Research Capsule

Signal Transduction by the SRC Family of Tyrosine Protein Kinases in Hemopoietic Cells

Joseph B. Bolen

Signal Transduction Laboratory, Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000

Signal transduction collectively encompasses the diverse biochemical reactions involved in modulating programmed cellular responses to external and internal stimuli. Among the cellular enzymes involved in this complex process, the TPKs appear to play key roles in mediating signaling events that aid in the regulation of cell growth and differentiation. The TPKs can be divided into two major groups on the basis of their predicted structures. The first TPK group contains those that possess extracellular domains which generally function to bind peptide hormones. Examples of TPKs included in this group are the receptors for epidermal growth factor and platelet-derived growth factor (reviewed in Ref. 1). The second TPK group contains those that lack extracellular domains and are categorized as the nonreceptor TPKs. Members of this group include the src family of TPKs as well as the members of the les/fps and abl gene families (reviewed in Refs. 2–4). The basic physiological function of the receptor TPKs to transduce peptide hormone binding into alterations in cellular metabolism is well established. However, only within the last 3 years has significant progress been made toward understanding potential physiological functions of at least a few members of the src family of nonreceptor TPKs. Normal physiological regulators of the les/fps and abl TPKs have not yet been reported.

The purpose of this review is to briefly discuss the recent evidence that implicates several members of the src-related TPKs as signaling components in cells of hemopoietic origin. A note of caution—this is a dynamic area of research, and the models presented in most cases represent working hypotheses that are destined, at best, for significant clarification.

The src Family of Tyrosine Protein Kinases

Expression and Potential Physiological Regulation. The src family currently consists of the eight members listed in Fig. 1. The first four family members listed, c-src, c-yes, Lyn, and Lyn, are expressed in a variety of cell and tissue types, with most cells expressing more than one family member. The lck, hck, c-fgr, and blk genes display a more limited pattern of expression and are found primarily in different types of cells of hemopoietic origin.

The predicted relationship between domains of a generic src family member is shown in Fig. 2A (for more detailed reviews, see Refs. 2 and 4). The different domains have been defined through genetic analysis of the c-src gene, although comparison of sequences indicates that all src family members are similarly organized. The sequences required for myristylation and stable membrane association are located at the amino terminus of the enzyme with the myristate covalently bound to the glycine residue at position 2 (reviewed in Ref. 5). Immediately adjacent to this domain is the roughly 50–80-amino acid “unique” region, where the sequences between src family members diverge most dramatically. It is this domain that likely governs the specific interactions between src family TPKs and other cellular proteins. The next approximately 160 amino acids contain the SH3 and SH2 (SH for src homology) regions that define two sequence motifs which are shared with other nonreceptor TPKs as well as with several additional cellular proteins (6–10). Genetic analysis suggests that the SH3 and SH2 sequences are important for regulating src family TPK activity (2, 4), although these regions may possess additional distinguishable functions (6, 11–13). The SH3 domain may aid in the localization of these enzymes to cytoskeletal components adjacent to the plasma membrane (14), whereas the SH2 domain represents a phosphotyrosine binding region (15). The majority of the carboxy-terminal half of src TPKs represents the catalytic or kinase domain, which is the region of highest src family homology (this would be the src homology 1 or SH1 domain). Included in this domain are the sites of ATP-divalent cation binding, autophosphorylation, and the “regulatory” tyrosine phosphorylation site near the carboxy terminus.

We do not currently have sufficient information on the participation of src family members in defined metabolic pathways to draw conclusions with regard to how versatile the physiological control of these enzymes may be. It is possible that the activity of src family members can be regulated by a variety of mechanisms which could differ from cell to cell and vary within the same cell during different programmed responses. One model for the regulation of src family TPK enzyme activity in normal cells is shown in Fig. 2B. This model is based upon previous genetic studies of various TPKs as well as our limited experiences with CD4-p56 in T-cells, B-cell antigen receptor-p56 in B-lymphocytes, and FcRγ-p56 in basophils/mast cells (see below). The natural state of the membrane-associated enzyme (bound to some transmembrane surface receptor or protein complex) would be one in which low basal TPK activity is maintained through the interaction of the carboxy-terminal tyrosine phosphate with the enzyme’s own SH2/SH3 domain (15). Engagement of the cell sur-
### Cell Type

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<th>Src family member</th>
<th>Molecular weight (kD)</th>
<th>T-cell</th>
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<th>NK-cell</th>
<th>Monocyte</th>
<th>Granulocyte</th>
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Fig. 1. Relative expression of src family members in selected cells of hemopoietic origin. The expression pattern and relative abundance of a given family member was determined by immune-complex tyrosine protein kinase assays. *, alternative splicing of mRNA or alternative start sites yielding multiple isozymes (for details, see reviews in Refs. 2–4).

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face protein(s) associated with the enzyme would require receptor dimerization, thereby coapproximating the associated nonreceptor TPKs. In theory, such dimers could be homodimers or dimers/multimers of heterologous receptors bound to other src family enzymes or potentially other TPKs which allow TPK-TPK interaction. Analogous to the receptor TPKs, coapproximation would stimulate intermolecular tyrosine autophosphorylation, resulting in a conformational change and thereby increasing the TPK activity of the enzyme and allowing for selected substrates to bind to the activated enzyme. At this point, the substrates (or other nonsubstrate proteins) could bind to unique sequences or to SH2 or SH3 sequences or could recognize the autophosphorylation site. Events that would then turn off this activated enzyme could be the internalization of the enzyme-receptor complex or the actions of a phosphotyrosyl phosphatase dephosphorylating the phosphotyrosine at the autophosphorylation site. Once autophosphorylation has occurred, the state of phosphorylation at the carboxy terminus would be irrelevant until the autophosphorylation was removed. At this point, the carboxy-terminal regulatory site would be required to be in the phosphorylated state in order to once again associate with the SH2/SH3 region. As this regulatory site of phosphorylation is likely not to be an autophosphorylation site, a second TPK is required for this reaction (16).  

An important prediction of this model is that activation would not necessitate the dephosphorylation of the "regulatory" tyrosine prior to altering the activity of the enzyme. There is little current physiological evidence that phosphotyrosyl phosphatases normally act to turn on receptor-associated src family TPKs during ligand-mediated activation, although it is important to note that accurate assessment of the phosphorylation state of the responsive enzymes is difficult. However, mitosis-spe-

cific tyrosine 527 dephosphorylation of pp60<sup>c-src</sup> has been reported (reviewed in Ref. 17), raising the possibility that phosphotyrosyl phosphatases might function to regulate src family TPKs in a cell cycle-specific manner independent of surface receptor status. As the manner through which the activity of these phosphatases is regulated is not understood, it is possible that examples of receptor-mediated phosphotyrosyl phosphatase activation of nonreceptor TPKs will be forthcoming.

### Involvement in Hemopoietic Signal Transduction

Currently, the best characterized example of a src family member being involved in physiological signal transduction is the product of the lck gene in T-lymphocytes. However, recent evidence suggests that the product of the lyn gene (referred to as either p59<sup>hm</sup> or p60<sup>hm</sup>) functions as a mediator of TCR signaling (18) and forms a complex with TCR subunits (19). Furthermore, other members of the src family have been found to associate with several other cell surface receptors (see below). These emerging observations imply that additional members of the src family of TPKs will soon share the lymphocyte signal transduction spotlight with p56<sup>ck</sup>.

The product of the <i>lck</i> gene, p56<sup>ck</sup>, forms a noncovalent complex with the surface glycoproteins CD4 and CD8 (reviewed in Refs. 20 and 21) (Fig. 3). This interaction involves sequences (roughly amino acids 10–32) in the unique amino-terminal region of p56<sup>ck</sup> and a small linear domain in the cytoplasmic region of CD4 and CD8α. The observations that complex integrity requires free sulphydryls and that site-specific mutation of specific lck or CD4/CD8 cysteines abolishes the capacity for complex formation (22, 23) have been interpreted to suggest that the complex may rely on bimolecular binding to a metal ion. Physiologically, the state of serine/threonine phosphorylation of CD4, but interestingly not of CD8α, appears to modulate and/or mediate CD4-p56<sup>ck</sup> complex stoichiometry (24).

The lck TPK is apparently capable of participating in T-cell signal transduction pathways mediated through the

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1. J. S. Brugge, personal communication.
TCR as well as through the high affinity IL-2 receptor (Fig. 3). Whereas the CD4-p56\textsuperscript{kk} complex in T-cells is capable of TCR-independent signaling when properly manipulated (25), the more realistic physiological role that this complex plays is that of a transient component of the TCR (reviewed in Refs. 21 and 26). Indeed, recent evidence indicates that the CD4-p56\textsuperscript{kk} complex should be functionally considered as an important coreceptor in TCR-mediated signal transduction (27, 28). Additionally, this complex may play an important role in T-cell development in the thymus (29, 30). The activation of p56\textsuperscript{kk} TPK activity following IL-2 addition to T-cells suggests that p56\textsuperscript{kk} may aid in the signaling from the IL-2 receptor (31). In this case, the response of p56\textsuperscript{kk} appears to be independent of interactions with CD4 or CD8. As there is no direct evidence that p56\textsuperscript{kk} is capable of direct association with either the \(\alpha\)- or \(\beta\)-subunit of the IL-2 receptor, the mechanism underlying the observed p56\textsuperscript{kk} enzyme activation is obscure. Current evidence suggests that a TPK distinct from p56\textsuperscript{kk} may, in fact, associate with the \(\beta\)-subunit of the IL-2 receptor, although the identity of this kinase is not known.\textsuperscript{4} The observed functional relationship between IL-2 receptor signaling and p56\textsuperscript{kk} may, however, provide an explanation of why p56\textsuperscript{kk} is also expressed at high levels in CD4\text--CD8\text-- natural killer cells, which are similar to T-cells in that they require IL-2 for growth.

The product of the \textit{lyn} gene also has been implicated in T-cell signal transduction events. In T-cells, p60\textsuperscript{pp} has been shown to communoprecipitate with TCR components (19). The interaction between p60\textsuperscript{pp} and TCR is different from that described above for p56\textsuperscript{kk}-CD4/CD8, since communoprecipitation of p60\textsuperscript{pp} by anti-TCR antibodies requires that the cells be solubilized with mild detergents such as digitonin (19). This implies that the TCR-p60\textsuperscript{pp} interaction is less stable than the p56\textsuperscript{kk}-CD4 bimolecular complex and may be an indication that the p60\textsuperscript{pp}-TCR complex requires the interactions of several different proteins. Although a direct functional response by p60\textsuperscript{pp} to TCR engagement has not been documented, recent evidence suggests that transgenic overexpression of enzymatically active p60\textsuperscript{pp} in thymocytes renders the TCR hyperresponsive, whereas transgenic expression of an enzymatically inactive form of p60\textsuperscript{pp} results in inhibition of TCR-mediated signaling (18). Interestingly, expression of pp60\textsuperscript{pp} (a mutated, constitutively hyperactive version of pp60\textsuperscript{pp} in T-cells also results in hyperresponsive TCR signaling (32), even though pp60\textsuperscript{pp} is not normally expressed in T-cells or thymocytes. Similar to the situation in T-cells, signal transduction mediated through the B-cell antigen receptor also appears to involve members of the src family of TPKs (Fig. 4). Normal murine splenic B-cells express three members of the src family: p56\textsuperscript{bk}, p56\textsuperscript{hm}, and p60\textsuperscript{pp} (33). Of these kinases, the product of the \textit{blk} gene is expressed exclusively in B-cells (34), thereby suggesting a potentially cell type-specific function for this enzyme. Evidence to support the view that TPKs are involved in B-cell receptor signaling stems from reports that engagement of surface

\textsuperscript{4} I. D. Horak, personal communication.
immunoglobulin results in rapid tyrosine phosphorylation of B-cell proteins (35, 36), including components of the immunoglobulin receptor itself (37). The initial evidence implicating src TPKs in this process was the observation that p56

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The FccRI receptor expressed on the surface of basophils and mast cells plays a critical role in mediating immediate allergic reactions. The function of this multicomponent receptor is to specifically bind the Fc portion of IgE and to transmit signals following binding of an allergen to the surface-bound IgE (for a review, see Ref. 39) (Fig. 5). Signal transduction mediated by the FccRI receptor is thought to be a consequence of receptor-receptor interactions induced by multivalent allergen binding and results in the release of histamine and proteases as well as the synthesis of prostaglandins and leukotrienes. As with the other receptor systems described above, phosphorylation of cellular proteins on tyrosine residues appears to be an immediate consequence of allergen binding (40). In the rat basophilic leukemia cell model system, antibodies directed against the α- or β-subunits of the FccRI coimmunoprecipitate p56

Furthermore, the TPK activity of the receptor-

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is elevated immediately following receptor engagement by antigen or antibodies. The mouse mast cell line PT-18 is another cell type that expresses FccRI and has been used as a model system for studying receptor signaling. In these cells, tyrosine phosphorylation is also observed as a consequence of receptor engagement, but no p56

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family, pp60cyc, p62C-src, and p56lck, have recently been found to tightly associate with the platelet membrane glycoprotein CD36. Furthermore, similar complexes have been found in other CD36-expressing nucleated cells. Given that anti-CD36 antibodies can induce many of the physiological changes that occur in platelet activation, including aggregation and secretion, these observations imply that CD36 may represent as yet another signal-transducing surface receptor which couples to multiple members of the src family of TPKs.

Perspective
The diverse nature of the receptors to which members (in some cases, multiple members) of the src family of TPKs have been either structurally and/or functionally associated with the src family. The Src model of p56lck interacting with CD4 and CD8 may be the exception rather than the rule. This group of TPKs may more commonly recognize tertiary or quaternary structures of multicomponent cell surface receptors rather than individual linear amino acid sequences or metal-binding elements. Given this possibility, it is likely that many more receptor-src family interactions will be identified.

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References