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Minireview

Regulation of the Src protein tyrosine kinase

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Abstract Members of the Src family of protein tyrosine kinases are involved in a variety of cellular processes, including cell growth, cell differentiation and neuronal signalling. N-terminal to the catalytic domain, Src family members contain a Src homology 2 (SH2) domain, a Src homology 3 (SH3) domain, and a unique domain, all capable of protein-protein interactions. Negative regulation by phosphorylation of a conserved tyrosine residue at the C-terminal tail of the molecules is characteristic of this family of enzymes. Phosphorylation of this residue causes the intramolecular interactions of the SH2 domain with the tail, and of the SH3 domain with an as yet undefined region, probably within the catalytic domain. Enzymatically active Src family kinases, on the other hand, are phosphorylated at a tyrosine in the middle of the catalytic domain and phosphorylation of this residue is a prerequisite for high activity. Regulators of these enzymes may thus act by altering the phosphorylation state of the two key tyrosine residues or by interfering with the regulatory intramolecular interactions, either by direct binding or by modification of the interfaces involved.

Key words: Src protein tyrosine kinase; SH3 domain; SH2 domain; Regulation of enzyme activity; Autophosphorylation site

1. Structure

There are at least nine members of Src family kinase in mammals and birds: Src, Fyn, Yes, Lyn, Hck, Fgr, Lck, Blk and Yrk. Sequence identities among the different family members exceed 70%. Expression of Src, Fyn and Yes is ubiquitous, while other members of the family have a more restricted expression pattern, several being expressed only in cells of the hematopoietic system (reviewed in [1,2]). Within the first 15 N-terminal residues, all Src family members bear a signal for myristylation and most also one for palmitoylation (reviewed in [3], Fig. 1), which direct the association of these proteins with cellular membranes. The following 40–70 amino acids are poorly conserved among the different members. This region, termed the unique domain, has in some cases been shown to participate in the interaction with other proteins (reviewed in [4]). C-terminal to the unique domain is the SH3 domain, a structure of about 60 residues capable of protein-protein interaction with regions of proteins which form left-handed polyproline type II helices (reviewed in [5–7]). The three-dimensional structure of several SH3 domains of Src family members is known, and the molecular details of their interaction with peptides have been elucidated [8,9]. Several proteins able to interact with the SH3 domain of Src family members have been identified (reviewed in [7,10]). Next to the SH3 domain is the SH2 domain, comprising about 100 residues. The SH2 domain is a high-affinity binding unit for phosphotyrosine-containing proteins (reviewed in [5,7]), and is frequently found in proteins involved in signal transduction [11]. The SH2 domains of Src family members mediate interactions with receptor-type protein tyrosine kinases and with proteins present in focal adhesions, and may also mediate the interaction with protein substrates of Src kinases [11,12]. The structure of the SH2 domain of several Src family members has been solved [13–17] and provides a molecular basis to the understanding of the observed binding specificity [18,19]. C-terminal to the SH2 domain is the catalytic domain, which is very similar to, though bearing some distinct features from, those of other protein tyrosine kinases, and is reviewed elsewhere [20,21]. No structure of a protein kinase of the Src family is yet available. The catalytic domain is followed by a short 'tail' containing a conserved tyrosine residue, a site of regulatory phosphorylation characteristic of the Src family of proteins (see below).

2. Function

In recent years we have seen a rapid improvement in our understanding of the function of Src family members. Proteins of this family have been found associated with several cell surface receptors, some with tyrosine kinase activity, others with no obvious catalytic function (reviewed in [4,22,23]). In many cases, activation of these receptors causes an increase in the activity of Src family members. A direct functional involvement of Src family members in intracellular signalling through these receptors has been demonstrated in several cases (reviewed in [22,23]). A genetic approach to the function of Src family kinases has become possible through homologous recombination at the loci of genes coding for Src family members in mice (reviewed in [24]). Surprisingly, mice defective for any single Src family gene show relatively minor phenotypes or no detectable phenotype at all, suggesting partial functional compensation among Src family members. Nevertheless, the mutant mice clearly demonstrated the involvement of given Src family members in specific functions. Fyn −/− mice, for example, show defective T-cell receptor signalling in thymocytes, abnormal development of the hippocampus and impairment of long-term potentiation [25–27]. Recent reports show that the phenotype of double and triple mutants is more than additive, suggesting that in some cases, Src family members indeed overlap functionally [28,29].

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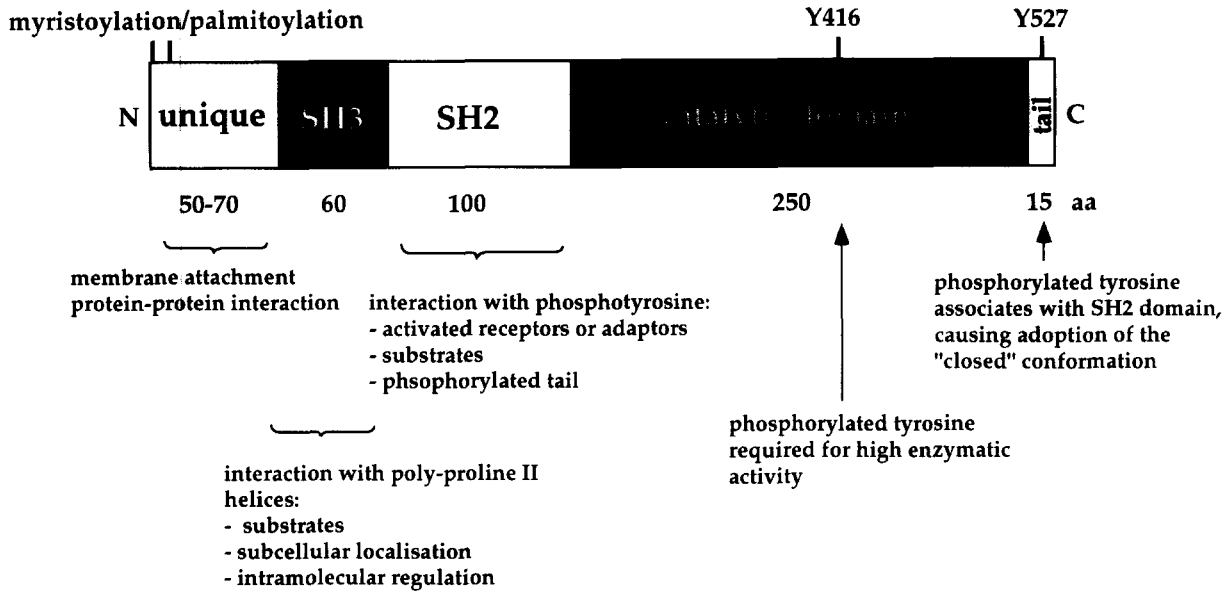

Fig. 1. Topology of the Src family of protein tyrosine kinases. Approximate numbers of amino acids comprising the various domains in Src family members are indicated. The sites of myristoylation (Gly2) and palmitoylation (cysteine residues within the first 10 residues) are shown, as well as two major regulatory phosphorylation sites. See text for details. Abbreviations: unique, unique domain; SH3, Src homology 3 domain; SH2, Src homology 2 domain; aa, amino acid; N, N-terminus; C, C-terminus.

3. Regulation

All Src family members are negatively regulated by phosphorylation of a conserved tyrosine in the tail at the C-terminus of the molecules (reviewed in [30,31], see also [32]). Src itself has been the most widely studied, and will therefore be discussed here as a paradigm for all Src family kinases. Mutation of Tyr527, the regulatory tyrosine in the tail of chicken c-Src, to phenylalanine is sufficient to convert the proto-oncogene to an oncogene, and dephosphorylation or mutation of this residue is accompanied by an at least 10-fold increase in kinase activity [33]. Members of a related but distinct class of protein tyrosine kinases, the Csk family, currently consisting of two members, Csk itself and Ctk/Ntk/Matk/Hyl/Lsk, are responsible for this phosphorylation (reviewed in [21]). Inactivation of the Csk gene by homologous recombination in the mouse has shown that Csk is largely responsible for phosphorylation of Src in the embryo (reviewed in [24,31]). Phosphatases specifically acting on Tyr527, on the other hand, have not been identified yet. Hanafusa and colleagues originally proposed a model according to which the negative effect of phosphorylation of Tyr527 was mediated by an intramolecular interaction between the phosphorylated tail and the SH2 domain of the same molecule [34]. Indeed, mutations in the Src SH2 can activate the kinase, and in some cases cause Src to transform fibroblasts [35–37]. Much experimental evidence for this interaction has been provided over the years [38–42].

From mutational analysis there is also evidence for a negative role of the SH3 domain in Src regulation. Some mutations derived from the v-Src SH3 domain, when transplanted into c-Src, are sufficient to partially activate the enzyme [43,44] and a deletion of the SH3 domain rendered c-Src able to transform chicken embryo fibroblasts [37]. It was later found by several groups that the activity of Src alleles mutated in their SH3 domain, unlike the activity of wild-type Src, was not affected by C-terminal phosphorylation when coexpressed along with Csk in yeast [42,45,46]. Peptide binding experiments revealed that the SH3 domain was necessary in order for the phosphorylated tail to interact stably with the SH2 domain [42]. A so-called snail model for the regulated state of Src was derived, according to which the phosphorylated tail interacts with the SH2 domain while the nearby SH3 domain interacts with some other part of the molecule [42]. In Src, proline-rich motives and therefore potential SH3 domain interaction sites, are found in the unique domain and at the end of the catalytic domain. We have mutated both regions and found that neither is involved in regulation by C-terminal phosphorylation (G.S.F., unpublished results). On the other hand we have recently shown that the same conserved residues involved in the interaction of the SH3 domain with other proteins participate in intramolecular regulation, implying that the function of the SH3 domain in Src regulation requires the same ligand-binding surface [47]. The crystal structure of the SH3 and SH2 domains of Lck published by Eck et al. rules out the possibility that the ligand binding surface of the SH3 interacts with the SH2 domain *in cis*, but rather points to a possible interaction between the SH2 domain and the SH3 domain of two Src molecules *in trans* [17]. We have purified Src to homogeneity in its repressed state, phosphorylated at Tyr527, and found that it runs as a monomer on sizing columns, arguing against the existence of regulated dimers (A. Weijland, G.S.F., S.A. Courtneidge and R. Wierenga, unpublished results). With what portion of the molecule then does the SH3 domain interact ? If we exclude the unique and the SH2 domains, we must conclude that the SH3 domain interaction site is in an exposed region of the catalytic domain. While there is no obviuos proline-rich region to candidate, on the surface of globular proteins, left-handed polyproline type II helices might form even in the absence of any proline residue [48], making it possible to the Src SH3 to bind. Moreover, the affinity of the SH3 with the putative interaction

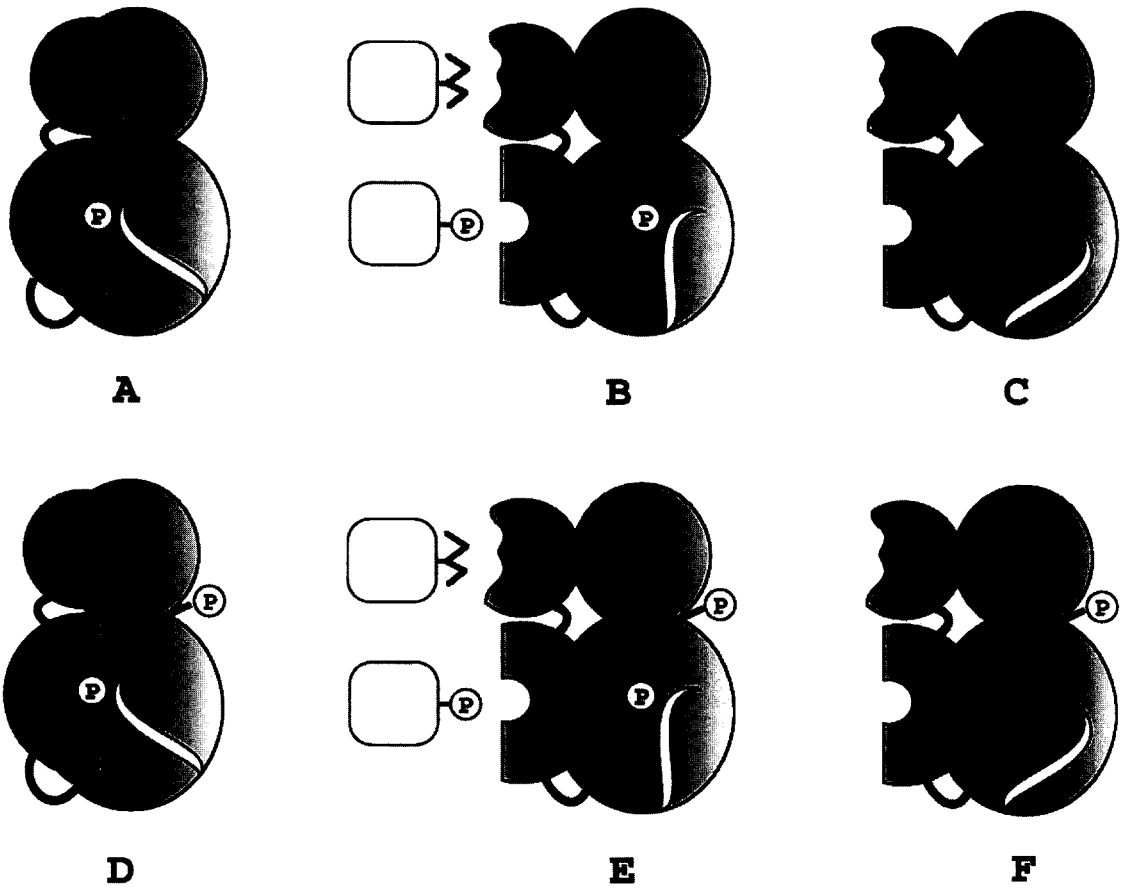

Fig. 2. Models of active and inactive forms of Src. The figure shows cartoons of our current models of Src regulation. The unique domain is not shown. The catalytic domain is drawn as a bilobal structure consisting of the smaller ATP-binding lobe and of the larger substrate-binding lobe. The SH2 domain is shown as a hemisphere with a cavity to accommodate a phosphotyrosine residue. The SH3 domain is also drawn as a hemisphere with a zig-zag ligand-binding surface. The C-terminal tail is drawn in white. The activation loop is drawn as a short black line in the cleft between the small ATP-binding lobe and the large substrate-binding lobe of the kinase domain. The letter 'P' indicates a phosphorylated tyrosine. The letter 'Y' stands for tyrosine. The two white boxes represent a protein containing a poly-proline type II helix, able to bind with high affinity to the Src SH3 domain, and a protein phosphorylated on tyrosine, able to bind with high affinity to the Src SH2 domain. (A–D) Inactive forms of Src. (E,F) Active forms of Src. (A) Regulated Src. Phosphorylated Tyr527 interacts with the SH2 domain, the SH3 domain interacts with an as yet unidentified region in the catalytic domain, possibly within the small lobe. This is the so-called closed conformation. Tyr416 is not phosphorylated. The activity is low. (B) Proteins able to interact with high affinity with the SH3 and/or the SH2 domains keep Src in the 'open' conformation, despite phosphorylation at Tyr527. The kinase activity, however, is low because Tyr416 is unphosphorylated, possibly due to the action of a phosphatase. The 'opening' effect may also be induced by phosphorylation of the interaction surfaces of the SH3 and SH2 domains. (C) Tyr527 is not phosphorylated and therefore Src is in the 'open' conformation. Absence of Tyr416 phosphorylation keeps the kinase inactive. (D) Despite phosphorylation at Tyr416, Src is inactive because phosphorylation at Tyr527 causes Src to adopt the 'closed' conformation. This conformation is dominant over the state of the activation loop. (E) Active Src. Association of the SH3 and/or the SH2 domains of Src with proteins binding with high affinity prevents the intramolecular interactions of these domains. The kinase activity is derepressed. Tyr416 is phosphorylated. As in B, the 'opening' effect may also be induced by phosphorylation of the interaction surfaces of the SH3 and SH2 domains. (F) In the absence of phosphorylation of Tyr527 in the tail, there is no intramolecular association with the SH2 domain. The kinase is derepressed. Tyr416 is phosphorylated. See text for details.

site should be rather weak, the interaction being already kinetically favored by its intramolecular nature and because of the necessity of being easily reversed. Accordingly, the affinity of the Src SH2 domain for a peptide modelled on the tail of Src is quite weak [19,40,49]. Further mutagenesis studies and the solution of the structure of Src in the 'closed' conformation will allow identification of the SH3 interaction site.

Activated forms of Src, like v-Src or Src expressed in yeast in the absence of Csk, are phosphorylated at Tyr416 in the so-called activation loop, a region essential for the activity of several serine/threonine and tyrosine kinases and a site of regulatory phosphorylation (reviewed in [50,21], see also [51,52]). While Tyr416 is clearly a site of autophosphorylation *in trans*, it may also be phosphorylated by kinases other than Src. Pinna and co-workers have purified an activity from spleen, distinct from any known Src family member, able to specifically phosphorylate the 'autophosphorylation' site of Src family kinases [53]. When introduced in cells lacking Csk, where Src family kinases are activated, or even in cells lacking both endogenous Src and Csk, a catalytically defective form of Src is phosphorylated at Tyr416 ([54] and A. Imamoto and P. Soriano, personal communication), suggesting that Src family members might activate each other, or be activated by yet other kinases. Mutation of Tyr416 to phenylalanine seriously impairs the ability of activated c-Src to transform cells, but has only very little effect on the transformation potential of v-Src

(reviewed in [21,30]). In vitro, the difference in the specific kinase activity between wild-type Src and Src Y416F is less than five-fold. While this discrepancy is still unexplained at the moment, Y416F Src is at least 30-fold less active than wild-type Src when expressed in yeast (M. Koegl, S.A. Courtneidge and G.S.F., manuscript submitted), suggesting that the role of Tyr416 is currently underestimated. Moreover it is clear from a number of studies, including mass spectrographic analysis, that phosphorylations at Tyr416 and at Tyr527 are not mutually exclusive ([55], A. Weijland, G.S.F., S.A. Courtneidge and R. Wierenga, unpublished results).

4. Modulation

The data on Src regulation discussed so far suggest that there are several ways in which the activity of Src can be modulated. Phosphorylation of Tyr527 causes a rearrangement of the molecule associated with a severe reduction in enzyme activity ('closed' conformation, Fig. 2A). In this conformation, both the SH2 domain and the SH3 domain are engaged intramolecularly and are therefore not accessible for interaction with other proteins. Dephosphorylation of Tyr527 is certainly sufficient to destabilize this conformation and de-repress the enzyme ('open' conformation, Fig. 2F). However, if Src is not phosphorylated at Tyr416, the enzymatic activity is low (Fig. 2C). Interactions of heterologous proteins with high affinity for the Src SH2 domain will also result in destabilisation of the repressed state, activating the enzyme ([40], Fig. 2E). Such an event might occur at recruitment of Src to activated tyrosine kinase receptors and has been mimicked with phosphorylated peptides modelled on the PDGF receptor and the CSF-1 receptor Src binding sites [49]. Recent results have shown that SH2-domain-containing tyrosine kinases display a substrate specificity that matches the binding preferences of their SH2 domain [12], suggesting that once a substrate is phosphorylated by Src, it might cause the activation of more Src molecules in a positive feedback loop.

Activation of Src by proteins binding to its SH3 domain, on the other hand, has not yet been demonstrated (Fig. 2E). Such a mechanism might, however, operate during the localization of Src to focal adhesions [56], sites where Src is presumed to be active and phosphorylate, among other proteins, focal adhesion kinase (FAK). If, at focal adhesions, Src requires constant anchoring through the SH3 domain, freezing the enzyme in the 'open' conformation, a possible requirement for down-regulation of Src activity might be fulfilled by phosphatases acting on Tyr416 (Fig. 2 B). Our model predicts that modification of the ligand-binding surfaces of either the SH2 or the SH3 domains would result in destabilization of the closed form. Such a modification has recently been described by Broome and Hunter, who found that after PDGF stimulation, mouse Src became phosphorylated at Tyr138 in the SH3 domain, resulting in an impairment of the binding potential of the SH3 domain (M. Broome and T. Hunter, personal communication). Mutation of Tyr136, the corresponding residue in chicken Src, to alanine, abolishes regulation of Src by C-terminal phosphorylation [47], raising the possibility that phosphorylation of Tyr136 (Tyr138) would indeed activate Src.

In summary there seem to be several ways in which Src may adopt the 'open' conformation and possibly also several ways in which further regulatory constraints can be superimposed on the open conformation. While we might have identified some of the basic regulatory mechanisms, their role within the cell remains to be elucidated.

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