Determinants of Substrate Recognition in Nonreceptor Tyrosine Kinases

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ABSTRACT

Cytoplasmic tyrosine kinases do not occur as isolated catalytic domains. Instead, each kinase family possesses a characteristic array of additional domains that are appended to the catalytic domain. The combination and the arrangement of these modular domains are important in kinase regulation and function. This Account describes how the noncatalytic regions of Src family tyrosine kinases are involved in enzyme regulation, substrate selection, and multisite phosphorylation.

Introduction

Tyrosine kinases are signaling enzymes that regulate growth, differentiation, and apoptosis in mammalian cells.[1] The fidelity of these processes depends on specific interactions between tyrosine kinases and their substrates. Tyrosine phosphorylation promotes the subsequent formation of specific multiprotein complexes that are critical in signal transduction. Tyrosine kinases are normally under tight control and have low basal activity; they are activated transiently in response to specific stimuli. Inappropriate activation of tyrosine kinases (by overexpression, mutation, or chromosomal rearrangement) can wreak havoc in a cell; deregulated tyrosine kinases play a role in many forms of human cancer.[2,3] There are approximately 100 tyrosine kinases in the human genome, and they fall into two groups: transmembrane receptor kinases (such as the epidermal growth factor receptor) and nonreceptor tyrosine kinases (such as c-Src).[4,5] Receptor tyrosine kinases consist of an extracellular ligand-binding domain, a single membrane-spanning sequence, and an intracellular kinase domain. Ligand binding to receptor tyrosine kinases promotes dimerization, tyrosine kinase activation, and autophosphorylation of the intracellular kinase domain.[5,6]

In mammals, there are 10 families of nonreceptor tyrosine kinases.[4] These enzymes possess catalytic domains that are closely related to those of receptor kinases. In addition, nonreceptor tyrosine kinases invariably contain noncatalytic regions that are important in kinase activation and substrate specificity.[5] Src-family nonreceptor tyrosine kinases contain Src homology 3 (SH3) and Src homology 2 (SH2) domains N-terminal to their kinase catalytic domains[7−9] (Figure 1). These modular protein domains, originally identified in v-Src, are present in a variety of proteins involved in cell signaling, including other protein kinases, phospholipases, protein phosphatases, lipid kinases, and noncatalytic adaptor proteins.[10,11] SH2 and SH3 domains mediate specific protein−protein interactions: SH2 domains recognize short peptide motifs containing phosphotyrosine, and SH3 domains bind to proline-rich sequences. SH2 and SH3 domains have a variety of functions in signal transduction. In various systems, SH2 and SH3 domains have been shown to recruit substrates to adjacent catalytic domains, act as adapters to promote complex formation, or modulate catalytic activity. In this review, I will discuss how nonreceptor tyrosine kinases recognize their substrates, with particular emphasis on how the various domains contribute to Src-family kinase specificity.

Catalytic Domain Specificity

One potential source of specificity for a tyrosine kinase is clearly the catalytic domain itself; kinase active sites might only accommodate certain amino acid sequences. Early work on the intrinsic specificity of protein kinases focused on the cAMP-dependent protein kinase (PKA), a Ser/Thr kinase. Short peptides that reproduced the phosphorylation site on pyruvate kinase, a natural PKA substrate, were efficiently phosphorylated by PKA, provided crucial basic side chains at P-2 and P-3 (relative to Ser/Thr) were present.[12] These studies gave rise to the notion of consensus sequences (Arg-Arg-X-Ser, in the case of PKA), which are defined as a set of common sequence elements that are necessary and sufficient for kinase recognition. The existence of consensus sequences for some kinases supports the idea that kinase active sites are customized to accept certain peptide sequences. The acidic residues of PKA that interact electrostatically with P-2 and P-3 were identified by chemical modification[13] and by mutagenic analysis of yeast PKA.[14] These residues were confirmed to interact with bound peptides from the crystal structure of PKA in a complex with a peptide inhibitor.[15] Many other Ser/Thr protein kinases have residues at equivalent positions that play a role in peptide substrate specificity.[16]

Are there consensus sequences for tyrosine kinases? Our group[17,18] and other groups[19,20] have pursued the answer to this question using combinatorial peptide libraries. In this method, a library of peptides is synthesized in which residues near tyrosine are randomized. A tyrosine kinase is allowed to select its preferred substrate (or substrates) from the library. We have used a variety of methods to identify the phosphorylated sequences that

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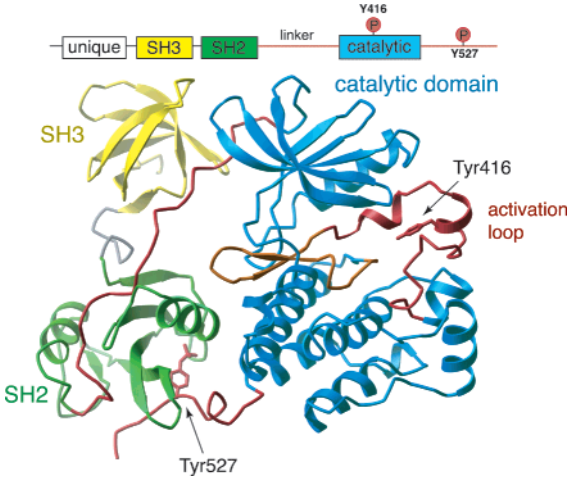

FIGURE 1. Top: domain structure of Src-family tyrosine kinases. Bottom: the three-dimensional structure of the Src family kinase Hck.[54] The SH3 domain is yellow, the SH2 domain is green, and the catalytic domain is blue. Phosphorylation sites at Tyr416 (in the activation loop) and Tyr527 (in the C-terminal tail) are indicated.

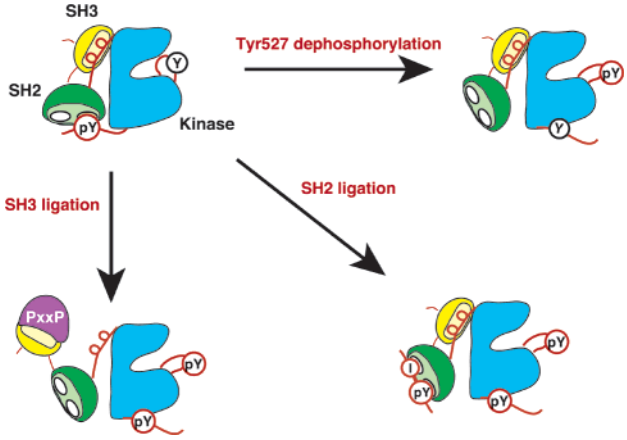

FIGURE 2. Mechanisms for Src kinase activation. The inactive form of the kinase is depicted at the upper left. Three processes can lead to increased autophosphorylation at Tyr416 and enhanced catalytic activity: Tyr527 dephosphorylation, SH2 ligation, or SH3 ligation. pY represents phosphorylated tyrosine.

are selected from peptide libraries.[17,18] Other powerful techniques have been devised to screen peptide libraries with increased diversity.[19,20] The results from the various methods are generally consistent, and they show that individual tyrosine kinases do possess preferred substrate sequences (for example, Src prefers the Glu-Glu-Ile-Tyr-Gly-Glu-Phe sequence). These preferences, however, are not very stringent. Tyrosine kinases can often tolerate amino acid substitutions in these peptides without drastic consequences. Moreover, the preferred sequences for different tyrosine kinases are not very different from each other. In general, while the catalytic domains do target certain types of sequences, they are too lax in their preferences to explain the in vivo specificity observed in kinase signaling pathways.

Role of Noncatalytic Domains in Tyrosine Kinase Regulation

Recent work on tyrosine kinase signaling has shown that there is an additional mechanism that governs specificity in vivo. The subcellular distribution of tyrosine kinases and the timing of their activation ensure that they will only encounter certain potential substrates.[21] For nonreceptor tyrosine kinases such as Src, the amino-terminal noncatalytic regions play a key role in specificity for at least three reasons: (i) they aid in the proper subcellular localization of the enzymes, which places the enzymes in the vicinity of certain cellular substrates; (ii) the SH2 and SH3 domains make important protein−protein interactions with potential substrates; and (iii) the amino terminal domains are involved in the selective activation of Src family tyrosine kinases. This imparts another level of specificity, since the enzymes are dormant until the correct substrate is in proximity to the catalytic domain.

In collaboration with Dr. John Kuriyan's laboratory, we carried out biochemical experiments on Src family kinase regulation using Hck, a Src-kinase expressed in hematopoietic cells. Hck contains the typical layout of domains of a Src family kinase (Figure 1). For all Src kinases, phosphorylation at a conserved C-terminal tyrosine (Tyr527, using Src numbering) produces an intramolecular interaction between the phosphorylated tail and the SH2 domain that inhibits kinase activity.[7,9] Src kinases are activated by autophosphorylation at Tyr416 in the activation loop, a mobile segment in the catalytic domain that controls the activity of many protein kinases. We studied the autoinhibited form of Hck that was phosphorylated at Tyr527. Dr. Kuriyan and his colleagues solved the three-dimensional structure of this form of Hck.[22] This structure, and the structure of c-Src,[23] confirmed that the SH2 domain binds the C-terminal tail containing phosphorylated Tyr527. The SH3 domain binds a polyproline type II helix in the linker region between the SH2 domain and the catalytic domain (Figure 1).

We showed that intramolecular binding of both the SH2 and SH3 domains inhibits Hck activity, and disruption of either of the interactions leads to enzymatic activation. We first showed the importance of the SH3 domain in autoinhibition by experiments with HIV Nef protein. Addition of Nef, a specific high-affinity ligand for the SH3 domain of Hck, caused maximal activation of the enzyme.[24] Nef promotes rapid autophosphorylation of Hck at Tyr416 in the activation loop. A mutant of Nef that lacks the polyproline sequence did not activate Hck, confirming the importance of SH3 binding. Furthermore, a mutant form of Hck with a weakened intramolecular SH3 interaction (W260A) has high constitutive kinase activity and is not activated further by Nef binding.[25] Binding of Nef to the SH3 domain of Hck in transfected cells also activates Hck and leads to cell transformation.[26] These results suggest that SH3 displacement is a potent mechanism for activation of Src kinases. More than 15 proteins have subsequently been reported by other laboratories to activate Src kinases by SH3 displacement. Our biochemical results on Hck, and the results of experiments on a variety of Src-family kinases by other investigators, suggest

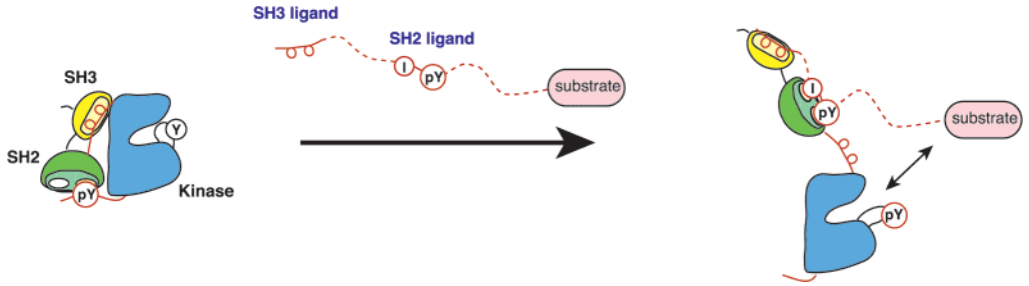

FIGURE 3. Coupling of Src-family kinase activation to substrate recognition. The catalytic domain (blue) is inhibited by intramolecular interactions between the SH3 domain (yellow) and the linker region and between the SH2 domain (green) and phosphorylated Tyr527. Ligands for the SH3 or SH2 domains can disrupt the intramolecular interactions and activate the kinase. Many of the best Src substrates contain ligands for the SH3 and/or SH2 domain. In this case, the ligands concomitantly activate the kinase and tether the substrate to the enzyme, facilitating phosphorylation.

that there are at least three ways to activate a resting Src kinase (Figure 2): (i) by SH3 displacement; (ii) by SH2 displacement; or (iii) by dephosphorylation of Tyr527. As described below, these activation steps are important in selective signaling by Src-family tyrosine kinases.

The intramolecular interactions that maintain Src kinases in the inactive state are of relatively low affinity. For example, the SH2 domain ligand in the C-terminal tail of Hck (pYQQQ) does not conform well to the sequence of the high-affinity SH2 ligand pYEEI. However, the intramolecular ligands for the SH2 and SH3 domains possess an entropic advantage in binding, as they are present on the same polypeptide. We hypothesized that the intramolecular SH2 binding site must be lower in affinity so that exogenous ligands can compete for binding to the SH2 domain.[27] This model predicts that if the intramolecular interactions were of very high affinity, the enzyme would be resistant to activation. To test this idea, we engineered a mutant form of Hck with the high-affinity pYEEI sequence in the C-terminal tail. This pYEEI-Hck mutant could not be activated by exogenous SH2 ligands.[27] We conclude that the intramolecular interactions in Src-family kinases must be strong enough to maintain the catalytic domain in an inactive conformation, but weak enough to allow for activation by exogenous ligands. These findings are likely to be relevant to other signaling systems that are regulated by autoinhibition, such as guanine nucleotide exchange proteins,[28] WASP family proteins,[29] and protein kinase C.[30]

Role of Noncatalytic Domains in Substrate Recognition

In addition to their negative regulatory roles in Src-family tyrosine kinases, the SH2 and SH3 domains have a positive role in kinase signaling. This role was first appreciated from experiments on Src kinases containing deletions or mutations in the Src homology regions (reviewed in ref 7). These experiments revealed that SH2- and SH3-mediated interactions with other proteins assist the kinases in recognizing certain cellular substrates. For example, the Src SH3 domain recognizes a proline-rich motif in the actin-associated protein AFAP-110; this interaction leads to phosphorylation of AFAP-110 by the catalytic domain of Src.[31] The importance of the SH2 and SH3

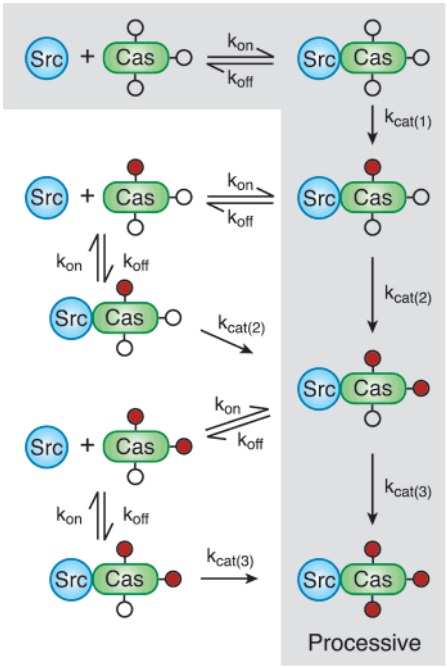

FIGURE 4. Multisite phosphorylation of Cas by Src can follow either a processive (shaded) mechanism or a nonprocessive mechanism. Unphosphorylated tyrosines on Cas are represented by open circles, and phosphorylated sites are represented by red circles. For simplicity, only three tyrosines are shown, although Cas contains approximately 15 potential Src phosphorylation sites.

domains in substrate targeting has also been established for the Src substrates Cas,[32,33] FAK,[34] and Sin.[35] Substitution of the Abl SH2 domain with heterologous SH2 domains results in the phosphorylation of alternative substrates in vivo.[36] These observations suggest that the substrate specificity of a nonreceptor tyrosine kinase is dependent on the specificity of its associated SH3 and SH2 domains.

The dual role for SH2 and SH3 domains of Src family kinases suggests a model that couples enzyme regulation to substrate recognition (Figure 3). In this model, binding of substrates by the SH3 and/or SH2 domains targets Src to potential substrates and concomitantly activates the catalytic domain. This mechanism ensures that Src kinases are only active when in the correct cellular environment, and when associated with their proper substrates. We[27,37] and others[34,35,38] have obtained evidence in support of this

Table 1. Phosphorylation of Src Substrates Containing SH2 Ligands[a]

Peptide	Sequence	Relative k_{cat}/K_m
Y(11)F	RRLEDAIYAAGGGGGEPPQFEEIG	1.0
Y(11)pY	RRLEDAIYAAGGGGGEPPQpYEEIG	9.6
Y(7)pY	RRLEDAIYAAGEPPQpYEEIG	3.7
Y(3)pY	RRLEDAIYAPQpYEEIG	0.5
Y(3)F	RRLEDAIYAPQFEEIG	1.6
pY(12)Y	EPQpYEEIGGGGGEDAIYARRG	7.3

[a] The substrate sequence (Glu-Asp-Ala-Ile-Tyr) is shown in red, and the SH2 domain binding sequence (phospho Tyr-Glu-Glu-Ile) is shown in blue. The mutated SH2 binding sequence is Phe-Glu-Glu-Ile. The number in parentheses in the peptide name indicates the length of the spacer between phosphotyrosine (or phenylalanine) and tyrosine. The kinetic measurements were carried out using C-terminally dephosphorylated (activated) Hck.[37]

model, in experiments with purified proteins as well as in intact cells. A corollary of this model is that the best in vivo substrates for Src kinases would be those proteins containing SH2- or SH3-binding sites (to activate the kinase) in addition to suitable substrate sequences.

Many of these proteins are phosphorylated at multiple sites ("hyperphosphorylated") by Src family kinases. In general, multiple phosphorylations of a substrate can occur by either a processive or a nonprocessive (distributive) mechanism.[39,40] In a distributive mechanism, each phosphorylation of the substrate results from a separate collision between enzyme and substrate. In a processive mechanism, the kinase remains bound to its substrate until it completes all of the phosphorylations, and therefore only one collision between kinase and substrate is required (Figure 4). The dual phosphorylation of MAP kinase by MAPKK has been shown to proceed by a distributive mechanism.[39,40] For Src family kinases, the possibility exists that protein substrates would remain bound via SH3/SH2-mediated interactions through multiple rounds of phosphorylation.[33] Binding would raise the effective local concentration of individual phosphorylation sites and increase the efficiency of phosphorylation.

We have used synthetic peptide model systems to test for processive phosphorylation by tyrosine kinases. In the first set of experiments, we asked whether the presence of a high-affinity Src SH2 binding sequence on a substrate enhances its phosphorylation. We prepared peptides containing the optimal Src SH2 binding sequence (pYEEI) linked to a substrate sequence derived from peptide library studies (EDAIY) by a variable-length glycine linker.[37] We also prepared control peptides in which the pTyr was replaced by Phe to eliminate SH2 binding (Table 1). These experiments showed that the presence of the SH2 binding sequence increases substrate phosphorylation (Figure 5A). For enhanced phosphorylation to occur, there must be a minimum distance of approximately seven amino acids between the SH2 binding sequence and the phosphorylatable tyrosine (Table 1). Kinetic experiments

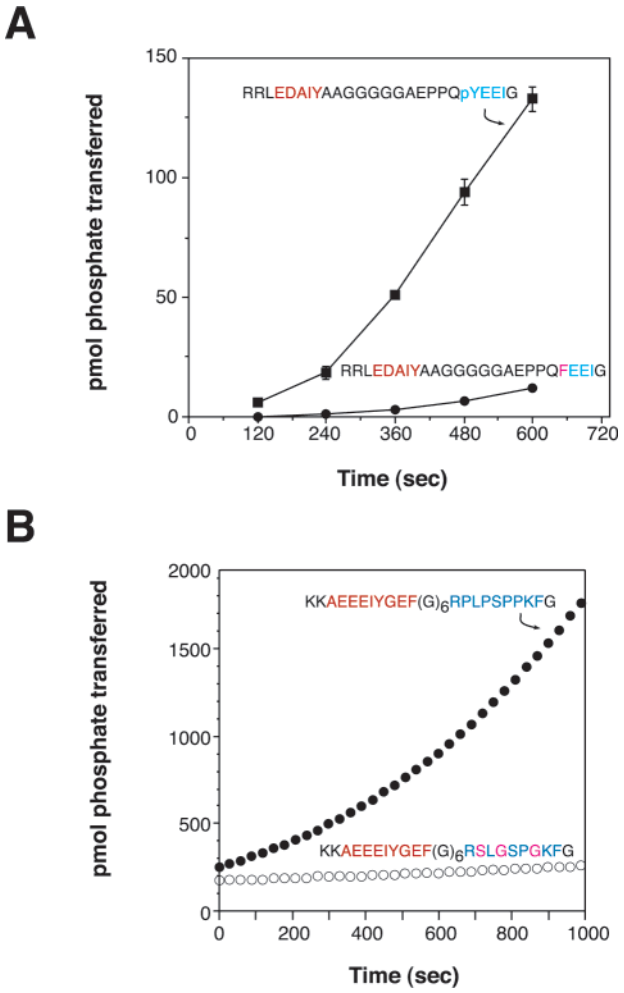

FIGURE 5. Enhanced phosphorylation of substrates containing SH2 ligands (panel A) and SH3 ligands (panel B) by Hck. The sequences of the peptides are given above the curves. Red type indicates the substrate sequence, and blue type indicates the SH3 or SH2 ligand sequence. Residues changed for control peptides are indicated in magenta. The rates of phosphorylation were determined using the phosphocellulose paper assay in panel A[37] and with the spectrophotometric assay in panel B.[42]

showed that the pTyr-containing peptides were phosphorylated with a 10-fold lower K_m than their Phe-containing counterparts.[37] These experiments are consistent with results that show the inclusion of an SH2 binding sequence improves the inhibitory potency of an active site-directed inhibitor.[41] In a parallel set of experiments, we showed that the addition of an SH3 domain ligand to a substrate increases the efficiency of its phosphorylation by Hck (Figure 5B). The SH3 domain ligand promotes kinase activation and also decreases the K_m values for such substrates.[42]

These experiments confirm a basic principle of processive phosphorylation by Src kinases: the presence of an SH3/SH2 ligand on a substrate improves its phosphorylation. However, as depicted in Figure 4, multisite phosphorylation could proceed by either a processive or a distributive mechanism, and the experiments described above do not distinguish between these possibilities. To test more rigorously for processive phosphorylation, we

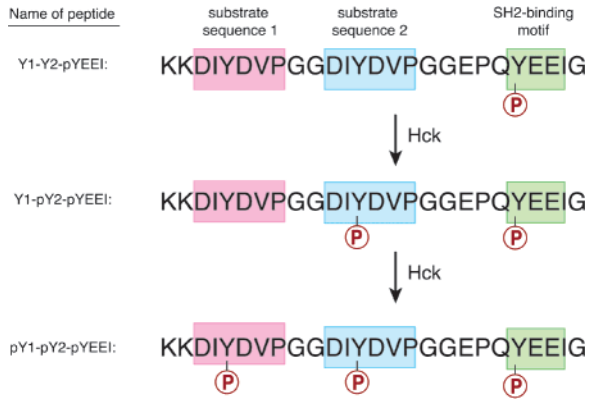

FIGURE 6. Peptide model for processive phosphorylation by Src. The synthetic peptide contains the SH2 binding sequence pYEEI and two copies of a substrate sequence derived from Cas. Substrate sequence 2 is phosphorylated first by Hck,[42] followed by substrate sequence 1, as pictured. In this figure, phosphotyrosine is indicated with a red circle.

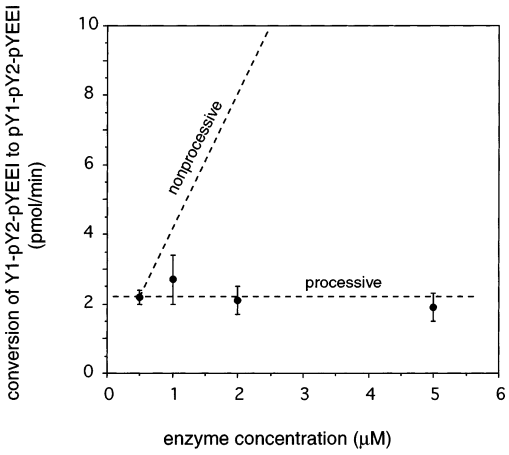

FIGURE 7. Phosphorylation of the model substrate (pictured in Figure 6) follows a processive mechanism.[42] The peptide was incubated with Hck and [^{32}P]-labeled ATP in a pulse reaction to allow phosphorylation of substrate sequence 2. This was followed by a chase reaction carried out in the presence of excess unlabeled ATP. The rate of conversion to the final product was measured by HPLC and scintillation counting. The data points are shown in closed circles (with standard deviation), and the predictions for processive and nonprocessive mechanisms are shown by dashed lines.

created a model peptide with multiple phosphorylation sites (Figure 6). This peptide contains the high-affinity SH2 binding sequence pYEEI at the C-terminus, and two substrate sequences derived from phosphorylation sites on p130^{Cas}. Using this peptide, we were able to follow the production of all intermediate phosphorylated species during reactions with the Src family kinase Hck. First, we addressed the order of phosphorylation of the two N-terminal tyrosine residues. We incubated the substrate with Hck and ATP and analyzed the reaction products by HPLC. The results showed that the "middle" tyrosine (substrate sequence 2 in Figure 6) is strongly favored for the first phosphorylation event.[42] At later time points, this intermediate was converted to the triply phosphorylated peptide (pY1−pY2−pYEEI).

Ordered phosphorylation is consistent with a processive mechanism, but the true hallmark of processive phosphorylation is that phosphorylation of all substrate motifs requires only one interaction between enzyme and substrate. To test for processive phosphorylation, we carried out pulse-chase experiments using radiolabeled ATP. We first incubated Hck and the peptide substrate with [γ-^{32}P]ATP under conditions that generated the doubly phosphorylated intermediate (Y1−pY2−pYEEI). Next, we carried out "chase" reactions with unlabeled ATP and measured the rate of conversion of intermediate Y1−pY2−pYEEI to the final product pY1−pY2−pYEEI. We performed these reactions at different concentrations of Hck, because the processive and distributive mechanisms make different predictions about the dependence on enzyme concentration. Since enzyme and substrate remain bound in a processive mechanism, the rate of progression should be independent of enzyme concentration. In contrast, in a distributive (nonprocessive) mechanism in which each phosphorylation event depends on enzyme−substrate binding (Figure 4), the rate should depend on enzyme concentration. The results, shown in Figure 7, indicate that conversion from intermediate to final product was independent of enzyme concentration, consistent with a processive mechanism.[42] In a separate set of experiments, we showed that production of the fully phosphorylated form of the peptide follows classical Michaelian kinetics. These results are also indicative of a processive mechanism.[42]

Our results with synthetic peptides show that Src family kinases have the capability of operating in the processive mode. Is this the case for intact proteins as well? A Src substrate that is believed to be phosphorylated processively is p130^{Cas}, a protein found mainly in focal adhesions.[33] Cas is a large, multifunctional protein that was originally identified because it is heavily tyrosine-phosphorylated in cells transformed by the v-Src or v-Crk oncogenes.[43] The central "substrate domain" of Cas contains 15 potential phosphorylation sites with the consensus YXXP (Figure 8). At the C-terminus, Cas contains a Src SH2 binding sequence (pYDYV) and a Pro-rich sequence that interacts with the SH3 domains of Src kinases.[32,38] Mutations in this region result in impaired phosphorylation of Cas in vivo.[32]

We have studied the mechanism of processive phosphorylation of Cas using pulse-chase experiments similar to those described above for the peptides. Purified Cas and Src were incubated with [γ-^{32}P]ATP for a brief pulse period, followed by a chase with an excess of unlabeled ATP. These studies showed that ^{32}P-containing Cas became progressively phosphorylated with unlabeled ATP and migrated with a higher apparent molecular weight (Figure 8).[44] These results imply that multiple sites are phosphorylated by Src under these conditions. To distinguish between processive and nonprocessive mechanisms, we carried out experiments at different substrate and enzyme concentrations, as described above for the peptides. The results were most consistent with a processive mechanism.[44] In contrast, the Cas mutant lacking the SH3 binding site (CasPPX) was phosphorylated poorly by Src,

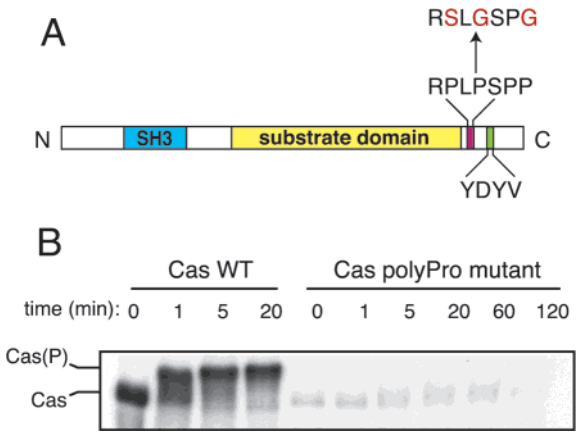

FIGURE 8. Phosphorylation of Cas. Top: the domain structure of Cas. The SH3- and SH2-binding sequences at the C-terminus of Cas are indicated. The mutated SH3 binding sequence is shown above the figure. Bottom: a pulse-chase reaction shows that Src phosphorylates Cas at multiple sites. The polyPro mutant form of Cas shows reduced phosphorylation.[44]

and the results were consistent with a nonprocessive mechanism (Figure 8). Thus, an initial interaction between the SH3 domain of Src and the C-terminal polyproline sequence of Cas appears to "prime" the reaction for processive phosphorylation. Polyproline sequences in other hyperphosphorylated proteins may play analogous roles.

Implications for Substrate Specificity

As described above, tyrosine kinase specificity is governed by the intrinsic specificity of the catalytic domain and by the spatial and temporal organization of the kinase signaling pathway. Processive phosphorylation of proteins such as Cas illustrates how these effects combine to target important cellular substrates. Molecules such as Cas are preferred substrates for nonreceptor tyrosine kinases because they (i) contain SH3/SH2 ligand sequences and can activate the kinases at the right time and place, (ii) contain good substrate sequences, and (iii) remain complexed with the kinases through multiple rounds of phosphorylation (Figure 3). This coupling of activation and substrate recognition ensures that Src will only be active when it is near the appropriate cellular targets.

This process also indicates that in many cases the activation of nonreceptor tyrosine kinases is accomplished by relocalization to a cellular site containing high concentrations of potential substrates with SH2 or SH3 domain ligands. Activation of Src-family kinases is often accompanied by changes in subcellular localization.[7] For example, autophosphorylation of the focal adhesion kinase FAK creates a binding site for the SH2 domain of Src. Src is relocalized to focal adhesions, where it phosphorylates a number of focal adhesion proteins.[34,45] Recognition of these proteins (and desorption from FAK) is presumably driven by their high affinity for Src's SH3 and SH2 domains, and by their abundance. Many important Src family kinase substrates contain polyproline sequences that bind (or have the potential to bind) Src SH3 domains.[7] This suggests that novel Src substrates could be identified as SH3 binding proteins.

We adopted this strategy to search for proteins that interact with the Src-family kinase Hck.[46] Hck is expressed predominantly in myeloid cells such as monocytes,[8] where it has been implicated in a wide variety of signaling pathways. However, relatively few substrates or effectors of Hck have been identified. In our earlier work on Hck, we showed that an HIV accessory protein, Nef, was able to activate Hck by SH3 domain displacement.[24] In our recent experiments, we sought to identify the normal cellular proteins that activate Hck in this manner. We activated the monocytic cell line U937 with phorbol-12-myristate-13-acetate (PMA) to induce the cells to differentiate to a more macrophage-like phenotype, and then we used immobilized Hck SH3 domain to isolate binding proteins from U937 lysates. Proteins that bound specifically to the SH3 domain were identified by mass spectrometry in collaboration with Dr. Roland Annan. This study identified proteins known to bind to Hck (Cbl and Sam68), proteins known to interact with other Src family kinases (Sos, ASAP1, hnRNP K, CMS/CD2-associated protein, p85, and SLP-76), and a large number of proteins that have not previously been reported to interact with Src kinases.[46] We confirmed several of these interactions by direct in vitro binding assays and by coexpression in mammalian cells. One of the binding partners for the SH3 domain of Hck that we identified in our screen was the mammalian ortholog of the *C. elegans* gene, *ced*-12.[47] Mammalian CED-12 (ELMO1) has been shown to be involved in phagocytosis and cell migration.[47] Because Hck is strongly implicated in phagocytosis, ELMO1 may be an important downstream component of this Hck signaling pathway.

Coda: Are Tyrosine Kinase Catalytic Domains Important in Specificity?

The results discussed above confirm the importance of the Src SH3 domain in substrate targeting. Broadly speaking, it is clear that the catalytic domains of tyrosine kinases do not operate in isolation but, instead, are dependent on associations with noncatalytic regions (such as SH3 and SH2 domains) to localize the kinases near good substrates. This leads to the question: do tyrosine kinase catalytic domains play any role in selective signaling? Or, to take an extreme version of the contrasting view, do the catalytic domains act as promiscuous catalysts, phosphorylating whatever proteins are presented by the regulatory regions? (Of course, "promiscuity" is a relative term. To adapt an infamous definition of human promiscuity, any kinase might be classified as "promiscuous" if it phosphorylates more proteins than some other kinase.)

For some tyrosine kinases, the intrinsic specificity of the catalytic domain has been demonstrated to be important for faithful signal transduction. For example, a Met918-to-Thr mutation that alters substrate specificity of the RET receptor tyrosine kinase leads to multiple endocrine neoplasia type 2B.[19] Csk, the kinase that phos-

phorylates the C-terminus of Src family kinases, does not require its SH3 and SH2 domains to recognize Src.[48] For most tyrosine kinases, however, the catalytic domain must act in concert with other domains to select its substrates. Phosphorylation of Crk by Abl tyrosine kinase depends primarily on an interaction between the SH3 domain of Crk and a proline-rich region on Abl,[49] but the efficiency is increased by proper catalytic domain recognition.[50] The SH2 domains of Abl family kinases can also dictate substrate specificity[36] and are responsible for multisite phosphorylation of substrates.[51,52] In the case of the Btk tyrosine kinase, which lacks SH3 and SH2 domains, the tandem PH and Tec homology domains are required for recognition of protein substrates.[53] Each family of nonreceptor tyrosine kinases displays a unique array of noncatalytic domains (e.g., PH and JH domains, Pro-rich sequences, integrin, and actin-binding motifs). Results from the Src family suggest that these noncatalytic domains will be critical determinants of substrate recognition in most nonreceptor tyrosine kinases.

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