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Structure and Function of Syk Protein-Tyrosine Kinase[1]

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Non-receptor type of protein-tyrosine kinase Syk contains 2 Src homology 2 (SH2) domains in tandem and multiple autophosphorylation sites. Syk is activated upon binding of tandem SH2 domains to immunoreceptor tyrosine-based activating motif (ITAM) and plays an essential role in lymphocyte development and activation of immune cells. Syk is critical for tyrosine phosphorylation of multiple proteins which regulate important pathways leading from the receptor, such as Ca^{2+} mobilization and mitogen-activated protein kinase (MAPK) cascades. Recent findings reveal that expression of Syk appears to be involved in a wide variety of cellular functions and pathogenesis of malignant cancer. These observations have demonstrated that Syk is a key molecule that controls multiple physiological functions in cells.

Key words: genetic analysis, ITAM, PTK, Syk, ZAP-70.

The non-receptor type protein-tyrosine kinases (PTKs) are associated with cell surface receptors to amplify receptor activated signals inside the cells. More than 30 mammalian non receptor type PTKs has been identified and divided into 11 distinct subfamilies based upon functional domains and sequence motifs. Among them, Src family (Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr), Tec family (Itk, Btk, Tec, Bmx, Rlk), JAK family (Jak1, Jak2, Tyk2, Jak3), Csk family (Csk, Ctk), Fes family (Fes, Fer), Abl family (Abl, Arg), Fak family (Fak, CAKβ/Pyk2/RAFTK), and Syk family PTKs (Syk, ZAP-70) have been well characterized. Multiple specialized domains, such as Src homology 2 (SH2), SH3, SH4, plextrin homology, Jak homology, or cdc42-binding domains are identified in non-receptor PTKs. These regions generally mediate regulation of PTK activity and interaction with other molecules. The most striking unique feature of Syk family PTKs is the presence of two SH2 domains (*1*).

Syk ($p72^{syk}$), one of the non-receptor type PTKs, was isolated from porcine spleen cDNA library by using oligonucleotides designed according to the partial sequence of a purified, protease-cleaved 40 kDa fragment of the kinase (*1–3*). Thereby it was named Syk after **S**pleen Tyrosine (**Y**) **K**inase. Originally cytosolic PTKs, CPTK71 and PTK72 were isolated by protein purification from platelets and thymus, respectively (*4–6*) At present, those PTKs are known to be identical to Syk by cross reactivity with Syk-specific antibodies. Human, mouse and rat Syk cDNAs were then isolated and human *SYK* locus was mapped to chromosome 9 at band q22. An alternatively spliced form of Syk was found in both normal and tumor cells. In this article, we will summarize the structural basis of Syk function and essential roles of Syk in physiological cell signaling.

I. Structure of Syk.

Tandem SH2 domains. Syk family PTKs Syk and ZAP-70 contain tandem SH2 and C-terminal kinase domains, interrupted by two interdomains: A and B (Fig. 1) (*1*, *7–9*). Tandem SH2 domains of Syk family PTKs selectively bind to diphosphorylated immunoreceptor tyrosine-based activating motif (ITAM) [(Asp/Glu)-X-X-Tyr-X-X-Leu-$(X)_{6-8}$-Tyr-X-X-Leu] of the cytoplasmic region of immune-receptors, such as T-cell receptor (TCR), B-cell receptor (BCR), Fc receptors, and NK cell receptors.

The overall topology of the N-terminal half of Syk that is presented by the crystal structure is similar to its counterpart in ZAP-70 (*8*) but quite different from that of the tyrosine phosphatase SHP-2 which also contains two SH2 domains (*9*) The tandem SH2 domains bind to diphosphorylated ITAM head to tail, *i.e.*, the N-terminal pTyr-X-X-Leu of ITAM binds to the C-terminal SH2 domain of Syk and *vice-versa* Interdomain A forms a helical coiled-coil structure, which is important in protein-protein interactions. Surprisingly, conformational flexibility in the architecture of tandem SH2 domains in Syk provides up to six different conformations which, however, are not found in ZAP-70. Each SH2 domain of Syk contains a "complete" pocket that binds to phosphotyrosyl residues whereas the N-terminal SH2 domain of ZAP-70 is an "incomplete" SH2 domain. The net positive charge in the pTyr binding pocket of C-terminal SH2 domain of Syk is higher than that in ZAP-70 (*9*). This suggestion is consistent with the evidence that C-terminal SH2 domain of Syk retains substantial binding affinity for ITAM (*10*, *11*). These findings about tandem SH2 domains may provide the molecular basis for the ubiquitous involvement of Syk in a variety of signal transduction pathways (*9*).

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Abbreviations PTKs, protein-tyrosine kinases; SH2, Src homology 2, ITAM, immunoreceptor tyrosine-based activating motif; MAPK, mitogen-activated protein kinase, TCR, T-cell receptor; BCR, B-cell receptor; PLC-γ, phospholipase C-γ, JNK, c-Jun NH_2-terminal kinase; PI 3-K, phosphatidylinositol 3-kinase, FcRγ, Fc receptor γ-subunit, PLD, phospholipase D

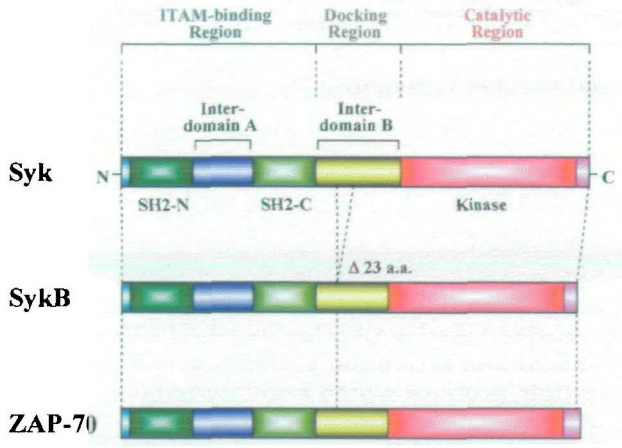
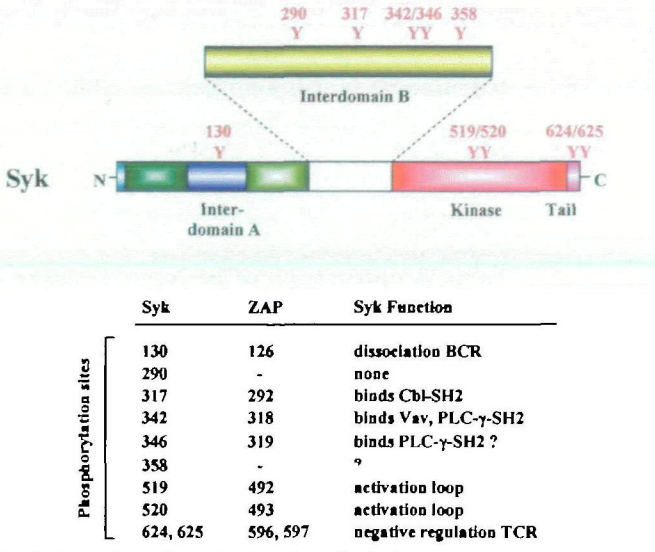


Fig 1. **Structure of Syk family protein-tyrosine kinases.** Mammalian animals have two members of Syk family PTK, Syk, and ZAP-70 SykB is an alternatively spliced form of Syk, missing 23 amino acids from interdomain A. Ligands for Syk activation are diphosphorylated ITAM of immune receptors bound to tandem SH2 domains (SH2-N and SH2-C) Increase in Syk catalytic activity results in autophosphorylation of tyrosine residues in interdomain B to create docking sites for interaction with substrates.



	Syk	ZAP	Syk Function
Phosphorylation sites	130	126	dissociation BCR
	290	-	none
	317	292	binds Cbl-SH2
	342	318	binds Vav, PLC-γ-SH2
	346	319	binds PLC-γ-SH2 ?
	358	-	?
	519	492	activation loop
	520	493	activation loop
	624, 625	596, 597	negative regulation TCR

Fig 2 **Autophosphorylation sites in Syk.** At present 10 tyrosine residues are identified as putative autophosphorylation sites in Syk. Mutational analysis has revealed that phosphorylation of tyrosines in interdomain B of Syk family PTK has important consequences for downstream signals following immune receptor activation. Two tyrosines in the activation loop of kinase domain are necessary for Syk activation.

Interdomain B has five putative autophosphorylation sites. This linker segment acts as a docking region that provides phosphotyrosyl residues for binding to SH2 domains of other signaling molecules.

Autophosphorylation sites. Syk is tyrosine phosphorylated by autophosphorylation (*1*). Ten major autophosphorylation sites have been identified by an *in vitro* kinase reaction using recombinant Syk expressed in insect cells (*12*). Each phosphotyrosine residue performs a distinct function by interacting with a different molecule (Fig 2) The numbering of the specific tyrosine residues below is based on the amino acids sequence of murine Syk (*12*).

Tyr^{130} is located within interdomain A. Tyr^{130} and Tyr^{317} are rapidly phosphorylated in an *in vitro* kinase reaction using Syk from activated B cells. Tyr^{130} is thought to play a role in mediating both the activation of Syk and its release from the antigen receptor (*13*)

Tyr^{290} is located within the interdomain B. SykB, an alternatively spliced variant of Syk and ZAP-70, lack a short insert including Tyr^{290}. This inserted sequence is necessary for maximal Syk function in immune receptor signaling. However, alteration of Tyr^{290} does not affect the function of Syk in FcεRI-mediated signaling suggesting that the phosphorylation of Tyr^{290} could not be critical for Syk function (*14*).

Tyr^{317} is located in the middle of interdomain B. Tyr^{292}, the corresponding site in ZAP-70, is a direct binding site for the SH2 domain of the negative regulator c-Cbl (*15*). Cells expressing ZAP-70 mutant of Tyr^{292} demonstrate a hyperactive phenotype in TCR signaling. Phosphorylation of Syk Tyr^{317} negatively regulates signal transduction in B cells and mast cells. One possible mechanism underlying this phenomenon may be the loss of binding site for Cbl (*16–19*).

Tyr^{342} is required for the direct binding of Syk to the SH2 domain of Vav1 and for the phosphorylation of Vav1 (*20*). A corresponding site in ZAP-70, Tyr^{315}, is necessary for tyrosine phosphorylation of Vav1 in TCR signaling. In COS cell overexpression system, point mutation of either Tyr^{342} or Tyr^{346} eliminates direct interaction of Syk and phospholipase C-γ (PLC-γ). Subsequently, genetic studies have shown that adapter molecules such as LAT, Gads, SLP-76 or BLNK are required to connect Syk family PTKs to PLC-γ in immune cells (*21–24*).

A specific function of Tyr^{346} has not yet been reported. Corresponding site in ZAP-70, Tyr^{319}, is rapidly tyrosine phosphorylated after TCR cross linkage and binds to SH2 domain of Src family PTK Lck. Phosphorylation of Tyr^{319} in ZAP-70 is required for TCR-mediated tyrosine phosphorylation of LAT, PLC-γ, Ca^{2+} mobilization, NF-AT activation and Ras activation (*25, 26*).

Tyr^{358} is lightly phosphorylated by autophosphorylation *in vitro* (*12*). There is no information concerning the function of Tyr^{358}.

Tyr^{519} and Tyr^{520} (Tyr^{518} and Tyr^{519} in porcine Syk, Tyr^{525} and Tyr^{526} in human Syk) are located in the activation loop of kinase domain. A study using specific anti-Syk activation loop phosphotyrosine antibodies suggests that FcεRI aggregation on mast cells induces the phosphorylation of Tyr^{519} and Tyr^{520} in the Syk activation loop (*27*). Phosphorylation of Tyr^{519} and Tyr^{520} is necessary for immune receptor–mediated activation of Syk and the propagation of downstream signaling (*11, 28*).

Aggregation of chimeric transmembrane protein bearing Syk alone suffices to trigger Ca^{2+} mobilization (*29*). Syk is capable of autophosphorylation of Tyr^{519} and Tyr^{520} *in vitro* independent of Src family PTKs (*12*). In immune receptor signaling Syk plays a major role in the phosphorylation of its activation loop tyrosines *in vivo* while Src family PTK probably makes only a minor contribution (*27, 30*). Src family PTK might participate in initial tyrosine phosphorylation of Tyr^{317}, Tyr^{342}, and Tyr^{346} in interdomain B (*30*). In contrast, ZAP-70 could not catalyze autophosphorylation in its activation loop (*31*). Phosphorylation of ZAP-70 Tyr^{493}

(corresponds to Syk Tyr^{520}) by Lck is critical for TCR-mediated activation of ZAP-70, Ca^{2+} mobilization and Ras pathways in T cells (*32*).

Clustered tyrosine residues Tyr^{623}, Tyr^{624}, and Tyr^{625} are located at the C-terminal tail of Syk. Tyr^{624} and Tyr^{625} are phosphorylated *in vitro* (*12*). Expression of Syk chimera with mutations on these sites showed enhanced functional activity in TCR signaling. The mechanism of this gain of function remains unclear (*33*).

Visualization of Syk. In rat basophilic leukemia cell line RBL-2H3, FcεRI aggregation results in the translocation of green fluorescent protein (GFP)–tagged tandem SH2 domains of Syk from the cytosol (where it is distributed uniformly) to the plasma membrane and to the glycolipid enriched microdomains (GEMs) (*34*). In B cells, GFP-tagged full-length Syk (Syk-EGFP) is localized in both the nuclear and the cytoplasmic compartments. BCR aggregation leads to the recruitment of Syk-EGFP to the plasma membrane, however, Syk is not associated with the internalized receptor in B cells (*35*).

SykB: an alternatively spliced form of Syk. SykB, an alternatively spliced form of Syk, was found both in normal and in tumor cells (*14*, *36*, *37*). SykB lacks 23 amino acids including Tyr^{290} in interdomain B (Fig. 1). This linker insert is also absent from the closely related enzyme ZAP-70 Although *in vitro* catalytic activities of Syk and SykB expressed in COS cells are similar, SykB is less efficient rather than Syk at coupling to phosphorylated ITAM. Perhaps SykB can mediate productive immune receptor signaling at higher ligand concentrations during the process of hematopoietic cell development (*14*).

II. Role of Syk in lymphocyte development and antigen receptor signaling

T cells. Homozygous *syk* mutant mice suffered severe hemorrhaging as embryos and died perinatally. Analysis of $syk^{-/-}$ lymphoid cells from reconstitution chimeras showed that Syk deletion impaired pro-B to pre-B transition and thus the clonal expansion of pre-B cells (*38*, *39*). Mature T cells bearing αβ T-cell receptors developed in a normal fusion both functionally and numerically (*38*)

Syk is expressed in $CD4^-CD8^-$ double-negative (DN) and $CD4^+CD8^+$ double positive (DP) thymocytes, but level of Syk expression is markedly reduced in $CD4^+$ and $CD8^+$ single positive thymocytes and peripheral T cells, while ZAP-70 is expressed throughout thymocyte development and in peripheral T cells (*40*). Mice deficient in either Syk or ZAP-70 have no defect in the formation of DP thymocyte However, in mice lacking both Syk and ZAP-70, DN thymocytes fail to develop to the DP stage (*41*). This process is usually associated with pre-TCR signaling. Therefore, although Syk appears not to be essential for positive selection during T-cell development, Syk and ZAP-70 may have functional redundancy in pre-TCR signaling.

In T cells Syk, but not ZAP-70, is able to mediate TCR signaling independently of CD45 and Lck (*42*). This might be explained thus that Syk mediates TCR-triggered tyrosine phosphorylation of CD3ζ. Syk could regulate the initiation of TCR signaling by phosphorylation of ITAM in TCR (*43*). These findings suggest that Syk and ZAP-70 play distinct roles in TCR signaling.

Development of Vγ3 dendric epidermal T cells (DETCs) and γδ intra epithelial lymphocytes (IELs) are dependent on ZAP-70, although the number of some γδ T cells is reduced in mice deficient in Syk (*44*).

NK cells. Killer-cell inhibitory receptor (KIR) family without immunoreceptor tyrosine-based inhibitory motif (ITIM) and non-inhibitory C-type lectin family such as Ly49D and Ly49H are coupled with DAP12 (*45*). DAP12 is one of the ITAM-bearing immune receptor subunits, such as CD3ζ, Igα, and FcRγ. Phosphorylated DAP12 binds to Syk and ZAP-70 leading to the activation of NK cells. Analysis of $syk^{-/-}$ NK cells from reconstitution chimera showed that Syk is not essential for NK cell differentiation and is dispensable for crucial NK cell effector functions, including natural cytotoxicity, lymphokine-activated killer cell activity and cytokine production (*46*). $syk^{-/-}$ NK cells express ZAP-70 and there may be functional redundancy between Syk and ZAP-70 in NK cells receptor signaling.

B cells. Syk is expressed throughout B cell development (*40*). Analysis of $syk^{-/-}$ B cells showed that disruption of *syk* gene impairs B cell differentiation at the pro-B to pre-B transition and at the maturation of immature B cells into recirculating cells (*38*, *39*, *47*). The functional role of Syk was observed initially in studies on BCR signaling. Antigen receptor stimulation results in the phosphorylation of ITAM in Igα and Igβ by activation of Src family PTK *via* CD45. Subsequent association of phosphorylated ITAM with SH2 domain of Syk leads to the activation of Syk by autophosphorylation (*11*, *48*, *49*) Disruption of *syk* gene in B cells provided the evidence that Syk is critical for BCR-mediated tyrosine phosphorylation of adaptor molecules BLNK/BASH and BCAP, which couple the receptor activating signals to the downstream events (*50–53*). SH3P7 is phosphorylated by Syk and Src family PTKs, and is an adaptor protein which links BCR signaling to the cytoskeleton (*54*). HS1 is synergistically phosphorylated by Syk and Src-family PTK Lyn, and is translocated to nuclei (*55*). Because of its regulation of adaptor molecules, Syk is essential for Ca^{2+} mobilization, c-Jun NH_2-terminal kinase (JNK) pathway, and phosphatidylinositol 3-kinase (PI 3-K) activation in B cells.

III. Essential role of Syk in hematopoietic cell functions

Fc receptor signaling. Aggregation of high affinity IgE receptor (FcεRI) results in the sequential activation of PTKs Lyn and Syk (*56*, *57*). Syk is activated upon association with tyrosine phosphorylated γ and β subunits of FcεRI (*58–60*). SH2 domain–mediated targeting, but not localization, of Syk in the plasma membrane is critical for Syk activation (*61*). Reconstitution studies using Syk-negative variant of RBL-2H3 cells and genetic analysis using $syk^{-/-}$ bone marrow derived basophils revealed that expression of Syk is critical for FcεRI-mediated Ca^{2+} mobilization, degranulation, production of cytokines and arachidonic acid metabolism (Fig. 3) (*62*, *63*). Peripheral blood basophils that fail to degranulate in response to FcεRI cross-linking lack the expression of Syk (*64*). Syk is necessary for FcεRI-mediated tyrosine phosphorylation of LAT, SLP-76, PLC-γ1, PLC-γ2, Vav, Cbl, and Pyk2, and activation of ERK and JNK (*18*, *22*, *62*, *63*, *65–68*). Moreover, Syk regulates Btk and Btk activates PKCβI which is involved in the JNK pathway for cytokine production in FcεRI signaling in basophils (*69*). These observations have demonstrated that Syk is essential for propagating intracellular

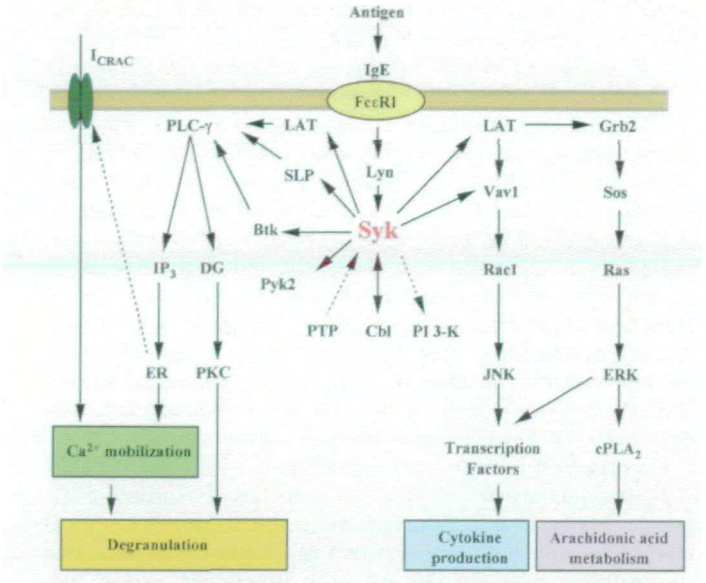


Fig 3 **Essential role of Syk in FcεRI-mediated mast cells/basophils signaling.** Syk is essential for propagating intracellular signals following FcεRI aggregation Syk is critical for FcεR-mediated Ca^{2+} mobilization, degranulation, production of cytokines, and arachidonic acid metabolites.

signals following FcεRI aggregation (Fig. 3).

Syk is critical for FcγRs signaling in macrophages and neutrophils. *syk*$^{-/-}$ macrophages are defective in phagocytosis induced by FcγR but show normal phagocytosis in response to complement (*70*, *71*) *syk*$^{-/-}$ neutrophils are incapable of generating reactive oxygen intermediates in response to FcγR engagement (*71*). Moreover, Fc receptor γ-subunit (FcRγ)–mediated activation of Syk is necessary for FcR transport from endosomes to lysosomes and MHC class II restricted antigen presentation (*72*)

Cytokine signaling. Cytokine stimulation activates several different families of PTKs. Syk is associated with and activated by the IL-2, IL-3, erythropoietin, and granulocyte colony-stimulating factor (G-CSF) receptors (*73–75*). IL-2–mediated activation of Syk is observed in normal human T and B cells that express IL-2R, but not in cells from Jak3-deficient patients indicating that IL-2–mediated activation of Syk is dependent on Jak3 (*76*). However, *syk*$^{-/-}$ T cells proliferate normally in response to IL-2 demonstrating that Syk is not essential for IL-2 signaling. Also IL-2-induced STAT phosphorylation does not require Syk Moreover, there is no reduction in the number of erythrocytes, leukocytes, or platelets in the peripheral blood of *syk*$^{-/-}$ fetuses. *syk*$^{-/-}$ fetal liver progenitors respond normally to IL-3 and G-CSF indicating that Syk is not essential for signaling by these cytokines (*38*).

Role of Syk in platelets. In platelets Syk is tyrosine phosphorylated and activated by collagen (*77*). Genetic analysis showed that both FcRγ and Syk were essential for platelet activation and secretion by collagen (*78*). Platelets from those patients who lack collagen receptor glycoprotein VI (GPVI, p62) show selective deficiency in collagen-induced tyrosine phosphorylation of FcRγ, Syk, PLC-γ, platelet aggregation and secretion (*79*). GPVI is required for the expression of FcRγ on the cell surface, and Fyn and Lyn are associated with GPVI-FcRγ complex and may contribute to tyrosine phosphorylation of ITAM in FcRγ to recruit and activate Syk kinase (*80*, *81*). Thus, Syk is essential for collagen receptor signaling through an interaction with FcRγ in platelets In addition, collagen causes Ca^{2+} mobilization in *syk*$^{-/-}$ megakaryocytes although the response is attenuated. Collagen appears to mediate Ca^{2+} mobilization *via* GPVI and integrin $\alpha_2\beta_1$ in megakaryocytes (*82*).

Syk is activated upon stimulation by various ligands which act *via* G-protein coupled receptors such as thrombin, platelets activating factor, thromboxane A_2, and ADP, and is translocated to cytoskeleton (*82–87*). Genetic studies have revealed that Syk is not necessary for platelet activation by thrombin (*78*).

Syk is activated by outside-in signaling of soluble fibrinogen through $\alpha_{IIb}\beta_3$ and complexed with tyrosine phosphorylated β_3 (*88*, *89*). Genetic analysis showed that *syk*$^{-/-}$ platelets exhibit a partial defect in fibrinogen-$\alpha_{IIb}\beta_3$ binding by ADP and epinephrine. Therefore, Syk does play a role in inside-out $\alpha_{IIb}\beta_3$ signaling, achieving maximal $\alpha_{IIb}\beta_3$ activation. Alternatively, activation of Syk upon stimulation of G-protein coupled receptors leads to integrin $\alpha_{IIb}\beta_3$ exposure during cellular shape change (*90*). However, lack of Syk did not affect the ability of platelets to adhere to immobilized fibrinogen, and neither did it have an effect on $\alpha_{IIb}\beta_3$ mediated primary hemostasis, as assessed by tail bleeding times (*91*).

Wiscott-Aldrich syndrome (WAS) and X-linked thrombocytopenia are caused by mutations of the WAS protein (WASP). CrkL associates with WASP and Syk, and may be a novel adaptor for both molecule to translocate to the cytoskeleton in platelets (*92*).

IV. Events downstream of Syk

Syk substrates. Elevation of Syk enzymatic activity is induced by a variety of physiological ligands. A list of Syk substrates/downstream molecules *in vivo* and *in vitro* is shown in Table I. Cytoplasmic region of erythrocyte band 3 protein is frequently used as an exogenous substrate for *in vitro* kinase reaction of Syk (*93*). Catalytic specificity of Syk kinase, as assessed by phage display study, suggests that Syk exhibited a distinct substrate requirement for aspartic acid in position −1 and glutamate in position +1 (Asp-Tyr-Glu) (*94*)

Syk kinase-interacting proteins (SKIP) were identified by the yeast two-hybrid system N-terminal half of c-Cbl, Vav, and 3BP2 were isolated as SKIP, and Syk-SKIP interactions were dependent on Syk kinase activity. One of those molecules, 3BP2, binds to the Syk kinase domain *via* its SH2 domain. Expression of 3BP2 in T cells promotes IL-2 gene activation (*95*).

MAPK activation. In addition to Ca^{2+} mobilization, a critical role of Syk in immune receptor–mediated activation of mitogen-activated protein kinase (MAPK) is well characterized. Expression of Syk is essential for FcεRI-mediated activation of extracellular signal–regulated kinase (ERK) and JNK in mast cells (*62*, *63*, *96*). Adaptor molecule LAT, a substrate of Syk, associates with Grb2 which may contribute to the recruitment and localization of guanine nucleotide exchanging factors and is necessary for immune receptor-mediated ERK and JNK activation. In B cells, BLNK is essential for coupling Syk to Rac1-JNK pathway (*24*).

Autophosphorylation of Tyr^{342} (Tyr^{341} in porcine Syk) in

TABLE I. **Syk substrates/downstream molecules.**

A. Syk (autophosphorylation)		(*1, 12*)
B Substrates for *in vitro* kinase reaction		
[Val^5]angiotensin II		(*3*)
histone H2B		(*1*)
myelin basic protein		(*133*)
erythrocyte band 3 protein		(*93*)
tubulin		(*106*)
C Syk downstream molecules		
1) Adaptor molecules		
LAT	linker for activation of T cells	(*22*)
SLP-76	SH2 domain–containing leukocyte protein of 76 kDa	(*65*)
BLNK	B cell linker protein	(*134*)
BCAP	B cell adaptor for PI 3-K	(*53*)
HS1	LckBP1 (Lck binding protein-1), SPY75	(*55*)
Shc	Src homology and collagen	(*135*)
TCR CD3ζ	subunit of TCR	(*43*)
3BP2	c-Abl SH3-interacting protein 2, SKIP-2	(*95*)
2) Enzymes		
Cbl	product of protooncogene *c-cbl*	(*16*)
PLC-γ	phospholipase C-γ	(*50*)
PLD	phospholipase D	(*101*)
Btk	Bruton's tyrosine kinase	(*69*)
Pyk2	related kinase to PYK1, proline-rich tyrosine kinase 1	(*68*)
Vav1	guanine nucleotide exchanging factor for Rac	(*66*)
PI 3-K	phosphatidylinositol 3-kinase	(*136*)
MAPK	mitogen-activated protein kinase, ERK and JNK	(*63*)
3) Cytoskeletal components		
tubulin		(*106*)
SH3P7	SH3 domain–containing protein 7	(*54*)

the linker region of Syk provides a direct docking site for SH2 domain of Vav1 (*20*). SH2 domain of Vav1 is required for its translocation to GEMs to interact with LAT, SLP-76, and Rac1 for JNK activation (*66*). In T cells, Syk and Rac1 cooperate in synergistic activation of JNK suggesting that Syk may generate another signal leading to potent JNK activation (*97*). Genetic analysis revealed that in mast cells Vav1 is essential for the regulation of PLC-γ and Ca^{2+} mobilization, in addition to Rac1-JNK pathway (*98*).

BCR-mediated p38 MAPK activation is abolished in Lyn and Syk double deficient cells, demonstrating that either Syk or Lyn alone may be sufficient to activate p38 MAPK (*99*). p21-activated kinase 1 (PAK1) activation requires Syk family kinase, but occurs independently of LAT and SLP-76 by TCR. TCR-mediated PAK1 activation may require focal adhesion signaling molecules (*100*)

Phosholipase D activation. Phosholipase D (PLD) is activated in response to a variety of extracellular ligands and plays a role in signal transduction through phosphatidylcholine hydrolysis to choline and phosphatidic acid. We previously showed that Syk and PLC-γ2 are essential for BCR-mediated PLD activation (*101*) In addition, treatment of many cell types with phorbol esters stimulates PLD activity implying regulation of the enzyme by protein kinase C. Recently, we have found that Syk-PLC-γ2 pathway is required for phorbol ester–induced PLD activation in B cells. Treatment of cells with phorbol ester induces activation and tyrosine phosphorylation of Syk through protein kinase C activation (*102*). Syk appears to play some role in cellular functions such as cytoskeletal reorganization by regulating PLD activity in both BCR and phorbol ester–mediated signaling in B cells.

Negative regulation of Syk. Syk is neither tyrosine phosphorylated nor associated with the cell surface receptors in resting cells. There must exist a mechanism to negatively regulate Syk activation in order to prevent constitutive signaling from Syk because ectopic expression of Syk results in its hyper phosphorylation. One possible means to negatively regulate Syk is dephosphorylation of Syk by specific protein-tyrosine phosphatases (PTPs). CD45 and SHP1 are candidate molecules which might negatively regulate Syk in resting cells although activation of Syk is independent of CD45 (*42, 49, 103–105*). Biochemical analysis suggests that Syk tyrosine phosphorylated by BCR stimulation is found predominantly in the soluble fraction of the cells. This implies that once activated, Syk is released from the immune receptor complex and is then free to associate with and phosphorylate soluble substrates (*106*). The unanswered question is whether Syk is regulated by a balance between phosphorylation (autophosphorylation) and dephosphorylation (unknown specific PTP) such as Ser/Thr kinase in metabolic systems.

Intra-molecular interaction might contribute to maintain the close inactive state of Syk in resting cells. The interdomain A of Syk family PTKs consisting of coiled-coil region, which is commonly involved in protein-protein interactions, may inhibit the catalytic activity of Syk kinase domain (*8, 9*). Antibodies against C-terminal region of Syk react with activated form of Syk indicating that tandem SH2 domains or interdomain A may mask or modify the accessibility of the C-terminus of the kinase domain at resting state (*107*).

One other possible mechanism depends on additional proteins such as a negative regulator. Cbl binds to linker region of ZAP-70 via its SH2 region. Cbl-deficiency markedly up-regulates the activity of ZAP-70 (*108, 109*). Overexpression of Cbl by vaccinia virus suppresses FcεRI-mediated cellular signaling in RBL-2H3 cells (*110*). Phosphorylation of Tyr^{317} of Syk negatively regulates signal transduction from the immune receptor because of the lack of interaction with Cbl (*16–18*). The RING domain of Cbl possesses ubiquitin-protein ligase function and is essential for negative-regulation of Syk (*19, 111*). Binding of Cbl to immune receptor associated PTK may cause ubiquitination of the immune receptor and PTK complex, which promotes endocytosis, lysosomal targeting and proteosome-mediated degradation of activated receptor complex (*112*).

Latent membrane protein 2A (LMP2A) of Epstein-Barr virus (EBV) binds to WW domain of E3 protein-ubiquitin ligase that ubiquitinate BCR-associated PTK Lyn and Syk. LMP2A serves as a molecular scaffold to recruit PTK and protein-ubiquitin ligase. This may explain the fact that LMP2A of EBV appears to function by inhibiting BCR signaling (*113*).

Inhibitor of Syk. Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) inhibits FcεRI-mediated IP_3 production, serotonin secretion, membrane ruffling and cell spreading by inhibiting Syk but not Lyn (*114*). Piceatannol has been widely used to study Syk function and results obtained with this inhibitor have been used to predict the role of Syk in various cell signaling events, however, this reagent can inhibit other kinases as well, in particular, FAK, Src, Jak1, rat PKC, and PKA (*91, 115, 116*). ER-27319 [3,4-dimethyl-10-(3-aminopropyl)-9-acridone oxalate] inhibits FcεRI-mediated degranulation, tyrosine phosphorylation of Syk,

but not Lyn in intact mast cells with a half-maximal inhibition (IC_{50}) at 10 μM (*117*).

A novel peptide library approach to identify specific inhibitors of ZAP-70 was reported. By screening more than 6 billion peptides, Phe-for-Tyr substituted version of ZAP-70 binding peptide, Lys-Leu-Ile-Leu-Phe-Leu-Leu-Leu was identified as a competitive inhibitor of ZAP-70. This peptide is a poor inhibitor of Lck and Syk. A membrane-permeant form of this peptide inhibits ZAP-70 in intact lymphocytes (*118*). Isolation of a specific inhibitor for Syk by the same technique has not been reported yet.

V. Role of Syk in oxidative stress and cell survival signaling

We have demonstrated that Syk is activated by oxidative stress following treatment of cells with H_2O_2. Although the receptor for oxidative stress has not been identified yet, oxidative stress-induced tyrosine phosphorylation of Syk is dependent on the presence of Src-family PTK Lyn in B cells (Fig. 4) (*119*). Mutational analysis suggests that this Syk activation is not simply explained by recruitment of Syk to ITAM (*120*).

Oxidative stress induces Ca^{2+} response and activation of ERK, JNK, and p38 in B cells. Our genetic studies using Syk-deficient DT40 B cells revealed that Syk mediates tyrosine phosphorylation of PLC-γ2, production of IP_3, and inducing Ca^{2+} release from intracellular stores. Furthermore, Syk is essential for an increase in tyrosine phosphorylation of cellular proteins and JNK activation following oxidative stress (*121*). BLNK is required for coupling Syk to PLC-γ2 in oxidative signaling as well as in that of BCR (Takano, T. and Yamamura, H., unpublished data).

We have recently reported that lack of Syk expression leads to an increase in caspase-9 activity and decrease of cell viability induced by H_2O_2-treatment in DT40 B cells. Moreover, Syk is required for oxidative stress–induced activation of PI 3-K-Akt survival pathway in B cells. Akt phosphorylates caspase-9 to inhibit its protease activity. Therefore, we have demonstrated that Syk modulates caspase-9 activity through the activation of Akt survival pathway and enhances cellular resistance to oxidative stress–induced apoptosis in B cells (*122*).

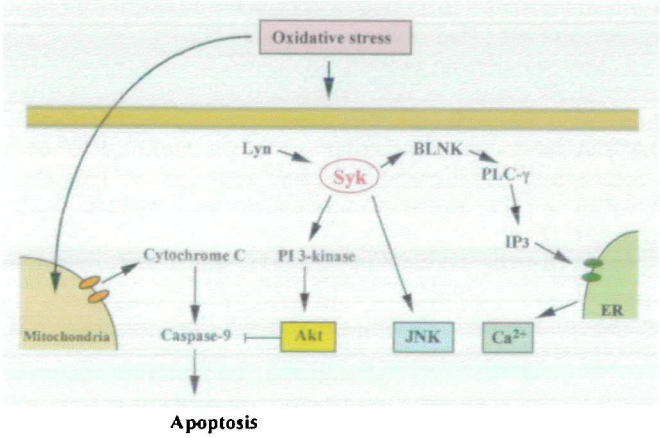


Fig. 4. **Essential role of Syk in stress signaling.** Syk is activated by oxidative stress. Expression of Syk is required for oxidative stress–mediated Ca^{2+} mobilization and JNK activation. Activated Syk modulates PI 3-K-Akt survival pathway to inhibit caspase-9 activity, preventing apoptosis

VI. Perspectives

Role of Syk in a wide variety of cells. Syk exhibits a very widespread expression pattern and is multifunctional in non-hematopoietic cells such as epithelial cells, hepatocytes, fibroblasts, neuronal cells, and breast tissue. Syk is involved in the differentiation of 3T3-L1 mouse embryonic fibroblasts to adipocytes and adipogenesis (*123*). Expression of Syk is observed in human nasal fibroblasts and level of Syk expression affects lipopolysaccharide-induced RANTES production and JNK activation in fibroblasts from nasal polyps (*124*). Our previous work in hepatocytes suggested that Syk plays an important role in signaling steps leading to MAPK activation by G-protein–coupled receptors (*125*). In the case of neuronal cells, we have found that adenovirus-mediated overexpression of Syk induces supernumerary neurite formation and ERK activation in embryonal carcinoma P19 cells (*126*).

Syk-deficient mice showed severe petechiae *in utero* and died shortly after birth (*38*, *39*). The mechanism of this bleeding, however, remains unknown Most recently, we have found that Syk is expressed in several vascular endothelial cell lines and this bleeding may be caused by a dysfunction in Syk-deficient vascular endothelial cells during embryogenesis (Yanagi *et al.*, *Blood*, in press). Our results indicate that Syk plays a critical role in endothelial cell functions, including morphogenesis, cell growth, migration and survival. Moreover, Syk may contribute in maintaining vascular integrity *in vivo* Thus, these findings demonstrate that Syk appears to play a general physiological role in a wide variety of cells, although Syk has been believed to be involved specifically in immune receptor signaling. Furthermore, Syk appears to be deeply implicated in cellular reorganization of vascular endothelial cells during angiogenesis. Therefore, Syk may be a potential target for inhibition of angiogenesis and tumor growth.

The analysis of Syk in immune cells revealed that Syk is essential for immune receptor–mediated Ca^{2+} mobilization and MAPK cascades. Similarly, Syk may contribute the increase in the intracellular free calcium and activation of MAPK in a wide variety of cells. In addition, Syk could modulate the functions that are not remarkable in immune cells, such as reorganization of cytoskeletons and cross-talk with other growth factor receptor–mediated signaling pathway to regulate cell growth and apoptosis by phosphorylating distinct substrates.

Implication of Syk in human disease. Dysfunction of ZAP-70 causes severe combined immunodeficiency (SCID). The peripheral T cell phenotype is characterized by abundant nonfunctional $CD4^+$ T cells and absence of $CD8^+$ T cells. Mutation of *ZAP-70* gene by insertion or single amino acid alteration results in destabilization of protein or expression of inactive kinase (*127–130*). Human congenital disease due to the mutation of *syk* gene has not been reported yet.

In myelodysplastic syndrome with t(9;12)(q22;p12), product of *TEL-syk* fusion gene is constitutively tyrosine phosphorylated. Expression of TEL-Syk protein transformed BaF3 cells from an IL-3 dependent to an IL-3 independent growth suggesting that TEL-Syk is a novel transforming protein in hematopoietic cells (*131*).

Syk expression is detected in normal breast tissue, benign breast tissue, and low-tumorigenic breast cancer cell lines However, Syk is low or undetectable in invasive

breast cancer. Transfection of Syk into Syk-negative breast cancer cell line inhibited its tumor growth and metastasis formation in athymic mice, as a consequence of aberrant mitosis and cytokinesis (*132*). Knockout mice lacking c-Cbl develop mammary epithelial hyperplasia which could be due to a loss of negative regulator of Syk in their breast tissues (*108*). This finding suggests that Syk is a potent modulator of epithelial cell growth and a potential tumor suppressor in human breast carcinomas (*132*). Thus, Syk controlling kinase activity may represent a novel approach to develop new cancer therapeutic strategies.

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