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# Src Protein Tyrosine Kinases in Stress Responses

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**A role of Src family protein tyrosine kinases (SFK) as mediators of receptor-ligand initiated responses is well established. Well documented, but less well understood is the role of SFK in cellular reaction to stresses. Evidence from the wide variety of experimental systems indicates that SFK mediate responses to all major classes of stress, including oxidation, DNA damage, mechanical impacts, and protein denaturing. SFK may be activated by stresses directly or via regulatory circuits whose identity is not yet fully understood. Depending on the cell type and the nature of activating stimulus, SFK may activate known downstream signaling cascades leading to cell survival, proliferation, cytoskeletal rearrangement, and apoptosis; the identity of these cascades is discussed. As in the case of receptor-initiated signaling, roles of individual SFK in various stress responses may be redundant or non-redundant. Although signals generated by different stresses are generally transduced via distinct SFK pathways, these pathways may overlap or exhibit crosstalk. In some cell types stress-induced activation of SFK promotes survival and inhibits apoptosis, whereas the opposite may be true for other cell types. Stress responses constitute a new and rapidly developing area of SFK-mediated signaling.**

Ligand-induced activation of receptor tyrosine kinases and cytosolic protein tyrosine kinases (PTK) leads to a variety of cellular responses via the activation or recruitment of a limited repertoire of signal transduction molecules. Excessive or deficient tyrosine kinase activity may lead to disease states such as cancer or growth defects. As the first oncogene described as well as the first PTK identified biochemically and genetically, Src serves as the prototype for a family of eight mammalian kinases. Src family kinases (SFK) are expressed widely, although their predominant expression occurs in blood cells. Typically, a cell expresses several members of the SFK. A wide range of ligands result in increased SFK activity. SFK may mediate signaling from transmembrane receptors lacking tyrosine kinase activity, or further amplify tyrosine phosphorylation events that follow engagement of receptor tyrosine kinases. Cellular responses that depend on SFK include cell cycle progression, survival, differentiation, adhesion, and migration. Altogether, these observations establish SFK as a critical signaling mediator for ligand-induced responses. Well documented, but less well understood is the role of SFK in mediating cellular reaction to stresses, be it oxidation, DNA damage, mechanical impacts, or protein denaturing. This review will address the activation of SFK by different stressors and the contribution of SFK to cellular stress responses.

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## Src Family Kinases and Their Structure

SFK emerge phylogenetically in vertebrates, therefore their functions are related to higher levels of metazoan organization. There are eight members of the Src family in mammalian cells: c-Src, c-Fgr, c-Yes, Fyn, Lck, Blk, Lyn, and Hck. Fyn and Lck are found predominantly in T cells, Lyn and Blk in B cells, and Lyn, Hck, and c-Fgr in non-lymphoid hematopoietic cells (Corey and Anderson, 1999). Src, Fyn, and Yes may also be found in endodermal and ectodermal tissues. Each consists of an N-terminal unique domain followed by two protein-binding domains found in a variety of signaling molecules, SH3 and SH2, and the C-terminal catalytic domain. Within the catalytic domain there is a positive autophosphorylation site (SrcY416) and a negative regulatory phosphorylation site (SrcY-527). The regulatory steps in SFK activation have been identified biochemically and structurally (Xu et al., 1999). In inactive state, a cytosolic PTK Csk (for C-terminal Src kinase) phosphorylates the C-terminal tyrosine in Src, which then engages into intramolecular bond with the SH2 domain. Furthermore, a polyproline-helix found between the SH2 domain and the catalytic domain associates intramolecularly with Src's SH3

domain. These two intra-molecular interactions force SFK into a folded and inactive state. Upon stimulation, tyrosine phosphatases (e.g. CD45 or SHP-1) hydrolyze the phosphate group at the negative regulatory site. The Src kinase unfolds, phosphorylates itself, and becomes "activated". Activation may also occur by binding other polyproline- and phosphotyrosine-containing proteins to the SH3 and SH2 domains of SFK (Bjorge et al., 2000).

## Src Kinases in Responses to Oxidative Stress

Oxidants include reactive oxygen species, heavy metals and other agents. Reactive oxygen species are generated endogenously by physiological oxidative processes in cells or exogenously by exposure to peroxides, ultraviolet (UV), or ionizing radiation (IR). Oxidants damage multiple molecules and structures in cells. Oxidative stresses activate Lck in T cells (Lander et al., 1992; Nakamura et al., 1993; Nakamura et al., 1996), Lyn and Hck in myeloid and breast cancer cells (Brumell et al., 1996; Qin et al., 1996; Robbins et al., 2000; Bezombes et al., 2001), Fyn in fibroblasts (Abe et al., 2000), and unspecified SFK in brain (Pei et al., 2000), heart (Ping et al., 1999; Takeishi et al., 1999), endothelium (Aoki et al., 1999), erythrocytes and neurons (Mallozzi et al., 1999).

Several observations indicate that oxidative agents may directly activate SFK. First, SFK activation is the earliest detectable response (Devary et al., 1992). Second, *in vitro* kinase activity of SFK is increased by treatment with $H_2O_2$ (Barchowsky et al., 1995) and peroxynitrite (Mallozzi et al., 1999). Third, activation of Src by $Hg^{2+}$, a thiol oxidative agent, overcomes the inhibitory effect of Y527 phosphorylation (Pu et al., 1996). SFK may be activated by oxidants indirectly, involving disruption of negative regulatory mechanisms that normally keep these kinases inactive (Brumell et al., 1996). One possible indirect mechanism of SFK activation could be the inhibition by oxidants of protein tyrosine phosphatases resulting in increased phosphorylation at the SFK autophosphorylation site (Heffetz et al., 1990; Whisler et al., 1995; Yurchak et al., 1996). Activation of Lck by $H_2O_2$ or the oxidant diamide increases tyrosine phosphorylation at both positive (Y394) and negative (Y505) regulatory sites (Nakamura et al., 1993; Hardwick and Sefton, 1997). Phosphorylation at Y394 may occur in the absence of Lck catalytic activity, implicating another PTK (Hardwick and Sefton, 1995). Irreversible activation of Lyn, but not Src or Hck, by peroxynitrite may proceed via generation of nitrotyrosines in SH2-binding regulatory proteins. These phosphotyrosine-mimicking nitrotyrosines may allow SH2-mediated docking of regulatory proteins to Lyn, resulting in its activation (Mallozzi et al., 2001a). Src and Hck are activated by peroxynitrite via reversible cysteine redox changes (Mallozzi et al., 2001b). Both Src and epidermal growth factor receptor (EGFR) are required for responses to $H_2O_2$ in endothelial cells, indicating possible role of cell surface receptors in SFK activation (Chen et al., 2001). Activation by $H_2O_2$ of membrane anchorage-defective, cytosolic Lck (Yurchak et al., 1996) argues, however, against the universal role of receptors in this process. In rabbit heart, activation of Src and Lck following ischemia and reperfusion is presumably mediated by the protein kinase C-epsilon PKC-ε, (Ping et al., 1999). Activation of Src in cardiomyocytes by both abnormally high and abnormally low oxygen concentrations suggests redox sensors acting upstream of Src (Aikawa et al., 1997). Activation of SFK by oxidants *in vivo* could be a complex process. For example, in pancreatic tumors, oxidative stress elevates both nitrotyrosine and phosphotyrosine content of Src, increases its tyrosine kinase activity, and alters its substrate binding affinity (MacMillan-Crow et al., 2000).

SFK trigger multiple pathways in the oxidative stress response. One involves phospholipase C (PLC) and PKC. In mouse embryonic fibroblasts (MEFs) treated with $H_2O_2$, PLCγ1 becomes rapidly tyrosine-phosphorylated. SFK-specific inhibitors prevent this phosphorylation, indicating a role for SFK (Wang et al., 2001). In *Xenopus* oocytes, $H_2O_2$-induced phosphorylation of PLCγ is also prevented by an SFK inhibitor (Sato et al., 2001). It is not clear whether SFK directly phosphorylate PLCγ or Src activates a downstream kinase that phosphorylates PLCγ. In chicken DT40 cells, oxidant-induced phosphorylation of PLCγ2 requires the non-SFK Syk (Ding et al., 2000). PLCγ1 gene knockout in MEFs results in hypersensitivity to hydrogen peroxide (Wang et al., 2001), whereas overexpression of PLCβ1 in NIH3T3 cells protects them from oxidant toxicity (Lee et al., 2000), underscoring the role of PLC in cell survival following oxidative stress. Tyrosine phosphorylation activates PLCγ isoforms; the active PLC is recruited to the membrane where it cleaves phosphatydylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (DAG), two molecules that act as secondary messengers. $IP_3$ stimulates the release of internal $Ca^{2+}$. Calcium release activates calcium-dependent signal transducers and enzymes that may have a role in cell survival following stress (Servitja et al., 2000; Hook and Means, 2001), and causes changes in mitochondrial metabolism, which may have a role in apoptosis (Harman and Maxwell, 1995; McConkey and Orrenius, 1996; Suzuki et al., 1997; Chakraborti et al., 1999). DAG activates several isoforms of PKC, a serine-threonine protein kinase involved in multiple cellular processes (Nishizuka, 1995). Although DAG is presumed to be the most important physiological stimulus for PKC isoforms, PKC can be activated directly by Src-mediated tyrosine phosphorylation, by-passing the PLCγ step (Konishi et al., 1997). PKC can be also activated by $Ca^{2+}$ (Rasmussen et al., 1984), presumably via Src-PLCγ-$IP_3$ loop. In some cell lines activation of PKC by

oxidative stress is independent of SFK, suggesting the existence of alternative regulatory mechanisms (Whisler et al., 1994). Activation of PKC may lead to diverse cellular responses including survival, proliferation, and apoptosis. One of the downstream effectors of PKC in the oxidative stress response in mouse 3T3 fibroblasts and COS-7 cells is the serine protein kinase PKD. Pharmacological data show that activation of PKD by $H_2O_2$ depends completely on PKC and partially on SFK and PLC (Waldron and Rozengurt, 2000). PKC regulates fundamental cellular processes via signaling pathways that are not well understood, in part because multiple isoforms of this kinase may either play redundant roles or trigger distinct pathways leading to physiologically opposite responses (reviewed in Dempsey et al., 2000; Musashi et al., 2000).

Other SFK-dependent pathways of oxidative stress involve MAP kinase cascades. Oxidants activate all three families of MAP kinases, p38 MAPK, ERK, and JNK (Seko et al., 1997; Schieke et al., 1999). Depending on the family, cell type, and the nature of oxidative stress, activation of MAP kinases may or may not require SFK. Activation of ERK requires Src (Abe et al., 1997) or other SFK (Aikawa et al., 1997). SFK-dependent activation of ERK1/2 by $H_2O_2$ in cardiomyocytes is not affected by PKC inhibitors or $Ca^{2+}$ chelators, but is abolished by inhibition of Ras or Raf, implicating these upstream activators of ERK (Aikawa et al., 1997). Ras and Raf have been also shown to mediate SFK-dependent oxidative signaling in other cell types (Devary et al., 1992). Upstream of Ras, the ERK pathway could be regulated by heterotrimeric G proteins $G_i$ and $G_o$. $H_2O_2$ appears to modify $G_\alpha$ with subsequent $G_{\beta\gamma}$ dissociation. Free $G_{\beta\gamma}$ activates the ERK cascade in Src- and PI3-kinase-dependent manner (Nishida et al., 2000). The downstream target of ERK in oxidative stress response could be ribosomal S6 kinase p90 RSK (Takeishi et al., 1999). Similarly to ERK, JNK activation by oxidants may require SFK. Activation of JNK by $H_2O_2$ in endothelial cells is decreased by tyrosine kinase inhibitors and dominant-negative Src, as well as EGFR antisense oligonucleotides (Chen et al., 2001). Activation of JNK in MCF-7 or IMR90 cells under hypoglycemia, a condition resulting in oxidative stress, is mediated by Lyn (Liu et al., 1997; Robbins et al., 2000). However, in MEFs derived from triple Src-Yes-Fyn knockout mice, activation of JNK by UV-induced oxidative stress is completely normal (Amdjadi and Sefton, 2000). The downstream targets of JNK in the oxidative stress response are transcription factors c-Jun and AP-1 (Chen et al., 2001). No role for SFK has been reported so far in activation of p38 MAPK by oxidative stresses. Signaling pathways downstream of MAP kinases have been recently reviewed by Pearson et al. (2001).

## Src Kinases in Response to DNA Damage

Genotoxic agents such as IR, UV, chemotherapeutic drugs and endogenous cellular processes such as respiration, transcription, or DNA replication may cause several types of DNA damage. This comprises double- and single-strand breaks, crosslinks, mismatches, and base modifications. To adequately respond to DNA damage, cells possess distinct detection and execution mechanisms specific for particular types of lesions (reviewed in Zhou and Elledge, 2000; Ronen and Glickman, 2001; Wood et al., 2001).

Two SFK members, Src and Lyn, have been shown to play a role in DNA damage signaling. An epithelial cell line transfected with v-Src becomes more resistant to DNA crosslinking drug cis-platin than vector-transfected cells. v-Src does not confer cross-resistance to other genotoxic agents; therefore it acts in a damage type-specific manner (Masumoto et al., 1999). This specificity is corroborated by unchanged resistance to IR of RAT-1 fibroblasts upon transfection with v-Src (Shimm et al., 1992). The cis-platin-resistant phenotype of v-Src does not depend on Ras, PI 3-kinase, or PKC (Masumoto et al., 1999). Src-dependent activation of the transcription factor AP-1 by cis-platin in RPMI-8322 melanoma cells does not require the Raf-MAP kinase pathway, whereas activation by oxidative stress following UV irradiation does (Rasmussen et al., 1984). These findings indicate a novel non-conventional pathway downstream of Src. Activation of JNK by the alkylating drug methyl methanesulfonate (MMS) is attenuated in Src-deficient MEFs, pointing to a possible role of Src as an activator of JNK in genotoxic response (Liu et al., 1996b). The mechanism of Src activation by cis-platin and MMS is not known.

Lyn is activated following treatment with genotoxic agents of different damage type specificities, including IR (Kharbanda et al., 1994a), alkylating drugs mitomycin C (Kharbanda et al., 1994b) and MMS (A.G., unpublished), nucleotide analog Ara-C (Yuan et al., 1995), and cis-platin (Singh and Sodhi, 1998). Mechanisms of this activation are not well understood. Activation of Lyn by IR does not occur in human lymphoblastoid cells derived from Ataxia Telangiectasia patients (Yan et al., 2000). These cells lack a functional ATM, a kinase believed to act as a sensor of double strand breaks (Zhou and Elledge, 2000). Accordingly, a signal for Lyn activation by IR is initiated by damaged DNA and proceeds through ATM. IR activates nuclear, but not cytoplasmic Lyn (Kharbanda et al., 1996), arguing for primary role of nuclear events. However, activation of Lyn by IR occurs normally in enucleated human B cell precursors and can be abrogated by an oxygen radical scavenger. This observation points to IR-generated oxygen radicals in the cytoplasm as activators of Lyn (Xiao et al., 1996). More studies are needed to resolve this controversy.

Several kinases have been identified as downstream

targets of Lyn in the DNA damage response. In the nucleus, DNA damage-activated Lyn binds to and inhibits two cyclin-dependent kinases, Cdc2 (Kharbanda et al., 1996) and Cdk2 (Yuan et al., 1996), which may be associated with DNA damage mitotic checkpoints. Lyn constitutively associates with and regulates the catalytic subunit of DNA-dependent protein kinase (Kumar et al., 1998). Lyn regulates JNK activation by various genotoxic agents via MEKK1-MKK7 pathway, providing connection with general stress responses (Yoshida et al., 2000). Association of Lyn leads to the inhibition of the protein tyrosine phosphatase SHP-1 (Yoshida et al., 1999) indicating a putative negative feedback loop. To date, no links have been established between Lyn and DNA repair enzymes.

**Src Kinases in Responses to Mechanical Stresses**

Within a living organism, cells are routinely subjected to mechanical impacts that may compromise cellular integrity or serve as stimuli that elicit physiological responses. An example of the latter is response to flow, pressure, and stretch in control of circulation by blood vessel cells (reviewed in Lehoux and Tedgui, 1998; Traub and Berk, 1998; Langille, 2001). Mechanical stress reactivity is not exclusively confined to the cardiovascular system; other cell types also respond to mechanical stimuli, suggesting general occurrence of these responses (reviewed in Banes et al., 1995). Osmotic shock response can be regarded as one of the mechanical stress responses because its primary driving force is change in cell volume (Lang et al., 1998).

Responses to mechanical perturbations occur via multiple signaling pathways (reviewed in Banes et al., 1995; Yamazaki et al., 1998). In rabbit aorta smooth muscle cells, SFK inhibitors partially prevent responses to stretch from intraluminal pressure (Birukov et al., 1997). In cultured endothelial cells of different species expression of dominant-negative Src (Jalali et al., 1998) or treatment with SFK inhibitors (Wittstein et al., 2000; Davis et al., 2001) significantly attenuate at least some aspects of response to fluid shear stress. Similarly, responses to elevated intraluminal pressure in rat mesenteric small arteries (Wesselman et al., 2001) and to stretch in human mesangial cells (Gruden et al., 1997) are attenuated or completely obliterated by SFK inhibitors. In human umbilical vein endothelial cells, Src activity is increased by fluid shear stress (Takahashi and Berk, 1996) and uniaxial cyclic stretch (Sokabe et al., 1997; Naruse et al., 1998). In guinea pig heart, Src is activated by pressure overload (Takeishi et al., 2001). In rat fetal lung cells, Src is activated by mechanical strain (Liu et al., 1996a). Stretch-induced orientation of rat fibroblasts requires Src activation (Sai et al., 1999). In cultured keratinocytes, monolayer wounding strongly and rapidly activates Src not only in damaged cells, but in adjacent cells as well (Yamada et al., 2000). In chicken embryonic fibroblasts Src activity increases following forced detachment from matrix (Maher, 2000). In osteoblasts, SFK mediate several aspects of response to mechanical strain or microgravity (Granet et al., 2001). Osmotic stress responses in smooth muscle cells are sensitive to pharmacological inhibition of SFK (Koh et al., 2001). In human neutrophils, hypertonic shrinkage activates two SFK, Fgr and Hck, and inhibition of these kinases with genistein abrogates physiological responses to this stress (Krump et al., 1997). In CHO cells, hyperosmolarity has dual effect on SFK: it activates Fyn, but inhibits Src (Kapus et al., 1999). Hypotonic swelling in lymphocytes activates Lck (Lepple-Wienhues et al., 2000). These examples show the wide occurrence of SFK-mediated mechanosensory events in a variety of cell types.

How mechanical stresses activate SFK is not yet fully understood, but the role of focal adhesions in this process is well established. Mechanical stresses and adhesion to matrix may activate the same downstream responses in a non-additive manner (Takahashi and Berk, 1996), and focal adhesion components are required for both responses (Ishida et al., 1996; Li et al., 1997). Arising in a variety of cell types, focal adhesions are specialized cell surface structures facilitating cell-matrix contacts. Their formation and dissociation may be affected by mechanical disturbances (Langille, 2001). Membrane-spanning integrins play a pivotal role in triggering focal adhesion assembly and signaling. Formation of focal adhesions at contact points involves association of integrins with extracellular matrix (ECM) proteins outside the cell and cytoskeletal proteins inside the cell. SFK and focal adhesion kinase (FAK) play a co-ordinated role in recruiting other signaling molecules such as PI 3-kinase and adaptor proteins. Binding of integrins to ECM proteins not merely anchors cells, but can affect signaling for cytoskeletal rearrangements required for migration and proliferation (reviewed in Schlaepfer et al., 1999; Sastry and Burridge, 2000). Integrin engagement presumably generates intrinsic conformational changes, which are transmitted to signaling molecules, causing their activation or deactivation, depending on the nature of stimulus (reviewed in Longhurst and Jennings, 1998; Schlaepfer and Hunter, 1998). Adhesion to the ECM proteins (Wennerberg et al., 2000) or treatment with anti-integrin antibodies (McLean et al., 2000), as well as shear stress (Li et al., 1997), trigger autophosphorylation of FAK at tyrosine 397 that creates a high affinity binding site for the SH2 domain of Src (Guan and Shalloway, 1992; Hanks et al., 1992; Schaller et al., 1994; Calalb et al., 1995; Wennerberg et al., 2000). Binding to FAK activates Src (Schlaepfer and Hunter, 1997), presumably by unfolding its closed and inactive conformation. Low affinity constitutive association between the SH3 domain of Src and the

proline- rich domain of FAK (Hauck et al., 2001) may also contribute to this process. The activated Src-FAK complex recruits Cas and other substrates, followed by phosphorylation of these proteins by Src kinase activity (Okuda et al., 1999; Ruest et al., 2001). Src-dependent phosphorylation of FAK at residues other than Y397 may activate FAK (McLean et al., 2000). In a putative positive feedback loop phosphorylated Cas can activate Src by binding its SH3 and SH2 domains (Burnham et al., 2000). The picture is further complicated by the fact that Src kinase activity is required for efficient integrin-mediated autophosphorylation of FAK at tyrosine 397 (Salazar and Rozengurt, 2001), although this tyrosine residue is not a substrate of Src (McLean et al., 2000). The critical importance of SFK in focal adhesion signaling is underscored by almost complete lack of tyrosine phosphorylation in fibroblasts derived from triple Src-Fyn-Yes knockout mice following integrin engagement (Klinghoffer et al., 1999; Salazar and Rozengurt, 2001); single knockout cells have normal responses (Bockholt and Burridge, 1995). SFK activation by mechanical stresses may not be solely confined to focal adhesions. It could occur in cytoskeletal structures (Kuppuswamy et al., 1997) and caveolae (Volonte et al., 2001), two other subcellular entities that recruit SFK. Mechanical stimulation of SFK may be mediated by stress-activated $Ca^{2+}$ channels (Naruse et al., 1998). In summary, activation of SFK by mechanical stresses may depend on events in focal adhesions as well as other cellular structures.

A subset of complex downstream signaling pathways of SFK in mechanical stress responses is likely the same or similar to those of ECM adhesion responses. These pathways include ERK2 activation through a non-linear network of regulatory circuits operating via FAK, Grb2, Shc, PI 3-kinase, and Ras, and leading to gene expression, proliferation, and motility; JNK activation via adaptor proteins Cas, Crk, and Nck leading to transcriptional stress response; phosphorylation of cytoskeletal proteins (e.g. paxillin) mediated by Cas, Crk, small G protein Rac, and PI 3-kinase leading to cytoskeletal rearrangement, and down-regulation of the small G protein RhoA leading to focal adhesion dissociation and cell migration. These pathways have been reviewed in detail elsewhere (Hanks and Polte, 1997; Longhurst and Jennings, 1998; Schlaepfer et al., 1999; Sastry and Burridge, 2000). Other pathways of mechanical stress response emanating from SFK may be different from those of adhesion responses. One example is shrinkage-induced Src-dependent phosphorylation of caveolin (Volonte et al., 2001). Another example is Fyn-mediated phosphorylation of FER, a Fes-related PTK, which in turn phosphorylates the actin-binding protein contractin, thus triggering cytoskeletal rearrangement in shrinkage response (Kapus et al., 1999; Kapus et al., 2000). An important downstream event in the shrinkage response is the activation of the $Na^+/H^+$ exchanger NHE1, a key regulator of cell volume. The evidence for SFK as mediators of NHE1 activation is contradictory. In human neutrophils, hypertonia-induced activation of NHE1 is prevented by the SFK inhibitor genistein (Krump et al., 1997), however, in CHO cells another SFK inhibitor, PP2, fails to prevent activation of NHE1 under similar conditions (Kapus et al., 1999). In lymphocytes, Lck activated by hypotonic swelling opens the membrane outwardly rectifying chloride channel ORCC, whose action tends to equalize inward and outward osmotic pressure (Lepple-Wienhues et al., 2000).

**Src Kinases in Response to Heat Shock**

Heat shock and several other stresses including exposure to solvents or proteasome inhibitors cause accumulation of denatured cellular proteins. The most studied aspects of protein-denaturing stress are transcriptional induction and function of heat shock proteins (HSP). Belonging to several groups based on their sequence homology and mechanism of action, these proteins serve as molecular chaperones to correctly fold denatured polypeptides (reviewed in Feldman and Frydman, 2000; Mathew et al., 2000; Sherman and Goldberg, 2001). Cell cycle- and proliferation-dependent expression of HSP points to their role in growth processes not only under stress, but also under normal conditions (reviewed in Helmbrecht et al., 2000).

Although it is widely believed that denatured proteins within cell initiate the heat shock response (reviewed in (Morimoto, 1998), the identity of denatured protein sensors and their signaling pathways remain elusive. Heat shock transcription factors (HSF) responsible for the transcriptional up-regulation of HSP genes can be directly activated by protein-denaturing agents (Mosser et al., 1990; Goodson and Sarge, 1995; Larson et al., 1995; Zhong et al., 1998), thus circumventing the necessity for upstream activators. However, these findings do not exclude indirect activation of HSF via signaling cascades. If such cascades exist, SFK, whose activity may be increased by heat shock, are likely to play a role. Heat shock rapidly activates Src in NIH 3T3 fibroblasts (Lin et al., 1997) and in K562 myeloid cells (Han et al., 2000). Following activation by heat shock, Src phosphorylates and activates the PI 3-kinase (Lin et al., 1997; Maroni et al., 2000), which could in turn activate downstream kinases AKT and GSK3 (Maroni et al., 2000), or ERK and ribosomal S6 kinase p70 (Lin et al., 1997). Interestingly, ERK activation by heat shock is dependent on the EGFR (Lin et al., 1997). Another target of Src in heat shock response is JNK (Han et al., 2000; Maroni et al., 2000). MAP kinase pathways may lead to HSF activation and actin polymerization (Han et al., 2000), or other aspects of heat shock response.

One interesting connection occurs between SFK and HSP90. Although association with HSP90 negatively regulates kinase activity of Src (Oppermann et al.,

1981), it is critical for Src's membrane targeting (Xu and Lindquist, 1993) and correct folding (Scholz et al., 2001) of the latter. Pharmacological inhibition of HSP90 by benzoquinone drugs herbimycin A or geldanamycin reverses transformed phenotype of oncogenic Src (Whitesell et al., 1994) and targets misfolded Src for degradation by proteasome (An et al., 2000). These observations may implicate HSP90 as a critical regulator of SFK in heat shock response and other cellular processes.

**Src Kinases in Other Stresses**

SFK are activated by photodynamic treatment whose mechanism of action may involve reactive oxygen (Xue et al., 1997); crosslinking of proteins by glyoxal, a compound generated in cells by a reaction between glucose and reactive oxygen (Akhand et al., 1999); bacterial invasion, which is likely to activate mechanical stress response (Esen et al., 2001); and xenobiotics (Vogel et al., 2000).

**Redundant versus Non-redundant Roles of Src Kinases in Stress Responses**

As discussed above, different SFK members are involved in a wide variety of stress response signaling pathways. This raises the question whether individual SFK have specific or redundant roles. Despite their structural similarity, there are likely functional differences among the SFK. The same SFK member may be involved in different responses, depending on the cell type. Of the three SFK present in MCF7 cells, Lyn is activated by oxidative stress, whereas Fyn and Lck are not (Robbins et al., 2000). Hck has been reported to be the major SFK activated by the oxidative agent mercuric chloride (Blackburn et al., 1999), although another report shows that Src can be activated as well (Pu et al., 1996). Whereas various SFK may be activated by $H_2O_2$ (Barchowsky et al., 1995; Hardwick and Sefton, 1995; Yurchak et al., 1996; Hardwick and Sefton, 1997), the downstream effect on Ras and p90RSK is greatly diminished in Fyn-deficient, but not in Src-deficient MEFs (Abe et al., 2000). Src, but not other SFK, is coupled to the oxidant-induced activation of BMK1 (Abe et al., 1997). In rabbit heart, the oxidative stress caused by ischemic pre-conditioning activates Src and Lck, but not Fyn, Fgr, Yes, Lyn, or Blk (Ping et al., 1999). Peroxynitrite acts promiscuously, as all individual kinases studied, Src, Lyn, Fyn, Hck, and Fgr are activated by this oxidant (Mallozzi et al., 1999; MacMillan-Crow et al., 2000). However, unlike other SFK, Lyn is activated by peroxynitrite in a fashion that depends on its SH2 domain (Mallozzi et al., 2001a). Lyn, but not Src, Fyn, or Lck is activated by IR (Kharbanda et al., 1994a) mitomycin C, nitrogen mustard, or cisplatin (Kharbanda et al., 1994b) in HL-60 cells. Fyn is activated whereas Src is inhibited in CHO cells during hypertonic shrinkage, and of all SFK in MEFs Fyn is the major kinase whose activity is required for the phosphorylation of contractin in response to shrinkage (Kapus et al., 1999). In focal adhesion signaling, Src, Yes, and Fyn are redundant, as none of the respective single knockouts has dramatic effect (Bockholt and Burridge, 1995), whereas in the triple knockout the focal adhesion signaling is almost completely wiped out (Klinghoffer et al., 1999). Src, but not Fyn, associates with the cytoskeleton in pressure-overloaded myocardium (Kuppuswamy et al., 1997). These data show that individual SFK may have unique mode of activation and specific targets in stress responses, although in many instances these kinases are redundant.

**Is There a General Src Kinase-mediated Response to Stress?**

SFK role in responses to different types of stresses suggests the scenario whereby various damage stimuli converge on SFK, which activate a general stress response pathway. The idea of a generalized SFK-dependent stress response is at least partially valid because different types of damage seem to activate the same transcriptional stress response via SFK and the JNK-c-Jun-AP-1 cascade. Other examples of components shared by different response pathways are: EGFR that is required for Src activation by both oxidative stress and heat shock, caveolin that is phosphorylated by Src in response to a variety of stressors, PI 3-kinase that is engaged in mechanical and heat shock pathways, and ERK that is a downstream effector of SFK in oxidative, heat shock, and mechanical responses. These examples may indicate cross-talk between the pathways; on the other hand they may reflect specific usages of the same signaling component in different cell types. These considerations notwithstanding, the data described above clearly demonstrate the existence of non-overlapping or only partially overlapping pathways that relay signals specific for particular types of stress. Indeed, PLC and PKC are major targets of SFK in oxidative responses, whereas FAK, Cas, Crk and cytoskeletal proteins are the major targets of SFK in mechanical responses. None of these targets have been implicated in genotoxic or protein-denaturing pathways of SFK. Src activation by oxidative stress is not accompanied by phosphorylation of focal adhesion substrates, FAK and paxillin, in neuroblastoma cells (Li et al., 1998), indicating some degree of insulation between the oxidative and mechanical pathways. It should be noted that experimental evidence implicating the same signaling molecules in different stress pathways should be treated with caution because many real life stressors are not "pure". Thus, oxygen radicals damage DNA by modifying bases, and denature proteins by oxidizing disulfide bonds. IR, in

addition to breaking DNA, generates oxygen radicals. UV generates both oxygen radicals and pyrimidine dimers in DNA. Therefore, each of these stressors may simultaneously activate more than one pathway.

## Outcome of Src Kinase Activation: Survival or Apoptosis?

Cells damaged by stresses either survive or die; the death could be due to necrosis or apoptosis. Necrosis is a non-physiological death resulting from too much damage; this process does not depend on signal transduction. By contrast, survival and apoptosis are the two alternative physiological programs activated by signaling networks. SFK have prominent role in deciding whether the survival or the apoptotic program is activated following damage by stresses. A body of evidence correlates stress-induced SFK activation with pro-survival and anti-apoptosis. Conditional inactivation of v-Src in cells transformed with this oncogene results in apoptosis (Johnson et al., 2000; Carragher et al., 2001). SFK inhibitors enhance the apoptotic response to the oxidative stress in cultured cardiomyocytes (Aikawa et al., 1997) and Uvr-1 cells (Hiwasa et al., 1999), and decrease survival of L5178Y cells following photodynamic treatment (Xue et al., 1997). v-Src may increase resistance to IR in some cell lines (Shimm et al., 1992). Splenocytes from mice deficient in Src homology protein tyrosine phosphatase SHP-1, a functional antagonist of SFK, are resistant to IR-induced apoptosis (Hsu et al., 2001). Other studies, however, suggest the opposite. Src may generate apoptotic signal if its survival pathways are obliterated (Webb et al., 2000). In T cells, Lck is required for IR-induced apoptosis (Belka et al., 1999). In the chicken B cell line DT40 Lyn mediates apoptosis induced by topoisomerase II inhibitors (Maruo et al., 1999) and UV (Qin et al., 1997). Lyn's activation has been correlated with DNA damage-induced apoptosis in other cell lines (Yoshida et al., 1999; Yoshida et al., 2000). Although not directly relevant to stress response, an insight into receptor-dependent SFK-mediated signaling may be useful for understanding the outcomes of SFK activation by stresses. In a variety of cell lines, mostly hematopoietic, cytokine receptor-mediated activation of Src (Kulik et al., 1997; Abu-Ghazaleh et al., 2001; Rosen et al., 2001), Lyn (Katagiri et al., 1996; Wei et al., 1996; Yousefi et al., 1996; Pazdrak et al., 1998; Mou and Linnekin, 1999; Dahl et al., 2000; Bates et al., 2001), Fgr (Katagiri et al., 1996), Fyn (Utting et al., 2001), Lck (al-Ramadi et al., 1998) and unspecified SFK (Endo et al., 2001; Nishio et al., 2001; Xing et al., 2001) promotes survival and inhibits apoptosis. However, in T lymphocytes, Lck and Fyn have been implicated in signal transduction originating from cell death receptors (Migita et al., 1995; Atkinson et al., 1996; Schlottmann et al., 1996; Gonzalez-Garcia et al., 1997), T cell receptor (Ricci et al., 2001), and other inducers of apoptosis (Migita et al., 1995; Corbeil et al., 1996; Manna and Aggarwal, 2000; Manna et al., 2000). Other studies question the positive role of Lck in ligand-induced apoptosis (Schraven and Peter, 1995; Latinis and Koretzky, 1996). Pro-apoptotic role of SFK has been reported in peripheral blood lymphocytes (Matache et al., 2001), eosinophils (Simon et al., 1998), and B cell lines (Shan et al., 2000). Receptor-mediated and SFK-dependent regulation of proliferation, survival, and apoptosis in hematopoietic cells and B cells has been reviewed elsewhere (Corey and Anderson, 1999; Hsueh and Scheuermann, 2000). Taken together, these data show that SFK are capable of promoting either survival or apoptosis, depending on particular cell type, specific kinase, and the nature of stimulus.

The pro-survival and anti-apoptotic signaling pathways of Src have been studied in some detail. One of the pro-survival pathways of SFK in the oxidative stress response involves PLCγ1 (Wang et al., 2001). Known anti-apoptotic pathways of SFK include inhibition of BAD and transcriptional induction of Bcl-2, positive and negative apoptotic proteins, via ERK and RSK (Wang et al., 2001) and transcriptional induction of the Bcl-2 family protein Mcl-1 together with the transcriptional activator STAT3 (Epling-Burnette et al., 2001). Lyn may negatively regulate genotoxic apoptosis by directly inhibiting the apoptotic effector GADD34 (Grishin et al., 2001). Little is known about ultimate targets of SFK-dependent pathways in induction or promotion of apoptosis. Interestingly, apoptotic process itself could impinge on SFK. Fyn and Lyn undergo caspase-dependent cleavage in apoptotic cells that removes their N-terminal membrane anchors, thus shifting them to the cytosol (Luciano et al., 2001). Cytosolic SFK retain their enzymatic activity, however, they may become physiologically inactive because of the failure to interact with their signaling partners (Yurchak et al., 1996).

## Concluding Remarks

During the past several years much information has accumulated on the involvement of SFK in cellular responses to stresses. This information is yet rather fragmentary, and it poses more questions than gives answers. However, there is no doubt that it identifies a new and interesting area of SFK-mediated signal transduction. Several key questions in this area are likely to be addressed in the near future. Identities of stress sensors and their connections will help understanding mechanisms of SFK activation by stresses. Downstream pathways of various SFK in different cell lines and tissues, and in response to diverse stimuli will be studied in depth, and new pathways will be likely discovered. These studies will provide insights into relationship between stress response pathways and other signaling networks of SFK, pathway com-

partmentalization and cross-talk. Finally, the process of making decision between life and death following stress will be explored in more detail, which may reveal new connections between SFK and key effector proteins of survival and apoptosis.

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