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The Human and Mouse Complement of SH2 Domain Proteins—Establishing the Boundaries of Phosphotyrosine Signaling

Resource

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Summary

SH2 domains are interaction modules uniquely dedicated to the recognition of phosphotyrosine sites and are embedded in proteins that couple protein-tyrosine kinases to intracellular signaling pathways. Here, we report a comprehensive bioinformatics, structural, and functional view of the human and mouse complement of SH2 domain proteins. This information delimits the set of SH2-containing effectors available for PTK signaling and will facilitate the systems-level analysis of pTyr-dependent protein-protein interactions and PTK-mediated signal transduction. The domain-based architecture of SH2-containing proteins is of more general relevance for understanding the large family of protein interaction domains and the modular organization of the majority of human proteins.

Introduction

Selective protein-protein interactions are important in organizing the regulatory processes of eukaryotic cells and are commonly mediated by modular protein domains, for which the prototype is the Src homology 2 (SH2) domain, a conserved sequence that controls signaling by protein tyrosine kinases (PTK). SH2 domains typically bind specific phosphotyrosine (pTyr)-containing motifs (Figure 1) and thereby couple activated PTKs to intracellular pathways that regulate many aspects of cellular communication in metazoans (Chervitz et al., 1998; Hunter, 2000; Pawson and Nash, 2000).

The intimate relationship between tyrosine kinases and SH2 domains is supported by their coordinate emergence during eukaryotic evolution. Unicellular fungi such as yeast lack conventional PTKs and SH2 domains. However, pTyr binding SH2 domains are features of some amoebozoa. Notably, the social amoeba *Dictyostelium discoidum* contains 12 proteins with SH2 domains, including polypeptides similar to STAT transcription factors and Cbl E3 protein-ubiquitin ligases, as well as dual specificity protein kinases with an N-terminal SH2 domain (SHKs) and proteins with unusual architectures in which the SH2 domain is linked to leucine rich repeats or to an F box and ankyrin repeats (Eichinger et al., 2005). Such amoebae lack conventional PTKs but have a large set of tyrosine kinase-like (TKL-group) kinases, with the potential for tyrosine phosphorylation (Goldberg et al., 2006; Moniakis et al., 2001). Choanoflagellates are protazoa that may represent immediate predecessors of multicellular animals; one such organism, *Monosiga breicollis*, encodes not only SH2 domains but also bona fide PTKs and has the earliest identified example of an SH2 domain covalently linked to a tyrosine kinase domain (King et al., 2003). These observations suggest that the acquisition of pTyr-SH2 domain signaling facilitated metazoan evolution (Katzmann et al., 2004; Kawata et al., 1997).

SH2 domains represent the largest class of known pTyr-recognition domains (Pawson et al., 2001). In addition to SH2 domains, the PTB domains of docking proteins within the IRS, SHC, and DOK subclasses also bind specific pTyr-containing motifs on activated receptor tyrosine kinases (RTKs) and are consequently phosphorylated at sites that recruit SH2 domains (Yaffe, 2002). However, only about one quarter of the 79 human PTB domains have apparently acquired the capacity for pTyr-dependent recognition, while the majority of PTB domains recognize nonphosphorylated peptide ligands, or phosphoinositides (Schlessinger and Lemmon, 2003; Uhlik et al., 2005). In addition, the C2 domain of PKCδ binds a transmembrane Src-associated glycoprotein, CDCP1/SIMA135, in a pTyr-dependent manner, though this is likely an idiosyncratic property of this particular C2 family member (Benes et al., 2005). SH2 domains therefore appear to be uniquely dedicated to pTyr recognition and thus represent primary targeting and specificity elements in tyrosine kinase signaling.

To fully appreciate the signaling events initiated by tyrosine kinases, it is important to clarify the set of genes that encode SH2 domains in different species. Here, we describe the SH2 domains specified in the genomes of both human and mouse, combined with an analysis of the SH2 domain families and a catalog of current SH2 domain structures, human diseases, and mouse mutants. This reveals a number of hitherto unstudied SH2 domain proteins, as well as gaps in our overall understanding of SH2 domain structure and biological function. It sets a benchmark for a more global approach to studying pTyr-nucleated signaling events. Interactive figures and additional information may be found at http://sh2.uchicago.edu/.

Results and Discussion

The Human Complement of SH2 Domains

We combined several approaches to identify the complete nonredundant set of recognizable human SH2 domains and their corresponding proteins (see the Supplemental Data available with this article online). After removal of duplicates, splice variants, and pseudogenes, this analysis afforded a total of 120 SH2 domains contained in 110 distinct proteins, of which 10 have dual SH2 domains. These 110 human proteins are outlined in

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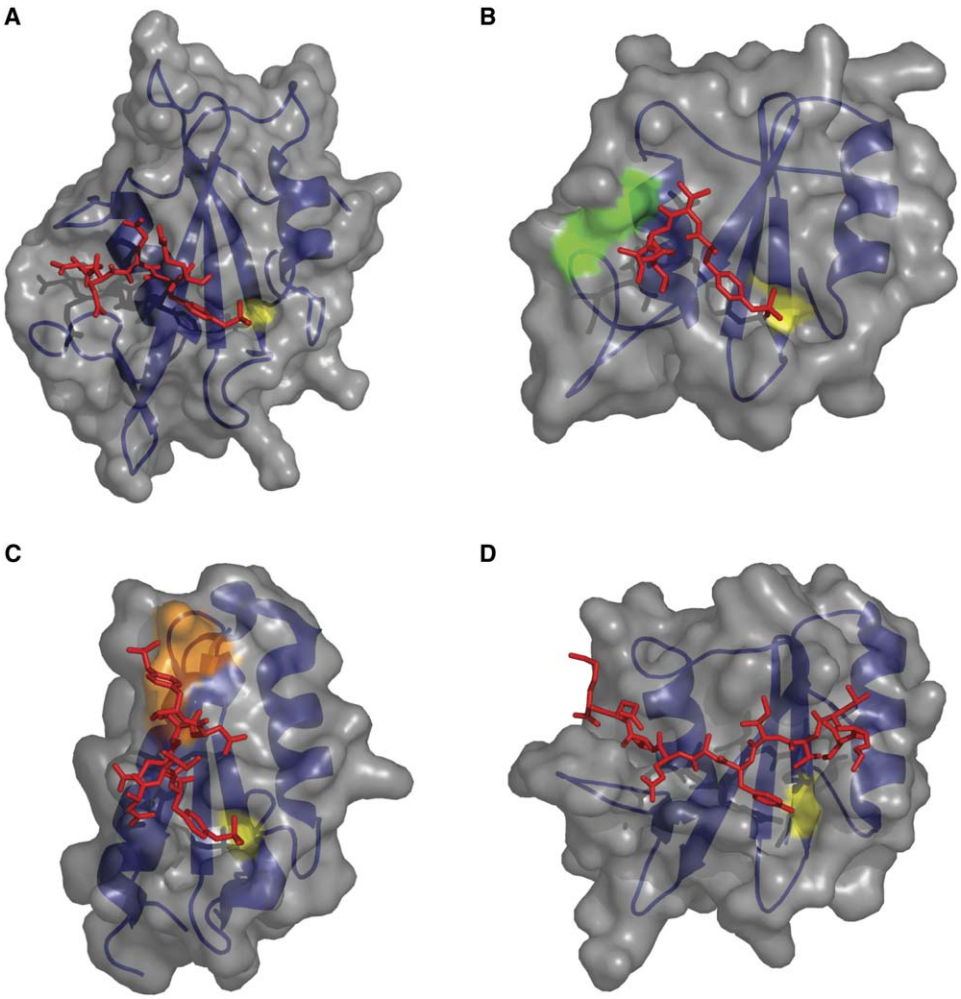

Figure 1. Modes of SH2 Binding

(A) Structure of the Src SH2 domain bound to a pTyr-Glu-Glu-Ile peptide (PDB, 1SPS) (Waksman et al., 1993). The surface of the SH2 domain is shown as a translucent gray skin and the secondary structural elements of the SH2 domain in blue. The αA helix is to the right and αB helix to the left. The Arg βB5 residue critical for pTyr binding is in yellow. The N-terminal pTyr of the peptide (red) occupies the pTyr binding pocket. The peptide runs over the central β sheet of the SH2 domain, the +1 and +2 glutamates contact the surface of the domain, and the side chain of the +3 Ile (to the left) fits in a hydrophobic pocket.

(B) Grb2 SH2 domain in complex with pYVNV (red) (PBD, 1BMB). Trp (green) stabilizes the β turn conformation essential for high affinity binding.

(C) Two phosphotyrosine binding pockets are present on a single SH2 domain of APS. A single APS SH2 molecule is bound to pYETDpY (red) peptide of the activation loop of INSR. The pTyr-1158 interacts with the Arg-438 (yellow), while Lys-455 and Lys-457 (orange) create a second binding pocket for pTyr-1162 (PDB, 1RQQ).

(D) SH2D1A/SAP in complex with a nonphosphorylated SLAM peptide KSLTIYAQVQK (red) (PBD, 1D4T). Ribbon and surface diagrams were generated using Pymol.

Table S1 along with the chromosomal location of each corresponding gene, a list of aliases or alternate symbols, and information on their mouse homologs. This is somewhat more than previous numbers; an early survey of the human genome identified 87 SH2 domain-containing proteins (Venter et al., 2001), and a more recent study identified 108 SH2 domains in 98 proteins (Jones et al., 2006). The present work clarifies previous curation efforts that generated similar total numbers but suffered from issues of redundancy and incomplete annotation (Huang et al., 2004; Lander et al., 2001).

Mouse homologs were independently determined and the closest relatives established by BLAST, supported by information from UniGene (Wheeler et al., 2005) and manual examination of genetic loci, revealing a list of 120 SH2 domains in 110 proteins. The gene encoding SH2D1C (or ERT) is a functional gene in mouse, though it appears to be a pseudogene in humans. Murine SH2D1C is a signal regulator related to SH2D1B/EAT2 and SH2D1A/SAP, though its expression appears highly restricted to natural killer (NK) cells, in which it acts as a negative regulator of antigen signaling (Roncagalli et al., 2005). The human gene encoding SH2D3A appears to be absent in the rodent lineage, though it is present in primates. Human diseases associated with genetic lesions identified as corresponding to SH2 domain-containing proteins were assembled from OMIM (Hamosh et al., 2005) and from the literature (Table 1). A survey of targeted gene disruptions in mouse models was obtained from the literature (Table 2). These data

Table 1. Human Diseases Associated with Mutations in SH2-Encoding Genes

Name	Summary of Mutations	Phenotype
ABL1	t(9;22) translocation results in fusion with the BCR gene	Chronic myelogeneous leukemia (CML), acute lymphoblastic leukemia (ALL), myelogenous leukemia (AML) (de Klein et al., 1982; Heisterkamp et al., 1983)
ABL2	t(1;12)(q25;p13) translocation with ETV6/TEL	Acute myeloid leukemia (Cazzaniga et al., 1999)
BLNK	Base pair substitution of a splice donor site results in reduction or loss of BLNK transcripts	Human immundeficiency (Minegishi et al., 1999)
	Complete loss or drastic reduction of expression due to incorporation of alternative exons	Pre-B-cell acute lymphoblastic leukemia (ALL) (Jumaa et al., 2003)
BTK	Missense mutations, deletions, or splice site mutations	X-linked agammaglobulinemia (XLA) (Rawlings et al., 1993)
CBL	Translocation with MLL	Acute myeloid leukemia (Fu et al., 2003)
	t(4;11)(q21;q23)	Acute leukemia
	t(11;14)(q23;q32)	B cell lymphoma
	t(11;22)(q23;q12)	Ewing sarcoma (Savage et al., 1991)
CRK	Deletions of 17p13.3	Isolated lissencephaly sequence (ILS) to Miller-Dieker syndrome (MDS) (Cardoso et al., 2003)
ITK	t(5;9)(q33;q22) translocation to SYK	Peripheral T cell lymphoma (Streubel et al., 2005)
JAK2	t(9;12)(p24;p13) fusion with TEL, results in a constitutive kinase	Acute lymphoblastic leukemia (Lacronique et al., 1997)
	t(9;15;12)(p24;q15;p13) fusion with TEL and ETV6	Chronic myelogenous leukemia (Peeters et al., 1997)
	Dominant gain-of-function mutation V617F	Polycythemia vera and myeloproliferative disorders (James et al., 2005; Kralovics et al., 2005)
JAK3	Nucleotide insertion, substitution, or deletion resulting in a frame shift or premature termination	SCID, lymphopenia (Russell et al., 1995)
LCK	Translocation of the t(1;7)(p34;q34) that fuses LCK and TCRB	T cell acute lymphoblastic leukemia (T cell ALL) (Burnett et al., 1991)
	Decrease expression of p56(lck), likely due to alternative splicing of exon 7	SCID (Goldman et al., 1998)
PTPN11	Missense mutations altering amino acids D61 at the N-SH2 domain, resulting in gain of function (Tartaglia et al., 2001)	Noonan syndrome (Tartaglia et al., 2001)
	Missense mutation in exons 7, 12, and 13	Multiple lentigines (ML)/Leopard syndrome (LS) (Digilio et al., 2002)
	95% of mutations are in exon 3 or a defect in exon 13 affecting the protein tyrosine phosphatase domain resulting in a gain of function	Juvenile myelomonocytic leukemia (JMML) (Tartaglia et al., 2003)
RASA1	Nonsense mutations within the SH2 domain	Basal cell carcinoma (Friedman et al., 1993)
	A 2 bp deletion results in a frameshift and a premature stop codon; a missense mutation in the PH domain	Capillary malformation-arteriovenous malformation (Eerola et al., 2003)
SH2D1A	Mutations or deletions cause the protein to not fold and function correctly	X-linked lymphoproliferative syndrome (XLP) (Sayos et al., 1998)
SH3BP2	Point mutations in exon 9 affect three amino acids within the six amino acid sequence (RSPPDG)	Cherubism (Ueki et al., 2001)
SHIP2	Mutation or deletions, SNPs	Type 2 diabetes, hypertension (Marion et al., 2002)
SRC	Truncating mutation in the SRC codon 531	Advanced colon cancer (Irby et al., 1999)
STAT1	Nucleotide substitutions, homozygous deletions generating a premature stop codon	Susceptibility to mycobacterial and viral disease (Dupuis et al., 2001)
STAT5B	Homozygous A630P mutation	Growth hormone insensitivity with immunodeficiency (Kofoed et al., 2003)
	t(15;17)(q11.2; q21.1) translocation with RARA	Acute promyeloyctic leukemia (APL) (Arnould et al., 1999)
SYK	t(9;12)(q22;p12) translocation with TEL	Myelodysplastic syndrome (MDS) (Kuno et al., 2001)
	t(5;9)(q33;q22) translocation with ITK	Peripheral T cell lymphoma (Streubel et al., 2005)
TYK2	SNPs resulting in amino acid substitutions in the JAK homology (JH) regions	Systemic lupus erythematosus (SLE) (Sigurdsson et al., 2005)
ZAP70	A single base substitution mutation in a splice acceptor site	SCID (T cell defect) (Chan et al., 1994; Elder et al., 1994)

provide a census of SH2 domain-containing proteins encoded in the human genome.

The SH2-ome

SH2 domains are incorporated into proteins with a range of biochemical properties. We have classified SH2-containing proteins into 11 functional categories, based on their modular domain composition (Figure 2). Taken together, this information delimits the physiological SH2-containing targets available for pTyr signaling in human cells. Since the functional output from any given PTK likely depends on the subset of SH2 signaling proteins it recruits, either directly or through phosphorylated scaffold proteins, these data define the predominant pathways through which PTKs modify cellular behavior.

The groups of SH2 proteins indicate that pTyr-dependent interactions channel PTK signals into defined, if diverse, areas of cellular regulation. These include tyrosine phosphorylation itself (through cytoplasmic PTKs and tyrosine phosphatases), the control of phospholipid metabolism (by phosphatidylinositol 3′-kinase, PLCγ and inositol phosphatases), the regulation of small

Table 2. Mouse Knockouts of SH2 Domain Proteins

Knockout	Phenotype	Organs and Cell Types Affected	Source
Abl1	Death 1–2 weeks postbirth	Thymus, spleen	(Schwartzberg et al., 1991; Tybulewicz et al., 1991)
Abl2	Multiple behavioral abnormalities	Brain	(Koleske et al., 1998)
Aps	Viable and fertile; hypoinsulinemia	B1 lymphocytes, adipocytes	(Iseki et al., 2004; Minami et al., 2003)
Bks	Normal	Hepatocytes	(Minoguchi et al., 2003)
Blk	Normal	None	(Texido et al., 2000)
Blnk	High incidence of pre-B cell lymphoma	B lymphocytes	(Flemming et al., 2003; Jumaa et al., 2003)
Bmx	Normal	Endocardium and arteries, endothelial cells	(Rajantie et al., 2001)
Btk	XID phenotype	B lymphocytes	(Kerner et al., 1995; Khan et al., 1995)
Cbl	Thymic phenotype	Thymus, T lymphocytes	(Naramura et al., 1998)
Cblb	Autoimmune encephalomyelitis	T lymphocytes	(Chiang et al., 2000)
Cblc	Normal	None	(Griffiths et al., 2003)
Crk	Normal	None	(Imaizumi et al., 1999)
Crkl	Partial embryonic lethality	Thymus	(Guris et al., 2001; Peterson et al., 2003)
Csk	Embryonic lethality at E10–E12	Neural tube	(Imamoto and Soriano, 1993; Nada et al., 1993)
Dapp1	Normal	B lymphocytes	(Fournier et al., 2003; Han et al., 2003)
Fer	Healthy and fertile (kinase-inactivating mutation)	None	(Craig et al., 2001)
Fes	Viable; abnormal innate immunity	Bone marrow, myeloid cells	(Hackenmiller et al., 2000)
Fgr	Defective lung inflammation	Tissue and airway eosinophilia	(Lowell et al., 1994)
Frk	Normal	None	(Chandrasekharan et al., 2002)
Fyn	Suckling abnormality	Olfactory bulb, hippocampus	(Yagi et al., 1993)
Gads	Healthy	T lymphocytes	(Yoder et al., 2001)
Grap	Viable, fertile, and healthy	Lymphocytes	(Shen et al., 2002)
Grb2	Embryonic lethality	Endoderm	(Cheng et al., 1998)
Grb10	30% larger in size than wild-type	Brain, liver	(Charalambous et al., 2003)
Grb14	Glucose tolerance improvement	Liver, skeletal muscle	(Cooney et al., 2004)
Hck	Normal	Macrophages	(Lowell et al., 1994)
Itk	Normal	T lymphocytes	(Liao and Littman, 1995)
Jak1	Perinatally lethal, nursing defect	Neurons, fibroblasts, T and B lymphocytes	(Rodig et al., 1998)
Jak2	Embryonic lethality	Hematopoeitic cells	(Neubauer et al., 1998; Parganas et al., 1998)
Jak3	Immunodeficient; lymphopenia	Thymus, spleen, lymph nodes	(Nosaka et al., 1995; Thomis et al., 1995)
Lck	Thymic atrophy	Fetal thymus	(Molina et al., 1992)
Lnk	Splenomegaly, defects in hematopoietic lineages	Hematopoietic progenitor cells, spleen	(Takaki et al., 2000; Velazquez et al., 2002)
Lyn	Hyperglobulinemic, glomerulonephritis	B lymphocytes, mast cells	(Hibbs et al., 1995; Nishizumi et al., 1995)
Matk	Normal	None	(Hamaguchi et al., 1996)
Mist	Normal	None	(Utting et al., 2004)
Nck1	Normal	None	(Bladt et al., 2003)
Nck2	Normal	None	(Bladt et al., 2003)
Pik3r1	X-linked immunodeficiency; hypoglycemia	B lymphocytes	(Fruman et al., 1999; Suzuki et al., 1999)
Pik3r2	Hypoglycemia	Muscle	(Ueki et al., 2002)
Plcγ1	Embryonic lethality between E8.5 and E9.0	N/A	(Ji et al., 1997)
Plcγ2	Viable, B cell deficiency	B lymphocytes	(Wang et al., 2000)
Ptpn6	Immunity and hematopoiesis defects	T lymphocytes	(Shultz et al., 1993; Van Zant and Shultz, 1989)
Ptpn11	Die peri-implantation	Trophoblast stem cell survival, node, notochord	(Arrandale et al., 1996; Saxton et al., 1997; Yang et al., 2006)
Rasa1	Embryonic lethality by E10.5	Vascular system, endothelial cells	(Henkemeyer et al., 1995)
Rin1	Normal	Amygdala	(Dhaka et al., 2003)
Sh2b	Growth retardation, fertility defect, insulinemia	Testis, ovaries, skeletal muscle, liver and fat	(Duan et al., 2004; Ohtsuka et al., 2002)
Sh2d1a	Recapitulates X-linked lymphoproliferative disease	T and (B) lymphocytes	(Crotty et al., 2003; Czar et al., 2001; Wu et al., 2001)
Sh2d2a	Susceptible to Lupus-like autoimmune disease	T lymphocytes	(Rajagopal et al., 1999)
Shc1	Embryonic lethality by E11.5	Heart	(Lai and Pawson, 2000)
Shc2	Normal	Sensory neurons	(Sakai et al., 2000)
Shc3	Normal	None	(Sakai et al., 2000)
Ship1	Fail to thrive with splenomegaly	Spleen, lymphoid, erthryoid, myeloid cells	(Helgason et al., 1998)
Ship2	Resistant to dietary obesity and growth affects	Skeletal muscle, liver	(Elchebly et al., 1999; Sleeman et al., 2005)

Table 2. *Continued*

Knockout	Phenotype	Organs and Cell Types Affected	Source
Slap	Fertile and healthy	T lymphocytes	(Lowell et al., 1994; Sosinowski et al., 2001)
Slp76	Impaired viability; defective thymocyte development	T lymphocytes	(Pivniouk et al., 1998)
Socs1	Fatty degeneration of the liver	Liver, thymocytes	(Starr et al., 1998)
Socs2	Gigantism	Most visceral organs	(Metcalf et al., 2000)
Socs3	Embryonic lethality between E11 and E13	Placental, blood vessel	(Marine et al., 1999; Metcalf et al., 2000; Roberts et al., 2001)
Socs5	Normal	None	(Brender et al., 2004)
Socs6	Normal but weigh 10% less than wild-type	N/A	(Krebs et al., 2002)
Socs7	Healthy and fertile but die from hydrocephalus	Brain	(Krebs et al., 2004)
Src	Defective bone remodeling and osteoporosis	Bone osteoclast	(Soriano et al., 1991)
Srms	Normal	None	(Kohmura et al., 1994)
Stat1	Normal but sensitive to infection	T lymphocytes, macrophages	(Meraz et al., 1996)
Stat2	Exhibit multiple defects in immune response	Fibroblasts	(Park et al., 2000)
Stat3	Embryonic lethality between E6.5 and E7.5	Visceral endoderm, T lymphocytes	(Takeda et al., 1997)
Stat4	Normal	T lymphocytes	(Kaplan et al., 1996)
Stat5a	Normal	Mammary glands	(Liu et al., 1997)
Stat5b	Decrease growth in males	Liver, mammary glands, adipose tissue	(Teglund et al., 1998)
Stat6	Normal	T and B lymphocytes	(Shimoda et al., 1996)
Syk	Perinatal lethality and blocked B cell development	B lymphocytes	(Cheng et al., 1995; Turner et al., 1995)
Tec	Normal	B lymphocytes	(Ellmeier et al., 2000)
Tns1	Normal	Kidney, muscle myoblast	(Lo et al., 1997)
Txk	Death by 102 days	Splenocytes	(Schaeffer et al., 1999)
Tyk2	Normal	T lymphocytes	(Karaghiosoff et al., 2000; Shimoda et al., 2000)
Vav1	Normal	T lymphocytes	(Zhang et al., 1994)
Vav2	Normal	None	(Tedford et al., 2001)
Vav3	Normal	None	(Fujikawa et al., 2003)
Yes	Normal	None	(Stein et al., 1994)
Zap70	SCID	T lymphocytes	(Negishi et al., 1995)

Family Knockouts

Gene	Phenotype	Organs or Cells Affected	Reference
Abl1/2	Neurulation defects	Neural tube	(Koleske et al., 1998)
Aps/Sh2b	Body weight increase, obesity, hyperglycemia	Adipocytes	(Li et al., 2006)
Btk/Tec	Defective lymphocyte development	B lymphocytes	(Ellmeier et al., 2000)
Cbl/Cblb	Embryonic lethality at E10	T lymphocytes	(Naramura et al., 2002)
Fgr/Hck	Immunodeficiency	Macrophages	(Lowell et al., 1994)
Fgr/Src	Src defects	Osteoclasts	(Lowell et al., 1996)
Hck/Src	Two thirds die at birth; osteopetrosis	Hematopoietic cells	(Lowell et al., 1996)
Itk/Txk	Immunological defects	T lymphocytes	(Schaeffer et al., 1999)
Lck/Fyn	T cell development defect	T lymphocytes, dendritic cells	(van Oers et al., 1996)
Nck1/2	Embryonic lethality at E9.5	Notochord	(Bladt et al., 2003)
Shc2/3	Normal but defective neural development	Superior cervical ganglia (SCG) neurons	(Sakai et al., 2000)
Stat5a/b	Infertile female mice	Ovaries, mammary glands	(Teglund et al., 1998)
Syk/Zap70	T cell development defect	Thymocytes	(Cheng et al., 1997)
Vav1/2/3	Defective lymphocyte development	T and B lymphocytes	(Fujikawa et al., 2003)

Targeted gene disruptions have been completed for a large subset of SH2 proteins, yielding a wide range of phenotypes. In some cases, compensatory mechanisms have required the disruption of multiple members of a family in order to expose a phenotype.

GTPases (including Ras, Rho, Rap, and Rab family members) by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), gene expression directed by STAT transcription factors, ubiquitylation mediated by Cbl E3 protein-ubiquitin ligases, and cytoskeletal organization by tensin proteins. A group of SH2/SH3 proteins act as adaptors (i.e., Grb2, Crk, and Nck), each of which appear to target a set of SH3 binding proteins with related functions (Pawson et al., 2004). Nck recruits cytoskeletal regulators such as N-Wasp and Pak serine/threonine kinases (Buday et al., 2002; Rivera et al., 2004); Grb2 binds Sos and Gab1 (Gu and Neel, 2003; Lock et al., 2000; Rozakis-Adcock et al., 1993; Takenawa et al., 1998), involved in MAPK/PI3K signalling; and Crk targets GEFs for Rap and Rac GTPases that control adhesion (Feller, 2001).

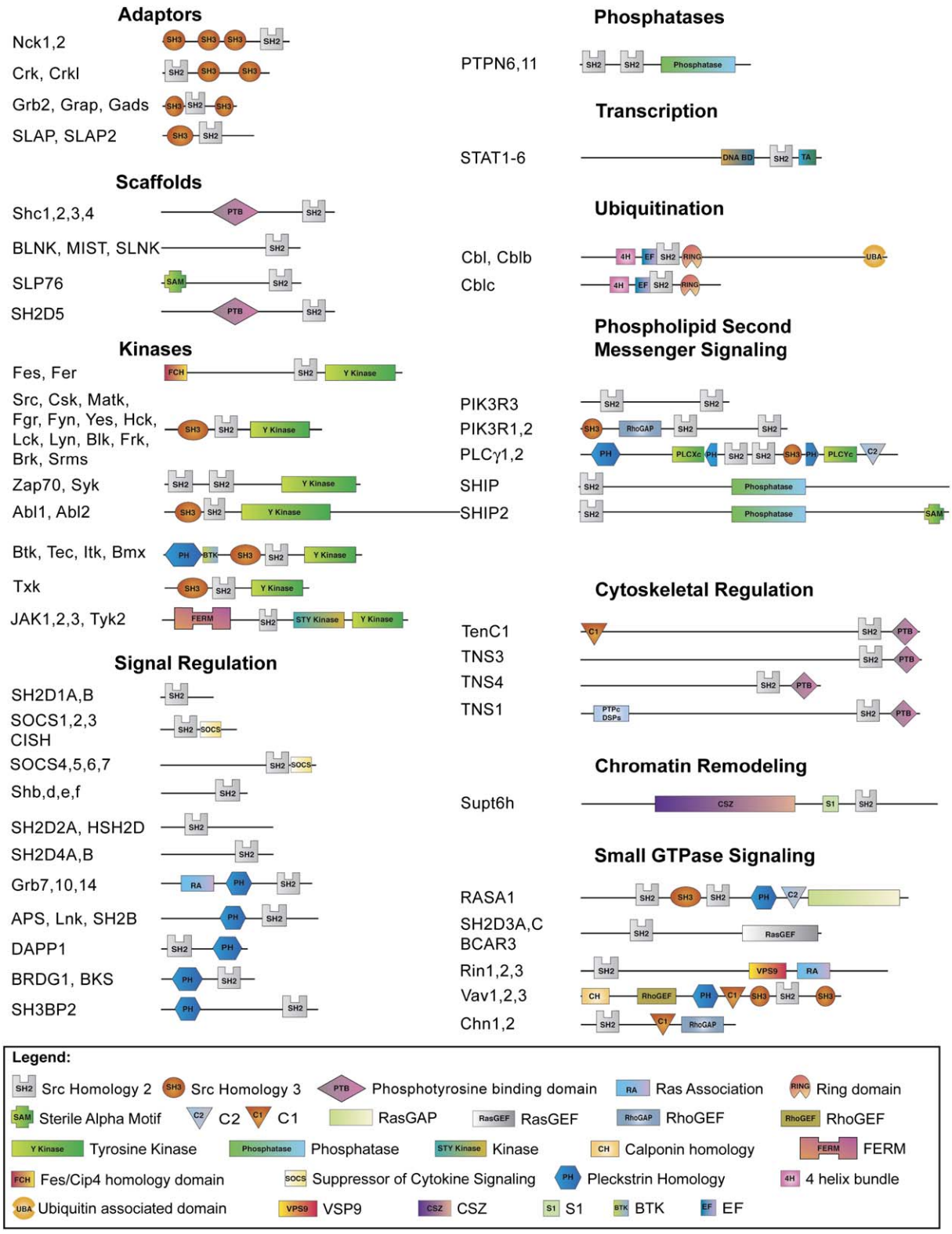

Figure 2. Modular Domain Organization of SH2-Containing Proteins

Classification and domain composition of the 110 nonredundant human SH2 domain-containing proteins identified in human and mouse by Pfam (Bateman et al., 2004) and SMART (http://smart.embl-heidelberg.de/). More information on SH2-containing proteins can be found at http://sh2.uchicago.edu/. More information on the individual domains portrayed can be found at http://www.mshri.on.ca/pawson/domains.html and http://smart.embl-heidelberg.de/.

In addition, a significant number of SH2 proteins regulate the duration and location of PTK signaling. For example, SOCS proteins are transcriptionally induced by JAK-STAT signals and provide an inhibitory feedback both by blocking JAK tyrosine kinase activity and by promoting ubiquitylation (Ilangumaran et al., 2004). Cbl proteins induce pTyr-dependent receptor multiubiquitylation and thus create binding sites for endocytic proteins with ubiquitin interaction motifs, involved in receptor trafficking (Haglund and Dikic, 2005). SH2 proteins can also directly interact with active sites of RTKs, exemplified by the SH2 domain of the APS protein, which simultaneously homodimerizes and binds the phosphorylated activation loop of the insulin receptor (INSR), potentially stabilizing the active state of the receptor (Hu et al. 2003). Conversely, the SH2 domain of the related Grb14 protein is proposed to bind the phosphorylated INSR activation loop but has a 45 residue sequence (BPS region) located between the SH2 and PH domains that antagonizes receptor activity by acting as a pseudosubstrate (Depetris et al., 2005). Thus, SH2 proteins, in addition to acting as "on" or "off" regulators of intracellular biochemical pathways, can modify the kinetics, activity, and substrate specificity of tyrosine kinase signals.

Given the large repertoire of distinct interaction and catalytic domains in the human proteome, it is evident that SH2 domains are associated with a focused subset of modules, most notably SH3, PTB, PH, kinase, phosphatase, GEF, and GAP domains (Figure 2). This may reflect the requirement that SH2 proteins initially act near the plasma membrane to engage specific signaling networks that promote cell growth, differentiation, morphology, and metabolism, although proteins such as STAT transcription factors subsequently move to other subcellular compartments. By virtue of their modular design, SH2 domains can be artificially joined to domains from which they are normally segregated, to create aberrant signaling pathways (Howard et al., 2003). Presumably, such nonphysiological combinations were selected against in the course of evolution to insulate PTKs from inducing counterproductive intracellular responses.

In this analysis, we have not considered shorter peptide motifs, which can serve as ligands for interaction domains and thereby extend the binding properties of SH2 domain proteins.

Multiple Alignments and the SH2 Tree

Both Pfam and SMART HMMs identify SH2 sequences that are somewhat smaller than the full folded domain, revealed by corresponding structural information. The C-terminal region of the SH2 structural domain is often excluded from the sequence identified by bioinformatic means, and this can eliminate important elements, notably β strand G. Although this C-terminal region of the SH2 domain is less well conserved than either the N-terminal or central core sequences, it is important for the formation of the ligand binding pocket and for the stable folding of the intact domain. Thus, we extended the protein sequence from the Pfam- and SMART-identified SH2 domains by some 20 amino acids on both the N and C termini to obtain a sequence group for multiple sequence alignment. The 120 SH2 domain sequences were aligned with ClustalW (1.8), and this alignment was manually refined based on structural information (Figure S1). From the raw ClustalW data, we generated an unrooted neighbor-joining tree represented as a dendrogram (Figure 3). This tree was then color coded such that the branch colors represent the functional classes of the SH2-domain-containing proteins identified in Figure 2. Those SH2 domains for which one or more structures exist are highlighted in red.

Uncharacterized SH2 Domain Proteins

A small number of SH2 domains have not previously been described or have been only briefly mentioned in the literature. LOC284948 is a member of the family of SH2 scaffold proteins that includes SLP-76, BLNK, and MIST/CLNK. We propose naming the LOC284948 gene product SLNK for SH2 linker protein related to BLNK, consistent with the nomenclature currently utilized in this family. SH2D5 (LOC400745) is an uncharacterized protein that has similar domain architecture to the Shc family of scaffolding proteins, containing a PTB domain followed by an SH2 domain. Despite this architectural similarity, SH2D5 bears minimal sequence resemblance to the Shc family. The Shc family of adaptor proteins does have a bona fide fourth family member that has been reported as Rai-like protein (RaLP) but is of unknown function. We propose terming this protein Shc4 (or ShcD), consistent with the current nomenclature in this family.

Related to HSH2D and SH2D2A are two additional SH2 proteins, which the HUGO-HGNC has named SH2D4A and SH2D4B. Each member of this family of four proteins contains a single SH2 domain in the absence of other recognizable modules, and we have classified them as signal regulatory proteins, though little is known of their cellular roles. SH2D4A and SH2D4B, while highly similar to one another, are distinct from HSH2D and SH2D2A in both the sequences of their SH2 domains, as well as in the position of the SH2 domain within the primary sequence, and may therefore need to be considered as a distinct subclass.

Supt6h is an archaic protein that is conserved from yeast to man (Chiang et al., 1996). It contains the most primitive example of an SH2-like sequence and the only such sequence in yeast. Unlike the majority of other SH2 proteins, Supt6h has not expanded in number in more complex organisms, and it contains a variant SH2 domain, which in our hands does not bind to pTyr-containing peptides (B.A.L. and P.D.N., unpublished data). Nonetheless, the evolutionary conservation of the Supt6h SH2 domain argues that it serves a functional role.

SH2 Domain Proteins in Development and Disease

SH2 domain proteins have been extensively studied for their roles in invertebrate and mammalian development, and a number have been identified as altered in hereditary or sporadic human diseases. The genes for 81 SH2-containing proteins have been disrupted in mice (Table 2). Several SH2 domain proteins are critical for early to midembryogenesis, including Grb2, RasGAP, Csk, Shp2, Shc1, PLC-γ1, Jak2, and Stat-3, while other proteins are first required later in development or in specific tissues postnatally. In this latter category are proteins with primary functions in more specialized cell types,

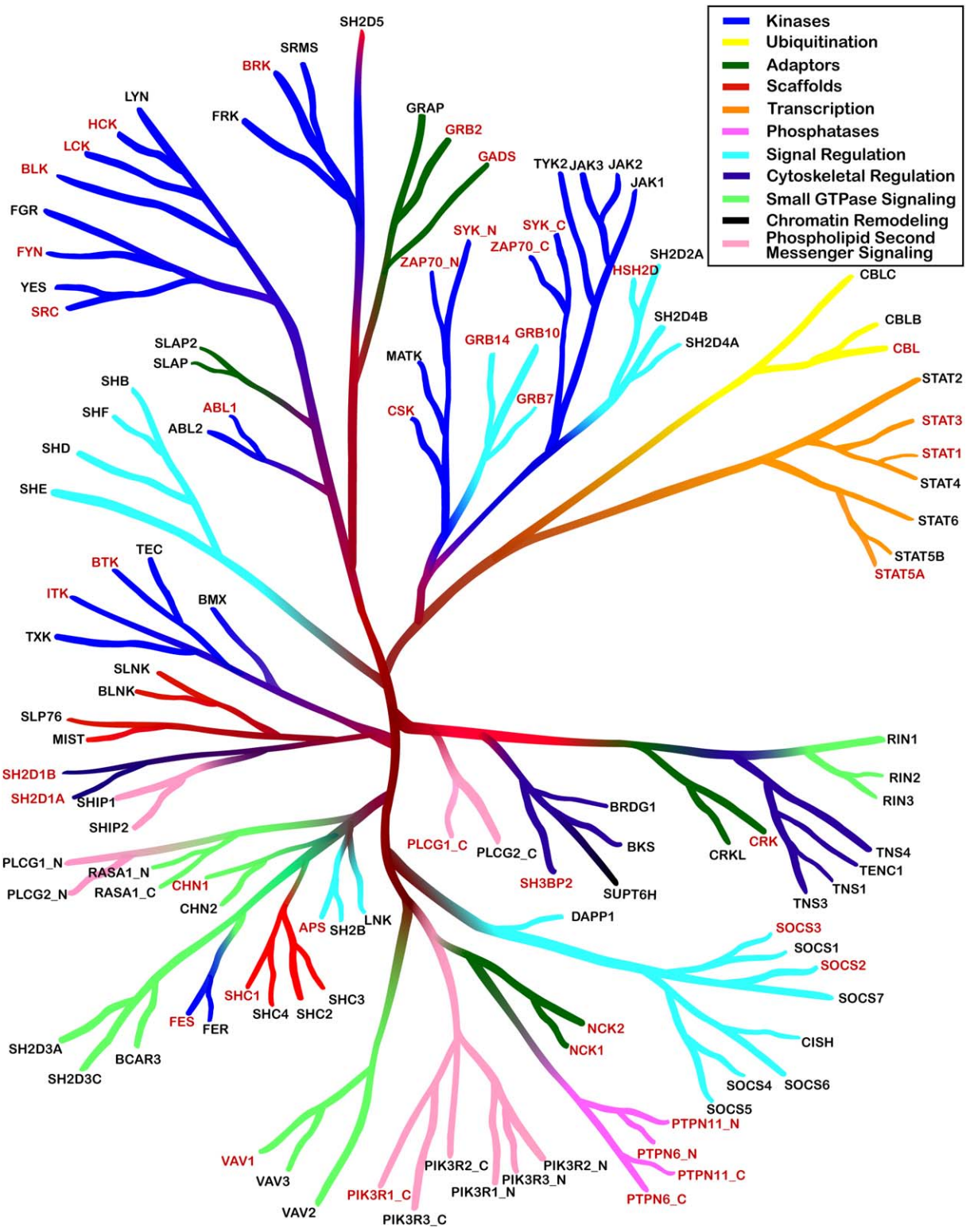

Figure 3. Dendrogram of the Human Complement of SH2 Domains

The dendrogram, modified for ease of viewing, shows the sequence similarity between the SH2 domains indicated in Table S1. The initial branching pattern was built from a neighbor-joining tree derived from a clustalW protein sequence alignment of the domains. This was modified by reference to other alignment and tree-building methods (hmmalign and parsimony trees) and by pairwise alignment of SH2 domains. The curved layout was created manually. The overall branching pattern is more informative than that produced by any single automated method, though many branch lengths are semiquantitative. Branches of the tree are colored according to the presumed function of the protein in which each SH2 domain is embedded according to the legend provided. The protein name is indicated in red if one or more structures exist as PDB files (indicated in Table S2). An interactive version of this dendrogram is available at http://sh2.uchicago.edu/.

such as those of the immune system. For example, Lck, ZAP-70, Slp-76, and Gads are interconnected proteins in the signaling network downstream of the T cell antigen receptor, and their corresponding genes are required for thymic development and functional signaling in thymocytes (Arpaia et al., 1994; Molina et al., 1992; Pivniouk et al., 1998; Yoder et al., 2001). Several SH2 proteins have overlapping functions with closely related family members; Table 2 indicates 14 cases in which combined inactivation of two or more family members reveals a more severe phenotype than observed in mice with individual gene mutations. For example, individual Vav1$^{-/-}$, Vav2$^{-/-}$, or Vav3$^{-/-}$ mice have minimal phenotypic abnormalities (Fujikawa et al., 2003; Stein et al., 1994; Tedford et al., 2001), while the Vav1/2/3 triple knockout mice have significant defects in lymphocytic cell development (Fujikawa et al., 2003).

With respect to disease, known mutations in the genes for 18 distinct SH2 domain proteins contribute to human disorders, including cancers and leukemias, developmental disorders, diabetes, and immunodeficiencies (Table 1). These can arise from either loss- or gain-of-function mutations in SH2 domains. In the former group, mutations that affect the peptide binding properties of SH2 proteins such as SH2D1A/SAP or Btk induce immunodeficiencies. An example of the latter class is provided by the tyrosine phosphatase Shp2, which has two N-terminal SH2 domains preceding the catalytic domain. Shp2 enzymatic activity is controlled through an intramolecular interaction between the N-terminal (N−) SH2 domain and the active site of the phosphatase domain, which inactivates both domains (Hof et al., 1998). The binding of tyrosine-phosphorylated peptides to the SH2 domains activates the catalytic domain and juxtaposes it to its targets, resulting in the stimulation of the ERK/MAP kinase pathway. In Noonan syndrome (NS), a developmental disorder characterized by congenital heart disease, skeletal defects, and cognitive impairment, this autoinhibitory regulation is disrupted by mutations in the gene for human Shp2 (PTPN11) (Tartaglia et al., 2001). The resulting substitutions cluster at the SH2 domain-phosphatase interface, causing a loss of autoinhibition without obvious adverse effect on catalytic or pTyr binding properties. Mutations that affect the same Shp2 residues but introduce less conservative amino acid substitutions are associated with myeloid leukemias (i.e., juvenile myelomonocytic leukemia), possibly because they elicit a more potent activation of Shp2 (Bentires-Alj et al., 2004).

SH2 domains, and SH2-mediated interactions, can also be critical functional components of chimeric human oncoproteins such as Bcr-Abl (Zhang et al., 2001) and contribute to the specific action of kinase inhibitors such as imatinib/Gleevec (Nagar et al., 2003). Mutation of SH2 domain binding sites can also contribute to the oncogenicity of human RTKs, as in the case of the Met RTK in human lung cancers, which is upregulated due to loss of the Cbl SH2 binding site and consequent decreased ubiquitination (Kong-Beltran et al., 2006).

Structural and Functional Diversity

The first structures of SH2 domains appeared in 1992 (Booker et al., 1992; Overduin et al., 1992; Waksman et al., 1992), and subsequent analysis has revealed both conserved and variable features of SH2 domain interactions. We located 169 structures covering 43 SH2 domains in 38 proteins (Supplemental Data), many of which were solved with peptide or synthetic ligands. In several cases, the structures encompass larger regions of the SH2-containing proteins or the SH2 domain ligand and thus provide molecular insight into the regulatory properties of SH2-mediated interactions. Table S2 summarizes the current state of SH2 structures, and SH2 domains for which structures exist are highlighted in the dendrogram (Figure 3), indicating a number of subfamilies of SH2 domains that are underrepresented in terms of structural understanding. The structural features of SH2 domains, and their interactions with both phosphopeptides and small molecules, have been extensively reviewed (Bradshaw and Waksman, 2002; Machida and Mayer, 2005), and we confine our comments to a few salient points.

SH2 domains contain approximately 100 amino acids, which usually form an N-terminal α helix (αA) and a central antiparallel β sheet (strands βA–βD), followed by a smaller β sheet (βD′, βE, βF), a second α helix (αB), and a C-terminal β strand (βG). This creates a bipartite structure in which the central β sheet separates a conserved pTyr binding pocket from a more variable binding surface that typically engages residues C-terminal to the pTyr. SH2 domains are therefore configured to bind a four to seven residue tyrosine-based peptide, dependent on phosphorylation of the tyrosine (which yields about half the free energy of binding) and the presence of C-terminal amino acids that can be accommodated by the specificity pocket. In a physiological context, these short phosphopeptide motifs are components of larger polypeptides, such as activated RTKs or phosphorylated scaffolds (Kuriyan and Cowburn, 1997).

The preferred selectivity of SH2 domains for peptide ligands has been explored by in vitro approaches such as the probing of degenerate phosphopeptide libraries (Songyang et al., 1994), as summarized in Table S3. These data yield position-specific scoring matrices that can be exploited to predict potential SH2 domain binding sites (Yaffe et al., 2001) (see Scansite at http://scansite.mit.edu) (Obenauer et al., 2003) and can be compared to experimentally identified SH2 recruitment sites (for example, see the Phospho.ELM database at http://phospho.elm.eu.org/) (Diella et al., 2004).

Although SH2 domains are generally considered to be relatively uniform in their ligand binding properties, recent data indicate that they show considerable versatility, in part because they can bind phosphopeptides in several different modes (Figure 1). Most commonly, (1) the phosphopeptide binds as an extended strand that crosses the central β sheet to present at least three C-terminal residues to the more variable specificity pocket. For example, the specificity pocket of the C-terminal SH2 domain of phospholipase C (PLC)-γ1 forms a hydrophobic cleft (Pascal et al., 1994), and PLC-γ1 SH2 domains therefore bind motifs in which the pTyr is followed by a run of hydrophobic residues (i.e., pYIILPDP in the β-PDGF receptor). Even in this simple mode, there are complexities, since the PLC-γ1 C-terminal SH2 domain also binds tightly (K_d = 70 nM) to a doubly phosphorylated peptide found in the activated Syk tyrosine kinase (pYESPpYAD). In this latter complex,

the PLC-γ1 SH2 domain undergoes a significant conformational change to create a secondary pTyr binding site in the specificity pocket (Groesch et al., 2006). (2) Some SH2 domains bind longer phosphopeptides in an extended conformation, through the recognition of both N- and C-terminal residues (relative to the pTyr), as in the case of the SH2D1A SH2 domain, which is unique in being able to bind a nonphosphorylated tyrosine-based peptide with relatively high affinity (Li et al., 1999; Poy et al., 1999). The SOCS3 SH2 domain has an extended N-terminal region that forms a hydrophobic pocket for Val at the peptide −2 position and thus binds tightly to a pTyr peptide from the IL-6 receptor (K_d = 150 nM) (Babon et al., 2006). In contrast, (3) pTyr-X-Asn peptides bound to the Grb2 SH2 domain traverse the β sheet but are forced into a β turn by a bulky Trp residue at position EF1 of the SH2 domain (Rahuel et al., 1996). The phosphorylated INSR activation loop, which engages the APS SH2 domain (4), is structurally constrained by virtue of its integration with the kinase domain. One of the exposed IRK pTyr residues (pTyr-1158) occupies the conventional pTyr binding pocket of the APS SH2 domain, but the activation loop then makes a sharp turn to run parallel to the β sheet and positions pTyr-1162 at the +4 position for interaction with two Lys residues in the βD strand (Hu et al., 2003).

SH2 domains can also mediate idiosyncratic protein-protein interactions through binding surfaces that are distinct from the conventional phosphopeptide recognition region (Figure 4). As a consequence, an individual SH2 domain can potentially bind multiple partners. Some SH2 domains, such as that of SH2D1A, have secondary binding surfaces that engage specific SH3 domains and can thereby act as self-contained adaptors that link tyrosine-phosphorylated ligands to SH3 domain proteins. In T cells, SH2D1A engages the SLAM receptor through the conventional ligand binding surface but recruits the SH3 domain of the Fyn tyrosine kinase through a distinct basic surface centered around Arg-78 of the SH2 domain. This stimulates Fyn kinase activity and targets it to phosphorylate tyrosine residues in the tail of the receptor, which consequently recruit SH2 proteins that constrain T cell activation (Latour et al., 2001, 2003). Similarly, the Crk SH2 domain can bind the Abl SH3 domain through a proline-rich loop (Figure 4B). In a further example of noncanonical interactions, the SH2 domains of Grb10, Grb14, APS, and likely Grb7 and SH2-B form noncovalent homodimers through a conserved dimer interface in the αB helix (Figure 4) (Depetris et al., 2005; Hu et al., 2003; Stein et al., 2003) while simultaneously engaging the phosphorylated activation loop of a RTK.

The covalent joining of interaction domains can potentially yield new specificities and affinities. Thus, the tandem SH2 domains of PI3K p85, ZAP-70, Syk, Shp-2, and phospholipase C-γ1 each bind with substantially enhanced affinity to specific bisphosphorylated tyrosine-based motifs, corresponding to their appropriate biological partners (Ottinger et al., 1998). In a related fashion, the Cbl SH2 domain is embedded in a larger structural module that also contains a four-helix bundle and an EF hand and engages residues both N and C terminal to the pTyr of a phosphopeptide ligand. Indeed, peptide residues in the −5 and −6 positions contact the four-helix bundle of the extended Cbl SH2 domain (Hu and Hubbard, 2005).

Phosphotyrosine-Independent SH2 Domain Interactions

As noted above, the Supt6h SH2 domain likely lacks pTyr binding activity. This may apply to a number of other SH2 domains, such as those predicted on the basis of sequence in JAK tyrosine kinases. One JAK family member, TYK2, has a His in place of the critical ArgβB5 that coordinates the phosphate group of pTyr. Although the related SH2 domains in human JAK1, JAK2, and JAK3 all have an Arg at βB5, they are divergent in the conserved N-terminal region of the SH2 encompassing βA, αA, βB, and βC, and substitution of ArgβB5 in the JAK1 SH2 domain does not alter JAK1 localization or function (Radtke et al., 2005). However, an intact SH2 is required for JAK1 to bind to cytokine receptors, suggesting that JAK SH2 domains may lack conventional pTyr binding properties but are nonetheless structurally important for receptor recognition. Indeed, modeling of JAK2 suggests that the SH2 domain may contact the N-terminal FERM domain (Giordanetto and Kroemer, 2002). Similarly, the SH2 domains of Rin2 (HisβB5) and SH2D5 (TrpβB5) also lack ArgβB5 and may also not bind pTyr ligands, though little is presently known about the function of the SH2 domain in either protein.

Some SH2 domains may also bind nonpeptide ligands. The SH2 domains of phosphatidylinositol (PI) 3′-kinase can bind PI(3,4,5)P_3, which interferes with recognition of pTyr-containing peptides and may thereby provide a feedback inhibition of SH2-phosphoprotein interactions (Rameh et al., 1995). Similarly, the Src SH2 domain can also bind PIP_3 (Rameh et al., 1995), as well as sulfogalactose (Lingwood et al., 2005), in addition to phosphopeptides.

These results are consistent with the notion that modules now prevalent in the human proteome, such as SH2 domains, may have been selected during the course of evolution for their ability to acquire new binding modes. Interestingly, the three-dimensional SH2 fold appears to be more ancient than the conventional SH2 domain itself. The *E. coli* biotin ligase, BirA, contains a structural region analogous to SH2 domains, though it shares no apparent sequence homology to known SH2 domains (Russell and Barton, 1993). The SH2-like region of BirA binds phosphate, likely in the form of ATP, and biotin on a binding face corresponding to that used by SH2 domains to bind phosphopeptides. While the lack of sequence similarity makes an evolutionary link uncertain, this does hint at the diverse functional applications of the SH2 fold.

Conclusions

Our analysis of SH2 domain proteins is complementary to existing proteome-wide information regarding protein-tyrosine kinases (Manning et al., 2002) and tyrosine phosphatases (Alonso et al., 2004; Andersen et al., 2004). These data sets provide an inventory of specific gene/protein families involved in generating and interpreting pTyr signals and are therefore useful for a systems-level understanding of signal transduction. A number of experimental and bioinformatics approaches have the potential to illuminate these issues. By understanding

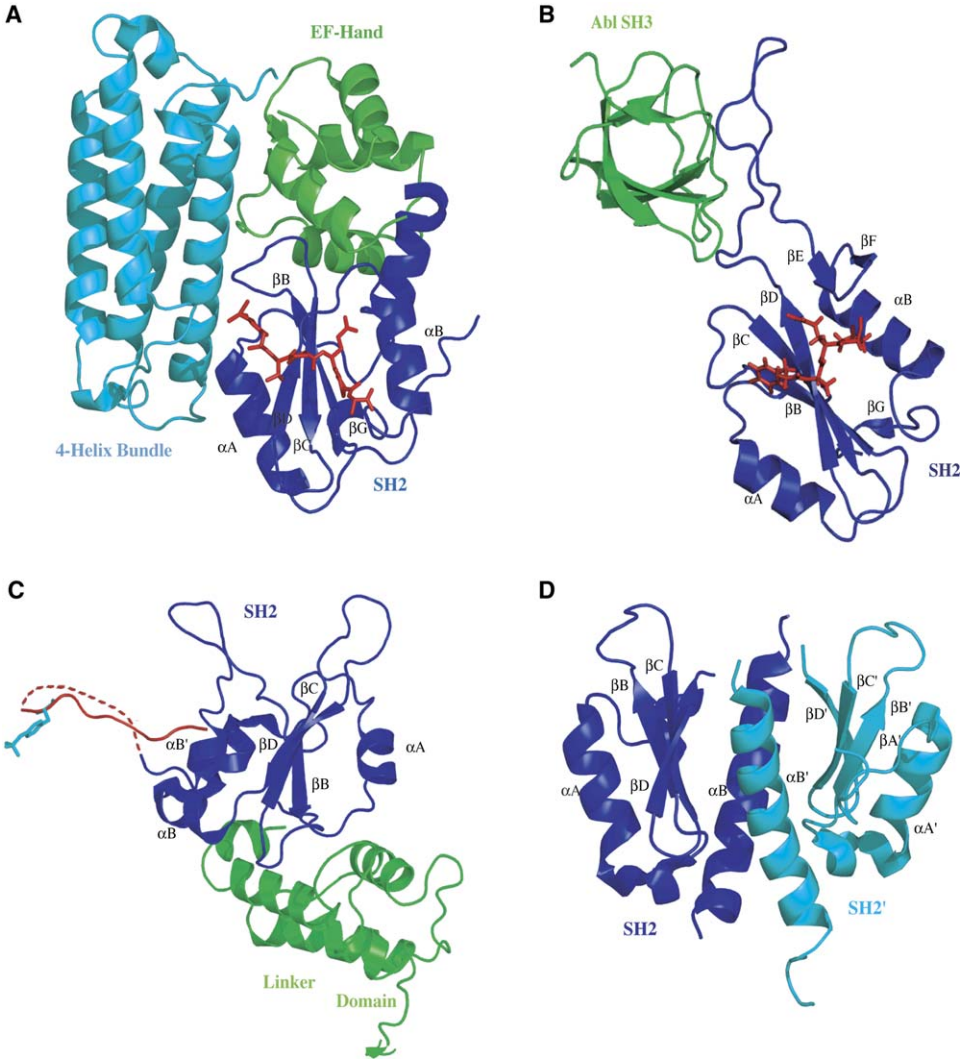

Figure 4. Variety and Complexity in SH2 Domain Structures

Structural ribbon diagrams of SH2 domains and associated peptide ligands or binding partners.

(A) Structure of the phosphotyrosine binding region of Cbl with the SH2 domain indicated in dark blue, the EF hand in green, and the four-helix bundle in cyan and a peptide corresponding to a portion of ZAP-70 (GpYTPEPA) represented as a ball and stick model painted orange modified from PDB 2CBL (Meng et al., 1999).

(B) The SH2 domain of the Crk adaptor protein in a ternary complex with a Crk pY221 phosphopeptide (pYAQPS) via the Crk SH2 phosphotyrosine binding pocket and the SH3 domain of Abl via an interaction between the proline-rich loop between βD and βE of the Crk SH2 and the Abl SH3 domain modified from PDB 1JU5 (Donaldson et al., 2002).

(C) The atypical structure of the SH2 domain of STAT-1 (blue) and the STAT-1 linker region (green). The tyrosine-phosphorylated (cyan) C-terminal segment (orange) promotes STAT-1 dimerization. Modified from PDB 1BF5 (Chen et al., 1998).

(D) The dimerization of APS SH2 domains through contacts in αB. Individual APS SH2 domains are indicated in cyan and blue. Modified from PDB 1RPY (Hu et al., 2003).

the substrate specificity of kinases and the phosphopeptide binding properties of SH2 domains, it is possible to predict potentially relevant signaling complexes (Johnson and Hunter, 2005; Joughin et al., 2005; Obenauer and Yaffe, 2004). In addition, mass spectrometry-based approaches allow a more comprehensive analysis of actual pTyr sites, as well as quantitative investigation of tyrosine phosphorylation events and SH2-mediated interactions (Blagoev et al., 2003, 2004). Similarly, chip-based techniques, in which extensive sets of domains or phosphopeptides are arrayed and probed with potential binding partners, facilitate a broad analysis of the affinities with which SH2 domains bind specific pTyr sites on activated RTKs or scaffolds (Jones et al., 2006; Stoevesandt et al., 2005). Taken with expression data and functional analysis, these varied approaches provide a means to explore the complex responses of normal and disease cells to pTyr-SH2 signals and for profiling cancer cells (Nollau and Mayer, 2001). The SH2 domain is one of around 100 families of interaction domains, each of which can be found in tens or hundreds of copies in human proteins; these therefore represent a prevalent feature of the human proteome. Generating comprehensive data regarding the biochemical properties of these domains, and the organization and functions of the proteins in which

they reside, will provide an important level of information for understanding cellular behavior.

Experimental Procedures

Method of Data Retrieval and SH2 Identification

The Pfam HMM and SMART HMM domain descriptions (Bateman et al., 2004; Bateman and Haft, 2002; Letunic et al., 2004; Schultz et al., 1998) were used to search the protein sequence data available from Uniprot, Ensembl, and NCBI databases (Apweiler et al., 2004; Bairoch et al., 2005; Hubbard et al., 2005). This was enhanced with a search of translated cDNA and genomic sequence data (including predicted ORFs) from the Pawson lab in-house database (COBRA) using RPS-BLAST searches using the SMART, Pfam, and CDD profiles for the SH2 domain (Marchler-Bauer et al., 2003; Pandit et al., 2004). This complete set contained a significant degree of sequence duplication and redundancy. The overpopulated domain set was filtered for identity and for proteins identified as having identical genetic loci or representing sequence polymorphisms/sequencing errors of the same protein. Duplications, splice variants, and pseudogenes were manually removed following detailed inspection and comparisons of sequence similarity. This resulted in a nonredundant set of 120 SH2 domains contained in 110 distinct proteins (Table S1). The specific genetic locus responsible for encoding each SH2 protein was determined by BLAST analysis of SH2 domain cDNA against the human genomic sequences. Entrez Gene identifiers were determined and a list of alternate symbols and names compiled from these sources as well as from available literature (Maglott et al., 2005). Mouse SH2 domains were determined independently in a similar manner, and homologs between mouse and human SH2 domains were determined as closest relatives by BLAST, supported by information from UniGene (Wheeler et al., 2005). While mice also have 120 SH2 domains in 110 proteins, the set is not entirely overlapping, as SH2D1C (ERT) is present in mouse but is a pseudogene in human while the human gene encoding SH2D3A appears to be absent in the rodent lineage, though it is conserved in primates.

Database Release Dates and Versions

UniProtKB Release 6, February 7, 2006; NCBI data, February 7, 2006; PDB files, February 7, 2006; PFAM definition of the SH2 domain, HMMER2.0 [2.3.2]; ACC:PF00017.12, Saturday, October 22, 2005, 15:17:03.

Identification of SH2 Domain Structures

A combination of BLAST and HMM was used to interrogate sequence extracted from the PDB files for SH2 domains using the Nash lab in-house database to establish a list of SH2 domain structures. This was supported by extensive searches of the literature and manual updating of novel structures.

Supplemental Data

Supplemental data include one figure and three tables and can be found with this article online at http://www.molecule.org/cgi/content/full/22/6/851/DC1/.

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