Oncogene (1998) 17, 1463–1468

http://www.stockton-press.co.uk/onc

The many faces of Src: multiple functions of a prototypical tyrosine kinase

Pamela L Schwartzberg

Laboratory of Genetic Disease Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892-4472 USA

c-*src* was first isolated as the normal cellular homologue of v-*src*, the transforming gene of Rous Sarcoma virus (Stehelin *et al*., 1976). As the first proto-oncogene described and one of the first molecules demonstrated to have tyrosine kinase activity, Src has provided a prototype for understanding signal transduction involving tyrosine phosphorylation. Comparison between c-*src* and activated or transforming mutants of Src including v-*src*, combined with recent data on the structure of Src family kinases has provided new insight into their regulation. In this review, I will discuss the function of the various domains of Src in light of these mutational and structural studies.

Keywords: Src; tyrosine kinase; PDGF-R, integrin

Structure of Src

Src is a member of a family of non-receptor tyrosine kinases defined by a common modular structure (Brown and Cooper, 1996) (Figure 1). The amino-termini of all family members are myristoylated, a fatty acid modification that is determined by the first seven amino-acids of the molecule and causes association with the plasma membrane (Resh, 1994). Downstream of the myristoylation sequence lies a short (50–80 amino-acid) sequence that varies highly among the various family members and is termed the unique domain.

The next section of the protein consists of two distinct regions shared not only with other Src family kinases, but also with a variety of unrelated proteins involved in signal transduction (Pawson and Gish, 1992). These are the Src Homology or SH3 and SH2 domains. The importance of these domains was first suggested by mutants in Src and in non-Src family tyrosine kinases that displayed a variety of host range and conditional transforming phenotypes (Parsons and Weber, 1989; Pawson and Gish, 1992). It was subsequently demonstrated that these regions encode protein interaction domains. SH3 domains interact with specific proline-rich sequences that fold in a left-handed helix (Cicchetti *et al*., 1992; Ren *et al*., 1993). Interestingly, SH3 domains can bind to proline-rich stretches in opposite orientations, suggesting that structure is more important than actual protein sequence for binding recognition (Feng *et al*., 1994).

The SH2 domains in turn, bind to phosphorylated tyrosine residues in the context of specific amino-acid sequences (Pawson and Gish, 1992; Songyang *et al*., 1993, 1994). Using peptide libraries, a consensus binding sequence for Src's SH2 domain was determined to be pYEEI (Songyang *et al*., 1993). Interestingly, specific *in vivo* interactions may not fit this consensus sequence – the SH2 domain of Src binds to the Platelet Derived Growth Factor receptor at the sequence pYIpYV (Mori *et al*., 1993). In this case, phosphorylation of the tyrosine at the +2 position may facilitate binding in the absence of acidic residues.

The C-terminal half of the molecule consists of the kinase or SH1 domain. Certain residues within this domain are strictly conserved among all kinases and are important for the binding of ATP and the phosphotransfer reaction (Hunter and Cooper, 1985). Mutation of these residues can inactive the kinase (Parsons and Weber, 1989). Other residues within the kinase domain contribute to the enzymatic efficiency. In particular, the major autophosphorylation site is located within the kinase domain in a region termed the activation loop, that has been shown to contain phosphorylated residues in the activated version of many kinases (Smart *et al*., 1981). Mutation of the autophosphorylation site leads to decreased kinase activity (Kmiecik and Shalloway, 1987; Piwnica-Worms *et al*., 1987). Mutation of residues 378 or 441 leads to a constitutively activated kinase (Levy *et al*., 1986) however the mechanism of this activation is unclear.

Finally, near the end of the Src family kinases is a conserved tyrosine residue (tyrosine 527 in Src), that is critical for the regulation of the molecule. This tyrosine provides one of the key regulatory features for Src and is a defining feature of the Src family kinases (Brown and Cooper, 1996).

Regulation of the Src kinase

The demonstration that c-*src* was the normal cellular counterpart of the v-*src* transforming gene lead to an active comparison of differences between the two molecules. One of the defining mutations in all versions of v-*src* is the truncation and replacement of sequences encoding the COOH terminus so that Y527 is deleted (Parsons and Weber, 1989). The finding that either dephosphorylation or mutation of Y527 constitutively activates the c-Src kinase and enables it to transform cells, helped implicate this residue as a key regulatory element (Cartwright *et al*., 1987; Courtneidge, 1985; Kmiecik and Shalloway, 1987). Evidence suggests that, when phosphorylated,

this tyrosine interacts intramolecularly with Src's own SH2 domain, thereby inactivating the kinase. It has been postulated that the SH2-Y527 interaction would either distort or cover the kinase domain, leading to its inactivation in this 'closed conformation'. Phosphorylation of Y527 is carried out by another kinase, the Cellular Src Kinase, CSK (Nada *et al*., 1991), and accordingly, targeted deletion of CSK in mice causes constitutive activation of the Src family kinases (Imamoto and Soriano, 1993). In further support of this model, mutations in the SH2 domain also activate the c-Src kinase, as would be expected by disrupting this intramolecular interaction (Parsons and Weber, 1989).

Mutation of the SH3 domain also leads to activation of the Src kinase and indeed, v-*src* also contains two point mutations within the SH3 domain (Parsons and Weber, 1989). Until recently, however, the mechanism of inhibition of kinase activity by the SH3 domain remained unclear. Initial speculation led to the suggestion that the SH3 domain aided in the binding of the SH2 domain to Tyrosine 527. SH3 mutants display decreased phosphorylation of Y527, suggesting that this tyrosine is not bound by the SH2 domain and is therefore more accessible to cellular phosphatases (Parsons and Weber, 1989). While that interpretation is correct in part, recent crystallographic data provides a more detailed understanding of these interactions (Figure 2) (Sicheri *et al*., 1997; Xu *et al*., 1997; Yamaguchi and Hendrickson, 1996). It is now clear that the kinase domain remains exposed, even in the 'closed conformation' when the SH2 domain binds phosphorylated Y527 (Figure 2). However, in addition to this interaction, the SH3 domain exhibits a separate intramolecular contact with the initial region of the kinase domain. Inactivation of the kinase, thus, may result from torsional constraint where the dual binding of the SH2 and SH3 domains prevents free movement within the kinase domain. Mutation of either protein interaction domain frees this constraint and probably leads to decreased stability of the other inhibitory interactions. Interestingly, displacement of the SH3-linker interaction by a separate SH3 binding protein (as with Nef and the SH3 domain of Hck) leads to a more profound activation of the kinase than does displacement of the SH2 domain by a phosphorylated tyrosine peptide or displacement of the SH3 domain with a polyproline sequence (Moarefi *et al*., 1997), suggesting that *in vivo* contacts with proteins may strongly influence binding of both domains.

Thus, both the SH2 and SH3 domains contribute to the regulation of the kinase via intramolecular interactions and disruption of these interactions may be a major mechanism for activation of Src. Furthermore, since other kinases have shown similar regulatory activity of the SH3 and SH2 domains (i.e. fps and abl) (Pawson and Gish, 1992), it is likely that these domains may play similar roles in regulating their kinase activity despite the lack of an equivalent regulatory tyrosine near the COOH terminus. It is worth noting that, not only does this mechanism of regulation allow precise regulation of kinase activity, it also regulates interactions between the SH2 and SH3 domains and other molecules, providing multiple levels of control for the Src family kinases.

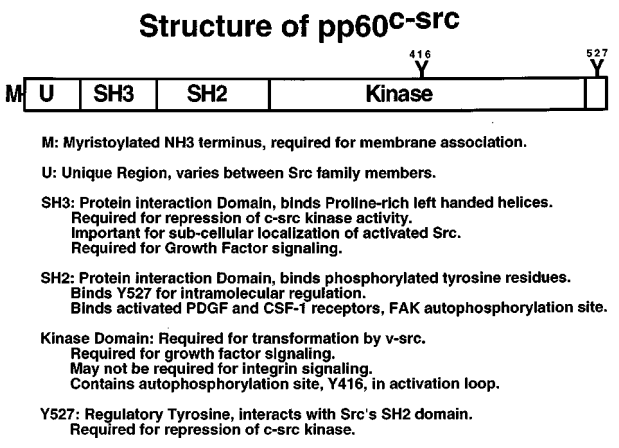

Figure 1 Domain structure of pp60$^{c\text{-}src}$

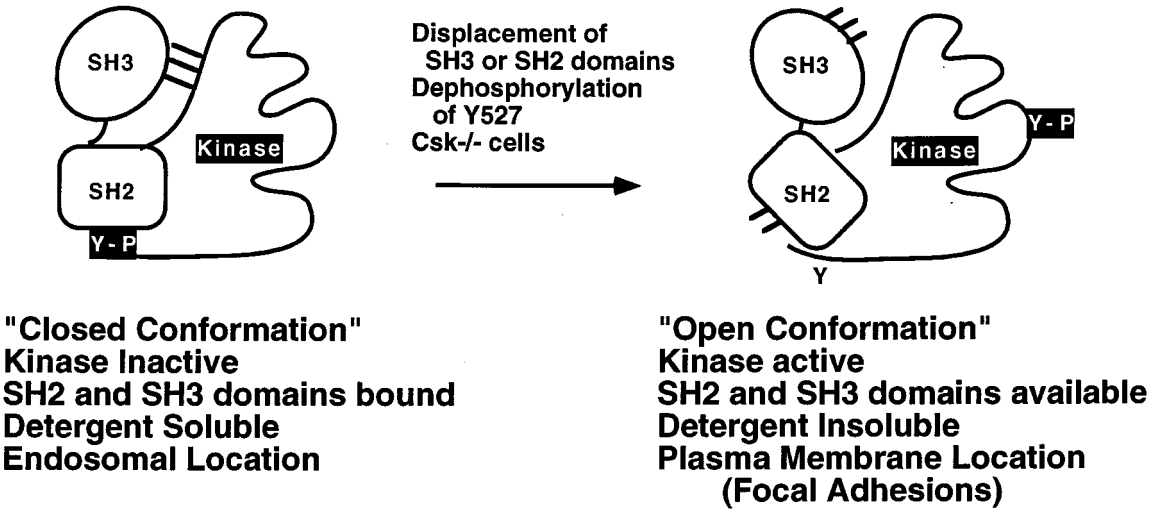

Figure 2 Activation of pp60$^{c\text{-}src}$ changes, protein localization and intramolecular interactions

Function of the Src domains in intracellular signaling

There are currently at least nine members of the Src family of kinases (Brown and Cooper, 1996). These proteins can be divided into two classes – those with wide patterns of expression such as Src, Fyn and Yes, and those with more restricted patterns of expression, such as Lck, Hck, Fgr, Blk and Lyn. Perhaps due to the overlapping patterns of expression and potential functional redundancy of these kinases, evaluation of Src function through gene targeting in mice has produced only limited information. Although Src is expressed in most cell-types, targeted disruption of Src leads to only one major phenotype – osteopetrosis or a failure to resorb bone due to an intrinsic defect in osteoclasts (Boyce *et al*., 1992; Soriano *et al*., 1991). Osteoclasts express high levels of Src and the lack of this molecule leads to dramatic defects in the ability of this cell-type to resorb bone and organize its actin cytoskeleton (Horne *et al*., 1992; Lowell *et al*., 1996; Neff *et al*., 1996; Schwartzberg *et al*., 1997). However, the nature of the defect(s) in signal transduction in these cells remains unclear.

Understanding of Src's function has therefore come from several sources, including biochemical examination of signaling pathways in which the Src kinase has been demonstrated to be activated, comparison of these pathways in cells derived from wildtype and *src*−/− gene-targeted mice, and use of mutant Src molecules to manipulate Src activity in these pathways. Two of the best studied pathways are signaling downstream of growth factor and integrin adhesion receptors. In the next section, I will address the function of the different modular domains of Src with special emphasis on these two signaling pathways.

Kinase domain

Src was first defined as a tyrosine kinase and several lines of evidence strongly support a critical role for the kinase activity of Src for it's function. Mutations that inactivate the kinase prevent transformation of fibroblasts by v-*src* (Jove and Hanafusa, 1987; Parsons and Weber, 1989). Kinase-inactive mutants of c-*src* can act as dominant-negative molecules, blocking signaling in several assay systems. Perhaps best studied is signaling from certain tyrosine kinase growth factor receptors, such as the receptors for Platelet Derived Growth Factor (PDGF) and Colony Stimulating Factor-1 (CSF-1). Upon binding of ligand these receptors activate their own intrinsic tyrosine kinase activity leading to autophosphorylation of tyrosines on their intracellular portions (Schlessinger and Ullrich, 1992). At least one of these phosphorylated tyrosines can bind to the SH2 domain of the Src family members, Src, Fyn and Yes, and transiently activate these kinases (Kypta *et al*., 1990; Ralston and Bishop, 1985). Overexpression of kinase-inactive versions of Src or Fyn will block growth factor-induced entry into S-phase after serum starvation (Broome and Hunter, 1996; Roche *et al*., 1995; Twamley-Stein *et al*., 1993). Presumably, these molecules act in a dominant-negative fashion by binding to the activated receptor via their SH2 domains, but failing to phosphorylate downstream targets. Thus, the kinase activity of Src is essential for triggering downstream pathways leading to cell division from these receptors, apparently in a pathway that has been shown to be mediated by myc (Barone and Courtneidge, 1995). Other pathways that are blocked by kinase-inactive Src include signaling from certain G-protein coupled receptors, and response to oxidative and UV stress (Devary *et al*., 1992; Luttrell *et al*., 1996; Mukhopadhyay *et al*., 1995). It should be noted that these dominant-negative effects are observed under conditions where these molecules are over-expressed (5–20-fold) and may not come under full regulation of the CSK kinase. In several of these pathways, dominant-negative activities have been demonstrated with double mutants that were not only kinase inactive, but also contained mutations of Y527, and therefore were also in an 'open conformation' (Mukhopadhyay *et al*., 1995). In these cases, the dominant-negative activities of these molecules may act more potently, due to increased availability of the SH2 and SH3 domains.

While the kinase activity of Src family members appears to be critical for these pathways, kinase activity may not be strictly essential for all of Src's normal functions. In particular, signaling from integrin receptors involves Src family kinases, but may not require Src's kinase activity. Upon crosslinking of integrins, Src is transiently activated and is found in a detergent insoluble fraction, enriched in focal adhesions, the sites where integrins help organize cytoskeletal and signaling proteins in response to adhesion (Kaplan *et al*., 1995). Fibroblasts derived from *src*−/− mice exhibit a defect in initial cell spreading (Kaplan *et al*., 1995), defects in phosphorylation of an adaptor molecule pp130cas (Bockholt and Burridge, 1995; Hamasaki *et al*., 1996; Vuori *et al*., 1996) and a 10-fold attenuation of MAPK activation in response to plating on fibronectin (Schlaepfer *et al*., 1997), indicating that Src is critical in pathways downstream of integrin crosslinking.

In contrast to the previously described signaling pathways, these integrin-mediated responses are not blocked by kinase-inactive Src. In fact, defects in integrin signaling in *src*−/− cells are actually complemented, in part, by kinase-inactive molecules. For example, either a kinase-inactive point mutant of Src or a truncated molecule that removes the entire kinase domain will complement the cell-spreading defect (Kaplan *et al*., 1995); these molecules will also increase pp130cas phosphorylation in response to fibronectin (Schlaepfer *et al*., 1997 and P Schwartzberg, unpublished observations). A truncated Src molecule lacking the kinase domain has also been demonstrated to restore partial activation of MAPK (Mitogen Activated Protein Kinase) in response to plating on fibronectin (Schlaepfer *et al*., 1997). Thus, in integrin signaling, Src appears to more closely resemble an adaptor molecule that may function by recruiting or activating other tyrosine kinases. Such a proposed role for Src has also been postulated in the increased release of catecholamine in response to Src expression in Vaccinia infected adrenal chromaffin cells, where both wildtype and kinase inactive Src cause similar phenotypes (Ely *et al*., 1994). In these pathways, proper protein interactions may be more critical than kinase activity for Src function. Combined with the previously described data on growth factor signaling,

these results suggest that Src may play different roles in the context of different signaling systems.

We have extended these analyses of c-*src* function to transgenic animals and have found parallel results in a very different cell-type. Osteoclast-specific expression of kinase-inactive Src at physiologic levels can partially rescue the osteopetrosis in *src*−/− mice with improved bone histology, despite relatively poor rescue of cell morphology (Schwartzberg *et al*., 1997). The similarity of this kinase-independent rescue with rescue of integrin signaling suggests that this may be one of the major pathways aberrant in the *src*−/− osteoclast. Supporting this idea, several groups have now found defects in phosphorylation of substrates in response to integrin cross-linking in osteoclasts.

However, higher expression of a kinase-inactive point mutant or expression of a truncated Src molecule actually exacerbates osteopetrosis in mice by inducing osteoclasts to undergo apoptosis (P Schwartzberg, L Xing, B Boyce and HE Varmus, manuscript in preparation). Both mutants prevent growth factor signaling in fibroblasts and we suggest that these data argue that Src is involved in multiple signaling pathways in osteoclasts as well as in fibroblasts. Expression of kinase-inactive molecules may contribute to certain pathways (integrin signaling), yet block others (i.e. growth factor signaling), emphasizing the multiple functions residing in this one molecule.

Critical downstream targets of the Src kinase remain controversial and again, probably depend on the particular signaling pathway that activates the kinase. Many proposed substrates of c-Src come from studies of proteins phosphorylated by the activated viral Src protien (Brown and Cooper, 1996). In the case of growth factor signaling, downstream targets remain unclear, but a variety of proteins phosphorylated by v-Src, including PI3K, SHC, and PLC-γ are potential substrates in this pathway.

In the case of integrin mediated signaling, the Focal Adhesion Kinase is a substrate for the Src kinase (Schaller *et al*., 1994; Schlaepfer and Hunter, 1996). Additionally, a whole host of cytoskeletal proteins such as vinculin, paxillin, tensin, and cortactin as well as adaptor molecules such as pp130cas may also be phosphorylated by Src (Bockholt and Burridge, 1995; Brown and Cooper, 1996; Hamasaki *et al*., 1996; Vuori *et al*., 1996). However, as that kinase activity of Src may not be strictly required in this pathway, other kinases may involved in the direct phosphorylation of these molecules.

A consensus sequence for Src phosphorylation has been determined by peptide-library scans – interestingly this sequence is similar to the consensus sequence for SH2 binding by Src, suggesting that targets for the kinase may in turn bind Src's SH2 domain (Songyang *et al*., 1994). Recent techniques to clone target molecules phosphorylated by Src may open new vistas in finding critical downstream targets. However, they may just add to an ever growing list of molecules that Src is able to phosphorylate. For example, it is clear that a number of receptors and channels can be phosphorylated by Src family members and that this phosphorylation can lead to altered activity (Holmes *et al*., 1996; Yu *et al*., 1997). The role and importance of phosphorylation by Src will need to be determined in each individual pathway.

Role of the SH3 and SH2 protein interaction domains

Several lines of evidence indicate that the SH3 and SH2 protein interaction domains are crucial to the normal function of Src as well as to the activation of v-Src. Interaction of the SH2 domain has been thought to be important for initiating interactions with other molecules in signal transduction cascades, thereby activating Src family kinases. As discussed above, binding to tyrosine kinase growth factor receptors is mediated by the binding of the Src SH2 domain to tyrosine-phosphorylated residues on the receptor cytoplasmic tail, and is probably critical for activating Src kinase and transmitting downstream signals. Overexpression of SH2 domains alone can act as a dominant-negative block to signaling from these receptors (Twamley-Stein *et al*., 1993). SH2 interactions have also been shown to be important for interactions with a number of other proteins. In integrin mediated signal transduction, Src can bind to the autophosphorylation site of FAK via its SH2 domain (Schaller *et al*., 1994). This interaction leads to the phosphorylation of FAK by Src and the subsequent recruitment of adaptor molecules such as GRB2, initiating a pathway leading to MAPK activation (Schlaepfer and Hunter, 1996).

SH3 domains also play critical roles in transmission of intracellular signals. In the growth factor signaling pathways described above, mutants affecting the SH3 domain also have a dominant-interfering phenotype, probably by failing to interact with downstream targets (Broome and Hunter, 1996; Erpel *et al*., 1996). Isolation of interacting molecules through two-hybrid interaction strategies or peptide-library screens has lead to a number of interesting downstream molecules, including SHC, PI3K p85 subunit and SIN (Alexandropoulos and Baltimore, 1996; Liu *et al*., 1993; Weng *et al*., 1994). Two other molecules that bind to the Src SH3 domain are SAM68 (Src Associated in Mitosis) and hnRNPK, both of which are found in the nucleus (Fumagalli *et al*., 1994; Taylor and Shalloway, 1994; Weng *et al*., 1994). The interaction with these potential nuclear substrates may relate to Src's proposed role in the cell-cycle during M phase when Src kinase activity is transiently elevated by a mechanism independent of SH2-Y527F displacement (Shalloway *et al*., 1992). How these nuclear interactions relate to other signaling pathways remains unclear. One further pathway that is regulated by SH3 inte-ractions is the regulation of the potassium channel – interactions with this channel have been shown to be mediated by the Src SH3 domain (Holmes *et al*., 1996).

Protein localization

The protein-protein interactions of SH3 domains also contribute to the correct sub-cellular localization of Src. Activated or 'open conformation' Src is not only associated with an increase in kinase activity, but also with an accompanying change in protein localization (Kaplan *et al*., 1995). Src in its 'closed conformation' is constitutively found associated with endosomal membranes in a detergent soluble fraction. Activation of Src is accompanied by a translocation of Src to a detergent insoluble fraction – this translocation

appears to be independent of kinase activity per se but depends on the 'open' conformation of Src, as demonstrated by mutational analyses. For example, mutants of c-*src* that remove or alter Y527, including v-*src*, are found in detergent insoluble fractions and are associated with the membrane, particularly in focal adhesions (Goldberg *et al*., 1980; Hamaguchi and Hanafusa, 1987). However, a double mutant that alters Y527 and is also kinase inactive, is still detergent insoluble, suggesting independence from kinase activation (Kaplan *et al*., 1994). Furthermore, a truncated molecule, Src251, that removes the entire kinase domain, leaving the amino-terminal half of the molecule, is very strongly associated with focal adhesions. This interaction has been shown to be dependent on an intact SH3 domain, arguing that the SH3 domain is a key element in localizing Src to the detergent insoluble membrane associated fraction. However, the kinase domain itself may also play a role in some of these interactions – mutation of the SH3 domain in the context of full-length or activated (Y527F) Src does not necessarily prevent association with the cytoskeleton, suggesting critical interactions and possible functional redundancy of localization functions with other parts of the molecule (Fukui *et al*., 1991; Okamura and Resh, 1994 and P Schwartzberg, unpublished observations). The exact nature of Src's localization may also depend on the pathway by which it has been activated – activation of Src by PDGF is associated with an increase in detergent-insolubility and a translocation of Src to the plasma membrane, but not necessarily to focal adhesions. It is clear that the protein interaction domains, especially the SH3 domain, are not only important for protein-protein interactions, but also for the correct localization of Src molecules in signaling complexes.

Function of the unique domain

Functional properties of the amino-terminal unique region have not been well defined, nor has crystallographic data been published. It has been assumed that this domain may be required for specific interactions between particular Src family kinases and downstream targets. Recent studies of Src's interaction with and phosphorylation of the NMDA receptor support this view – phosphorylation is dependent on the Src unique region (Yu *et al*., 1997). Activation of Src during M phase is accompanied by phosphorylation of Src on certain Ser and Thr residues within the unique domain – mutation of these residues can lower activation of Src during M phase, but does not eliminate it (Shalloway *et al*., 1992). Mutation of this domain can also reduce transformation by v-*src*, but this requires large mutations that may also alter the SH3 domain (Parsons and Weber, 1989). Further study of this domain in the context of normal cellular functions of Src will be revealing.

Myristoylation

Myristoylation is mediated by signals within the first seven amino-acids of Src and is required for association of Src with membranes (Resh, 1994). In addition, several of the other Src family members palmitoylate cysteine residues near their amino-terminus. Mutations that prevent myristoylation prevent transformation by v-*src* and presumably would interfere with the normal cellular functions of Src family members. Mutations that alter the palmitoylation status of Lck, prevent its function in T-cell receptor signaling (Kabouridis *et al*., 1997). However, the description of a myristoylation mutant of MARCKS (Myristoylated Alanine Rich C Kinase Substrate) that still complements certain functions of MARCKS deficiency, paves the way for the possibility of signaling pathways independent of fatty acid modification (Swierczynski *et al*., 1996). Once again, these observations point to multiple functions within signaling proteins, further contributing to levels of genetic redundancy.

Conclusions

Src has provided a wealth of information on signal transduction – yet our understanding of Src's own function within a given cell remains unclear. In part, this may result from redundancy with other Src family members – particularly Yes and Fyn, which are also very widely expressed. Further adding to this confusion is the fact that, within a given cell, Src can be involved in many signaling pathways. I have concentrated on signaling from tyrosine kinase growth factor receptors and integrin adhesion receptors, since these are relatively well studied pathways. However, Src has clearly been implicated in many different pathways including those in response to G protein coupled serpentine receptors and stress, as well as those regulating receptor and channel activity. The complexity of Src functions and interactions may depend on each particular pathway in which it is involved – so that for some, phosphorylation of downstream targets is crucial, while in others, adaptor functions may define Src's critical activity. What is clear is that the more we learn about Src, the more we continue to learn about the function and regulation of signaling molecules in general.

References

Alexandropoulos K and Baltimore D. (1996). *Genes Dev.,* **10,** 1341 – 1355.

Barone MV and Courtneidge SA. (1995). *Nature,* **378,** 509 – 512.

Bockholt SM and Burridge K. (1995). *Cell. Adhes. Commun.,* **3,** 91 – 100.

Boyce B, Yoneda T, Lowe C, Soriano P and Mundy G. (1992). *J. Clin. Invest.,* **90,** 1622 – 1627.

Broome MA and Hunter T. (1996). *J. Biol. Chem.,* **271,** 16798 – 16806.

Brown MT and Cooper JA. (1996). *Biochim. Biophys. Acta,* **1287,** 121 – 149.

Cartwright CA, Eckhart W, Simon S and Kaplan PL. (1987). *Cell,* **49,** 83 – 91.

Cicchetti P, Mayer BJ, Thiel G and Baltimore D. (1992). *Science,* **257,** 803 – 806.

Courtneidge SA. (1985). *EMBO J.,* **4,** 1471–1477.
Devary Y, Gottlieb A, Smeal T and Karin M. (1992). *Cell,* **71,** 1081–1091.
Ely CM, Tomiak WM, Allen CM, Thomas L, Thomas G and Parsons SJ. (1994). *J. Neurochem.,* **62,** 923–933.
Erpel T, Alonso G, Roche S and Courtneidge SA. (1996). *J. Biol. Chem.,* **271,** 16807–16812.
Feng S, Chen JK, Yu H, Simon JA and Schreiber SL. (1994). *Science,* **266,** 1241–1247.
Fukui Y, O'Brien MC and Hanafusa H. (1991). *Mol. Cell. Biol.,* **11,** 1207–1213.
Fumagalli S, Totty NF, Hsuan JJ and Courtneidge SA. (1994). *Nature,* **368,** 871–874.
Goldberg AR, Krueger JG and Wang E. (1980). *Cold Spring Harb. Symp. Quant. Biol.,* **44,** 991–1005.
Hamaguchi M and Hanafusa H. (1987). *Proc. Natl Acad. Sci. USA,* **84,** 2312–2316.
Hamasaki K, Mimura T, Morino N, Furuya H, Nakamoto T, Aizawa S, Morimoto C, Yazaki Y, Hirai H and Nojima Y. (1996). *Biochem. Biophys. Res. Commun.,* **222,** 338–343.
Holmes TC, Fadool DA, Ren R and Levitan IB. (1996). *Science,* **274,** 2089–2091.
Horne W, Neff L, Chatterjee D, Lomri A, Levy J and Baron R. (1992). *J. Cell. Biol.,* **119,** 1003–1013.
Hunter T and Cooper JA. (1985). *Annu. Rev. Biochem.,* **54,** 897–930.
Imamoto A and Soriano P. (1993). *Cell,* **73,** 1117–1124.
Jove R and Hanafusa H. (1987). *Annu. Rev. Cell Biol.,* **3,** 31–56.
Kabouridis PS, Magee AI and Ley SC. (1997). *EMBO J.,* **16,** 4983–4998.
Kaplan KB, Bibbins KB, Swedlow JR, Arnaud M, Morgan DO and Varmus HE. (1994). *EMBO J.,* **13,** 4745–4756.
Kaplan KB, Swedlow JR, Morgan DO and Varmus HE. (1995). *Genes Dev.,* **9,** 1505–1517.
Kmiecik TE and Shalloway D. (1987). *Cell,* **49,** 65–73.
Kypta RM, Goldber Y, Ulug ET and Courtneidge SA. (1990). *Cell,* **62,** 481–492.
Levy JB, Iba H and Hanafusa H. (1986). *Proc. Natl Acad. Sci. USA,* **83,** 4228–4232.
Liu X, Marengere LE, Koch CA and Pawson T. (1993). *Mol. Cell. Biol.,* **13,** 5225–5232.
Lowell CA, Niwa M, Soriano P and Varmus HE. (1996). *Blood,* **87,** 1780–1792.
Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ and Lefkowitz RJ. (1996). *J. Biol. Chem.,* **271,** 19443–19450.
Moarefi I, LaFevre-Bernt M, Sicheri F, Huse M, Lee CH, Kuriyan J and Miller WT. (1997). *Nature,* **385,** 650–653.
Mori S, Ronnstrand L, Yokote K, Engstrom A, Courtneidge SA, Claesson-Welsh L and Heldin CH. (1993). *EMBO J.,* **12,** 2257–2264.
Mukhopadhyay D, Tsiokas L, Zhou XM, Foster D, Brugge JS and Sukhatme VP. (1995). *Nature,* **375,** 577–581.
Nada S, Okada M, MacAuley A, Cooper JA and Nakagawa H. (1991). *Nature,* **351,** 69–72.
Neff L, Amling M and Baron R. (1996). *J. Bone Miner. Res.,* **11,** S290.
Okamura H and Resh MD. (1994). *Oncogene,* **9,** 2293–2303.
Parsons JT and Weber MJ. (1989). *Curr. Top. Microbiol. Immunol.,* **147,** 79–127.
Pawson T and Gish GD. (1992). *Cell,* **71,** 359–362.
Piwnica-Worms H, Saunders KB, Roberts TM, Smith AE and Cheng SH. (1987). *Cell,* **49,** 75–82.
Ralston R and Bishop JM. (1985). *Proc. Natl Acad. Sci. USA,* **82,** 7845–7849.
Ren R, Mayer BJ, Cicchetti P and Baltimore D. (1993). *Science,* **259,** 1157–1161.
Resh MD. (1994). *Cell,* **76,** 411–413.
Roche S, Koegl M, Barone MV, Roussel MF and Courtneidge SA. (1995). *Mol. Cell. Biol.,* **15,** 1102–1109.
Schaller MD, Hildebrand JD, Shannon JD, Fox JW, Vines RR and Parsons JT. (1994). *Mol. Cell. Biol.,* **14,** 1680–1688.
Schlaepfer DD, Broome MA and Hunter T. (1997). *Mole. Cell. Biol.,* **17,** 1702–1713.
Schlaepfer DD and Hunter T. (1996). *Mol. Cell. Biol.,* **16,** 5623–5633.
Schlessinger J and Ullrich A. (1992). *Neuron,* **9,** 383–391.
Schwartzberg P, Ling L, Hoffman O, Lowell C, Garrett L, Boyce B and Varmus HE. (1997). *Genes Dev.,* **11,** 2835–2844.
Shalloway D, Bagrodia S, Chackalaparampil I, Shenoy S, Lin PH and Taylor SJ. (1992). *Ciba Found. Symp.,* **170,** 248–265; discussion 265–275.
Sicheri F, Moarefi I and Kuriyan J. (1997). *Nature,* **385,** 602–609.
Smart JE, Oppermann H, Czernilofsky AP, Purchio AF, Erikson RL and Bishop JM. (1981). *Proc. Natl. Acad. Sci. USA,* **78,** 6013–6017.
Songyang Z, Blechner S, Hoagland N, Hoekstra MF, Piwnica-Worms H and Cantley LC. (1994). *Curr. Biol.,* **4,** 973–982.
Songyang Z, Shoelson SE, Chaudhuri M, Gish G, Pawson T, Haser WG, King F, Roberts T, Ratnofsky S, Lechleider RJ *et al.* (1993). *Cell,* **72,** 767–778.
Soriano P, Montgomery C, Geske R and Bradley A. (1991). *Cell,* **64,** 693–702.
Stehelin D, Varmus HE, Bishop JM and Vogt PK. (1976). *Nature,* **260,** 170–173.
Swierczynski SL, Siddhanti SR, Tuttle JS and Blackshear PJ. (1996). *Dev. Biol.,* **179,** 135–147.
Taylor SJ and Shalloway D. (1994). *Nature,* **368,** 867–871.
Twamley-Stein GM, Pepperkok R, Ansorge W and Courtneidge SA. (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 7696–7700.
Vuori K, Hirai H, Aizawa S and Ruoslahti E. (1996). *Mol. Cell. Biol.,* **16,** 2606–2613.
Weng Z, Thomas SM, Rickles RJ, Taylor JA, Brauer AW, Seidel-Dugan C, Michael WM, Dreyfuss G and Brugge JS. (1994). *Mol. Cell. Biol.,* **14,** 4509–4521.
Xu W, Harrison SC and Eck MJ. (1997). *Nature,* **385,** 595–602.
Yamaguchi H and Hendrickson WA. (1996). *Nature,* **384,** 484–489.
Yu X-M, Askalan R, Keil GJ and Salter MW. (1997). *Science,* **275,** 674–678.