifs were originally discovered in the beta subunit of heterotrimeric G proteins (325). Since then they have been found in various other proteins. WD 40 motifs represent regions of approximately forty amino acids. Their characteristic feature is that they always terminate with Trp-Asp (WD). WD-40 motifs are always arranged in tandem with sequence similarity among them. In the past few years a number of reports indicated that WD-40 motifs of F-box containing proteins bind their target proteins in a serine/threonine phosphorylation-dependent manner (317). F-box proteins are the ones responsible for recognizing phosphorylated substrates and linking them to E3 ubiquitin ligases. The biochemical data accumulated on F-box-protein interactions with their substrates suggests that it is WD-40 repeat regions of F-box proteins that mediate F-box protein binding to the substrate (326,327,328,329). Unlike for 14-3-3, WW and FHA domains, for WD-40 domains no experiment has been performed to provide us with structural proof that WD-40 indeed binds substrates in phospho-serine/threonine dependent manner.

11. DIRECT EFFECTS: SERINE/THREONINE PHOSPHORYLATION OF AN ADAPTOR PROTEIN RECRUITS IN A BINDING PARTNER FOR IT.

11.1. Paxillin

Paxillin was initially discovered as a tyrosine phosphorylated protein from Src transformed cells (330). Paxillin is localized to focal adhesions, where it serves as a scaffold for a wide variety of signaling and structural proteins. The ability of paxillin to bind various proteins is attributed to several protein-protein interaction domains - i.e., LD motifs and SH2 and SH3 binding motifs in the

amino terminus and four LIM domains in the carboxy terminus. LD motifs are conserved eight amino acid leucine rich sequences (LDXLLXXL), serving as binding sites for a number of paxillin binding partners such as FAK, vinculin, and PYK2. LIM domains are double zinc finger motifs approximately 50 amino acids long whose function is to serve as protein-protein binding modules. LIM domains of paxillin promote its localization to the plasma membrane *via* association with integrins. Paxillin is crucial for embryogenesis since paxillin knockout mice die on the seventh-eighth day of embryogenesis (160). Paxillin deficient cells display an inability to spread (161).

Tyrosine phosphorylation of paxillin and its functional significance has been extensively studied for some time, while serine/threonine phosphorylation has started to draw attention only recently. Paxillin is phosphorylated on serine residues *in vivo* in response to plating of cultured cells on vitronectin or fibronectin, and also in mitosis and in MCF-7 cells in response to heregulin stimulation (331,332,333,334). The identity of serine/threonine kinases that phosphorylate paxillin *in vivo* is unknown although it is known that paxillin is serine phosphorylated in response to PKC activation following PMA treatment and treatment with a PKC inhibitor reduces paxillin serine phosphorylation (331). The fact that paxillin is phosphorylated upon adhesion of cells to ECM was confirmed by (335). Their work is cell type specific for EL4 cells, which represent murine thymoma cell line. These cells display an ability to adhere in response to PMA treatment. PMA treatment also results in serine phosphorylation of paxillin. The signaling cascade may proceed from PMA stimulation *via* MEK/ERK pathway to adhesion. If a specific inhibitor of MEK activation is added, there is a reduction in a number of adherent cells. ERK is one (though not only) kinase that is activated downstream of PKC in these cells. MEK/ERK inhibitor abolished PMA induced paxillin phosphorylation leading to the suggestion that it proceeds from PKC *via* MEK/ERK pathway to paxillin. Paxillin is a substrate for ERK2 *in vitro* (335). Recent data of Hashimoto and co-workers indicate that paxillin associates with PAK3 and serves as a substrate for this kinase *in vitro* (336).

Paxillin has four LIM domains in its carboxy terminus. GST-LIM2 and GST-LIM3 constructs were phosphorylated on threonine or serine, respectively, *in vitro* (whereas GST-LIM1 and GST-LIM4 were not). Site directed mutagenesis was employed to identify phosphorylation sites within LIM2 and LIM3 domains. LIM3 and LIM2 become phosphorylated during cell adhesion to fibronectin *in vivo*. Phosphorylations of LIM domains of paxillin promote localization of this protein to focal adhesions. Certain site-directed mutants of paxillin, which have serine or threonine mutated to alanine or valine, respectively, do not localize to focal adhesions as efficiently as a wild type, and as a result these cells adhere to fibronectin poorly. Serine/threonine phosphorylations of LIM domains bring paxillin to newly created focal adhesions (337).

Serine/threonine phosphorylation of paxillin also has a role in cell movement, which was described in a paper by Vadlamudi and colleagues (334). They found that invasiveness of MCF-7 cells is increased in response to heregulin exposure. Along with this, in response to heregulin treatment paxillin becomes phosphorylated on serine and threonine residues (334) as demonstrated by SDS-PAGE mobility shift, phosphoamino acid analysis and serine/threonine phosphatase versus tyrosine phosphatase treatments; the latter does not interfere with heregulin induced phosphorylation of paxillin (334). This group of researchers found that two pools of paxillin are present in MCF-7 cells: one of them is co-localized with focal adhesions, while the other is cytoplasmic. Heregulin treatment causes relocation of paxillin from focal adhesions to perinuclear areas of the cytoplasm accompanied by changes in cell shape. Serine/threonine phosphorylation of paxillin and accompanying cell scattering are dependent upon p38MAPK rather than p42/44MAPK pathway activation, which has been demonstrated by use of specific MEK inhibitors. Overall, these data indicate that heregulin treatment leads to paxillin relocation from focal adhesions that promotes disassembly of the latter, which is necessary for cell movement to occur (334).

11.2. Gab1

The name Gab1 stands for Grb2 associated binder 1. In response to insulin, cytokine or growth factor stimulation (HGF, EGF, NGF, PDGF) Gab1 becomes phosphorylated on tyrosine and binds the tyrosine-phosphorylated receptor directly, or indirectly *via* Grb2 in a phosphorylation dependent manner (338). Gab1 has an amino terminal PH domain that tethers Gab1 at the membrane *via* PIP3 interaction and carboxy terminal Met binding domain (MBD) that mediates direct interaction of Gab1 with c-Met, and also has binding sites *via* which it interacts with SH3 domain of Grb2. There are 21 tyrosine residues in between these two regions, which upon phosphorylation serve as docking sites for a number of binding partners. The list of Gab1 binding partners includes Grb2, cMet, p85 subunit of PI3K, PLC-gamma, Shc, Shp2 phosphatase, Crk and CrkL (339,340,338).

Phosphoamino acid analysis of Gab1 overexpressed with dominant negative or activated MEK shows that serine/threonine phosphorylation levels of Gab1 are elevated in response to co-expression with constantly active MEK. Gab1 binds phosphorylated ERK2 directly and phosphorylated by it; Gab1 also associates with phospho-Erk1 in response to MEK activation (341). Several years later this same group of authors followed with another report, which demonstrates that c-Met activation results in MAPK cascade activation, and Gab1 becomes phosphorylated by active ERK2 followed by recruitment of PI3K to the membrane (339). Consequently, Akt activation follows in response to PI3K activation as a result of an association with Gab1. They state that Gab1 has to be phosphorylated both on threonine and tyrosine residues to ensure for PI3K association (339).

Okadaic acid treatment of cultured cells results in serine/threonine phosphorylation of Gab1 (342). One of the effects of c-Met stimulation by HGF is cell scattering, which is the result of c-Met stimulated increased cell motility. Okadaic acid treatment of cultured cells prior to

HGF stimulation prevents cell scattering. Under these conditions Gab1 is found to be hyper-phosphorylated on serine and threonine residues and hypo-phosphorylated on tyrosine residues. The okadaic acid-promoted decrease in tyrosine phosphorylation of Gab1 is a consequence of its elevated serine/threonine phosphorylation which by some undetermined mechanism prevents c-Met from interacting with and phosphorylating Gab1 (342).

Serine/threonine phosphorylation results in Gab1 association with 14-3-3. The functional significance of this interaction remains to be determined, as it does not seem to interfere with Gab1/Met1 interactions and therefore sequester Gab1 from the membrane. Okadaic acid activates MAPK and PKC by inhibition of serine/threonine phosphatases. Using pharmacological agents that are known to be specific inhibitors of MAPK pathway or PKC kinase, they show that for serine/threonine phosphorylation of Gab1 in response to okadaic acid treatment is PKC dependent. Phorbol ester treatment results in a decrease of both electrophoretic mobility and tyrosine phosphorylation levels of Gab1. Gab1 is a substrate for conventional PKC isozymes *in vitro*. PKC inhibitor staurosporine treatment restores cell scattering inhibited by okadaic acid (342). The authors hypothesize that serine/threonine phosphorylation alters Gab1 conformation so that tyrosine residues cannot be presented for phosphorylation; as a consequence, the docking sites for binding partners are not being created which results in Gab1 inability to associate with them (342).

11.3. PECAM-1

Platelet endothelial cell adhesion molecule 1 (PECAM-1) is a transmembrane glycoprotein belonging to immunoglobulin family of adhesion molecules that is expressed only in hematopoietic and vascular cells. Upon phosphorylation of tyrosines within its ITAM motif it becomes a binding partner for PI3K, PLC-gamma, SHP1 and 2, Stat5a, and cell-cell junction proteins catenins beta and gamma (343,344,345). PECAM-1 is phosphorylated not only on tyrosine but also on serine and threonine residues. The levels of serine/threonine phosphorylation of PECAM-1 are elevated in response to LPS treatment of monocytes in a PKC dependent manner, which is demonstrated by the observation that PKC inhibitor treatment down regulates LPS induced serine/threonine phosphorylation of PECAM-1 (346). Along with PECAM-1 serine/threonine phosphorylation LPS induces transendothelial migration of monocytes. Phosphorylation of PECAM-1 on serine and threonine residues appears to be connected to cell migration, this conclusion comes from the fact that PKC inhibitor treatment blocks monocyte migration through the endothelial cell layer (346). Phosphatase inhibitor treatment up-regulates both PECAM-1 serine/threonine phosphorylation and transendothelial movement of monocytes. These data suggests that PECAM-1 may be involved in the regulation of monocyte movement (346).

PECAM-1 is able to associate with both beta and gamma catenins (343). Serine/threonine phosphorylation of PECAM-1 has been shown to down regulate its ability to associate with gamma catenin (344). PECAM1 is a substrate for PKC *in vitro* and phosphorylated on serine/threonine residues in response to PKC activation *in vivo* (344). In response to PKC activation by DAG a decrease in PECAM-1 interaction with gamma catenin is observed, whereas PKC inhibitor bisindolylmaleimide treatment promotes this interaction. PKC, therefore, may be the kinase responsible for serine/threonine phosphorylation of PECAM-1 *in vivo* and PKC dependent serine/threonine phosphorylation of PECAM-1 may be a mechanism of regulation PECAM-1/gamma catenin interactions. Experiments of Ilan and colleagues suggest that one of the functions of PECAM-1 may be to bring catenins to adherence junctions (344); PECAM-1 interaction with gamma catenin is thought to link PECAM-1 to the cytoskeleton *via* vimentin and therefore participate in transduction of signals that regulate cell migration (344).

11.4. Sos

In response to growth factor stimulation receptor tyrosine kinases will autophosphorylate on tyrosine residues, creating binding sites for the adaptor proteins Grb2 and Shc. They, in turn, recruit a protein named Son of sevenless (Sos) to the membrane. Sos protein is a guanine nucleotide exchange factor for Ras. Once at the membrane, Sos promotes GDP to GTP exchange on Ras, activating it. Sos links growth factor receptor activation to Ras stimulation (347). It was determined several years ago that Sos1 is phosphorylated in response to EGFR stimulation (348). Phosphoamino acid analysis determined that Sos is phosphorylated predominantly on serine and to the lesser extent on threonine residues, while no tyrosine phosphorylation was detected (349). It was observed that the C terminus of Sos contains potential sites for MAPK phosphorylation. Indeed, using phosphotryptic-mapping analysis it was demonstrated that Sos is a substrate for MAP kinase both *in vitro* and *in vivo*, and the sites of phosphorylation were identified (350,351). Since it has been determined that Sos protein is serine/threonine phosphorylated, researchers have been trying to elucidate the functional significance of Sos phosphorylation. Rozakis-Adcock and co-workers demonstrated that serine/threonine phosphorylation of Sos down regulates Sos-Grb2 association with EGFR and Shc although does not affect Sos interaction with GST-fused Grb2 (349). Corbalan-Garcia and colleagues discovered that in response to being phosphorylated Sos dissociates from Grb2. The data accumulated by the two groups must be somewhat controversial, which might be explained by the differences in experimental setup and also by the fact that these groups of authors used Sos from different species (either human or murine) in their experiments. Both groups agree that MAPK dependent Sos phosphorylation serves as a feedback mechanism of Ras/MAPK pathway regulation. Sos is phosphorylated in MAPK cascade dependent manner by active MAP kinase and supposedly by other serine/threonine kinases that are activated downstream of MAPK pathway (349,350).

In T cells the MAPK cascade is activated in response to TCR ligation. Both TCR activation and PMA stimulation result in serine/threonine phosphorylation of

Sos-1; this is demonstrated by reduced mobility of Sos-1 derived from PMA stimulated cells (352). The mobility shift is lost if phosphatase treatment has been applied to Sos-1 from PMA treated cells. In T cells serine/threonine phosphorylation of Sos-1 is not MAPK cascade dependent, this conclusion comes from the observation that MEK inhibitor treatment does not result in decrease in serine/threonine phosphorylation levels of Sos-1 promoted by TCR engagement. Mutation of seven potential MAPK consensus sites does not diminish ability of Sos-1 to be phosphorylated on serine/threonine residues either (352).

It is well known that prolonged exposure to PMA results in depletion of cellular pool of PKC due to its subsequent ubiquitination (353,354). When cells that had undergone 16-hour PMA treatment were subjected to TCR stimulation no serine/threonine phosphorylation of Sos was detected. The same result was observed in cultured cells pre-treated with specific PKC inhibitors (352). Therefore, PKC may be a Sos kinase *in vivo*. Phosphorylation of Sos did not lead to its uncoupling from Grb2 under described conditions, which is in agreement with previous data of Rozakis-Acock and co-workers. Zhao and colleagues did not attempt to clarify the functional significance of Sos phosphorylation (352).

11.5. Cbl

c-Cbl is an adaptor protein that is ubiquitously expressed and known to be phosphorylated on tyrosine residues (102). Cbl has several modular domains including a zinc ring finger motif, SH2 and SH3 binding motifs and leucine zipper motif. These modules facilitate the association of Cbl with 40 target proteins, among which are receptor and non-receptor tyrosine kinases, E2 ubiquitination enzyme, p85 subunit of PI3 kinase, and adaptor proteins such as Grb2, Nck, and Crk. Cbl has the ability to form dimers *via* its carboxy-terminal leucine zipper motif. T cell receptor activation leads to tyrosine phosphorylation of Cbl and its transient association with CrkL and the p85 subunit of PI3K (355,102). P85 binds Cbl directly but it cannot bind CrkL, unless it associated with Cbl that brings it into a close proximity with CrkL. Cbl has to be tyrosine phosphorylated to perform this function. Cbl, therefore, act as a scaffold that brings PI3K and CrkL together (355).

Liu and co-workers found that TCR ligation leads to serine/threonine phosphorylation of Cbl upon which Cbl becomes associated with 14-3-3 (356). Cbl interaction with 14-3-3 is direct and occurs only in response to TCR (356) or CD43 (357) ligation; while in unstimulated cells there is no Cbl-14-3-3 complex detected. 14-3-3 displaces PI3K from Cbl. Serine/threonine phosphorylation of Cbl has also been shown to down regulate its tyrosine phosphorylation and association with another partner, the adaptor protein CrkL (356). PMA treatment promotes serine/threonine phosphorylation of Cbl and its consequent association with 14-3-3. Pretreatment of cells with specific PKC inhibitors prior to PMA treatment results in an inability of PMA to induce serine/threonine phosphorylation of Cbl. Cumulatively, these data suggest that Cbl phosphorylation may be PKC dependent (358,355,357). PMA treatment also results in a decrease in tyrosine phosphorylation levels of Cbl and therefore promotes its dissociation from p85 and CrkL. Recent work of Pedraza-Alva and co-workers shows that CD43 receptor cross-linking causes Cbl association with Vav and PI3K; the latter is activated in response to this interaction event (357). It is noteworthy that Cbl does not have to be phosphorylated on tyrosine residues for described CD43 induced interactions to occur, as there is no tyrosine phosphorylation of Cbl detectable under those conditions. Pedraza-Alva and colleagues attempted to gain an insight into the functional significance of serine/threonine phosphorylation of Cbl (357). It has been known for some time that overexpression of Cbl has an inhibitory effect on receptor-mediated MAP kinase activation. PKC dependent phosphorylation of Cbl followed by its association with 14-3-3 is thought to block the negative effect of Cbl on MAP kinase cascade activation (357). Serine/threonine phosphorylation of Cbl also negatively regulates Cbl tyrosine phosphorylation and therefore hampers its interaction with PI3K, CrkL and other phosphotyrosine binding partners. All the data on Cbl serine/threonine phosphorylation is T cell specific. Cumulatively, it leads to the conclusion that adaptor protein Cbl is phosphorylated on tyrosine and serine/threonine *via* two separate receptor-signaling pathways. Phosphorylation on either tyrosine or serine/threonine residues may enable Cbl to associate with different set of binding partners and therefore be engaged in either one of the two signaling cascades descending from the T cell receptor.

11.6. IRS1

Insulin receptor substrate 1 is a 165-185 kDa protein that associates with SH2 containing proteins in a phosphorylation dependent manner. IRS1 becomes phosphorylated on tyrosine residues by insulin receptor and consequently associates with PI3 kinase, activating the latter. PI3 kinase activation results in activation of Akt/PKB, which sends the signal further downstream. The physiological consequences of insulin receptor signaling are glucose uptake and glycogen synthesis. In response to PDGF (in a PI3K/Akt dependent manner) (359,360) or endothelin-1 (in a PKC and MAPK dependent manner) (360) stimulation IRS1 becomes phosphorylated on serine residues, four of which are MAPK consensus sites (361). Serine phosphorylation of IRS1 leads to the decrease in its tyrosine phosphorylation levels (359,360) and IRS1/PI3K complex disengagement (360,362) therefore inhibiting insulin receptor signaling. PDGR receptor stimulation results in inhibition of insulin receptor signaling, cased by inability of IRS-1 to be tyrosine phosphorylated and form a complex with PI3K. The IRS1 mutant, which has four serine residues substituted by alanines, retains the ability to be tyrosine phosphorylated in response to insulin treatment in the presence of PDGFR stimulation and associate with PI3K, activating it. In response to Akt activation IRS1 association with PI3K is abrogated. It is not known yet whether Akt phosphorylates IRS-1 directly or simply promotes IRS-1 phosphorylation by activating serine/threonine kinase(s) downstream of it (360). Whatever the mechanism might be, attenuation of insulin-receptor signaling by active Akt suggests a model for negative feedback loop where PI3 kinase, activated in

response to insulin-receptor stimulation *via* IRS-1 interaction, in turn activates Akt, which comes along and phosphorylates IRS-1 causing dissociation of IRS-1/PI3K complex (360).

Ogihara and colleagues (363) and Staubs and co-workers (359) observe serine/threonine phosphorylation of IRS1 in response to PI3K association promoted by insulin receptor activation. IRS1 is a substrate for PI3K *in vitro* (364,365). These data suggests that PI3 kinase itself may be responsible for an attenuation of insulin receptor signaling. Ogihara and co-workers also suggest that serine phosphorylation of IRS1 may lead to presentation of phosphotyrosines for tyrosine phosphatases therefore enabling attenuation of insulin signaling (363).

Decrease in tyrosine phosphorylation of IRS1 was also observed in response to PMA treatment of cultured cells (366). Activation of endogenous PKC by phorbol ester treatment inhibits insulin receptor-mediated tyrosine phosphorylation of IRS-1. It has not been demonstrated whether IRS1 is a substrate for PKC either *in vitro* or *in vivo* (366).

PKC is known to activate ERK1/2 in response to its activation. IRS1 is a substrate for ERK1/2 *in vitro* (367). IRS1 contains four potential MAPK sites of a consensus PXS/TP. PKC activates ERK1/2 in Raf-1 dependent manner. Both ERK1/2 and Raf activation result in decreased insulin receptor mediated tyrosine phosphorylation of IRS1 *in vivo*, resulting in diminished IRS1/PI3K association (367).

Both insulin and PDGF receptor activation lead to PI3 kinase activation by different mechanisms, which are responsible for different outcomes. IRS-1 is a key player in insulin receptor signaling as it activates PI3K *via* association with it and thereby triggers insulin-receptor mediated physiological events. PDGF receptor activates PI3 kinase *via* another pathway and causes PI3K dependent serine/threonine phosphorylation of IRS-1, interfering with insulin receptor signaling. Serine/threonine phosphorylation of IRS-1 was observed in response to activation of insulin receptor, which is in agreement with the statement that serine/threonine phosphorylation of IRS-1 may provide the means for a negative feedback regulation of insulin receptor signaling.

11.7. pShc66

Shc is a ubiquitously expressed adaptor protein, which plays an important role in growth factor receptor signaling. Upon tyrosine phosphorylation by ligand-activated receptor tyrosine kinases Shc becomes a docking molecule for Grb2 allowing Grb2 to bring Ras-GTP Sos to the membrane. Three isoforms of Shc are known – two of them, p46 Shc and p52 Shc are synthesized from the same transcript (starting sites differ), whereas p66 Shc is a result of alternative splicing (368,53). The three members of Shc family may have different biological functions (56,53). p66 Shc, for example, plays a role in regulation of oxidative stress response and life span in mice (369). Shc66 knockout mice have a prolonged life span and increased resistance to oxidative stress (369). p46 Shc and p52 Shc cannot substitute for the p66 defect in p66Shc-deficient mice. Oxidative stress response mediated by p66 Shc is serine phosphorylation dependent, which is demonstrated by the fact that non-phosphorylated p66Shc cannot revert mice to a normal phenotype (369). EGFR stimulation results in both tyrosine and serine/threonine phosphorylation of the p66Shc isoform in CHO cells, which is demonstrated by a mobility shift characteristic of serine/threonine phosphorylation. Moreover, p66Shc has to be phosphorylated on tyrosine prior to serine/threonine phosphorylation. Serine/threonine phosphorylation of p66Shc is MAPK cascade dependent, although the identity of the kinase is unknown. Upon serine/threonine phosphorylation the ability of p66Shc to bind EGFR is abolished, although it is still able to associate with Grb2. p66Shc competes with p52Shc for Grb2 as the amount of Grb2 in cells is limiting. Therefore, in response to serine/threonine phosphorylation p66Shc carries Grb2 away from the EGFR. Overexpression of p66 Shc inhibits ERK activation (370). These data suggest the existence of a negative feedback regulation of EGFR signaling. EGFR ligation leads to engagement of Shc/Grb2/Sos complex *via* which activation of Ras/Raf/MEK/ERK cascade occurs. Serine/threonine phosphorylation of p66Shc leads to disengagement of Shc/Grb2 complex from the EGFR leading to attenuation of the EGFR signaling (370). In mesangial cells p66Shc is serine/threonine phosphorylated in response to endothelin-1 receptor stimulation (371). p66 Shc serine/threonine phosphorylation occurs in response to ERK1/2 activation although the identity of kinase that phosphorylates Shc66 under these conditions is unknown. Serine phosphorylation of p66Shc brings it into proximity with three proteins two of which remain to be identified and the third is 14-3-3 (371). Function of p66Shc association with 14-3-3 remains unknown. As 14-3-3 is known to bind a wide variety of proteins, and the 14-3-3 dimer is capable of binding two different proteins at once, it may either bring p66Shc into proximity with some yet unidentified binding partner(s) for undetermined purpose or simply to sequester p66Shc from the membrane, preventing it from competing with two other Shc isoforms for Grb2 pool.

Taxol, an antitumor agent that has already been approved for treatment of breast, lung, and ovarian carcinomas, causes serine/threonine, but not tyrosine, phosphorylation of pShc66 in human lung carcinoma cells (372). Serine/threonine phosphorylated p66Shc prevents it from interacting with Grb2. Although phosphorylation of Raf-1 and ERK1/2 is observed simultaneously with p66Shc phosphorylation, these kinases are not responsible for serine/threonine phosphorylation of pShc66. Taxol-mediated serine phosphorylation of p66Shc is protein synthesis dependent, as cycloheximide treatment inhibits taxol-induced phosphorylation of p66Shc and Raf-1. These data lead to hypothesis that taxol-inducible p66Shc activation occurs in response to stimulation of yet unknown signaling pathway, different from the MAP kinase cascade (372).

Taxol is a microtubule-interacting agent that is capable of arresting cell in mitosis and it also has an apoptotic effect. Since the serine/threonine phosphorylation

of Shc reaches maximum at 9-18 hours after treatment, when cell may be in the mitotic stage, the authors speculate that Shc66 serine/threonine phosphorylation may be responsible for transducing an apoptotic signal (372) referring to the fact that Ser→Ala substitution of p66 Shc enhances resistance to apoptosis (369).

12. DIRECT EFFECTS: SERINE/THREONINE PHOSPHORYLATION OF AN ADAPTOR PROTEIN ALTERS ITS CONFORMATION AND SUBSEQUENT FUNCTION

12.1. AFAP-110

AFAP-110 was first identified as a 110-kDa protein that co-immunoprecipitated with activated Src (373). In normal cells AFAP-110 co-localizes with actin stress fibers and the cortical actin matrix. AFAP-110 has several protein-protein interaction modular domains such as WW domain, SH2 and SH3 binding motifs, serine threonine kinase interaction domain, pleckstrin homology (PH) domains, leucine zipper motif, and carboxy terminal actin binding domains (374). AFAP-110 binds actin filaments directly *via* its carboxy-terminal actin binding sequences, and it associates with active Src *via* SH2/SH3 interactions. The amino-terminal PH domain of AFAP-110 has been shown to facilitate its interactions with PKC (Qian et al, manuscript in preparation). AFAP-110 therefore may serve as an adaptor protein linking signaling proteins to actin filaments.

AFAP-110 has the capability to self-associate *via* interactions enabled by its carboxy-terminal leucine zipper motif. In non-transformed cells it exists in multiple forms including monomers, dimers, trimers, tetramers and even larger complexes. Upon co expression with active Src, AFAP-110 is detected as dimers, but not in larger complexes. Moreover, AFAP-110 self-association is diminished. These data indicate that active Src causes a conformational change in AFAP-110 responsible for alteration in leucine zipper motif mediated self-association (375).

AFAP-110 has an intrinsic ability to remodel actin filaments as its leucine zipper mutant overexpression causes actin filament disruption in cultured cells. This ability depends upon conformational changes in this protein, which affect its multimerization status. AFAP-110 is hyperphosphorylated in Src transformed cells, not only on tyrosine but also on serine and threonine residues (376). Recent data indicates that serine/threonine and not tyrosine phosphorylation of AFAP-110 is responsible for changes in its self-association (Baisden et al, in preparation). AFAP-110 is a substrate for PKC *in vitro* and serine/threonine phosphorylated in response to PKC activation *in vivo* (Qian et al, in preparation). Non-phosphorylated AFAP-110 cross-links actin filaments whereas in response to PKC phosphorylation in *vitro* it bundles them (Qian et al, in preparation). Serine/threonine phosphorylation of AFAP-110 alters its function by induction of AFAP-110 transition from actin-filament cross-linking to bundling protein. The change in AFAP-110 function occurs in response to phosphorylation-dependent alteration in its conformation and therefore the ability to self-associate. Recent evidence indicates that the site for PKC phosphorylation that mediates this change exists in the serine/threonine kinase interaction domain of AFAP-110 (377) (Cherezova and Flynn, unpublished observation). PKC activation induces motility structure formation such as lamellipodia and filopodia along with disappearance of actin stress fibers. Inside of lamellipodia, actin exists as a network of bundles. AFAP-110 therefore may be involved in PKC-dependent motility structure formation, acting as a protein mediating formation of actin meshwork underlying lamellipodia once phosphorylated on serine/threonine residues in PKC dependent manner.

12.2. Cortactin

Cortactin was initially identified as a tyrosine phosphorylated substrate of active Src (151). Cortactin has an amino terminal acidic (NTA) domain followed by a region of cortactin repeats; alpha-helical domain, proline rich motif containing tyrosines, serine and threonine residues in abundance and a carboxy-terminal SH3 domain (148). Cortactin is localized to cortical actin structures. Recently it has been found that cortactin is involved in regulating cell motility (378). Cortactin's structure along with its localization and ability to bind actin, suggests that this protein may be positioned to link signaling events such as growth factor receptor activation to actin filament reorganization(379). Cortactin is found to be phosphorylated in response to growth factor receptor stimuli. Phosphorylation therefore may play a role in regulation of its function (148).

Van Damme and co-workers in 1997 have shown that in response to EGF receptor stimulation of cortactin-overexpressing human squamous carcinoma cell line p80 cortactin is converted into p85 cortactin and this conversion is a result of phosphorylation (154). Phosphoamino acid analysis has shown that EGF-induced cortactin phosphorylation occurs predominantly on serine and threonine residues. Immunofluorescence analysis indicated that upon growth factor induced phosphorylation cortactin relocates from perinuclear space to cell-matrix adhesion zone.

Campbell and colleagues show that endogenous p80 cortactin is converted into the p85 form in response to EGF stimulation, confirming van Damme and co-workers data. They also show that cortactin phosphorylation is ERK dependent since pre-treatment with the specific MEK inhibitor PD98059 abolishes EGF-induced cortactin phosphorylation. Active MEK is sufficient to cause cortactin phosphorylation but insufficient to prevent basal phosphorylation of cortactin, which suggests that there are serine/threonine kinases other than MAPK that phosphorylate cortactin *in vivo*. Proline-rich region has been shown to contain phosphate acceptor sites, as it can be phosphorylated by Erk when expressed on its own (380). Although the role of serine/threonine phosphorylation of cortactin still remains to be determined, from data described above it is possible that phosphorylation of cortactin may regulate its subcellular localization. Campbell and co-workers speculate that the proline-rich

domain of cortactin may interact with its own SH3 domain and therefore keep cortactin in a "closed" conformation. Serine/threonine phosphorylation of a proline-rich domain will result in abrogation of this interaction resulting in cortactin transition into the "open" conformation (380). Alternatively, serine/threonine phosphorylation of the proline rich region of cortactin may disrupt its interaction with SH3 binding partners. The physiological significance of cortactin serine/threonine phosphorylation as well as its impact on conformation remains to be determined.

13. INDIRECT EFFECTS: SERINE/THREONINE PHOSPHORYLATION OF ANOTHER PROTEIN ENABLES IT TO BECOME A BINDING PARTNER FOR AN ADAPTOR PROTEIN

13.1. 14-3-3

14-3-3 proteins are small acidic proteins with a molecular weight around 30 kDa (381). The 14-3-3 proteins are cellular components of all cells types that have been examined, and they have been found in all vertebrates, invertebrates, plants, and fungi (382,383,384). The 14-3-3 family of proteins includes seven unique isoforms, named by Greek letters according to their elution order after reverse-phase high-performance liquid chromatography: beta/alpha, zeta/delta, eta, gamma, tau, sigma, and epsilon (381,385,386). 14-3-3 proteins are highly conserved among each other and across different species although they do have differences in some regions (386,387). All 14-3-3 proteins are able to dimerize, with the ability to form both homo- and heterodimers *in vitro* and *in vivo* (384,388). Dimerization occurs *via* hydrophobic interactions between highly conserved residues within amino-terminal parts of the molecules (381,385,386). Carboxy-termini of two molecules brought together by dimerization create a ligand binding amphipathic groove (389,316). Highly conserved residues form the inner surface of the binding groove, whereas variable residues create the outer surface. This groove may bind either two different molecules or bring together different regions of the same protein therefore serving as a scaffold or allosteric regulator (389,390). The variant residues of the outer surface of the groove may provide specific binding surfaces for different proteins. 14-3-3 proteins bind two well-defined structural motifs on target proteins. The first contains phosphoserine in the center with arginine, serine and proline in positions –3, –2, and +2, respectively. Another one is defined as short amino acid sequence containing phosphoserine in the center with arginine, aromatic residue, basic residue and proline at positions –4, -2, -1, +2 respectively (381,316,391). 14-3-3 dimers could bind to several phosphoserine-containing motifs on one protein or bring several target proteins together as a consequence of binding to conserved motifs found on different proteins. 14-3-3 dimers also may act as molecular links between proteins bound to the conserved groove and ones associated with variable outer surface, therefore performing a role of scaffolds that regulate specific protein-protein interactions (389). 14-3-3 proteins have over 60 in vivo binding partners (314). 14-3-3 proteins have been implicated in regulation of serine/threonine kinases such Raf and PKC (382,392,393,385). 14-3-3 proteins bind Raf and certain PKC isoforms directly and therefore serve to facilitate interactions between these signaling proteins (390). 14-3-3 brings Raf into proximity with Bcr as well (314). Raf is a key protein kinase in mitogen activated protein kinase cascade. Raf functions downstream of growth factor receptor and Src-family non-receptor tyrosine kinases and Ras and upstream of MAP kinase to transmit developmental and proliferative signals. 14-3-3 associates with Raf *in vivo* regardless of Raf subcellular localization or activation status (394,381,395,382). Overexpression of 14-3-3 proteins has been shown to contribute to Raf activation, although it is clear that 14-3-3 proteins are unable to activate Raf by themselves (395). Some other reports stated that the nature of 14-3-3 influence on Raf-1 is inhibitory (396,397). The data on Raf-14-3-3 interaction accumulated to date suggests the following model of Raf-14-3-3 interaction: Raf has three 14-3-3 binding sequences in its structure. 14-3-3 is bound to cytosolic Raf at amino-terminal and carboxy terminal binding sites; this association serves to keep Raf in an inactive, although activatable, state. Upon Raf activation by Ras the former undergoes translocation to the membrane. The 14-3-3 dimer is displaced from the amino terminal binding site on Raf, but it still associates with the carboxy terminal 14-3-3 binding site. Yet unidentified kinases come along and phosphorylate the serine in the center of the intermediate 14-3-3 binding site of Raf and 14-3-3 binds to it, stabilizing Raf in an active state (316,393). 14-3-3 therefore has a dual role in Raf regulation, it keeps Raf in an inactive state in the cytosol, but upon Ras-dependent Raf activation it stabilizes it in an active conformation. 14-3-3 proteins also have been shown to be both PKC substrates and regulatory proteins (398,399). 14-3-3 may bind to Raf and PKC at the same time, therefore bringing Raf to proximity with PKC to ensure phosphorylation of the former by the latter (381,400,390).

14-3-3 binding to a target protein often serves to keep that protein either in inactive state and/ or sequester it from binding partners. 14-3-3 binds Cdc25C phosphatase in a phosphorylation dependent manner *via* its RSXpSXP site –the perfect consensus for 14-3-3 binding. This interaction is responsible for keeping this protein inactive throughout G1, S and G2 phases of the cell cycle and also transports it from the nucleus to cytosol (401,402). 14-3-3 bind the apoptotic protein BAD in a phosphorylation dependent fashion. The function of this interaction is to sequester BAD in the cytosol and keep it from binding to Bcl2 and also possibly protecting it from phosphatases (403). 14-3-3 binds IRS-1 and therefore prevents its association with PI3K (404).

Dubois and colleagues show that two unidentified brain kinases phosphorylate 14-3-3 on the sites located at C terminus of 14-3-3, a region where PI3K, Raf and Cbl bind to 14-3-3 (405). Therefore, they speculate that phosphorylation regulates 14-3-3 interactions with binding partners. They also refer to the data of Aitken and colleagues that leads to suggestion that 14-3-3 alpha and delta, which are phosphoisoforms of 14-3-3 beta and zeta, respectively, may influence PKC activity more strongly than their non-phosphorylated counterparts (392). Later

that year they followed with another publication where they determined the identity of the brain kinase that phosphorylates Ser-233 of 14-3-3 as casein kinase 1 (406). Another kinase that utilizes 14-3-3 as a substrate is sphingosine dependent protein kinase SDK1 (407). The function of 14-3-3 phosphorylation remains to be determined.

13.2. p130Cas

Crk associated substrate p130Cas was discovered over a decade ago as a phosphotyrosine-containing binding partner for active Src and Crk (408). The Cas domain structure along with variety of binding partners, suggests that it acts as an adaptor protein. The domain structure from amino to carboxy terminus: SH3 domain, proline rich region, so-called "substrate-binding" domain that associates with adaptor proteins Crk, Nck and SHIP, serine rich motif and a carboxy-terminal region that interacts with different protein kinases and adaptor proteins (408). Cas is involved in regulation of cell motility, cytoskeleton integrity, growth regulation, apoptosis, and Src and Crk dependent transformation (408). Cas is localized to focal adhesions. where it associates with Crk, Src and FAK. FAK/Cas interaction occurs *via* proline-rich region of FAK and SH3 domain of Cas (409,410). Cas forms a complex with Crk at focal adhesions; Cas/Crk complexes influence cytoskeletal rearrangements in a Rac1-dependent manner (411,408). Focal adhesions are the sites where actin stress fibers are linked to extracellular matrix. Focal adhesions are also the sites of integrin signaling (333).

The data accumulated so far suggests that Cas is involved in the regulation of cytoskeletal integrity, cell-matrix adhesion and cell motility. In Cas knockout cells actin filaments are disorganized but formation of focal adhesions remains intact (412).

Cas, as well as FAK and paxillin, is phosphorylated on serine/threonine residues in mitosis and concomitantly dephosphorylated on tyrosine residues (333). Mitosis is the stage of the cell cycle when cells display actin filament disorganization and detachment from the substratum. Mitotic phosphorylation of focal adhesion proteins must be a way of cell detachment regulation. Serine phosphorylation of Cas and FAK during mitosis promotes their dissociation from one another. Mitotic FAK is able to reassociate with interphase Cas following serine/threonine phosphatase treatment, therefore FAK/Cas interaction does not depend on tyrosine phosphorylation of FAK as serine/threonine dephosphorylated mitotic FAK has no tyrosine phosphorylation. These data indicate that serine/threonine phosphorylation of FAK and not its tyrosine phosphorylation regulates FAK/Cas complex formation (333). Ma and co-workers confirm that serine/threonine phosphorylation of FAK down-regulates its ability to form a complex with Cas (413). The physiological significance of serine/threonine phosphorylation of Cas itself remains to be determined.

13.3. AP-2

Adaptor protein 2 is a multisubunit adaptor protein complex associated with endocytic clathrin coated vesicles. AP2 associates with proteins like EGF receptor substrate Eps15 and Epsin at the membrane. This association is disrupted in mitosis when both Eps15 and Epsin are phosphorylated on serine/threonine residues. Eps15 and Epsin are substrates for p34Cdc2 in vitro. Serine/threonine phosphorylation of epsin and Eps15 results in dissociation of these proteins from AP2 complex. This may provide a mechanism that inhibits clathrin-mediated endocytosis in the stage of mitosis (414,415).

14. ADAPTOR PROTEINS THAT ARE SERINE/THREONINE PHOSPHORYLATED BUT FUNCTION OF PHOSPHORYLATION REMAINS TO BE DETERMINED

14.1. Chat

Chat is a Cas/HEF-1 associated adaptor protein isolated by Sakakibara and Hattori last year (416). The function of Chat itself as well as the function of its phosphorylation has not been determined. Sakakibara and Hattori show that Chat binds the adaptor protein Cas directly, and this interaction is phosphorylation-independent (*in vitro* binding assay). Chat has multiple serine/threonine residues, four of which comprise potential MAPK sites (PXS/TP). Phosphorylation levels of Chat increase in response to growth factor treatment, and it is not due to tyrosine phosphorylation (there is a gel shift in response to GF treatment, but pTyr Ab does not react with the upper portion). MAP kinase may be the one responsible for GF mediated phosphorylation of Chat as MAPK inhibition results in the disappearance of the slower migrating band. Overexpression, but not phosphorylation, of Chat up-regulates JNK activity in a manner similar to that of Cas. Chat is a cytoplasmic protein, but in response to GF stimulation it moves to membrane ruffles where it colocalizes with actin (416).

14.2. NHERF

NHERF is a Na+/K+ exchange regulatory factor, which was discovered several years ago. Since then it became apparent that this is an adaptor protein. NHERF contains two PDZ domains in tandem where the majority of NHERF binding partners associate, along with the carboxy-terminal region that binds ERM proteins. NHERF proteins are localized to the plasma membrane, where in addition to already mentioned function as Na+/K+ exchange regulators they also regulate transmembrane receptors and are involved into growth factor receptor signaling (417).

In 1995, Weinman and colleagues suggested that NHERF may be a substrate for serine/threonine phosphorylation for they found potential sites for PKA phosphorylation in the C-terminus of NHERF (418). A more recent report by Hall and co-workers shows that NHERF is constitutively phosphorylated on serine/threonine residues, and that NHERF is a binding partner and an *in vitro* substrate for a G protein receptor coupled kinase GRK6A (419). The functional significance of NHERF phosphorylation remains to be determined.

NHERF proteins are able to dimerize. The physiological significance of this capability still remains to

be determined. The ability of NHERF proteins to dimerize is greatly diminished in response to okadaic acid treatment. It seems therefore that protein phosphorylation might be responsible for regulation of the ability of NHERF proteins to self-associate, although it is not known at this point whether phosphorylation of NHERF themselves or NHERF binding partners influences this effect (420).

14.3. Nck/Dock

Nck is an adaptor protein that was discovered in 1990 (421). Its domain structure suggests that it serves as an adaptor protein. Nck has three SH3 domains in tandem followed by an SH2 domain at the carboxy-terminus. Indeed, it binds a variety of target proteins such as protein kinases, adaptor and motor proteins, involved in regulation of actin filament integrity, DNA synthesis, transcription and translation as well as protein degradation (reviewed in (93)).

Two reports published in 1992 show that Nck is phosphorylated in growing cells and in response to PMA or growth factor stimulation of cultured cells (97,96). Phosphoamino acid analysis has demonstrated that Nck is phosphorylated predominantly on serine/threonine with one tyrosine phosphorylated peptide recovered. Neither the identity of serine/threonine kinase nor the physiological role of serine/threonine phosphorylation of Nck was determined (97,96).

14.4. Grb7

Grb7 is a 535 amino acid protein with similarity to Grb2, Shc and Nck. Grb7 family includes Grb7, Grb10 and Grb14 proteins. Grb7 proteins contain several protein-protein interaction modules such as proline-rich, PH, Ras-associating, and SH2 in their structure, which enable them to interact with a variety of signaling proteins and therefore serve as adaptors linking receptor activation to downstream signaling pathways (422). Grb7 is able to form homo- or heterodimers and larger complexes (423). Dimerization of Grb7-family proteins may serve to bring signaling proteins together into large complexes. The Grb7 family of adaptors has been implicated in the regulation of cell proliferation, cell motility, apoptosis and tumorigenesis (422).

The Grb7 family of adaptor proteins is constitutively phosphorylated on serine/threonine residues, and the level of their serine/threonine phosphorylation increases in response to EGFR and heregulin receptor stimulation (424,425,426). The functional significance of this phosphorylation remains to be determined.

15. ACKNOWLEDGEMENTS

Drs Lidia Cherezova, Amanda Gatesman contributed equally to this work.

16. REFERENCES

1. Clements J. L., N. J. Boerth, J. R. Lee, & G. A. Koretzky: Integration of T cell receptor-dependent signaling pathways by adapter proteins. *Annu Rev Immunol* 17, 89-108 (1999)

2. Uberall F., S. Kampfer, W. Doppler, & H. H. Grunicke: Activation of c-fos expression by transforming Ha-ras in HC11 mouse mammary epithelial cells is PKC-dependent and mediated by the serum response element. *Cell Signal* 6, 285-297 (1994)

3. Pawson T.: Protein modules and signalling networks. *Nature* 373, 573-580 (1995)

4. Kuriyan J. & D. Cowburn: Modular peptide recognition domains in eukaryotic signaling. *Annu Rev Biophys Biomol Struct* 26, 259-288 (1997)

5. Mayer B. J. & R. Gupta: Functions of SH2 and SH3 domains. *Curr Top Microbiol Immunol* 228, 1-22 (1998)

6. Feller S. M.: Crk family adaptors - signalling complex formation and biological roles. *Oncogene* 20, 6348-6371 (2001)

7. Mayer B. J., M. Hamaguchi, & H. Hanafusa: A novel viral oncogene with structural similarity to phospholipase C. *Nature* 332, 272-275 (1988)

8. Matsuda M., S. Tanaka, S. Nagata, A. Kojima, T. Kurata, & M. Shibuya: Two species of human CRK cDNA encode proteins with distinct biological activities. *Mol Cell Biol* 12, 3482-3489 (1992)

9. de Jong R., A. van Wijk, L. Haataja, N. Heisterkamp, & J. Groffen: BCR/ABL-induced leukemogenesis causes phosphorylation of Hef1 and its association with Crkl. *J Biol Chem* 272, 32649-32655 (1997)

10. Feller S. M., B. Knudsen, & H. Hanafusa: c-Abl kinase regulates the protein binding activity of c-Crk. *EMBO J* 13, 2341-2351 (1994)

11. Beitner-Johnson D. & D. LeRoith: Insulin-like growth factor-I stimulates tyrosine phosphorylation of endogenous c-Crk. *J Biol Chem* 270, 5187-5190 (1995)

12. Okada S. & J. E. Pessin: Insulin and epidermal growth factor stimulate a conformational change in Rap1 and dissociation of the CrkII-C3G complex. *J Biol Chem* 272, 28179-28182 (1997)

13. Okada S., M. Matsuda, M. Anafi, T. Pawson, & J. E. Pessin: Insulin regulates the dynamic balance between Ras and Rap1 signaling by coordinating the assembly states of the Grb2-SOS and CrkII-C3G complexes. *EMBO J* 17, 2554-2565 (1998)

14. Koval A. P., V. A. Blakesley, C. T. Roberts, Jr., Y. Zick, & D. LeRoith: Interaction in vitro of the product of the c-Crk-II proto-oncogene with the insulin-like growth factor I receptor. *Biochem J* 330 (Pt 2), 923-932 (1998)

15. Hashimoto Y., H. Katayama, E. Kiyokawa, S. Ota, T. Kurata, N. Gotoh, N. Otsuka, M. Shibata, & M. Matsuda: Phosphorylation of CrkII adaptor protein at tyrosine 221 by epidermal growth factor receptor. *J Biol Chem* 273, 17186-17191 (1998)

16. Nakashima N., D. W. Rose, S. Xiao, K. Egawa, S. S. Martin, T. Haruta, A. R. Saltiel, & J. M. Olefsky: The functional role of CrkII in actin cytoskeleton organization and mitogenesis. *J Biol Chem* 274, 3001-3008 (1999)

17. Escalante M., J. Courtney, W. G. Chin, K. K. Teng, J. I. Kim, J. E. Fajardo, B. J. Mayer, B. L. Hempstead, & R. B. Birge: Phosphorylation of c-Crk II on the negative regulatory Tyr222 mediates nerve growth factor-induced cell spreading and morphogenesis. *J Biol Chem* 275, 24787-24797 (2000)

18. Kain K. H. & R. L. Klemke: Inhibition of cell migration by Abl family tyrosine kinases through uncoupling of Crk-CAS complexes. *J Biol Chem* 276, 16185-16192 (2001)

19. Feller S. M., B. Knudsen, & H. Hanafusa: Cellular proteins binding to the first Src homology 3 (SH3) domain of the proto-oncogene product c-Crk indicate Crk-specific signaling pathways. *Oncogene* 10, 1465-1473 (1995)
20. Boussiotis V. A., G. J. Freeman, A. Berezovskaya, D. L. Barber, & L. M. Nadler: Maintenance of human T cell anergy: blocking of IL-2 gene transcription by activated Rap1. *Science* 278, 124-128 (1997)
21. Barber D. L., J. M. Mason, T. Fukazawa, K. A. Reedquist, B. J. Druker, H. Band, & A. D. D'Andrea: Erythropoietin and interleukin-3 activate tyrosine phosphorylation of CBL and association with CRK adaptor proteins. *Blood* 89, 3166-3174 (1997)
22. Chin H., T. Saito, A. Arai, K. Yamamoto, R. Kamiyama, N. Miyasaka, & O. Miura: Erythropoietin and IL-3 induce tyrosine phosphorylation of CrkL and its association with Shc, SHP-2, and Cbl in hematopoietic cells. *Biochem Biophys Res Commun* 239, 412-417 (1997)
23. Gelkop S. & N. Isakov: T cell activation stimulates the association of enzymatically active tyrosine-phosphorylated ZAP-70 with the Crk adapter proteins. *J Biol Chem* 274, 21519-21527 (1999)
24. Guris D. L., J. Fantes, D. Tara, B. J. Druker, & A. Imamoto: Mice lacking the homologue of the human 22q11.2 gene CRKL phenocopy neurocristopathies of DiGeorge syndrome. *Nat Genet* 27, 293-298 (2001)
25. Bustelo X. R.: Vav proteins, adaptors and cell signaling. *Oncogene* 20, 6372-6381 (2001)
26. Crespo P., K. E. Schuebel, A. A. Ostrom, J. S. Gutkind, & X. R. Bustelo: Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature* 385, 169-172 (1997)
27. Han J., B. Das, W. Wei, L. Van Aelst, R. D. Mosteller, R. Khosravi-Far, J. K. Westwick, C. J. Der, & D. Broek: Lck regulates Vav activation of members of the Rho family of GTPases. *Mol Cell Biol* 17, 1346-1353 (1997)
28. Movilla N. & X. R. Bustelo: Biological and regulatory properties of Vav-3, a new member of the Vav family of oncoproteins. *Mol Cell Biol* 19, 7870-7885 (1999)
29. Schuebel K. E., N. Movilla, J. L. Rosa, & X. R. Bustelo: Phosphorylation-dependent and constitutive activation of Rho proteins by wild-type and oncogenic Vav-2. *EMBO J* 17, 6608-6621 (1998)
30. Bustelo X. R.: Regulatory and signaling properties of the Vav family. *Mol Cell Biol* 20, 1461-1477 (2000)
31. Aghazadeh B., W. E. Lowry, X. Y. Huang, & M. K. Rosen: Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation. *Cell* 102, 625-633 (2000)
32. Katzav S., D. Martin-Zanca, & M. Barbacid: vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. *EMBO J* 8, 2283-2290 (1989)
33. Billadeau D. D., S. M. Mackie, R. A. Schoon, & P. J. Leibson: Specific subdomains of Vav differentially affect T cell and NK cell activation. *J Immunol* 164, 3971-3981 (2000)
34. Costello P. S., A. E. Walters, P. J. Mee, M. Turner, L. F. Reynolds, A. Prisco, N. Sarner, R. Zamoyska, & V. L. Tybulewicz: The Rho-family GTP exchange factor Vav is a critical transducer of T cell receptor signals to the calcium, ERK, and NF-kappaB pathways. *Proc Natl Acad Sci U S A* 96, 3035-3040 (1999)
35. Fischer K. D., A. Zmuldzinas, S. Gardner, M. Barbacid, A. Bernstein, & C. Guidos: Defective T-cell receptor signalling and positive selection of Vav- deficient CD4+ CD8+ thymocytes. *Nature* 374, 474-477 (1995)
36. Fischer K. D., Y. Y. Kong, H. Nishina, K. Tedford, L. E. Marengere, I. Kozieradzki, T. Sasaki, M. Starr, G. Chan, S. Gardener, M. P. Nghiem, D. Bouchard, M. Barbacid, A. Bernstein, & J. M. Penninger: Vav is a regulator of cytoskeletal reorganization mediated by the T- cell receptor. *Curr Biol* 8, 554-562 (1998)
37. Turner M., P. J. Mee, A. E. Walters, M. E. Quinn, A. L. Mellor, R. Zamoyska, & V. L. Tybulewicz: A requirement for the Rho-family GTP exchange factor Vav in positive and negative selection of thymocytes. *Immunity* 7, 451-460 (1997)
38. Wu J., S. Katzav, & A. Weiss: A functional T-cell receptor signaling pathway is required for p95vav activity. *Mol Cell Biol* 15, 4337-4346 (1995)
39. Li X., D. Sandoval, L. Freeberg, & R. H. Carter: Role of CD19 tyrosine 391 in synergistic activation of B lymphocytes by coligation of CD19 and membrane Ig. *J Immunol* 158, 5649-5657 (1997)
40. O'Rourke L. M., R. Tooze, M. Turner, D. M. Sandoval, R. H. Carter, V. L. Tybulewicz, & D. T. Fearon: CD19 as a membrane-anchored adaptor protein of B lymphocytes: costimulation of lipid and protein kinases by recruitment of Vav. *Immunity* 8, 635-645 (1998)
41. Weng W. K., L. Jarvis, & T. W. LeBien: Signaling through CD19 activates Vav/mitogen-activated protein kinase pathway and induces formation of a CD19/Vav/phosphatidylinositol 3- kinase complex in human B cell precursors. *J Biol Chem* 269, 32514-32521 (1994)
42. Finco T. S., T. Kadlecek, W. Zhang, L. E. Samelson, & A. Weiss: LAT is required for TCR-mediated activation of PLCgamma1 and the Ras pathway. *Immunity* 9, 617-626 (1998)
43. Kim H. H., M. Tharayil, & C. E. Rudd: Growth factor receptor-bound protein 2 SH2/SH3 domain binding to CD28 and its role in co-signaling. *J Biol Chem* 273, 296-301 (1998)
44. Zhang W., J. Sloan-Lancaster, J. Kitchen, R. P. Trible, & L. E. Samelson: LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* 92, 83-92 (1998)
45. Wu J., D. G. Motto, G. A. Koretzky, & A. Weiss: Vav and SLP-76 interact and functionally cooperate in IL-2 gene activation. *Immunity* 4, 593-602 (1996)
46. Onodera H., D. G. Motto, G. A. Koretzky, & D. M. Rothstein: Differential regulation of activation-induced tyrosine phosphorylation and recruitment of SLP-76 to Vav by distinct isoforms of the CD45 protein-tyrosine phosphatase. *J Biol Chem* 271, 22225-22230 (1996)
47. Raab M., A. J. da Silva, P. R. Findell, & C. E. Rudd: Regulation of Vav-SLP-76 binding by ZAP-70 and its relevance to TCR zeta/CD3 induction of interleukin-2. *Immunity* 6, 155-164 (1997)
48. Tuosto L., F. Michel, & O. Acuto: p95vav associates with tyrosine-phosphorylated SLP-76 in antigen-stimulated T cells. *J Exp Med* 184, 1161-1166 (1996)

49. Yablonski D., L. P. Kane, D. Qian, & A. Weiss: A Nck-Pak1 signaling module is required for T-cell receptor-mediated activation of NFAT, but not of JNK. *EMBO J* 17, 5647-5657 (1998)
50. Musci M. A., D. G. Motto, S. E. Ross, N. Fang, & G. A. Koretzky: Three domains of SLP-76 are required for its optimal function in a T cell line. *J Immunol* 159, 1639-1647 (1997)
51. Zeng L., P. Sachdev, L. Yan, J. L. Chan, T. Trenkle, M. McClelland, J. Welsh, & L. H. Wang: Vav3 mediates receptor protein tyrosine kinase signaling, regulates GTPase activity, modulates cell morphology, and induces cell transformation. *Mol Cell Biol* 20, 9212-9224 (2000)
52. Ravichandran K. S.: Signaling via Shc family adapter proteins. *Oncogene* 20, 6322-6330 (2001)
53. Luzi L., S. Confalonieri, P. P. Di Fiore, & P. G. Pelicci: Evolution of Shc functions from nematode to human. *Curr Opin Genet Dev* 10, 668-674 (2000)
54. Pelicci G., L. Dente, A. De Giuseppe, B. Verducci-Galletti, S. Giuli, S. Mele, C. Vetriani, M. Giorgio, P. P. Pandolfi, G. Cesareni, & P. G. Pelicci: A family of Shc related proteins with conserved PTB, CH1 and SH2 regions. *Oncogene* 13, 633-641 (1996)
55. Sakai R., J. T. Henderson, J. P. O'Bryan, A. J. Elia, T. M. Saxton, & T. Pawson: The mammalian ShcB and ShcC phosphotyrosine docking proteins function in the maturation of sensory and sympathetic neurons. *Neuron* 28, 819-833 (2000)
56. Migliaccio E., S. Mele, A. E. Salcini, G. Pelicci, K. M. Lai, G. Superti-Furga, T. Pawson, P. P. Di Fiore, L. Lanfrancone, & P. G. Pelicci: Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-fos signalling pathway. *EMBO J* 16, 706-716 (1997)
57. Pelicci G., L. Lanfrancone, F. Grignani, J. McGlade, F. Cavallo, G. Forni, I. Nicoletti, F. Grignani, T. Pawson, & P. G. Pelicci: A novel transforming protein (SHC) with an SH2 domain is implicated in mitogenic signal transduction. *Cell* 70, 93-104 (1992)
58. Ravichandran K. S., K. K. Lee, Z. Songyang, L. C. Cantley, P. Burn, & S. J. Burakoff: Interaction of Shc with the zeta chain of the T cell receptor upon T cell activation. *Science* 262, 902-905 (1993)
59. Gelderloos J. A., S. Rosenkranz, C. Bazenet, & A. Kazlauskas: A role for Src in signal relay by the platelet-derived growth factor alpha receptor. *J Biol Chem* 273, 5908-5915 (1998)
60. Yokote K., S. Mori, K. Hansen, J. McGlade, T. Pawson, C. H. Heldin, & L. Claesson-Welsh: Direct interaction between Shc and the platelet-derived growth factor beta-receptor. *J Biol Chem* 269, 15337-15343 (1994)
61. Campbell K. S., E. Ogris, B. Burke, W. Su, K. R. Auger, B. J. Druker, B. S. Schaffhausen, T. M. Roberts, & D. C. Pallas: Polyoma middle tumor antigen interacts with SHC protein via the NPTY (Asn-Pro-Thr-Tyr) motif in middle tumor antigen. *Proc Natl Acad Sci U S A* 91, 6344-6348 (1994)
62. Stephens R. M., D. M. Loeb, T. D. Copeland, T. Pawson, L. A. Greene, & D. R. Kaplan: Trk receptors use redundant signal transduction pathways involving SHC and PLC-gamma 1 to mediate NGF responses. *Neuron* 12, 691-705 (1994)
63. Blaikie P., D. Immanuel, J. Wu, N. Li, V. Yajnik, & B. Margolis: A region in Shc distinct from the SH2 domain can bind tyrosine- phosphorylated growth factor receptors. *J Biol Chem* 269, 32031-32034 (1994)
64. Blaikie P. A., E. Fournier, S. M. Dilworth, D. Birnbaum, J. P. Borg, & B. Margolis: The role of the Shc phosphotyrosine interaction/phosphotyrosine binding domain and tyrosine phosphorylation sites in polyoma middle T antigen- mediated cell transformation. *J Biol Chem* 272, 20671-20677 (1997)
65. Trub T., W. E. Choi, G. Wolf, E. Ottinger, Y. Chen, M. Weiss, & S. E. Shoelson: Specificity of the PTB domain of Shc for beta turn-forming pentapeptide motifs amino-terminal to phosphotyrosine. *J Biol Chem* 270, 18205-18208 (1995)
66. Zhou M. M., J. E. Harlan, W. S. Wade, S. Crosby, K. S. Ravichandran, S. J. Burakoff, & S. W. Fesik: Binding affinities of tyrosine-phosphorylated peptides to the COOH- terminal SH2 and NH2-terminal phosphotyrosine binding domains of Shc. *J Biol Chem* 270, 31119-31123 (1995)
67. Zhou M. M., K. S. Ravichandran, E. F. Olejniczak, A. M. Petros, R. P. Meadows, M. Sattler, J. E. Harlan, W. S. Wade, S. J. Burakoff, & S. W. Fesik: Structure and ligand recognition of the phosphotyrosine binding domain of Shc. *Nature* 378, 584-592 (1995)
68. Kavanaugh W. M. & L. T. Williams: An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. *Science* 266, 1862-1865 (1994)
69. van der G. P., S. Wiley, V. K. Lai, J. P. Olivier, G. D. Gish, R. Stephens, D. Kaplan, S. Shoelson, & T. Pawson: A conserved amino-terminal Shc domain binds to phosphotyrosine motifs in activated receptors and phosphopeptides. *Curr Biol* 5, 404-412 (1995)
70. Batzer A. G., P. Blaikie, K. Nelson, J. Schlessinger, & B. Margolis: The phosphotyrosine interaction domain of Shc binds an LXNPXY motif on the epidermal growth factor receptor. *Mol Cell Biol* 15, 4403-4409 (1995)
71. Harlan J. E., P. J. Hajduk, H. S. Yoon, & S. W. Fesik: Pleckstrin homology domains bind to phosphatidylinositol-4,5- bisphosphate. *Nature* 371, 168-170 (1994)
72. Harlan J. E., H. S. Yoon, P. J. Hajduk, & S. W. Fesik: Structural characterization of the interaction between a pleckstrin homology domain and phosphatidylinositol 4,5-bisphosphate. *Biochemistry* 34, 9859-9864 (1995)
73. van der G. P., S. Wiley, G. D. Gish, & T. Pawson: The Shc adaptor protein is highly phosphorylated at conserved, twin tyrosine residues (Y239/240) that mediate protein-protein interactions. *Curr Biol* 6, 1435-1444 (1996)
74. Gotoh N., A. Tojo, & M. Shibuya: A novel pathway from phosphorylation of tyrosine residues 239/240 of Shc, contributing to suppress apoptosis by IL-3. *EMBO J* 15, 6197-6204 (1996)
75. Gotoh N., M. Toyoda, & M. Shibuya: Tyrosine phosphorylation sites at amino acids 239 and 240 of Shc are involved in epidermal growth factor-induced mitogenic signaling that is distinct from Ras/mitogen-activated protein kinase activation. *Mol Cell Biol* 17, 1824-1831 (1997)
76. Pratt J. C., M. R. van den Brink, V. E. Igras, S. F. Walk, K. S. Ravichandran, & S. J. Burakoff: Requirement for Shc in TCR-mediated activation of a T cell hybridoma. *J Immunol* 163, 2586-2591 (1999)

77. Salcini A. E., J. McGlade, G. Pelicci, I. Nicoletti, T. Pawson, & P. G. Pelicci: Formation of Shc-Grb2 complexes is necessary to induce neoplastic transformation by overexpression of Shc proteins. *Oncogene* 9, 2827-2836 (1994)
78. Walk S. F., M. E. March, & K. S. Ravichandran: Roles of Lck, Syk and ZAP-70 tyrosine kinases in TCR-mediated phosphorylation of the adapter protein Shc. *Eur J Immunol* 28, 2265-2275 (1998)
79. Velazquez L., G. D. Gish, G. P. van der, L. Taylor, J. Shulman, & T. Pawson: The shc adaptor protein forms interdependent phosphotyrosine-mediated protein complexes in mast cells stimulated with interleukin 3. *Blood* 96, 132-138 (2000)
80. Barberis L., K. K. Wary, G. Fiucci, F. Liu, E. Hirsch, M. Brancaccio, F. Altruda, G. Tarone, & F. G. Giancotti: Distinct roles of the adaptor protein Shc and focal adhesion kinase in integrin signaling to ERK. *J Biol Chem* 275, 36532-36540 (2000)
81. Gu J., M. Tamura, R. Pankov, E. H. Danen, T. Takino, K. Matsumoto, & K. M. Yamada: Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. *J Cell Biol* 146, 389-403 (1999)
82. Habib T., J. A. Hejna, R. E. Moses, & S. J. Decker: Growth factors and insulin stimulate tyrosine phosphorylation of the 51C/SHIP2 protein. *J Biol Chem* 273, 18605-18609 (1998)
83. Biscardi J. S., A. P. Belsches, & S. J. Parsons: Characterization of human epidermal growth factor receptor and c-Src interactions in human breast tumor cells. *Mol Carcinog* 21, 261-272 (1998)
84. Pelicci G., L. Lanfrancone, A. E. Salcini, A. Romano, S. Mele, B. M. Grazia, O. Segatto, P. P. Di Fiore, & P. G. Pelicci: Constitutive phosphorylation of Shc proteins in human tumors. *Oncogene* 11, 899-907 (1995)
85. Stevenson L. E. & A. R. Frackelton, Jr.: Constitutively tyrosine phosphorylated p52 Shc in breast cancer cells: correlation with ErbB2 and p66 Shc expression. *Breast Cancer Res Treat* 49, 119-128 (1998)
86. Lai K. M. & T. Pawson: The ShcA phosphotyrosine docking protein sensitizes cardiovascular signaling in the mouse embryo. *Genes Dev* 14, 1132-1145 (2000)
87. Lowenstein E. J., R. J. Daly, A. G. Batzer, W. Li, B. Margolis, R. Lammers, A. Ullrich, E. Y. Skolnik, D. Bar-Sagi, & J. Schlessinger: The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 70, 431-442 (1992)
88. Buday L. & J. Downward: Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* 73, 611-620 (1993)
89. Egan S. E., B. W. Giddings, M. W. Brooks, L. Buday, A. M. Sizeland, & R. A. Weinberg: Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. *Nature* 363, 45-51 (1993)
90. Blenis J.: Signal transduction via the MAP kinases: proceed at your own RSK. *Proc Natl Acad Sci U S A* 90, 5889-5892 (1993)
91. Davis R. J.: Transcriptional regulation by MAP kinases. *Mol Reprod Dev* 42, 459-467 (1995)
92. Trub T., J. D. Frantz, M. Miyazaki, H. Band, & S. E. Shoelson: The role of a lymphoid-restricted, Grb2-like SH3-SH2-SH3 protein in T cell receptor signaling. *J Biol Chem* 272, 894-902 (1997)
93. Li W., J. Fan, & D. T. Woodley: Nck/Dock: an adapter between cell curface receptors and the actin cytoskeleton. *Oncogene* 20, 6403-6417 (2001)
94. Li W., J. Fan, & D. T. Woodley: Nck/Dock: an adapter between cell surface receptors and the actin cytoskeleton. *Oncogene* 6403-6417 (2001)
95. Chou M. M., J. E. Fajardo, & H. Hanafusa: The SH2- and SH3-containing Nck protein transforms mammalian fibroblasts in the absence of elevated phosphotyrosine levels. *Mol Cell Biol* 12, 5834-5842 (1992)
96. Li W., P. Hu, E. Y. Skolnik, A. Ullrich, & J. Schlessinger: The SH2 and SH3 domain-containing Nck protein is oncogenic and a common target for phosphorylation by different surface receptors. *Mol Cell Biol* 12, 5824-5833 (1992)
97. Meisenhelder J. & T. Hunter: The SH2/SH3 domain-containing protein Nck is recognized by certain anti-phospholipase C-gamma 1 monoclonal antibodies, and its phosphorylation on tyrosine is stimulated by platelet-derived growth factor and epidermal growth factor treatment. *Mol Cell Biol* 12, 5843-5856 (1992)
98. Park D. & S. G. Rhee: Phosphorylation of Nck in response to a variety of receptors, phorbol myristate acetate, and cyclic AMP. *Mol Cell Biol* 12, 5816-5823 (1992)
99. Li W. & H. She: The SH2 and SH3 adapter Nck: a two-gene family and a linker between tyrosine kinases and multiple signaling networks. *Histol Histopathol* 15, 947-955 (2000)
100. Langdon W. Y., J. W. Hartley, S. P. Klinken, S. K. Ruscetti, & H. C. Morse, III: v-cbl, an oncogene from a dual-recombinant murine retrovirus that induces early B-lineage lymphomas. *Proc Natl Acad Sci U S A* 86, 1168-1172 (1989)
101. Langdon W. Y., C. D. Hyland, R. J. Grumont, & H. C. Morse, III: The c-cbl proto-oncogene is preferentially expressed in thymus and testis tissue and encodes a nuclear protein. *J Virol* 63, 5420-5424 (1989)
102. Tsygankov A. Y., A. M. Teckchandani, E. A. Feshchenko, & G. Swaminathan: Beyond the RING: CBL proteins are multivalent adaptors. *Oncogene* 20, 6382-6402 (2001)
103. Meng W., S. Sawasdikosol, S. J. Burakoff, & M. J. Eck: Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. *Nature* 398, 84-90 (1999)
104. Bonita D. P., S. Miyake, M. L. Lupher, Jr., W. Y. Langdon, & H. Band: Phosphotyrosine binding domain-dependent upregulation of the platelet- derived growth factor receptor alpha signaling cascade by transforming mutants of Cbl: implications for Cbl's function and oncogenicity. *Mol Cell Biol* 17, 4597-4610 (1997)
105. Lupher M. L., Jr., K. A. Reedquist, S. Miyake, W. Y. Langdon, & H. Band: A novel phosphotyrosine-binding domain in the N-terminal transforming region of Cbl interacts directly and selectively with ZAP-70 in T cells. *J Biol Chem* 271, 24063-24068 (1996)

106. Lupher M. L., Jr., Z. Songyang, S. E. Shoelson, L. C. Cantley, & H. Band: The Cbl phosphotyrosine-binding domain selects a D(N/D)XpY motif and binds to the Tyr292 negative regulatory phosphorylation site of ZAP-70. *J Biol Chem* 272, 33140-33144 (1997)
107. Cory G. O., R. C. Lovering, S. Hinshelwood, L. MacCarthy-Morrogh, R. J. Levinsky, & C. Kinnon: The protein product of the c-cbl protooncogene is phosphorylated after B cell receptor stimulation and binds the SH3 domain of Bruton's tyrosine kinase. *J Exp Med* 182, 611-615 (1995)
108. Donovan J. A., R. L. Wange, W. Y. Langdon, & L. E. Samelson: The protein product of the c-cbl protooncogene is the 120-kDa tyrosine- phosphorylated protein in Jurkat cells activated via the T cell antigen receptor. *J Biol Chem* 269, 22921-22924 (1994)
109. Fukazawa T., K. A. Reedquist, T. Trub, S. Soltoff, G. Panchamoorthy, B. Druker, L. Cantley, S. E. Shoelson, & H. Band: The SH3 domain-binding T cell tyrosyl phosphoprotein p120. Demonstration of its identity with the c-cbl protooncogene product and in vivo complexes with Fyn, Grb2, and phosphatidylinositol 3-kinase. *J Biol Chem* 270, 19141-19150 (1995)
110. Keane M. M., O. M. Rivero-Lezcano, J. A. Mitchell, K. C. Robbins, & S. Lipkowitz: Cloning and characterization of cbl-b: a SH3 binding protein with homology to the c-cbl proto-oncogene. *Oncogene* 10, 2367-2377 (1995)
111. Meisner H., B. R. Conway, D. Hartley, & M. P. Czech: Interactions of Cbl with Grb2 and phosphatidylinositol 3'-kinase in activated Jurkat cells. *Mol Cell Biol* 15, 3571-3578 (1995)
112. Rivero-Lezcano O. M., J. H. Sameshima, A. Marcilla, & K. C. Robbins: Physical association between Src homology 3 elements and the protein product of the c-cbl proto-oncogene. *J Biol Chem* 269, 17363-17366 (1994)
113. Feshchenko E. A., W. Y. Langdon, & A. Y. Tsygankov: Fyn, Yes, and Syk phosphorylation sites in c-Cbl map to the same tyrosine residues that become phosphorylated in activated T cells. *J Biol Chem* 273, 8323-8331 (1998)
114. Blake T. J., M. Shapiro, H. C. Morse, III, & W. Y. Langdon: The sequences of the human and mouse c-cbl proto-oncogenes show v-cbl was generated by a large truncation encompassing a proline-rich domain and a leucine zipper-like motif. *Oncogene* 6, 653-657 (1991)
115. Robertson H., G. R. Hime, H. Lada, & D. D. Bowtell: A Drosophila analogue of v-Cbl is a dominant-negative oncoprotein in vivo. *Oncogene* 19, 3299-3308 (2000)
116. Bartkiewicz M., A. Houghton, & R. Baron: Leucine zipper-mediated homodimerization of the adaptor protein c-Cbl. A role in c-Cbl's tyrosine phosphorylation and its association with epidermal growth factor receptor. *J Biol Chem* 274, 30887-30895 (1999)
117. Levkowitz G., H. Waterman, E. Zamir, Z. Kam, S. Oved, W. Y. Langdon, L. Beguinot, B. Geiger, & Y. Yarden: c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* 12, 3663-3674 (1998)
118. Miyake S., M. L. Lupher, Jr., B. Druker, & H. Band: The tyrosine kinase regulator Cbl enhances the ubiquitination and degradation of the platelet-derived growth factor receptor alpha. *Proc Natl Acad Sci U S A* 95, 7927-7932 (1998)
119. Lee P. S., Y. Wang, M. G. Dominguez, Y. G. Yeung, M. A. Murphy, D. D. Bowtell, & E. R. Stanley: The Cbl protooncoprotein stimulates CSF-1 receptor multiubiquitination and endocytosis, and attenuates macrophage proliferation. *EMBO J* 18, 3616-3628 (1999)
120. Wang Y., Y. G. Yeung, & E. R. Stanley: CSF-1 stimulated multiubiquitination of the CSF-1 receptor and of Cbl follows their tyrosine phosphorylation and association with other signaling proteins. *J Cell Biochem* 72, 119-134 (1999)
121. Joazeiro C. A., S. S. Wing, H. Huang, J. D. Leverson, T. Hunter, & Y. C. Liu: The tyrosine kinase negative regulator c-Cbl as a RING-type, E2- dependent ubiquitin-protein ligase. *Science* 286, 309-312 (1999)
122. Levkowitz G., H. Waterman, S. A. Ettenberg, M. Katz, A. Y. Tsygankov, I. Alroy, S. Lavi, K. Iwai, Y. Reiss, A. Ciechanover, S. Lipkowitz, & Y. Yarden: Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell* 4, 1029-1040 (1999)
123. Waterman H., I. Alroy, S. Strano, R. Seger, & Y. Yarden: The C-terminus of the kinase-defective neuregulin receptor ErbB-3 confers mitogenic superiority and dictates endocytic routing. *EMBO J* 18, 3348-3358 (1999)
124. Deckert M., C. Elly, A. Altman, & Y. C. Liu: Coordinated regulation of the tyrosine phosphorylation of Cbl by Fyn and Syk tyrosine kinases. *J Biol Chem* 273, 8867-8874 (1998)
125. Lupher M. L., Jr., N. Rao, N. L. Lill, C. E. Andoniou, S. Miyake, E. A. Clark, B. Druker, & H. Band: Cbl-mediated negative regulation of the Syk tyrosine kinase. A critical role for Cbl phosphotyrosine-binding domain binding to Syk phosphotyrosine 323. *J Biol Chem* 273, 35273-35281 (1998)
126. Thien C. B. & W. Y. Langdon: EGF receptor binding and transformation by v-cbl is ablated by the introduction of a loss-of-function mutation from the Caenorhabditis elegans sli-1 gene. *Oncogene* 14, 2239-2249 (1997)
127. Miyake S., K. P. Mullane-Robinson, N. L. Lill, P. Douillard, & H. Band: Cbl-mediated negative regulation of platelet-derived growth factor receptor-dependent cell proliferation. A critical role for Cbl tyrosine kinase-binding domain. *J Biol Chem* 274, 16619-16628 (1999)
128. Lill N. L., P. Douillard, R. A. Awwad, S. Ota, M. L. Lupher, Jr., S. Miyake, N. Meissner-Lula, V. W. Hsu, & H. Band: The evolutionarily conserved N-terminal region of Cbl is sufficient to enhance down-regulation of the epidermal growth factor receptor. *J Biol Chem* 275, 367-377 (2000)
129. Ahmed Z., B. J. Smith, & T. S. Pillay: The APS adapter protein couples the insulin receptor to the phosphorylation of c-Cbl and facilitates ligand-stimulated ubiquitination of the insulin receptor. *FEBS Lett* 475, 31-34 (2000)
130. Andoniou C. E., N. L. Lill, C. B. Thien, M. L. Lupher, Jr., S. Ota, D. D. Bowtell, R. M. Scaife, W. Y. Langdon, & H. Band: The Cbl proto-oncogene product negatively regulates the Src-family tyrosine kinase Fyn by enhancing its degradation. *Mol Cell Biol* 20, 851-867 (2000)
131. Wang H. Y., Y. Altman, D. Fang, C. Elly, Y. Dai, Y. Shao, & Y. C. Liu: Cbl promotes ubiquitination of the T cell receptor zeta through an adaptor function of Zap-70. *J Biol Chem* 276, 26004-26011 (2001)

132. Barde Y. A.: Trophic factors and neuronal survival. *Neuron* 2, 1525-1534 (1989)
133. Goldfarb M.: Functions of fibroblast growth factors in vertebrate development. *Cytokine Growth Factor Rev* 7, 311-325 (1996)
134. Pawson T. & J. D. Scott: Signaling through scaffold, anchoring, and adaptor proteins. *Science* 278, 2075-2080 (1997)
135. Schlessinger J.: Cellular signaling by receptor tyrosine kinases. *Harvey Lect* 89, 105-123 (1993)
136. Kouhara H., Y. R. Hadari, T. Spivak-Kroizman, J. Schilling, D. Bar-Sagi, I. Lax, & J. Schlessinger: A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* 89, 693-702 (1997)
137. Lange-Carter C. A. & G. L. Johnson: Ras-dependent growth factor regulation of MEK kinase in PC12 cells. *Science* 265, 1458-1461 (1994)
138. Ong S. H., G. R. Guy, Y. R. Hadari, S. Laks, N. Gotoh, J. Schlessinger, & I. Lax: FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. *Mol Cell Biol* 20, 979-989 (2000)
139. Delahaye L., S. Rocchi, & E. Van Obberghen: Potential involvement of FRS2 in insulin signaling. *Endocrinology* 141, 621-628 (2000)
140. Dikic I., A. G. Batzer, P. Blaikie, A. Obermeier, A. Ullrich, J. Schlessinger, & B. Margolis: Shc binding to nerve growth factor receptor is mediated by the phosphotyrosine interaction domain. *J Biol Chem* 270, 15125-15129 (1995)
141. Obermeier A., H. Halfter, K. H. Wiesmuller, G. Jung, J. Schlessinger, & A. Ullrich: Tyrosine 785 is a major determinant of Trk--substrate interaction. *EMBO J* 12, 933-941 (1993)
142. Hadari Y. R., H. Kouhara, I. Lax, & J. Schlessinger: Binding of Shp2 tyrosine phosphatase to FRS2 is essential for fibroblast growth factor-induced PC12 cell differentiation. *Mol Cell Biol* 18, 3966-3973 (1998)
143. Ong S. H., Y. P. Lim, B. C. Low, & G. R. Guy: SHP2 associates directly with tyrosine phosphorylated p90 (SNT) protein in FGF-stimulated cells. *Biochem Biophys Res Commun* 238, 261-266 (1997)
144. Ong S. H., Y. R. Hadari, N. Gotoh, G. R. Guy, J. Schlessinger, & I. Lax: Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc Natl Acad Sci U S A* 98, 6074-6079 (2001)
145. Hadari Y. R., N. Gotoh, H. Kouhara, I. Lax, & J. Schlessinger: Critical role for the docking-protein FRS2 alpha in FGF receptor- mediated signal transduction pathways. *Proc Natl Acad Sci U S A* 98, 8578-8583 (2001)
146. Rodrigues G. A., M. Falasca, Z. Zhang, S. H. Ong, & J. Schlessinger: A novel positive feedback loop mediated by the docking protein Gab1 and phosphatidylinositol 3-kinase in epidermal growth factor receptor signaling. *Mol Cell Biol* 20, 1448-1459 (2000)
147. Melillo R. M., F. Carlomagno, G. De Vita, P. Formisano, G. Vecchio, A. Fusco, M. Billaud, & M. Santoro: The insulin receptor substrate (IRS)-1 recruits phosphatidylinositol 3- kinase to Ret: evidence for a competition between Shc and IRS-1 for the binding to Ret. *Oncogene* 20, 209-218 (2001)
148. Weed S. A. & J. T. Parsons: Cortactin: coupling membrane dynamics to cortical actin assembly. *Oncogene* 20, 6418-6434 (2001)
149. Weed S. A., A. V. Karginov, D. A. Schafer, A. M. Weaver, A. W. Kinley, J. A. Cooper, & J. T. Parsons: Cortactin localization to sites of actin assembly in lamellipodia requires interactions with F-actin and the Arp2/3 complex. *J Cell Biol* 151, 29-40 (2000)
150. Sparks A. B., J. E. Rider, N. G. Hoffman, D. M. Fowlkes, L. A. Quillam, & B. K. Kay: Distinct ligand preferences of Src homology 3 domains from Src, Yes, Abl, Cortactin, p53bp2, PLCgamma, Crk, and Grb2. *Proc Natl Acad Sci U S A* 93, 1540-1544 (1996)
151. Kanner S. B., A. B. Reynolds, R. R. Vines, & J. T. Parsons: Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases. *Proc Natl Acad Sci U S A* 87, 3328-3332 (1990)
152. Zhan X., C. Plourde, X. Hu, R. Friesel, & T. Maciag: Association of fibroblast growth factor receptor-1 with c-Src correlates with association between c-Src and cortactin. *J Biol Chem* 269, 20221-20224 (1994)
153. Okamura H. & M. D. Resh: p80/85 cortactin associates with the Src SH2 domain and colocalizes with v-Src in transformed cells. *J Biol Chem* 270, 26613-26618 (1995)
154. van Damme H., H. Brok, E. Schuuring-Scholtes, & E. Schuuring: The redistribution of cortactin into cell-matrix contact sites in human carcinoma cells with 11q13 amplification is associated with both overexpression and post-translational modification. *J Biol Chem* 272, 7374-7380 (1997)
155. Thomas S. M., P. Soriano, & A. Imamoto: Specific and redundant roles of Src and Fyn in organizing the cytoskeleton. *Nature* 376, 267-271 (1995)
156. Huang C., J. Liu, C. C. Haudenschild, & X. Zhan: The role of tyrosine phosphorylation of cortactin in the locomotion of endothelial cells. *J Biol Chem* 273, 25770-25776 (1998)
157. Hao Q. L., N. Heisterkamp, & J. Groffen: Isolation and sequence analysis of a novel human tyrosine kinase gene. *Mol Cell Biol* 9, 1587-1593 (1989)
158. Kim L. & T. W. Wong: Growth factor-dependent phosphorylation of the actin-binding protein cortactin is mediated by the cytoplasmic tyrosine kinase FER. *J Biol Chem* 273, 23542-23548 (1998)
159. Kapus A., K. Szaszi, J. Sun, S. Rizoli, & O. D. Rotstein: Cell shrinkage regulates Src kinases and induces tyrosine phosphorylation of cortactin, independent of the osmotic regulation of Na+/H+ exchangers. *J Biol Chem* 274, 8093-8102 (1999)
160. Turner C. E.: Paxillin and focal adhesion signalling. *Nat Cell Biol* 2, E231-E236 (2000)
161. Schaller M. D.: Paxillin: a focal adhesion-associated adaptor protein. *Oncogene* 20, 6459-6472 (2001)
162. Nakamura K., H. Yano, H. Uchida, S. Hashimoto, E. Schaefer, & H. Sabe: Tyrosine phosphorylation of paxillin alpha is involved in temporospatial regulation of paxillin-containing focal adhesion formation and F-actin

organization in motile cells. *J Biol Chem* 275, 27155-27164 (2000)
163. Petit V., B. Boyer, D. Lentz, C. E. Turner, J. P. Thiery, & A. M. Valles: Phosphorylation of tyrosine residues 31 and 118 on paxillin regulates cell migration through an association with CRK in NBT-II cells. *J Cell Biol* 148, 957-970 (2000)
164. Turner C. E. & J. T. Miller: Primary sequence of paxillin contains putative SH2 and SH3 domain binding motifs and multiple LIM domains: identification of a vinculin and pp125Fak-binding region. *J Cell Sci* 107 (Pt 6), 1583-1591 (1994)
165. Salgia R., J. L. Li, S. H. Lo, B. Brunkhorst, G. S. Kansas, E. S. Sobhany, Y. Sun, E. Pisick, M. Hallek, T. Ernst, & .: Molecular cloning of human paxillin, a focal adhesion protein phosphorylated by P210BCR/ABL. *J Biol Chem* 270, 5039-5047 (1995)
166. Schlaepfer D. D., C. R. Hauck, & D. J. Sieg: Signaling through focal adhesion kinase. *Prog Biophys Mol Biol* 71, 435-478 (1999)
167. Avraham H., S. Y. Park, K. Schinkmann, & S. Avraham: RAFTK/Pyk2-mediated cellular signalling. *Cell Signal* 12, 123-133 (2000)
168. Klinghoffer R. A., C. Sachsenmaier, J. A. Cooper, & P. Soriano: Src family kinases are required for integrin but not PDGFR signal transduction. *EMBO J* 18, 2459-2471 (1999)
169. Schaller M. D. & J. T. Parsons: pp125FAK-dependent tyrosine phosphorylation of paxillin creates a high- affinity binding site for Crk. *Mol Cell Biol* 15, 2635-2645 (1995)
170. Bellis S. L., J. T. Miller, & C. E. Turner: Characterization of tyrosine phosphorylation of paxillin in vitro by focal adhesion kinase. *J Biol Chem* 270, 17437-17441 (1995)
171. Birge R. B., J. E. Fajardo, C. Reichman, S. E. Shoelson, Z. Songyang, L. C. Cantley, & H. Hanafusa: Identification and characterization of a high-affinity interaction between v-Crk and tyrosine-phosphorylated paxillin in CT10-transformed fibroblasts. *Mol Cell Biol* 13, 4648-4656 (1993)
172. Salgia R., N. Uemura, K. Okuda, J. L. Li, E. Pisick, M. Sattler, R. de Jong, B. Druker, N. Heisterkamp, L. B. Chen, & .: CRKL links p210BCR/ABL with paxillin in chronic myelogenous leukemia cells. *J Biol Chem* 270, 29145-29150 (1995)
173. Feller S. M., G. Posern, J. Voss, C. Kardinal, D. Sakkab, J. Zheng, & B. S. Knudsen: Physiological signals and oncogenesis mediated through Crk family adapter proteins. *J Cell Physiol* 177, 535-552 (1998)
174. Sabe H., A. Hata, M. Okada, H. Nakagawa, & H. Hanafusa: Analysis of the binding of the Src homology 2 domain of Csk to tyrosine- phosphorylated proteins in the suppression and mitotic activation of c- Src. *Proc Natl Acad Sci U S A* 91, 3984-3988 (1994)
175. Bergman M., V. Joukov, I. Virtanen, & K. Alitalo: Overexpressed Csk tyrosine kinase is localized in focal adhesions, causes reorganization of alpha v beta 5 integrin, and interferes with HeLa cell spreading. *Mol Cell Biol* 15, 711-722 (1995)
176. Grgurevich S., A. Mikhael, & D. W. McVicar: The Csk homologous kinase, Chk, binds tyrosine phosphorylated paxillin in human blastic T cells. *Biochem Biophys Res Commun* 256, 668-675 (1999)
177. Ostergaard H. L., O. Lou, C. W. Arendt, & N. N. Berg: Paxillin phosphorylation and association with Lck and Pyk2 in anti-CD3- or anti-CD45-stimulated T cells. *J Biol Chem* 273, 5692-5696 (1998)
178. Keenan C. & D. Kelleher: Protein kinase C and the cytoskeleton. *Cell Signal* 10, 225-232 (1998)
179. Mellor H. & P. J. Parker: The extended protein kinase C superfamily. *Biochem J* 332 (Pt 2), 281-292 (1998)
180. Nishizuka Y.: The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature* 334, 661-665 (1988)
181. Schechtman D. & D. Mochly-Rosen: Adaptor proteins in protein kinase C-mediated signal transduction. *Oncogene* 20, 6339-6347 (2001)
182. Chang B. Y., M. Chiang, & C. A. Cartwright: The interaction of Src and RACK1 is enhanced by activation of protein kinase C and tyrosine phosphorylation of RACK1. *J Biol Chem* 276, 20346-20356 (2001)
183. Csukai M., C. H. Chen, M. A. De Matteis, & D. Mochly-Rosen: The coatomer protein beta'-COP, a selective binding protein (RACK) for protein kinase Cepsilon. *J Biol Chem* 272, 29200-29206 (1997)
184. Ron D., C. H. Chen, J. Caldwell, L. Jamieson, E. Orr, & D. Mochly-Rosen: Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins. *Proc Natl Acad Sci U S A* 91, 839-843 (1994)
185. Fong H. K., T. T. Amatruda, III, B. W. Birren, & M. I. Simon: Distinct forms of the beta subunit of GTP-binding regulatory proteins identified by molecular cloning. *Proc Natl Acad Sci U S A* 84, 3792-3796 (1987)
186. Rodriguez-Fernandez J. L. & E. Rozengurt: Bombesin, bradykinin, vasopressin, and phorbol esters rapidly and transiently activate Src family tyrosine kinases in Swiss 3T3 cells. Dissociation from tyrosine phosphorylation of p125 focal adhesion kinase. *J Biol Chem* 271, 27895-27901 (1996)
187. Chang B. Y., K. B. Conroy, E. M. Machleder, & C. A. Cartwright: RACK1, a receptor for activated C kinase and a homolog of the beta subunit of G proteins, inhibits activity of src tyrosine kinases and growth of NIH 3T3 cells. *Mol Cell Biol* 18, 3245-3256 (1998)
188. Newman P. J., M. C. Berndt, J. Gorski, G. C. White, S. Lyman, C. Paddock, & W. A. Muller: PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. *Science* 247, 1219-1222 (1990)
189. Cao M. Y., M. Huber, N. Beauchemin, J. Famiglietti, S. M. Albelda, & A. Veillette: Regulation of mouse PECAM-1 tyrosine phosphorylation by the Src and Csk families of protein-tyrosine kinases. *J Biol Chem* 273, 15765-15772 (1998)
190. Osawa M., M. Masuda, N. Harada, R. B. Lopes, & K. Fujiwara: Tyrosine phosphorylation of platelet endothelial cell adhesion molecule- 1 (PECAM-1, CD31) in mechanically stimulated vascular endothelial cells. *Eur J Cell Biol* 72, 229-237 (1997)
191. Jackson D. E., C. M. Ward, R. Wang, & P. J. Newman: The protein-tyrosine phosphatase SHP-2 binds platelet/endothelial cell adhesion molecule-1 (PECAM-1) and forms a distinct signaling complex during platelet aggregation. Evidence for a mechanistic link between

PECAM-1- and integrin-mediated cellular signaling. *J Biol Chem* 272, 6986-6993 (1997)
192. Sagawa K., T. Kimura, M. Swieter, & R. P. Siraganian: The protein-tyrosine phosphatase SHP-2 associates with tyrosine- phosphorylated adhesion molecule PECAM-1 (CD31). *J Biol Chem* 272, 31086-31091 (1997)
193. Famiglietti J., J. Sun, H. M. DeLisser, & S. M. Albelda: Tyrosine residue in exon 14 of the cytoplasmic domain of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) regulates ligand binding specificity. *J Cell Biol* 138, 1425-1435 (1997)
194. Lu T. T., M. Barreuther, S. Davis, & J. A. Madri: Platelet endothelial cell adhesion molecule-1 is phosphorylatable by c- Src, binds Src-Src homology 2 domain, and exhibits immunoreceptor tyrosine-based activation motif-like properties. *J Biol Chem* 272, 14442-14446 (1997)
195. DeLisser H. M., P. J. Newman, & S. M. Albelda: Molecular and functional aspects of PECAM-1/CD31. *Immunol Today* 15, 490-495 (1994)
196. Newman P. J.: The biology of PECAM-1. *J Clin Invest* 99, 3-8 (1997)
197. Cicmil M., J. M. Thomas, T. Sage, F. A. Barry, M. Leduc, C. Bon, & J. M. Gibbins: Collagen, convulxin, and thrombin stimulate aggregation-independent tyrosine phosphorylation of CD31 in platelets. Evidence for the involvement of Src family kinases. *J Biol Chem* 275, 27339-27347 (2000)
198. Newman D. K., C. Hamilton, & P. J. Newman: Inhibition of antigen-receptor signaling by Platelet Endothelial Cell Adhesion Molecule-1 (CD31) requires functional ITIMs, SHP-2, and p56(lck). *Blood* 97, 2351-2357 (2001)
199. Masuda M., M. Osawa, H. Shigematsu, N. Harada, & K. Fujiwara: Platelet endothelial cell adhesion molecule-1 is a major SH-PTP2 binding protein in vascular endothelial cells. *FEBS Lett* 408, 331-336 (1997)
200. Kelly M. E. & A. C. Chan: Regulation of B cell function by linker proteins. *Curr Opin Immunol* 12, 267-275 (2000)
201. Myung P. S., N. J. Boerthe, & G. A. Koretzky: Adapter proteins in lymphocyte antigen-receptor signaling. *Curr Opin Immunol* 12, 256-266 (2000)
202. Norian L. A. & G. A. Koretzky: Intracellular adapter molecules. *Semin Immunol* 12, 43-54 (2000)
203. Zhang W. & L. E. Samelson: The role of membrane-associated adaptors in T cell receptor signalling. *Semin Immunol* 12, 35-41 (2000)
204. Tang J., S. Sawasdikosol, J. H. Chang, & S. J. Burakoff: SLAP, a dimeric adapter protein, plays a functional role in T cell receptor signaling. *Proc Natl Acad Sci U S A* 96, 9775-9780 (1999)
205. Samelson L. E., S. C. Bunnell, R. P. Trible, T. Yamazaki, & W. Zhang: Studies on the adapter molecule LAT. *Cold Spring Harb Symp Quant Biol* 64, 259-263 (1999)
206. Tomlinson M. G., J. Lin, & A. Weiss: Lymphocytes with a complex: adapter proteins in antigen receptor signaling. *Immunol Today* 21, 584-591 (2000)
207. Law C. L., M. K. Ewings, P. M. Chaudhary, S. A. Solow, T. J. Yun, A. J. Marshall, L. Hood, & E. A. Clark: GrpL, a Grb2-related adaptor protein, interacts with SLP-76 to regulate nuclear factor of activated T cell activation. *J Exp Med* 189, 1243-1253 (1999)
208. Zhang W., R. P. Trible, & L. E. Samelson: LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. *Immunity* 9, 239-246 (1998)
209. Deckert M., S. Tartare-Deckert, J. Hernandez, R. Rottapel, & A. Altman: Adaptor function for the Syk kinases-interacting protein 3BP2 in IL-2 gene activation. *Immunity* 9, 595-605 (1998)
210. Songyang Z., S. E. Shoelson, J. McGlade, P. Olivier, T. Pawson, X. R. Bustelo, M. Barbacid, H. Sabe, H. Hanafusa, T. Yi, & .: Specific motifs recognized by the SH2 domains of Csk, 3BP2, fps/fes, GRB-2, HCP, SHC, Syk, and Vav. *Mol Cell Biol* 14, 2777-2785 (1994)
211. Finco T. S., D. Yablonski, J. Lin, & A. Weiss: The adapter proteins LAT and SLP-76 are required for T-cell activation. *Cold Spring Harb Symp Quant Biol* 64, 265-274 (1999)
212. Zhang W., R. P. Trible, M. Zhu, S. K. Liu, C. J. McGlade, & L. E. Samelson: Association of Grb2, Gads, and phospholipase C-gamma 1 with phosphorylated LAT tyrosine residues. Effect of LAT tyrosine mutations on T cell angigen receptor-mediated signaling. *J Biol Chem* 275, 23355-23361 (2000)
213. Zhang W., C. L. Sommers, D. N. Burshtyn, C. C. Stebbins, J. B. DeJarnette, R. P. Trible, A. Grinberg, H. C. Tsay, H. M. Jacobs, C. M. Kessler, E. O. Long, P. E. Love, & L. E. Samelson: Essential role of LAT in T cell development. *Immunity* 10, 323-332 (1999)
214. Fehling H. J., B. M. Iritani, A. Krotkova, K. A. Forbush, C. Laplace, R. M. Perlmutter, & H. von Boehmer: Restoration of thymopoiesis in pT alpha-/- mice by anti-CD3epsilon antibody treatment or with transgenes encoding activated Lck or tailless pT alpha. *Immunity* 6, 703-714 (1997)
215. Marie-Cardine A., H. Kirchgessner, E. Bruyns, A. Shevchenko, M. Mann, F. Autschbach, S. Ratnofsky, S. Meuer, & B. Schraven: SHP2-interacting transmembrane adaptor protein (SIT), a novel disulfide- linked dimer regulating human T cell activation. *J Exp Med* 189, 1181-1194 (1999)
216. Coggeshall K. M.: Inhibitory signaling by B cell Fc gamma RIIb. *Curr Opin Immunol* 10, 306-312 (1998)
217. Pfrepper K. I., A. Marie-Cardine, L. Simeoni, Y. Kuramitsu, A. Leo, J. Spicka, I. Hilgert, J. Scherer, & B. Schraven: Structural and functional dissection of the cytoplasmic domain of the transmembrane adaptor protein SIT (SHP2-interacting transmembrane adaptor protein). *Eur J Immunol* 31, 1825-1836 (2001)
218. Bruyns E., A. Marie-Cardine, H. Kirchgessner, K. Sagolla, A. Shevchenko, M. Mann, F. Autschbach, A. Bensussan, S. Meuer, & B. Schraven: T cell receptor (TCR) interacting molecule (TRIM), a novel disulfide- linked dimer associated with the TCR-CD3-zeta complex, recruits intracellular signaling proteins to the plasma membrane. *J Exp Med* 188, 561-575 (1998)
219. Kirchgessner H., J. Dietrich, J. Scherer, P. Isomaki, V. Korinek, I. Hilgert, E. Bruyns, A. Leo, A. P. Cope, & B. Schraven: The transmembrane adaptor protein TRIM regulates T cell receptor (TCR) expression and TCR-mediated signaling via an association with the TCR zeta chain. *J Exp Med* 193, 1269-1284 (2001)

220. Wardenburg J. B., C. Fu, J. K. Jackman, H. Flotow, S. E. Wilkinson, D. H. Williams, R. Johnson, G. Kong, A. C. Chan, & P. R. Findell: Phosphorylation of SLP-76 by the ZAP-70 protein-tyrosine kinase is required for T-cell receptor function. *J Biol Chem* 271, 19641-19644 (1996)
221. Yoder J., C. Pham, Y. M. Iizuka, O. Kanagawa, S. K. Liu, J. McGlade, & A. M. Cheng: Requirement for the SLP-76 adaptor GADS in T cell development. *Science* 291, 1987-1991 (2001)
222. Bubeck W. J., R. Pappu, J. Y. Bu, B. Mayer, J. Chernoff, D. Straus, & A. C. Chan: Regulation of PAK activation and the T cell cytoskeleton by the linker protein SLP-76. *Immunity* 9, 607-616 (1998)
223. Fang N., D. G. Motto, S. E. Ross, & G. A. Koretzky: Tyrosines 113, 128, and 145 of SLP-76 are required for optimal augmentation of NFAT promoter activity. *J Immunol* 157, 3769-3773 (1996)
224. Pivniouk V., E. Tsitsikov, P. Swinton, G. Rathbun, F. W. Alt, & R. S. Geha: Impaired viability and profound block in thymocyte development in mice lacking the adaptor protein SLP-76. *Cell* 94, 229-238 (1998)
225. Clements J. L., J. R. Lee, B. Gross, B. Yang, J. D. Olson, A. Sandra, S. P. Watson, S. R. Lentz, & G. A. Koretzky: Fetal hemorrhage and platelet dysfunction in SLP-76-deficient mice. *J Clin Invest* 103, 19-25 (1999)
226. Yablonski D., T. Kadlecek, & A. Weiss: Identification of a phospholipase C-gamma1 (PLC-gamma1) SH3 domain- binding site in SLP-76 required for T-cell receptor-mediated activation of PLC-gamma1 and NFAT. *Mol Cell Biol* 21, 4208-4218 (2001)
227. Liu S. K. & C. J. McGlade: Gads is a novel SH2 and SH3 domain-containing adaptor protein that binds to tyrosine-phosphorylated Shc. *Oncogene* 17, 3073-3082 (1998)
228. Liu S. K., N. Fang, G. A. Koretzky, & C. J. McGlade: The hematopoietic-specific adaptor protein gads functions in T-cell signaling via interactions with the SLP-76 and LAT adaptors. *Curr Biol* 9, 67-75 (1999)
229. Asada H., N. Ishii, Y. Sasaki, K. Endo, H. Kasai, N. Tanaka, T. Takeshita, S. Tsuchiya, T. Konno, & K. Sugamura: Grf40, A novel Grb2 family member, is involved in T cell signaling through interaction with SLP-76 and LAT. *J Exp Med* 189, 1383-1390 (1999)
230. Reth M. & J. Wienands: The maintenance and the activation signal of the B-cell antigen receptor. *Cold Spring Harb Symp Quant Biol* 64, 323-328 (1999)
231. Uckun F. M., A. L. Burkhardt, L. Jarvis, X. Jun, B. Stealey, I. Dibirdik, D. E. Myers, L. Tuel-Ahlgren, & J. B. Bolen: Signal transduction through the CD19 receptor during discrete developmental stages of human B-cell ontogeny. *J Biol Chem* 268, 21172-21184 (1993)
232. Kitanaka A., C. Ito, E. Coustan-Smith, & D. Campana: CD38 ligation in human B cell progenitors triggers tyrosine phosphorylation of CD19 and association of CD19 with lyn and phosphatidylinositol 3-kinase. *J Immunol* 159, 184-192 (1997)
233. Xiao J., Y. Messinger, J. Jin, D. E. Myers, J. B. Bolen, & F. M. Uckun: Signal transduction through the beta1 integrin family surface adhesion molecules VLA-4 and VLA-5 of human B-cell precursors activates CD19 receptor-associated protein-tyrosine kinases. *J Biol Chem* 271, 7659-7664 (1996)
234. Holsinger L. J., I. A. Graef, W. Swat, T. Chi, D. M. Bautista, L. Davidson, R. S. Lewis, F. W. Alt, & G. R. Crabtree: Defects in actin-cap formation in Vav-deficient mice implicate an actin requirement for lymphocyte signal transduction. *Curr Biol* 8, 563-572 (1998)
235. Sato S., A. S. Miller, M. C. Howard, & T. F. Tedder: Regulation of B lymphocyte development and activation by the CD19/CD21/CD81/Leu 13 complex requires the cytoplasmic domain of CD19. *J Immunol* 159, 3278-3287 (1997)
236. Rickert R. C., K. Rajewsky, & J. Roes: Impairment of T-cell-dependent B-cell responses and B-1 cell development in CD19-deficient mice. *Nature* 376, 352-355 (1995)
237. Engel P., L. J. Zhou, D. C. Ord, S. Sato, B. Koller, & T. F. Tedder: Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. *Immunity* 3, 39-50 (1995)
238. Otero D. C., S. A. Omori, & R. C. Rickert: Cd19-dependent activation of Akt kinase in B-lymphocytes. *J Biol Chem* 276, 1474-1478 (2001)
239. Buhl A. M., C. M. Pleiman, R. C. Rickert, & J. C. Cambier: Qualitative regulation of B cell antigen receptor signaling by CD19: selective requirement for PI3-kinase activation, inositol-1,4,5- trisphosphate production and Ca2+ mobilization. *J Exp Med* 186, 1897-1910 (1997)
240. Venkataraman C., P. J. Lu, A. M. Buhl, C. S. Chen, J. C. Cambier, & S. Bondada: CD72-mediated B cell activation involves recruitment of CD19 and activation of phosphatidylinositol 3-kinase. *Eur J Immunol* 28, 3003-3016 (1998)
241. Franke T. F., D. R. Kaplan, L. C. Cantley, & A. Toker: Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science* 275, 665-668 (1997)
242. Buhl A. M. & J. C. Cambier: Phosphorylation of CD19 Y484 and Y515, and linked activation of phosphatidylinositol 3-kinase, are required for B cell antigen receptor- mediated activation of Bruton's tyrosine kinase. *J Immunol* 162, 4438-4446 (1999)
243. Smith K. G. & D. T. Fearon: Receptor modulators of B-cell receptor signalling--CD19/CD22. *Curr Top Microbiol Immunol* 245, 195-212 (2000)
244. Bolland S. & J. V. Ravetch: Inhibitory pathways triggered by ITIM-containing receptors. *Adv Immunol* 72, 149-177 (1999)
245. Poe J. C., M. Fujimoto, P. J. Jansen, A. S. Miller, & T. F. Tedder: CD22 forms a quaternary complex with SHIP, Grb2, and Shc. A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux. *J Biol Chem* 275, 17420-17427 (2000)
246. Leprince C., K. E. Draves, R. L. Geahlen, J. A. Ledbetter, & E. A. Clark: CD22 associates with the human surface IgM-B-cell antigen receptor complex. *Proc Natl Acad Sci U S A* 90, 3236-3240 (1993)
247. Peaker C. J. & M. S. Neuberger: Association of CD22 with the B cell antigen receptor. *Eur J Immunol* 23, 1358-1363 (1993)
248. Ono M., H. Okada, S. Bolland, S. Yanagi, T. Kurosaki, & J. V. Ravetch: Deletion of SHIP or SHP-1 reveals two distinct pathways for inhibitory signaling. *Cell* 90, 293-301 (1997)

249. Wu Y., M. J. Nadler, L. A. Brennan, G. D. Gish, J. F. Timms, N. Fusaki, J. Jongstra-Bilen, N. Tada, T. Pawson, J. Wither, B. G. Neel, & N. Hozumi: The B-cell transmembrane protein CD72 binds to and is an in vivo substrate of the protein tyrosine phosphatase SHP-1. *Curr Biol* 8, 1009-1017 (1998)
250. Yang W., S. D. Mckenna, H. Jiao, M. Tabrizi, M. A. Lynes, L. D. Shultz, & T. Yi: SHP-1 deficiency in B-lineage cells is associated with heightened lyn protein expression and increased lyn kinase activity. *Exp Hematol* 26, 1126-1132 (1998)
251. Dustin L. B., D. R. Plas, J. Wong, Y. T. Hu, C. Soto, A. C. Chan, & M. L. Thomas: Expression of dominant-negative src-homology domain 2-containing protein tyrosine phosphatase-1 results in increased Syk tyrosine kinase activity and B cell activation. *J Immunol* 162, 2717-2724 (1999)
252. Yohannan J., J. Wienands, K. M. Coggeshall, & L. B. Justement: Analysis of tyrosine phosphorylation-dependent interactions between stimulatory effector proteins and the B cell co-receptor CD22. *J Biol Chem* 274, 18769-18776 (1999)
253. Tuscano J. M., A. Riva, S. N. Toscano, T. F. Tedder, & J. H. Kehrl: CD22 cross-linking generates B-cell antigen receptor-independent signals that activate the JNK/SAPK signaling cascade. *Blood* 94, 1382-1392 (1999)
254. Sato S., A. S. Miller, M. Inaoki, C. B. Bock, P. J. Jansen, M. L. Tang, & T. F. Tedder: CD22 is both a positive and negative regulator of B lymphocyte antigen receptor signal transduction: altered signaling in CD22-deficient mice. *Immunity* 5, 551-562 (1996)
255. Sato K. & A. Ochi: Superclustering of B cell receptor and Fc gamma RIIB1 activates Src homology 2-containing protein tyrosine phosphatase-1. *J Immunol* 161, 2716-2722 (1998)
256. Otipoby K. L., K. B. Andersson, K. E. Draves, S. J. Klaus, A. G. Farr, J. D. Kerner, R. M. Perlmutter, C. L. Law, & E. A. Clark: CD22 regulates thymus-independent responses and the lifespan of B cells. *Nature* 384, 634-637 (1996)
257. O'Keefe T. L., G. T. Williams, S. L. Davies, & M. S. Neuberger: Hyperresponsive B cells in CD22-deficient mice. *Science* 274, 798-801 (1996)
258. Nitschke L., R. Carsetti, B. Ocker, G. Kohler, & M. C. Lamers: CD22 is a negative regulator of B-cell receptor signalling. *Curr Biol* 7, 133-143 (1997)
259. Dolmetsch R. E., R. S. Lewis, C. C. Goodnow, & J. I. Healy: Differential activation of transcription factors induced by Ca2+ response amplitude and duration. *Nature* 386, 855-858 (1997)
260. Hashimoto A., H. Okada, A. Jiang, M. Kurosaki, S. Greenberg, E. A. Clark, & T. Kurosaki: Involvement of guanosine triphosphatases and phospholipase C-gamma2 in extracellular signal-regulated kinase, c-Jun NH2-terminal kinase, and p38 mitogen-activated protein kinase activation by the B cell antigen receptor. *J Exp Med* 188, 1287-1295 (1998)
261. Jiang A., A. Craxton, T. Kurosaki, & E. A. Clark: Different protein tyrosine kinases are required for B cell antigen receptor-mediated activation of extracellular signal-regulated kinase, c-Jun NH2-terminal kinase 1, and p38 mitogen-activated protein kinase. *J Exp Med* 188, 1297-1306 (1998)
262. Bolland S., R. N. Pearse, T. Kurosaki, & J. V. Ravetch: SHIP modulates immune receptor responses by regulating membrane association of Btk. *Immunity* 8, 509-516 (1998)
263. Astoul E., S. Watton, & D. Cantrell: The dynamics of protein kinase B regulation during B cell antigen receptor engagement. *J Cell Biol* 145, 1511-1520 (1999)
264. Jacob A., D. Cooney, S. Tridandapani, T. Kelley, & K. M. Coggeshall: FcgammaRIIb modulation of surface immunoglobulin-induced Akt activation in murine B cells. *J Biol Chem* 274, 13704-13710 (1999)
265. Tan J. E., S. C. Wong, S. K. Gan, S. Xu, & K. P. Lam: The adaptor protein BLNK is required for b cell antigen receptor- induced activation of nuclear factor-kappa B and cell cycle entry and survival of B lymphocytes. *J Biol Chem* 276, 20055-20063 (2001)
266. Ishiai M., M. Kurosaki, R. Pappu, K. Okawa, I. Ronko, C. Fu, M. Shibata, A. Iwamatsu, A. C. Chan, & T. Kurosaki: BLNK required for coupling Syk to PLC gamma 2 and Rac1-JNK in B cells. *Immunity* 10, 117-125 (1999)
267. Fu C. & A. C. Chan: Identification of two tyrosine phosphoproteins, pp70 and pp68, which interact with phospholipase Cgamma, Grb2, and Vav after B cell antigen receptor activation. *J Biol Chem* 272, 27362-27368 (1997)
268. Pappu R., A. M. Cheng, B. Li, Q. Gong, C. Chiu, N. Griffin, M. White, B. P. Sleckman, & A. C. Chan: Requirement for B cell linker protein (BLNK) in B cell development. *Science* 286, 1949-1954 (1999)
269. Kerner J. D., M. W. Appleby, R. N. Mohr, S. Chien, D. J. Rawlings, C. R. Maliszewski, O. N. Witte, & R. M. Perlmutter: Impaired expansion of mouse B cell progenitors lacking Btk. *Immunity* 3, 301-312 (1995)
270. Chan A. C., D. M. Desai, & A. Weiss: The role of protein tyrosine kinases and protein tyrosine phosphatases in T cell antigen receptor signal transduction. *Annu Rev Immunol* 12, 555-592 (1994)
271. Gergely J., I. Pecht, & G. Sarmay: Immunoreceptor tyrosine-based inhibition motif-bearing receptors regulate the immunoreceptor tyrosine-based activation motif-induced activation of immune competent cells. *Immunol Lett* 68, 3-15 (1999)
272. Plas D. R. & M. L. Thomas: Negative regulation of antigen receptor signaling in lymphocytes. *J Mol Med* 76, 589-595 (1998)
273. Saito T.: Negative regulation of T cell activation. *Curr Opin Immunol* 10, 313-321 (1998)
274. Thomas M. L.: Of ITAMs and ITIMs: turning on and off the B cell antigen receptor. *J Exp Med* 181, 1953-1956 (1995)
275. Unkeless J. C. & J. Jin: Inhibitory receptors, ITIM sequences and phosphatases. *Curr Opin Immunol* 9, 338-343 (1997)
276. Jones N. & D. J. Dumont: Recruitment of Dok-R to the EGF receptor through its PTB domain is required for attenuation of Erk MAP kinase activation. *Curr Biol* 9, 1057-1060 (1999)
277. Lemay S., D. Davidson, S. Latour, & A. Veillette: Dok-3, a novel adapter molecule involved in the negative regulation of immunoreceptor signaling. *Mol Cell Biol* 20, 2743-2754 (2000)
278. Martins B. M., B. Grimm, H. P. Mock, R. Huber, & A. Messerschmidt: Crystal structure and substrate binding

modeling of the uroporphyrinogen-III decarboxylase from nicotiana tabacum: Implications for the catalytic mechanism. *J Biol Chem* (2001)
279. Carpino N., D. Wisniewski, A. Strife, D. Marshak, R. Kobayashi, B. Stillman, & B. Clarkson: p62(dok): a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. *Cell* 88, 197-204 (1997)
280. Yamanashi Y. & D. Baltimore: Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok. *Cell* 88, 205-211 (1997)
281. Di Cristofano A., N. Carpino, N. Dunant, G. Friedland, R. Kobayashi, A. Strife, D. Wisniewski, B. Clarkson, P. P. Pandolfi, & M. D. Resh: Molecular cloning and characterization of p56dok-2 defines a new family of RasGAP-binding proteins. *J Biol Chem* 273, 4827-4830 (1998)
282. Noguchi T., T. Matozaki, K. Inagaki, M. Tsuda, K. Fukunaga, Y. Kitamura, T. Kitamura, K. Shii, Y. Yamanashi, & M. Kasuga: Tyrosine phosphorylation of p62(Dok) induced by cell adhesion and insulin: possible role in cell migration. *EMBO J* 18, 1748-1760 (1999)
283. Lock P., F. Casagranda, & A. R. Dunn: Independent SH2-binding sites mediate interaction of Dok-related protein with RasGTPase-activating protein and Nck. *J Biol Chem* 274, 22775-22784 (1999)
284. Yoshida K., Y. Yamashita, A. Miyazato, K. Ohya, A. Kitanaka, U. Ikeda, K. Shimada, T. Yamanaka, K. Ozawa, & H. Mano: Mediation by the protein-tyrosine kinase Tec of signaling between the B cell antigen receptor and Dok-1. *J Biol Chem* 275, 24945-24952 (2000)
285. Kashige N., N. Carpino, & R. Kobayashi: Tyrosine phosphorylation of p62dok by p210bcr-abl inhibits RasGAP activity. *Proc Natl Acad Sci U S A* 97, 2093-2098 (2000)
286. Tamir I., J. C. Stolpa, C. D. Helgason, K. Nakamura, P. Bruhns, M. Daeron, & J. C. Cambier: The RasGAP-binding protein p62dok is a mediator of inhibitory FcgammaRIIB signals in B cells. *Immunity* 12, 347-358 (2000)
287. Kawabuchi M., Y. Satomi, T. Takao, Y. Shimonishi, S. Nada, K. Nagai, A. Tarakhovsky, & M. Okada: Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. *Nature* 404, 999-1003 (2000)
288. Brdicka T., D. Pavlistova, A. Leo, E. Bruyns, V. Korinek, P. Angelisova, J. Scherer, A. Shevchenko, I. Hilgert, J. Cerny, K. Drbal, Y. Kuramitsu, B. Kornacker, V. Horejsi, & B. Schraven: Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. *J Exp Med* 191, 1591-1604 (2000)
289. Chen X. P., J. A. Losman, & P. Rothman: SOCS proteins, regulators of intracellular signaling. *Immunity* 13, 287-290 (2000)
290. Yoshimura A., T. Ohkubo, T. Kiguchi, N. A. Jenkins, D. J. Gilbert, N. G. Copeland, T. Hara, & A. Miyajima: A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *EMBO J* 14, 2816-2826 (1995)
291. Starr R., T. A. Willson, E. M. Viney, L. J. Murray, J. R. Rayner, B. J. Jenkins, T. J. Gonda, W. S. Alexander, D. Metcalf, N. A. Nicola, & D. J. Hilton: A family of cytokine-inducible inhibitors of signalling. *Nature* 387, 917-921 (1997)
292. Endo T. A., M. Masuhara, M. Yokouchi, R. Suzuki, H. Sakamoto, K. Mitsui, A. Matsumoto, S. Tanimura, M. Ohtsubo, H. Misawa, T. Miyazaki, N. Leonor, T. Taniguchi, T. Fujita, Y. Kanakura, S. Komiya, & A. Yoshimura: A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* 387, 921-924 (1997)
293. Naka T., M. Narazaki, M. Hirata, T. Matsumoto, S. Minamoto, A. Aono, N. Nishimoto, T. Kajita, T. Taga, K. Yoshizaki, S. Akira, & T. Kishimoto: Structure and function of a new STAT-induced STAT inhibitor. *Nature* 387, 924-929 (1997)
294. Minamoto S., K. Ikegame, K. Ueno, M. Narazaki, T. Naka, H. Yamamoto, T. Matsumoto, H. Saito, S. Hosoe, & T. Kishimoto: Cloning and functional analysis of new members of STAT induced STAT inhibitor (SSI) family: SSI-2 and SSI-3. *Biochem Biophys Res Commun* 237, 79-83 (1997)
295. Masuhara M., H. Sakamoto, A. Matsumoto, R. Suzuki, H. Yasukawa, K. Mitsui, T. Wakioka, S. Tanimura, A. Sasaki, H. Misawa, M. Yokouchi, M. Ohtsubo, & A. Yoshimura: Cloning and characterization of novel CIS family genes. *Biochem Biophys Res Commun* 239, 439-446 (1997)
296. Hilton D. J., R. T. Richardson, W. S. Alexander, E. M. Viney, T. A. Willson, N. S. Sprigg, R. Starr, S. E. Nicholson, D. Metcalf, & N. A. Nicola: Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc Natl Acad Sci U S A* 95, 114-119 (1998)
297. Auernhammer C. J., C. Bousquet, V. Chesnokova, & S. Melmed: SOCS proteins: modulators of neuroimmunoendocrine functions. Impact on corticotroph LIF signaling. *Ann N Y Acad Sci* 917, 658-664 (2000)
298. Favre H., A. Benhamou, J. Finidori, P. A. Kelly, & M. Edery: Dual effects of suppressor of cytokine signaling (SOCS-2) on growth hormone signal transduction. *FEBS Lett* 453, 63-66 (1999)
299. Pezet A., H. Favre, P. A. Kelly, & M. Edery: Inhibition and restoration of prolactin signal transduction by suppressors of cytokine signaling. *J Biol Chem* 274, 24497-24502 (1999)
300. Matsumoto A., M. Masuhara, K. Mitsui, M. Yokouchi, M. Ohtsubo, H. Misawa, A. Miyajima, & A. Yoshimura: CIS, a cytokine inducible SH2 protein, is a target of the JAK-STAT5 pathway and modulates STAT5 activation. *Blood* 89, 3148-3154 (1997)
301. Matsumoto A., Y. Seki, M. Kubo, S. Ohtsuka, A. Suzuki, I. Hayashi, K. Tsuji, T. Nakahata, M. Okabe, S. Yamada, & A. Yoshimura: Suppression of STAT5 functions in liver, mammary glands, and T cells in cytokine-inducible SH2-containing protein 1 transgenic mice. *Mol Cell Biol* 19, 6396-6407 (1999)
302. Yasukawa H., A. Sasaki, & A. Yoshimura: Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* 18, 143-164 (2000)

303. Verdier F., S. Chretien, O. Muller, P. Varlet, A. Yoshimura, S. Gisselbrecht, C. Lacombe, & P. Mayeux: Proteasomes regulate erythropoietin receptor and signal transducer and activator of transcription 5 (STAT5) activation. Possible involvement of the ubiquitinated Cis protein. *J Biol Chem* 273, 28185-28190 (1998)
304. Udy G. B., R. P. Towers, R. G. Snell, R. J. Wilkins, S. H. Park, P. A. Ram, D. J. Waxman, & H. W. Davey: Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proc Natl Acad Sci U S A* 94, 7239-7244 (1997)
305. Liu X., G. W. Robinson, K. U. Wagner, L. Garrett, A. Wynshaw-Boris, & L. Hennighausen: Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev* 11, 179-186 (1997)
306. Nakajima H., X. W. Liu, A. Wynshaw-Boris, L. A. Rosenthal, K. Imada, D. S. Finbloom, L. Hennighausen, & W. J. Leonard: An indirect effect of Stat5a in IL-2-induced proliferation: a critical role for Stat5a in IL-2-mediated IL-2 receptor alpha chain induction. *Immunity* 7, 691-701 (1997)
307. Teglund S., C. McKay, E. Schuetz, J. M. van Deursen, D. Stravopodis, D. Wang, M. Brown, S. Bodner, G. Grosveld, & J. N. Ihle: Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 93, 841-850 (1998)
308. Moriggl R., D. J. Topham, S. Teglund, V. Sexl, C. McKay, D. Wang, A. Hoffmeyer, J. van Deursen, M. Y. Sangster, K. D. Bunting, G. C. Grosveld, & J. N. Ihle: Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. *Immunity* 10, 249-259 (1999)
309. Yasukawa H., H. Misawa, H. Sakamoto, M. Masuhara, A. Sasaki, T. Wakioka, S. Ohtsuka, T. Imaizumi, T. Matsuda, J. N. Ihle, & A. Yoshimura: The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. *EMBO J* 18, 1309-1320 (1999)
310. Naka T., T. Matsumoto, M. Narazaki, M. Fujimoto, Y. Morita, Y. Ohsawa, H. Saito, T. Nagasawa, Y. Uchiyama, & T. Kishimoto: Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice. *Proc Natl Acad Sci U S A* 95, 15577-15582 (1998)
311. Starr R., D. Metcalf, A. G. Elefanty, M. Brysha, T. A. Willson, N. A. Nicola, D. J. Hilton, & W. S. Alexander: Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. *Proc Natl Acad Sci U S A* 95, 14395-14399 (1998)
312. Metcalf D., W. S. Alexander, A. G. Elefanty, N. A. Nicola, D. J. Hilton, R. Starr, S. Mifsud, & L. Di Rago: Aberrant hematopoiesis in mice with inactivation of the gene encoding SOCS-1. *Leukemia* 13, 926-934 (1999)
313. Muslin A. J., J. W. Tanner, P. M. Allen, & A. S. Shaw: Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. *Cell* 84, 889-897 (1996)
314. Tzivion G., Y. H. Shen, & J. Zhu: 14-3-3 proteins; bringing new definitions to scaffolding. *Oncogone* 6331-6338 (2001)
315. Yaffe M. B., K. Rittinger, S. Volinia, P. R. Caron, A. Aitken, H. Leffers, S. J. Gamblin, S. J. Smerdon, & L. C. Cantley: The structural basis for 14-3-3:phosphopeptide binding specificity. *Cell* 91, 961-971 (1997)
316. Fu H., R. R. Subramanian, & S. C. Masters: 14-3-3 proteins: structure, function, and regulation. *Annu Rev Pharmacol Toxicol* 40, 617-647 (2000)
317. Yaffe M. B. & A. E. Elia: Phosphoserine/threonine-binding domains. *Curr Opin Cell Biol* 13, 131-138 (2001)
318. Lu P. J., X. Z. Zhou, M. Shen, & K. P. Lu: Function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science* 283, 1325-1328 (1999)
319. Verdecia M. A., M. E. Bowman, K. P. Lu, T. Hunter, & J. P. Noel: Structural basis for phosphoserine-proline recognition by group IV WW domains. *Nat Struct Biol* 7, 639-643 (2000)
320. Hofmann K. & P. Bucher: The FHA domain: a putative nuclear signalling domain found in protein kinases and transcription factors. *Trends Biochem Sci* 20, 347-349 (1995)
321. Liao H., I. J. Byeon, & M. D. Tsai: Structure and function of a new phosphopeptide-binding domain containing the FHA2 of Rad53. *J Mol Biol* 294, 1041-1049 (1999)
322. Wang P., I. J. Byeon, H. Liao, K. D. Beebe, S. Yongkiettrakul, D. Pei, & M. D. Tsai: II. Structure and specificity of the interaction between the FHA2 domain of Rad53 and phosphotyrosyl peptides. *J Mol Biol* 302, 927-940 (2000)
323. Durocher D., J. Henckel, A. R. Fersht, & S. P. Jackson: The FHA domain is a modular phosphopeptide recognition motif. *Mol Cell* 4, 387-394 (1999)
324. Durocher D., I. A. Taylor, D. Sarbassova, L. F. Haire, S. L. Westcott, S. P. Jackson, S. J. Smerdon, & M. B. Yaffe: The molecular basis of FHA Domain:Phosphopeptide binding specificity and implications for phospho-dependent signaling mechanisms. *Mol Cell* 6, 1169-1182 (2000)
325. van der Voorn L & Ploegh HL: The WD-40 repeat. *FEBS Lett* 307, 131-134 (1992)
326. Fuchs S. Y., A. Chen, Y. Xiong, Z. Q. Pan, & Z. Ronai: HOS, a human homolog of Slimb, forms an SCF complex with Skp1 and Cullin1 and targets the phosphorylation-dependent degradation of IkappaB and beta-catenin. *Oncogene* 18, 2039-2046 (1999)
327. Orian A., H. Gonen, B. Bercovich, I. Fajerman, E. Eytan, A. Israel, F. Mercurio, K. Iwai, A. L. Schwartz, & A. Ciechanover: SCF(beta)(-TrCP) ubiquitin ligase-mediated processing of NF-kappaB p105 requires phosphorylation of its C-terminus by IkappaB kinase. *EMBO J* 19, 2580-2591 (2000)
328. Winston J. T., P. Strack, P. Beer-Romero, C. Y. Chu, S. J. Elledge, & J. W. Harper: The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. *Genes Dev* 13, 270-283 (1999)
329. Tsvetkov L. M., K. H. Yeh, S. J. Lee, H. Sun, & H. Zhang: p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27. *Curr Biol* 9, 661-664 (1999)
330. Glenney J. R., Jr. & L. Zokas: Novel tyrosine kinase substrates from Rous sarcoma virus-transformed cells are

present in the membrane skeleton. *J Cell Biol* 108, 2401-2408 (1989)
331. De Nichilo M. O. & K. M. Yamada: Integrin alpha v beta 5-dependent serine phosphorylation of paxillin in cultured human macrophages adherent to vitronectin. *J Biol Chem* 271, 11016-11022 (1996)
332. Bellis S. L., J. A. Perrotta, M. S. Curtis, & C. E. Turner: Adhesion of fibroblasts to fibronectin stimulates both serine and tyrosine phosphorylation of paxillin. *Biochem J* 325 (Pt 2), 375-381 (1997)
333. Yamakita Y., G. Totsukawa, S. Yamashiro, D. Fry, X. Zhang, S. K. Hanks, & F. Matsumura: Dissociation of FAK/p130(CAS)/c-Src complex during mitosis: role of mitosis-specific serine phosphorylation of FAK. *J Cell Biol* 144, 315-324 (1999)
334. Vadlamudi R., L. Adam, A. Talukder, J. Mendelsohn, & R. Kumar: Serine phosphorylation of paxillin by heregulin-beta1: role of p38 mitogen activated protein kinase. *Oncogene* 18, 7253-7264 (1999)
335. Ku H. & K. E. Meier: Phosphorylation of paxillin via the ERK mitogen-activated protein kinase cascade in EL4 thymoma cells. *J Biol Chem* 275, 11333-11340 (2000)
336. Hashimoto S., A. Tsubouchi, Y. Mazaki, & H. Sabe: Interaction of paxillin with p21-activated Kinase (PAK). Association of paxillin alpha with the kinase-inactive and the Cdc42-activated forms of PAK3. *J Biol Chem* 276, 6037-6045 (2001)
337. Brown M. C., J. A. Perrotta, & C. E. Turner: Serine and threonine phosphorylation of the paxillin LIM domains regulates paxillin focal adhesion localization and cell adhesion to fibronectin. *Mol Biol Cell* 9, 1803-1816 (1998)
338. Schaeper U., N. H. Gehring, K. P. Fuchs, M. Sachs, B. Kempkes, & W. Birchmeier: Coupling of Gab1 to c-Met, Grb2, and Shp2 mediates biological responses. *J Cell Biol* 149, 1419-1432 (2000)
339. Yu C. F., B. Roshan, Z. X. Liu, & L. G. Cantley: Erk regulates the hepatocyte growth factor-mediated interaction of gab1 and the phosphatidylinositol 3-kinase. *J Biol Chem* 276, 32552-32558 (2001)
340. Furge K. A., Y. W. Zhang, & G. F. Vande Woude: Met receptor tyrosine kinase: enhanced signaling through adapter proteins. *Oncogene* 19, 5582-5589 (2000)
341. Roshan B., C. Kjelsberg, K. Spokes, A. Eldred, C. S. Crovello, & L. G. Cantley: Activated ERK2 interacts with and phosphorylates the docking protein GAB1. *J Biol Chem* 274, 36362-36368 (1999)
342. Gual P., S. Giordano, S. Anguissola, P. J. Parker, & P. M. Comoglio: Gab1 phosphorylation: a novel mechanism for negative regulation of HGF receptor signaling. *Oncogene* 20, 156-166 (2001)
343. Ilan N., S. Mahooti, D. L. Rimm, & J. A. Madri: PECAM-1 (CD31) functions as a reservoir for and a modulator of tyrosine- phosphorylated beta-catenin. *J Cell Sci* 112 Pt 18, 3005-3014 (1999)
344. Ilan N., L. Cheung, E. Pinter, & J. A. Madri: Platelet-endothelial cell adhesion molecule-1 (CD31), a scaffolding molecule for selected catenin family members whose binding is mediated by different tyrosine and serine/threonine phosphorylation. *J Biol Chem* 275, 21435-21443 (2000)
345. Ilan N., L. Cheung, S. Miller, A. Mohsenin, A. Tucker, & J. A. Madri: Pecam-1 is a modulator of stat family member phosphorylation and localization: lessons from a transgenic mouse. *Dev Biol* 232, 219-232 (2001)
346. Shen Y., C. Sultana, M. Arditi, K. S. Kim, & V. K. Kalra: Endotoxin-induced migration of monocytes and PECAM-1 phosphorylation are abrogated by PAF receptor antagonists. *Am J Physiol* 275, E479-E486 (1998)
347. Paasinen-Sohns A. & E. Holtta: Cells transformed by ODC, c-Ha-ras and v-src exhibit MAP kinase/Erk-independent constitutive phosphorylation of Sos, Raf and c-Jun activation domain, and reduced PDGF receptor expression. *Oncogene* 15, 1953-1966 (1997)
348. Cherniack A. D., J. K. Klarlund, & M. P. Czech: Phosphorylation of the Ras nucleotide exchange factor son of sevenless by mitogen-activated protein kinase. *J Biol Chem* 269, 4717-4720 (1994)
349. Rozakis-Adcock M., G. P. van der, G. Mbamalu, & T. Pawson: MAP kinase phosphorylation of mSos1 promotes dissociation of mSos1-Shc and mSos1-EGF receptor complexes. *Oncogene* 11, 1417-1426 (1995)
350. Corbalan-Garcia S., S. S. Yang, K. R. Degenhardt, & D. Bar-Sagi: Identification of the mitogen-activated protein kinase phosphorylation sites on human Sos1 that regulate interaction with Grb2. *Mol Cell Biol* 16, 5674-5682 (1996)
351. Douville E. & J. Downward: EGF induced SOS phosphorylation in PC12 cells involves P90 RSK-2. *Oncogene* 15, 373-383 (1997)
352. Zhao H., Y. Y. Li, R. V. Fucini, S. E. Ross, J. E. Pessin, & G. A. Koretzky: T cell receptor-induced phosphorylation of Sos requires activity of CD45, Lck, and protein kinase C, but not ERK. *J Biol Chem* 272, 21625-21634 (1997)
353. Liu J. P.: Protein kinase C and its substrates. *Mol Cell Endocrinol* 116, 1-29 (1996)
354. Liu W. S. & C. A. Heckman: The sevenfold way of PKC regulation. *Cell Signal* 10, 529-542 (1998)
355. Fernandez B., M. P. Czech, & H. Meisner: Role of protein kinase C in signal attenuation following T cell receptor engagement. *J Biol Chem* 274, 20244-20250 (1999)
356. Liu Y. C., C. Elly, H. Yoshida, N. Bonnefoy-Berard, & A. Altman: Activation-modulated association of 14-3-3 proteins with Cbl in T cells. *J Biol Chem* 271, 14591-14595 (1996)
357. Pedraza-Alva G., S. Sawasdikosol, Y. C. Liu, L. B. Merida, M. E. Cruz-Munoz, F. Oceguera-Yanez, S. J. Burakoff, & Y. Rosenstein: Regulation of Cbl molecular interactions by the co-receptor molecule CD43 in human T cells. *J Biol Chem* 276, 729-737 (2001)
358. Liu Y. C., Y. Liu, C. Elly, H. Yoshida, S. Lipkowitz, & A. Altman: Serine phosphorylation of Cbl induced by phorbol ester enhances its association with 14-3-3 proteins in T cells via a novel serine-rich 14- 3-3-binding motif. *J Biol Chem* 272, 9979-9985 (1997)
359. Staubs P. A., J. G. Nelson, D. R. Reichart, & J. M. Olefsky: Platelet-derived growth factor inhibits insulin stimulation of insulin receptor substrate-1-associated phosphatidylinositol 3-kinase in 3T3-L1 adipocytes without affecting glucose transport. *J Biol Chem* 273, 25139-25147 (1998)
360. Li J., K. DeFea, & R. A. Roth: Modulation of insulin receptor substrate-1 tyrosine phosphorylation by an Akt/phosphatidylinositol 3-kinase pathway. *J Biol Chem* 274, 9351-9356 (1999)

361. Sun X. J., P. Rothenberg, C. R. Kahn, J. M. Backer, E. Araki, P. A. Wilden, D. A. Cahill, B. J. Goldstein, & M. F. White: Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 352, 73-77 (1991)
362. Mothe I. & E. Van Obberghen: Phosphorylation of insulin receptor substrate-1 on multiple serine residues, 612, 632, 662, and 731, modulates insulin action. *J Biol Chem* 271, 11222-11227 (1996)
363. Ogihara T., B. C. Shin, M. Anai, H. Katagiri, K. Inukai, M. Funaki, Y. Fukushima, H. Ishihara, K. Takata, M. Kikuchi, Y. Yazaki, Y. Oka, & T. Asano: Insulin receptor substrate (IRS)-2 is dephosphorylated more rapidly than IRS-1 via its association with phosphatidylinositol 3-kinase in skeletal muscle cells. *J Biol Chem* 272, 12868-12873 (1997)
364. Lam K., C. L. Carpenter, N. B. Ruderman, J. C. Friel, & K. L. Kelly: The phosphatidylinositol 3-kinase serine kinase phosphorylates IRS-1. Stimulation by insulin and inhibition by Wortmannin. *J Biol Chem* 269, 20648-20652 (1994)
365. Tanti J. F., T. Gremeaux, E. Van Obberghen, & Y. Marchand-Brustel: Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J Biol Chem* 269, 6051-6057 (1994)
366. De Fea K. & R. A. Roth: Protein kinase C modulation of insulin receptor substrate-1 tyrosine phosphorylation requires serine 612. *Biochemistry* 36, 12939-12947 (1997)
367. De Fea K. & R. A. Roth: Modulation of insulin receptor substrate-1 tyrosine phosphorylation and function by mitogen-activated protein kinase. *J Biol Chem* 272, 31400-31406 (1997)
368. Sasaoka T. & M. Kobayashi: The functional significance of Shc in insulin signaling as a substrate of the insulin receptor. *Endocr J* 47, 373-381 (2000)
369. Migliaccio E., M. Giorgio, S. Mele, G. Pelicci, P. Reboldi, P. P. Pandolfi, L. Lanfrancone, & P. G. Pelicci: The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402, 309-313 (1999)
370. Okada S., A. W. Kao, B. P. Ceresa, P. Blaikie, B. Margolis, & J. E. Pessin: The 66-kDa Shc isoform is a negative regulator of the epidermal growth factor-stimulated mitogen-activated protein kinase pathway. *J Biol Chem* 272, 28042-28049 (1997)
371. Foschi M., F. Franchi, J. Han, G. La Villa, & A. Sorokin: Endothelin-1 induces serine phosphorylation of the adaptor protein p66Shc and its association with 14-3-3 protein in glomerular mesangial cells. *J Biol Chem* 276, 26640-26647 (2001)
372. Yang C. P. & S. B. Horwitz: Taxol mediates serine phosphorylation of the 66-kDa Shc isoform. *Cancer Res* 60, 5171-5178 (2000)
373. Reynolds A. B., S. B. Kanner, H. C. Wang, & J. T. Parsons: Stable association of activated pp60src with two tyrosine- phosphorylated cellular proteins. *Mol Cell Biol* 9, 3951-3958 (1989)
374. Qian Y., J. M. Baisden, H. G. Zot, W. B. Van Winkle, & D. C. Flynn: The carboxy terminus of AFAP-110 modulates direct interactions with actin filaments and regulates its ability to alter actin filament integrity and induce lamellipodia formation. *Exp Cell Res* 255, 102-113 (2000)
375. Qian Y., J. M. Baisden, E. H. Westin, A. C. Guappone, T. C. Koay, & D. C. Flynn: Src can regulate carboxy terminal interactions with AFAP-110, which influence self-association, cell localization and actin filament integrity. *Oncogene* 16, 2185-2195 (1998)
376. Kanner S. B., A. B. Reynolds, H. C. Wang, R. R. Vines, & J. T. Parsons: The SH2 and SH3 domains of pp60src direct stable association with tyrosine phosphorylated proteins p130 and p110. *EMBO J* 10, 1689-1698 (1991)
377. Flynn D. C., T. H. Leu, A. B. Reynolds, & J. T. Parsons: Identification and sequence analysis of cDNAs encoding a 110-kilodalton actin filament-associated pp60src substrate. *Mol Cell Biol* 13, 7892-7900 (1993)
378. Patel A. S., G. L. Schechter, W. J. Wasilenko, & K. D. Somers: Overexpression of EMS1/cortactin in NIH3T3 fibroblasts causes increased cell motility and invasion in vitro. *Oncogene* 16, 3227-3232 (1998)
379. Wu H. & J. T. Parsons: Cortactin, an 80/85-kilodalton pp60src substrate, is a filamentous actin-binding protein enriched in the cell cortex. *J Cell Biol* 120, 1417-1426 (1993)
380. Campbell D. H., R. L. Sutherland, & R. J. Daly: Signaling pathways and structural domains required for phosphorylation of EMS1/cortactin. *Cancer Res* 59, 5376-5385 (1999)
381. Skoulakis E. M. & R. L. Davis: 14-3-3 proteins in neuronal development and function. *Mol Neurobiol* 16, 269-284 (1998)
382. Morrison D.: 14-3-3: modulators of signaling proteins? [comment]. *Science* 266, 56-57 (1994)
383. Burbelo P. D. & A. Hall: 14-3-3 proteins. Hot numbers in signal transduction. *Curr Biol* 5, 95-96 (1995)
384. Baldin V.: 14-3-3 proteins and growth control. *Prog Cell Cycle Res* 4, 49-60 (2000)
385. Aitken A., D. Jones, Y. Soneji, & S. Howell: 14-3-3 proteins: biological function and domain structure. *Biochem Soc Trans* 23, 605-611 (1995)
386. Reuther G. W. & A. M. Pendergast: The roles of 14-3-3 proteins in signal transduction. *Vitam Horm* 52, 149-175 (1996)
387. Aitken A., D. B. Collinge, B. P. van Heusden, T. Isobe, P. H. Roseboom, G. Rosenfeld, & J. Soll: 14-3-3 proteins: a highly conserved, widespread family of eukaryotic proteins. *Trends Biochem Sci* 17, 498-501 (1992)
388. Jones D. H., S. Ley, & A. Aitken: Isoforms of 14-3-3 protein can form homo- and heterodimers in vivo and in vitro: implications for function as adapter proteins. *FEBS Lett* 368, 55-58 (1995)
389. Marais R. & C. Marshall: 14-3-3 proteins: structure resolved, functions less clear. *Structure* 3, 751-753 (1995)
390. Van Der Hoeven P. C., J. C. Van Der Wal, P. Ruurs, M. C. Van Dijk, & J. Van Blitterswijk: 14-3-3 isotypes facilitate coupling of protein kinase C-zeta to Raf-1: negative regulation by 14-3-3 phosphorylation. *Biochem J* 345 Pt 2, 297-306 (2000)
391. Hausser A., P. Storz, G. Link, H. Stoll, Y. C. Liu, A. Altman, K. Pfizenmaier, & F. J. Johannes: Protein kinase C

mu is negatively regulated by 14-3-3 signal transduction proteins. *J Biol Chem* 274, 9258-9264 (1999)
392. Aitken A., S. Howell, D. Jones, J. Madrazo, H. Martin, Y. Patel, & K. Robinson: Post-translationally modified 14-3-3 isoforms and inhibition of protein kinase C. *Mol Cell Biochem* 149-150, 41-49 (1995)
393. Tzivion G., Z. Luo, & J. Avruch: A dimeric 14-3-3 protein is an essential cofactor for Raf kinase activity. *Nature* 394, 88-92 (1998)
394. Freed E., M. Symons, S. G. Macdonald, F. McCormick, & R. Ruggieri: Binding of 14-3-3 proteins to the protein kinase Raf and effects on its activation. *Science* 265, 1713-1716 (1994)
395. Freed E., F. McCormick, & R. Ruggieri: Proteins of the 14-3-3 family associate with Raf and contribute to its activation. *Cold Spring Harb Symp Quant Biol* 59, 187-193 (1994)
396. Rommel C., G. Radziwill, J. Lovric, J. Noeldeke, T. Heinicke, D. Jones, A. Aitken, & K. Moelling: Activated Ras displaces 14-3-3 protein from the amino terminus of c-Raf- 1. *Oncogene* 12, 609-619 (1996)
397. Clark G. J., J. K. Drugan, K. L. Rossman, J. W. Carpenter, K. Rogers-Graham, H. Fu, C. J. Der, & S. L. Campbell: 14-3-3 zeta negatively regulates raf-1 activity by interactions with the Raf-1 cysteine-rich domain. *J Biol Chem* 272, 20990-20993 (1997)
398. Acs P., Z. Szallasi, M. G. Kazanietz, & P. M. Blumberg: Differential activation of PKC isozymes by 14-3-3 zeta protein. *Biochem Biophys Res Commun* 216, 103-109 (1995)
399. Robinson K., D. Jones, Y. Patel, H. Martin, J. Madrazo, S. Martin, S. Howell, M. Elmore, M. J. Finnen, & A. Aitken: Mechanism of inhibition of protein kinase C by 14-3-3 isoforms. 14-3-3 isoforms do not have phospholipase A2 activity. *Biochem J* 299 (Pt 3), 853-861 (1994)
400. Bax B. & H. Jhoti: Protein-protein interactions. Putting the pieces together. *Curr Biol* 5, 1119-1121 (1995)
401. Peng C. Y., P. R. Graves, R. S. Thoma, Z. Wu, A. S. Shaw, & H. Piwnica-Worms: Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science* 277, 1501-1505 (1997)
402. Kolch W.: Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J* 351 Pt 2, 289-305 (2000)
403. Zha J., H. Harada, E. Yang, J. Jockel, & S. J. Korsmeyer: Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 87, 619-628 (1996)
404. Kosaki A., K. Yamada, J. Suga, A. Otaka, & H. Kuzuya: 14-3-3beta protein associates with insulin receptor substrate 1 and decreases insulin-stimulated phosphatidylinositol 3'-kinase activity in 3T3L1 adipocytes. *J Biol Chem* 273, 940-944 (1998)
405. Dubois T., S. Howell, B. Amess, P. Kerai, M. Learmonth, J. Madrazo, M. Chaudhri, K. Rittinger, M. Scarabel, Y. Soneji, & A. Aitken: Structure and sites of phosphorylation of 14-3-3 protein: role in coordinating signal transduction pathways. *J Protein Chem* 16, 513-522 (1997)
406. Dubois T., C. Rommel, S. Howell, U. Steinhussen, Y. Soneji, N. Morrice, K. Moelling, & A. Aitken: 14-3-3 is phosphorylated by casein kinase I on residue 233. Phosphorylation at this site in vivo regulates Raf/14-3-3 interaction. *J Biol Chem* 272, 28882-28888 (1997)
407. Megidish T., J. Cooper, L. Zhang, H. Fu, & S. Hakomori: A novel sphingosine-dependent protein kinase (SDK1) specifically phosphorylates certain isoforms of 14-3-3 protein. *J Biol Chem* 273, 21834-21845 (1998)
408. Bouton A. H., R. B. Riggins, & P. J. Bruce-Staskal: Functions of the adapter protein Cas: signal convergence and the determination of cellular responses. *Oncogene* 20, 6448-6458 (2001)
409. Polte T. R. & S. K. Hanks: Interaction between focal adhesion kinase and Crk-associated tyrosine kinase substrate p130Cas. *Proc Natl Acad Sci U S A* 92, 10678-10682 (1995)
410. Polte T. R. & S. K. Hanks: Complexes of focal adhesion kinase (FAK) and Crk-associated substrate (p130(Cas)) are elevated in cytoskeleton-associated fractions following adhesion and Src transformation. Requirements for Src kinase activity and FAK proline-rich motifs. *J Biol Chem* 272, 5501-5509 (1997)
411. Arai A., Y. Nosaka, H. Kohsaka, N. Miyasaka, & O. Miura: CrkL activates integrin-mediated hematopoietic cell adhesion through the guanine nucleotide exchange factor C3G. *Blood* 93, 3713-3722 (1999)
412. Honda H., H. Oda, T. Nakamoto, Z. Honda, R. Sakai, T. Suzuki, T. Saito, K. Nakamura, K. Nakao, T. Ishikawa, M. Katsuki, Y. Yazaki, & H. Hirai: Cardiovascular anomaly, impaired actin bundling and resistance to Src-induced transformation in mice lacking p130Cas. *Nat Genet* 19, 361-365 (1998)
413. Ma A., A. Richardson, E. M. Schaefer, & J. T. Parsons: Serine phosphorylation of focal adhesion kinase in interphase and mitosis: a possible role in modulating binding to p130(Cas). *Mol Biol Cell* 12, 1-12 (2001)
414. Chen H., V. I. Slepnev, P. P. Di Fiore, & P. De Camilli: The interaction of epsin and Eps15 with the clathrin adaptor AP-2 is inhibited by mitotic phosphorylation and enhanced by stimulation- dependent dephosphorylation in nerve terminals. *J Biol Chem* 274, 3257-3260 (1999)
415. Kariya K., S. Koyama, S. Nakashima, T. Oshiro, K. Morinaka, & A. Kikuchi: Regulation of complex formation of POB1/epsin/adaptor protein complex 2 by mitotic phosphorylation. *J Biol Chem* 275, 18399-18406 (2000)
416. Sakakibara A. & S. Hattori: Chat, a Cas/HEF1-associated adaptor protein that integrates multiple signaling pathways. *J Biol Chem* 275, 6404-6410 (2000)
417. Voltz J. W., E. J. Weinman, & S. Shenolikar: Expanding the role of NHERF, a PDZ-domain containing protein adapter, to growth regulation. *Oncogene* 6309-6314 (2001)
418. Weinman E. J., D. Steplock, Y. Wang, & S. Shenolikar: Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na(+)-H+ exchanger. *J Clin Invest* 95, 2143-2149 (1995)
419. Hall R. A., R. F. Spurney, R. T. Premont, N. Rahman, J. T. Blitzer, J. A. Pitcher, & R. J. Lefkowitz: G protein-coupled receptor kinase 6A phosphorylates the Na(+)/H(+) exchanger regulatory factor via a PDZ domain-mediated interaction. *J Biol Chem* 274, 24328-24334 (1999)
420. Shenolikar S., C. M. Minkoff, D. A. Steplock, C. Evangelista, M. Liu, & E. J. Weinman: N-terminal PDZ domain is required for NHERF dimerization. *FEBS Lett* 489, 233-236 (2001)

421. Lehmann J. M., G. Riethmuller, & J. P. Johnson: Nck, a melanoma cDNA encoding a cytoplasmic protein consisting of the src homology units SH2 and SH3. *Nucleic Acids Res* 18, 1048-(1990)
422. Han D. C., T. Shen, & J. Guan: The Grb7 family proteins: structure, interactions with other signaling molecules and potential cellular functions. *Oncogene* 20, 6315-6321 (20 A.D.)
423. Dong L. Q., S. Porter, D. Hu, & F. Liu: Inhibition of hGrb10 binding to the insulin receptor by functional domain-mediated oligomerization. *J Biol Chem* 273, 17720-17725 (1998)
424. Ooi J., V. Yajnik, D. Immanuel, M. Gordon, J. J. Moskow, A. M. Buchberg, & B. Margolis: The cloning of Grb10 reveals a new family of SH2 domain proteins. *Oncogene* 10, 1621-1630 (1995)
425. Daly R. J., G. M. Sanderson, P. W. Janes, & R. L. Sutherland: Cloning and characterization of GRB14, a novel member of the GRB7 gene family. *J Biol Chem* 271, 12502-12510 (1996)
426. Fiddes R. J., D. H. Campbell, P. W. Janes, S. P. Sivertsen, H. Sasaki, C. Wallasch, & R. J. Daly: Analysis of Grb7 recruitment by heregulin-activated erbB receptors reveals a novel target selectivity for erbB3. *J Biol Chem* 273, 7717-7724 (1998)
427. Larbolette O., B. Wollscheid, J. Schweikert, P. J. Nielsen, & J. Wienands: SH3P7 is a cytoskeleton adapter protein and is coupled to signal transduction from lymphocyte antigen receptors. *Mol Cell Biol* 19, 1539-1546 (1999)
428. Kessels M. M., A. E. Engqvist-Goldstein, D. G. Drubin, & B. Qualmann: Mammalian Abp1, a signal-responsive F-actin-binding protein, links the actin cytoskeleton to endocytosis via the GTPase dynamin. *J Cell Biol* 153, 351-366 (2001)
429. Kessels M. M., A. E. Engqvist-Goldstein, & D. G. Drubin: Association of mouse actin-binding protein 1 (mAbp1/SH3P7), an Src kinase target, with dynamic regions of the cortical actin cytoskeleton in response to Rac1 activation. *Mol Biol Cell* 11, 393-412 (2000)
430. Yokouchi M., T. Wakioka, H. Sakamoto, H. Yasukawa, S. Ohtsuka, A. Sasaki, M. Ohtsubo, M. Valius, A. Inoue, S. Komiya, & A. Yoshimura: APS, an adaptor protein containing PH and SH2 domains, is associated with the PDGF receptor and c-Cbl and inhibits PDGF-induced mitogenesis. *Oncogene* 18, 759-767 (1999)
431. Wakioka T., A. Sasaki, K. Mitsui, M. Yokouchi, A. Inoue, S. Komiya, & A. Yoshimura: APS, an adaptor protein containing Pleckstrin homology (PH) and Src homology-2 (SH2) domains inhibits the JAK-STAT pathway in collaboration with c-Cbl. *Leukemia* 13, 760-767 (1999)
432. Moodie S. A., J. Alleman-Sposeto, & T. A. Gustafson: Identification of the APS protein as a novel insulin receptor substrate. *J Biol Chem* 274, 11186-11193 (1999)
433. Mitchell P. J., E. A. Sara, & M. R. Crompton: A novel adaptor-like protein which is a substrate for the non-receptor tyrosine kinase, BRK. *Oncogene* 19, 4273-4282 (2000)
434. Watanabe S., H. Take, K. Takeda, Z. X. Yu, N. Iwata, & S. Kajigaya: Characterization of the CIN85 adaptor protein and identification of components involved in CIN85 complexes. *Biochem Biophys Res Commun* 278, 167-174 (2000)
435. Kirsch K. H., M. M. Georgescu, T. Shishido, W. Y. Langdon, R. B. Birge, & H. Hanafusa: The adapter type protein CMS/CD2AP binds to the proto-oncogenic protein c-Cbl through a tyrosine phosphorylation-regulated Src homology 3 domain interaction. *J Biol Chem* 276, 4957-4963 (2001)
436. Songyang Z., S. E. Shoelson, M. Chaudhuri, G. Gish, T. Pawson, W. G. Haser, F. King, T. Roberts, S. Ratnofsky, R. J. Lechleider, & .: SH2 domains recognize specific phosphopeptide sequences. *Cell* 72, 767-778 (1993)
437. Bausenwein B. S., M. Schmidt, B. Mielke, & T. Raabe: In vivo functional analysis of the daughter of sevenless protein in receptor tyrosine kinase signaling. *Mech Dev* 90, 205-215 (2000)
438. Holgado-Madruga M., D. R. Emlet, D. K. Moscatello, A. K. Godwin, & A. J. Wong: A Grb2-associated docking protein in EGF- and insulin-receptor signalling. *Nature* 379, 560-564 (1996)
439. Ingham R. J., M. Holgado-Madruga, C. Siu, A. J. Wong, & M. R. Gold: The Gab1 protein is a docking site for multiple proteins involved in signaling by the B cell antigen receptor. *J Biol Chem* 273, 30630-30637 (1998)
440. Myers M. G., Jr., Y. Zhang, G. A. Aldaz, T. Grammer, E. M. Glasheen, L. Yenush, L. M. Wang, X. J. Sun, J. Blenis, J. H. Pierce, & M. F. White: YMXM motifs and signaling by an insulin receptor substrate 1 molecule without tyrosine phosphorylation sites. *Mol Cell Biol* 16, 4147-4155 (1996)
441. White M. F. & L. Yenush: The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Curr Top Microbiol Immunol* 228, 179-208 (1998)
442. Lu Y., J. Brush, & T. A. Stewart: NSP1 defines a novel family of adaptor proteins linking integrin and tyrosine kinase receptors to the c-Jun N-terminal kinase/stress- activated protein kinase signaling pathway. *J Biol Chem* 274, 10047-10052 (1999)
443. Iseki M., S. Takaki, & K. Takatsu: Molecular cloning of the mouse APS as a member of the Lnk family adaptor proteins. *Biochem Biophys Res Commun* 272, 45-54 (2000)
444. Herrington J., M. Diakonova, L. Rui, D. R. Gunter, & C. Carter-Su: SH2-B is required for growth hormone-induced actin reorganization. *J Biol Chem* 275, 13126-13133 (2000)
445. Ahmed Z. & T. S. Pillay: Functional effects of APS and SH2-B on insulin receptor signalling. *Biochem Soc Trans* 29, 529-534 (2001)
446. Shlapatska L. M., S. V. Mikhalap, A. G. Berdova, O. M. Zelensky, T. J. Yun, K. E. Nichols, E. A. Clark, & S. P. Sidorenko: CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. *J Immunol* 166, 5480-5487 (2001)
447. Schuster V. & H. W. Kreth: X-linked lymphoproliferative disease is caused by deficiency of a novel SH2 domain-containing signal transduction adaptor protein. *Immunol Rev* 178, 21-28 (2000)
448. Morra M., O. Silander, S. Calpe, M. Choi, H. Oettgen, L. Myers, A. Etzioni, R. Buckley, & C. Terhorst: Alterations of the X-linked lymphoproliferative disease

gene SH2D1A in common variable immunodeficiency syndrome. *Blood* 98, 1321-1325 (2001)
449. Harrison S. C.: Peptide-surface association: the case of PDZ and PTB domains. *Cell* 86, 341-343 (1996)
450. Ochi F., T. Matozaki, T. Noguchi, Y. Fujioka, T. Yamao, T. Takada, M. Tsuda, H. Takeda, K. Fukunaga, Y. Okabayashi, & M. Kasuga: Epidermal growth factor stimulates the tyrosine phosphorylation of SHPS- 1 and association of SHPS-1 with SHP-2, a SH2 domain-containing protein tyrosine phosphatase. *Biochem Biophys Res Commun* 239, 483-487 (1997)
451. Guappone A. C., T. Weimer, & D. C. Flynn: Formation of a stable src-AFAP-110 complex through either an amino- terminal or a carboxy-terminal SH2-binding motif. *Mol Carcinog* 22, 110-119 (1998)
452. Baisden J. M., Y. Qian, H. G. Zot, & D. C. Flynn: The actin filament-associated protein AFAP-110 is an adaptor protein that modulates changes in actin filament integrity. *Oncogene* 20, 6435-6447 (2001)
453. Sakai R., A. Iwamatsu, N. Hirano, S. Ogawa, T. Tanaka, H. Mano, Y. Yazaki, & H. Hirai: A novel signaling molecule, p130, forms stable complexes in vivo with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner. *EMBO J* 13, 3748-3756 (1994)
454. Derry J. J., S. Richard, C. H. Valderrama, X. Ye, V. Vasioukhin, A. W. Cochrane, T. Chen, & A. L. Tyner: Sik (BRK) phosphorylates Sam68 in the nucleus and negatively regulates its RNA binding ability. *Mol Cell Biol* 20, 6114-6126 (2000)
455. Sanchez-Margalet V. & S. Najib: p68 Sam is a substrate of the insulin receptor and associates with the SH2 domains of p85 PI3K. *FEBS Lett* 455, 307-310 (1999)
456. Jevremovic D., D. D. Billadeau, R. A. Schoon, C. J. Dick, & P. J. Leibson: Regulation of NK cell-mediated cytotoxicity by the adaptor protein 3BP2. *J Immunol* 166, 7219-7228 (2001)
457. Marshall A. J., H. Niiro, C. G. Lerner, T. J. Yun, S. Thomas, C. M. Disteche, & E. A. Clark: A novel B lymphocyte-associated adaptor protein, Bam32, regulates antigen receptor signaling downstream of phosphatidylinositol 3-kinase. *J Exp Med* 191, 1319-1332 (2000)
458. Fu C., C. W. Turck, T. Kurosaki, & A. C. Chan: BLNK: a central linker protein in B cell activation. *Immunity* 9, 93-103 (1998)
459. Hashimoto S., A. Iwamatsu, M. Ishiai, K. Okawa, T. Yamadori, M. Matsushita, Y. Baba, T. Kishimoto, T. Kurosaki, & S. Tsukada: Identification of the SH2 domain binding protein of Bruton's tyrosine kinase as BLNK--functional significance of Btk-SH2 domain in B-cell antigen receptor-coupled calcium signaling. *Blood* 94, 2357-2364 (1999)
460. Tzeng S. R., M. T. Pai, F. D. Lung, C. W. Wu, P. P. Roller, B. Lei, C. J. Wei, S. C. Tu, S. H. Chen, W. J. Soong, & J. W. Cheng: Stability and peptide binding specificity of Btk SH2 domain: molecular basis for X-linked agammaglobulinemia. *Protein Sci* 9, 2377-2385 (2000)
461. Ohya K., S. Kajigaya, A. Kitanaka, K. Yoshida, A. Miyazato, Y. Yamashita, T. Yamanaka, U. Ikeda, K. Shimada, K. Ozawa, & H. Mano: Molecular cloning of a docking protein, BRDG1, that acts downstream of the Tec tyrosine kinase. *Proc Natl Acad Sci U S A* 96, 11976-11981 (1999)
462. Fusaki N., S. Tomita, Y. Wu, N. Okamoto, R. Goitsuka, D. Kitamura, & N. Hozumi: BLNK is associated with the CD72/SHP-1/Grb2 complex in the WEHI231 cell line after membrane IgM cross-linking. *Eur J Immunol* 30, 1326-1330 (2000)
463. Adachi T., H. Flaswinkel, H. Yakura, M. Reth, & T. Tsubata: The B cell surface protein CD72 recruits the tyrosine phosphatase SHP-1 upon tyrosine phosphorylation. *J Immunol* 160, 4662-4665 (1998)
464. Yu J., C. Riou, D. Davidson, R. Minhas, J. D. Robson, M. Julius, R. Arnold, F. Kiefer, & A. Veillette: Synergistic regulation of immunoreceptor signaling by SLP-76-related adaptor Clnk and serine/threonine protein kinase HPK-1. *Mol Cell Biol* 21, 6102-6112 (2001)
465. Nelms K., A. L. Snow, J. Hu-Li, & W. E. Paul: FRIP, a hematopoietic cell-specific rasGAP-interacting protein phosphorylated in response to cytokine stimulation. *Immunity* 9, 13-24 (1998)
466. Li Y., X. He, J. Schembri-King, S. Jakes, & J. Hayashi: Cloning and characterization of human Lnk, an adaptor protein with pleckstrin homology and Src homology 2 domains that can inhibit T cell activation. *J Immunol* 164, 5199-5206 (2000)
467. Huang X., Y. Li, K. Tanaka, K. G. Moore, & J. I. Hayashi: Cloning and characterization of Lnk, a signal transduction protein that links T-cell receptor activation signal to phospholipase C gamma 1, Grb2, and phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* 92, 11618-11622 (1995)
468. Takaki S., J. D. Watts, K. A. Forbush, N. T. Nguyen, J. Hayashi, J. Alberola-Ila, R. Aebersold, & R. M. Perlmutter: Characterization of Lnk. An adaptor protein expressed in lymphocytes. *J Biol Chem* 272, 14562-14570 (1997)
469. Sayos J., C. Wu, M. Morra, N. Wang, X. Zhang, D. Allen, S. van Schaik, L. Notarangelo, R. Geha, M. G. Roncarolo, H. Oettgen, J. E. De Vries, G. Aversa, & C. Terhorst: The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 395, 462-469 (1998)
470. Welsh M., Z. Songyang, J. D. Frantz, T. Trub, K. A. Reedquist, T. Karlsson, M. Miyazaki, L. C. Cantley, H. Band, & S. E. Shoelson: Stimulation through the T cell receptor leads to interactions between SHB and several signaling proteins. *Oncogene* 16, 891-901 (1998)
471. Karlsson T., Z. Songyang, E. Landgren, C. Lavergne, P. P. Di Fiore, M. Anafi, T. Pawson, L. C. Cantley, L. Claesson-Welsh, & M. Welsh: Molecular interactions of the Src homology 2 domain protein Shb with phosphotyrosine residues, tyrosine kinase receptors and Src homology 3 domain proteins. *Oncogene* 10, 1475-1483 (1995)
472. Hooshmand-Rad R., L. Lu, C. H. Heldin, L. Claesson-Welsh, & M. Welsh: Platelet-derived growth factor-mediated signaling through the Shb adaptor protein: effects on cytoskeletal organization. *Exp Cell Res* 257, 245-254 (2000)
473. Cocks B. G., C. C. Chang, J. M. Carballido, H. Yssel, J. E. De Vries, & G. Aversa: A novel receptor involved in T-cell activation. *Nature* 376, 260-263 (1995)

474. Spurkland A., J. E. Brinchmann, G. Markussen, F. Pedeutour, E. Munthe, T. Lea, F. Vartdal, & H. C. Aasheim: Molecular cloning of a T cell-specific adapter protein (TSAd) containing an Src homology (SH) 2 domain and putative SH3 and phosphotyrosine binding sites. *J Biol Chem* 273, 4539-4546 (1998)
475. Choi Y. B., C. K. Kim, & Y. Yun: Lad, an adapter protein interacting with the SH2 domain of p56lck, is required for T cell activation. *J Immunol* 163, 5242-5249 (1999)

Abbreviations: BLNK – B cell linker protein, Cpb/PAG – Csk-binding protein/phosphoprotein associated with GEMs, Crk/CrkL – Chicken tumor virus no. 10 regulator of kinase/Crk-like, Nck – Non-catalytic region of tyrosine kinase,Cbl – Casitas B lineage lymphoma protein,SLP-76 – SH2-domain-containing leukocyte protein of 76 kDa,LAT – Linker for activated T cells,SIT – SHP2-interacting transmembrane adaptor protein,SHP1 and 2 – SH2 domain-containing tyrosine phosphatases 1 and 2,SHIP – SH2 domain-containing inositol phosphatase,Gab – Grb2-associated binder,TRIM – T cell receptor interacting molecule,Dok – Downstream of kinases,Cas – Cellular apoptosis susceptibility,CLNK – Cytokine-dependent hematopoietic cell linker,Gads – Grb2-related adapter downstream of Shc,Grap – Grb2-related adaptor protein,IRS1 – Insulin receptor substrate 1,Grb2 – Growth factor receptor-bound substrate 2,Lnk – Linker of TCR pathways,Sap – SLAM-associated protein,Shb – Src homology 2 domain-containing transforming protein B,Shc – SH2 domain-containing transforming protein 1,SKAP55 – Src kinase-associated protein of 55 kDa,SLAP – Src-like adaptor protein,SLAP130/Fyb – SLP-76-associated phosphoprotein of 130 kDa/Fyn-binding protein,SLAM – Signaling lymphocytic activation molecule,Sos – son of sevenless,SOCS/JAB/CIS – Suppressors of cytokine signaling/Janus kinase binding protein/cytokine-inducible SH2 protein,STAT – Signal transducers and activators of transcription,JAK – Janus kinase,NSP – Novel SH2-containing protein 1,APS – Adaptor molecule containing PH and SH2 domains,SHPS1/2 – SHP2 substrate 1,PECAM1 – Platelet/endothelial cell adhesion molecule-1,Dos – Daughter of sevenless,FRS2/SNT1 – FGF receptor substrate 1/suc1-associated neurotrophic factor target protein 1,CMS/CD2AP – p130Cas ligand with multiple SH3 domains/CD2-associated protien,SH2D1A/SAP/DSHP – SH2 domain protein 1A/SLAM-associated protein/Duncan's disease SH2-protein,Abp1/HIP55/SH3P7 – Actin binding protein 1/HPK1-interacting protein of 55 kDa,BRDG1 – BCR downstream signaling 1,AFAP-110 – Actin filament associated protein of 110 kDa,SAM68 – Src-associated in mitosis of 68 kDa,RACKs – Receptor for activated C kinase

Key Words: Adaptor Protein, Phosphorylation, Signal Transduction, Review

Send correspondence to: Dr Daniel C. Flynn, The Mary Babb Randolph Cancer Center and the Department of Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV 26506-9300, Tel: 304-293-6966, Fax:304-293-7584, E-mail: dflynn@hsc.wvu.edu