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Mini-Review

# The Src-family Kinase Lyn in Immunoreceptor Signaling

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## Abstract

Effective regulation of immune-cell activation is critical for ensuring that the immune response, and inflammation generated for the purpose of pathogen elimination, are limited in space and time to minimize tissue damage. Autoimmune disease can occur when immunoreceptor signaling is dysregulated, leading to unrestrained inflammation and organ damage. Conversely, tumors can coopt the tissue healing and immunosuppressive functions of hematopoietic cells to promote metastasis and evade therapy. The Src-family kinase Lyn is an essential regulator of immunoreceptor signaling, initiating both proinflammatory and suppressive signaling pathways in myeloid immune cells (eg, neutrophils, dendritic cells, monocytes, macrophages) and in B lymphocytes. Defects in Lyn signaling are implicated in autoimmune disease, but mechanisms by which Lyn, expressed along with a battery of other Src-family kinases, may uniquely direct both positive and negative signaling remain incompletely defined. This review describes our current understanding of the activating and inhibitory contributions of Lyn to immunoreceptor signaling and how these processes contribute to myeloid and B-cell function. We also highlight recent work suggesting that the 2 proteins generated by alternative splicing of *lyn*, LynA and LynB, differentially regulate both immune and cancer-cell signaling. These principles may also extend to other Lyn-expressing cells, such as neuronal and endocrine cells. Unraveling the common and cell-specific aspects of Lyn function could lead to new approaches to therapeutically target dysregulated pathways in pathologies ranging from autoimmune and neurodegenerative disease to cancer.

**Key Words:** Lyn, autoimmunity, lupus, Src-family kinase, SFK, myeloid, signaling

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The immune system mediates a diverse set of tissue processes, killing pathogens and eliminating pathogen-infected cells, tolerating commensal microbiota (1), directing

organ development (2), eliminating apoptotic cells by efferocytosis (3–5), preventing and eliminating cancer-cell growth (6–8), modulating fibroblast function during

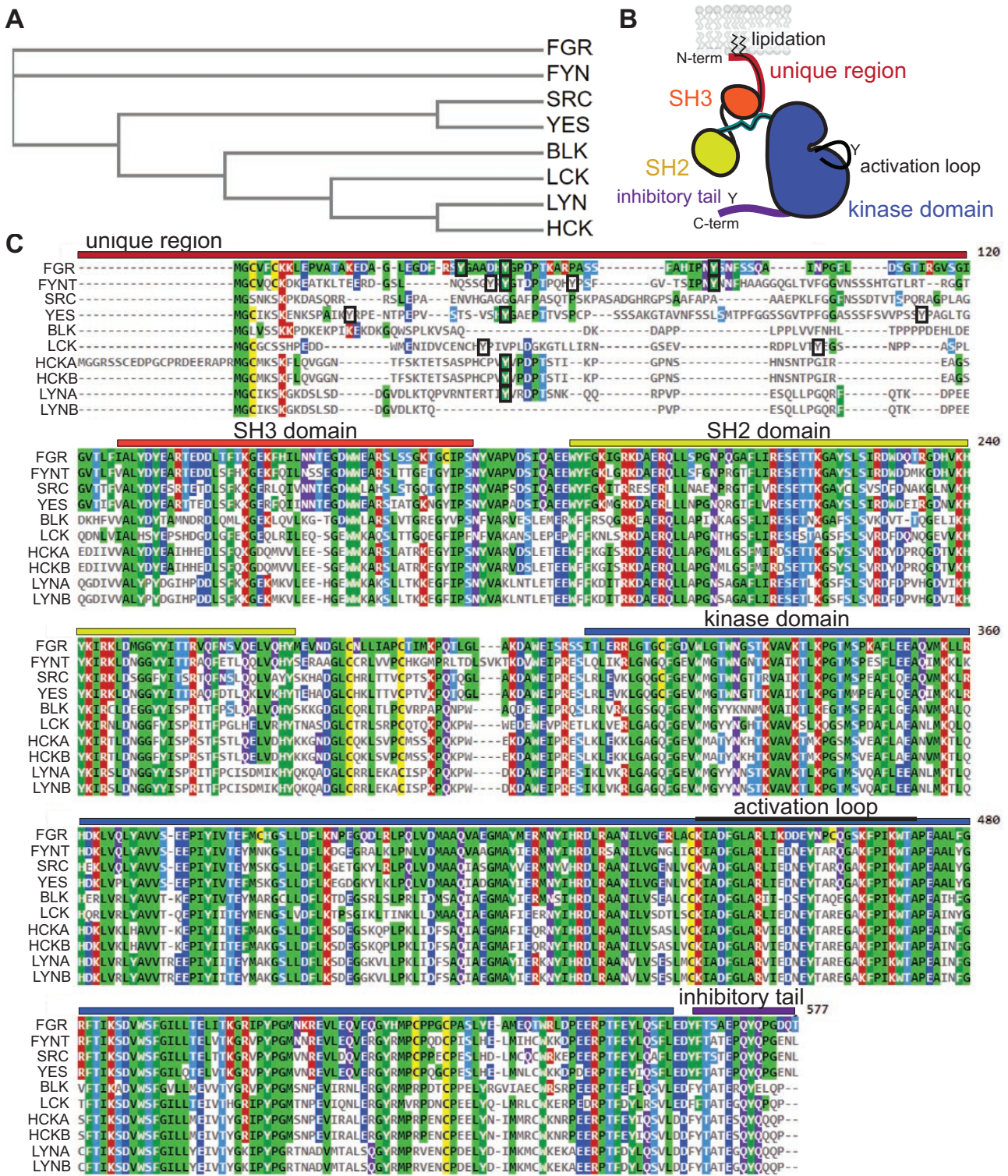
wound repair (9,10), and pruning neuronal synapses (11). Ensuring that immunoreceptor signaling is properly integrated and regulated is critical for directing these disparate functions and limiting inflammation in space and time to prevent excessive tissue damage. Autoimmune disease can develop when dysregulated immune activation leads to rampant inflammation and loss of tolerance (12-14). These conditions comprise a spectrum of disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis, and type I diabetes, in which the immune system mounts an improperly regulated response against self-antigens. Loss-of-function polymorphisms in SFKs, particularly in the *LYN* gene, are risk alleles for human SLE (15-18), suggesting that the Src-family kinase (SFK) Lyn may have an additional function in immunosuppressive signaling and protecting against autoimmune disease (16,19,20). Tumors can also invoke SFK-dependent pathways to subvert immune surveillance, blocking entry of some cells and coopting others to promote metastasis and protect tumors from immune and therapeutic destruction (21-24). This review describes the positive and negative functions of Lyn, with a spotlight on how its alternative splice variants LynA and LynB differentially regulate cell activation. Understanding how Lyn signaling regulates immune-cell activation could enable the development of therapies that selectively alter immune-cell activation thresholds to limit inflammation without eliminating antimicrobial function.

### Src-family Kinase Structure and Function

The Src-family kinases (SFKs) are evolutionarily and structurally conserved nonreceptor tyrosine kinases (25-27). They are also ancient, with a Src homolog and its negative regulator Csk dating to a common ancestor shared with choanoflagellates, which split from the future metazoans > 600 million years ago (28). In mammals, SFKs have duplicated and diversified (Fig. 1A) to provide universal survival and proliferation signals as well as cell- and tissue-specific activities. Due to their function in cell survival, proliferation, migration, and invasion, SFKs are potent oncogenes (30-32). Some members of the Src family (Src, Yes, and Fyn) are broadly expressed (33), whereas others are expressed in a cell-specific manner. In the immune system, for instance, T cells primarily express Lck and FynT; B cells primarily express Blk, Lyn, Hck, Fgr, and FynT (34); and myeloid cells express Lyn, Hck, Fgr and some FynT, Src, and/or Yes (26, 35). In addition to their canonical growth and survival functions, SFKs phosphorylate immunoreceptor-tyrosine-based activation motifs (ITAMs) within assembled T-cell receptors, B-cell receptors, and Fc receptors (36). The SFKs also initiate signaling from other classes of receptors, including integrins (37) and C-type lectins (36).

The SFKs share a canonical domain layout (Fig. 1B), with N-terminal palmitoylation/myristylation sites followed by a disordered or loosely ordered unique region, Src-homology (SH)3, and SH2 domains, and a 2-lobed kinase domain. Lipidation provides membrane anchoring and facilitates microlocalization within membrane subdomains (38,39). Catalytic activity is regulated by a rapid-cycle dynamic equilibrium—tyrosine phosphorylation and dephosphorylation of activating and inhibitory segments—that is controlled in a cell-specific manner by localization, local concentration, basal preactivation, and protein turnover (40-46). A close relative of the SFKs, Csk, phosphorylates the SFK C-terminal inhibitory tail (47,48). This phosphorylation creates an intramolecular binding site for the SFK SH2 domain, which sequesters the kinase away from other docking interactions and constrains the conformation of the kinase domain (25,47,49,50). Inhibitory-tail phosphorylation is reversed by phosphatases such as CD45 and CD148 (50,51). The kinase-domain activation loop is a site of autophosphorylation that opens and reorients the active site to facilitate substrate docking (52). Phosphorylation of the activation loop is reversed by phosphatases such as PTPN22 and CD45 (42,50). Activated SFKs may instead be polyubiquitinated and degraded (41,53-55).

The unique region is the least conserved segment of the SFKs. Although other SFK domains have been characterized structurally, most notably by X-ray crystallography and nuclear magnetic resonance spectroscopy, the unique region appears to be conformationally heterogeneous or disordered (56) and is thus not amenable to high-yield purification or atomic-resolution structural analysis. Nuclear magnetic resonance studies have indicated that the unique region may form loosely ordered, “fuzzy” interactions with the regulatory domains or kinase domain, through which phosphorylation, carbohydrate conjugation, or binding partners (55-58) could regulate kinase activity allosterically or alter protein-protein interactions (59,60). For example, the unique region of Lck contains a zinc-clasp structure that interacts with the T-cell coreceptors CD4 and CD8, a key nucleating interaction of the T-cell receptor–MHC signaling assembly (61,62). The relative lack of conservation with the unique region is exemplified by the locations of tyrosine residues in different Src family members (Fig. 1C). Src, Blk, and LynB lack unique-region tyrosines. There is no clear pattern of conservation in the other SFKs. Furthermore, one modestly conserved tyrosine residue (Y32 in Lyn isoform A numbering) does not have a conserved function: phosphorylation of LynA Y32 triggers polyubiquitination and degradation, whereas similarly situated tyrosine residues in Fgr, Fyn, Lck, and Hck lack this function (29,41,55).



**Figure 1.** Domains and sequences of the SRC family. (A) Phylogeny of human SFKs, showing divergence of FGR and FYN. Widely expressed SRC and YES are more closely related to each other, as are LYN and HCK. The lymphocyte-specific kinases LCK and BLK occupy separate branch points. Generated with EMBL-EBI Clustal Omega and Simple Phylogeny (29). (B) All SFKs have an N-terminal (N-term) unique region, with lipidation and protein-protein interaction sites, followed by SH3 and SH2 regulatory domains and a kinase domain. Their activities are controlled via a dynamic equilibrium of phosphorylation and dephosphorylation of tyrosine (Y) residues in their kinase-domain activation loop and C-terminal (C-term) inhibitory tail. (C) Amino-acid sequence alignment of representative human SFKs, showing approximate domain boundaries. Lack of conserved (colored) residues highlights unique-region divergence, including the translation start-site variants of HCK (isoforms A and B) and the alternatively spliced variants of LYN (isoforms A and B). The immune-cell form of FYN (isoform T) is also shown. Tyrosine-residue diversity in the unique region is highlighted (boxes). Generated with EMBL-EBI Clustal Omega and MView (29).

The SFK SH3 and SH2 domains are relatively well characterized, structurally and functionally (52,63). Both regulate kinase activity directly and serve as intramolecular or intermolecular docking sites for protein-protein interactions (64). The SH3 domain binds polyproline motifs (65), and the SH2 domain binds phosphotyrosine peptides (66). As a regulatory unit, the SH2-SH3 domains bind intramolecular ligands (the tyrosine-phosphorylated inhibitory tail and the linker PXXP region, respectively) to keep the kinase in a closed conformation. Upon inhibitory-tail dephosphorylation, the SH2-SH3 domains are released to form intermolecular interactions that nucleate signaling supercomplexes. For example, the SH3 domain of Lck in T cells binds a conserved polyproline motif in the adaptor protein LAT, while the Lck SH2 domain binds a phosphotyrosine segment in Zap70; these interactions form a bridge that helps assemble and organize the T-cell receptor signalosome (67). Upon activation and substrate binding, the SFK kinase domain transfers the  $\gamma$  phosphate of adenosine triphosphate (ATP) to a tyrosine on a substrate peptide, which can initiate or inhibit signaling by altering the structure of a target protein and/or by forming new protein-protein interactions (64).

### The SFK Lyn as A Negative Regulator of Immune Activation

Lyn kinase, expressed in B and myeloid immune cells, the nervous system, epithelial and endocrine cells, and many cancers, is of particular interest within the Src family as a master regulator of immune and nonimmune signaling thresholds and functions. The *LYN* gene was first identified in 1987 in a human placenta cDNA library probed for homologs of the Src family member Yes (68); its tyrosine kinase activity was demonstrated in vitro shortly thereafter (69). In 1991, Lyn was shown to be expressed in B cells, to co-immunoprecipitate with the B-cell antigen receptor (BCR), to become phosphorylated following BCR ligation (70), and to bind the noncatalytic subunit of phosphoinositide 3-kinase (PI3K) (70, 71). Lyn was also found to co-immunoprecipitate with the ITAM-coupled Fc $\epsilon$ R in mast cells, become activated following Fc $\epsilon$ R crosslinking, and provide activating phosphorylation to phospholipases (72-74). Finally, Lyn was reported to associate with the fatty-acid transport receptor CD36 in platelets and to become activated following ligation of granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor in monocytes (75-77).

In 1995, the development of gene targeting by homologous recombination in embryonic stem cells enabled the generation of Lyn knockout (KO) mice (19). Histology and cell-subsetting analyses of tissues from Lyn<sup>KO</sup> mice by

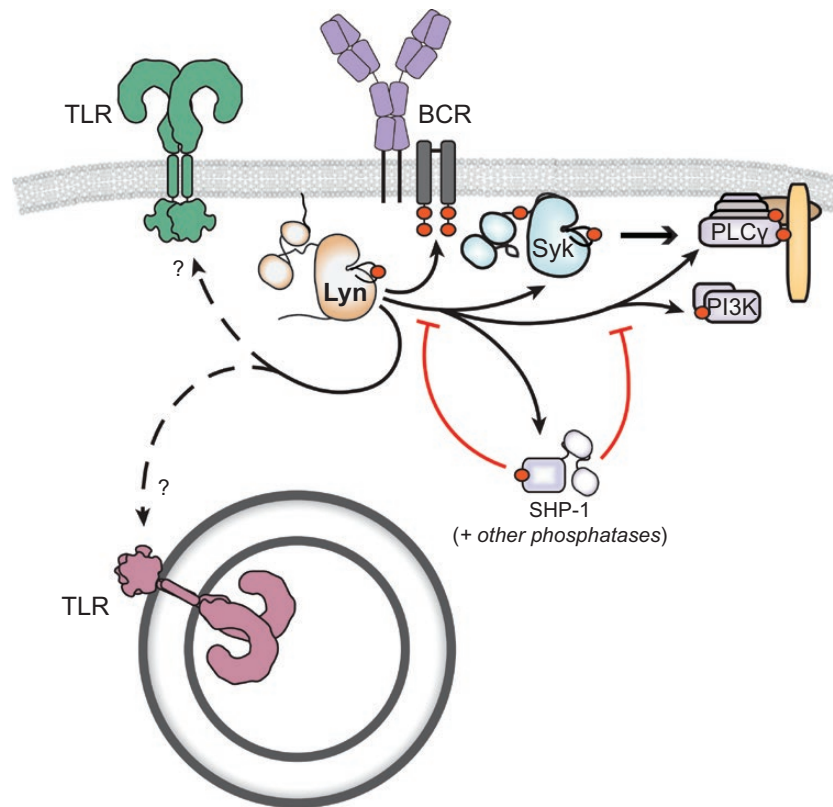
Ashley Dunn's group revealed that Lyn, perhaps uniquely within the Src family, can suppress immune-cell signaling and activation (Fig. 2). At roughly 10 months of age, Lyn<sup>KO</sup> mice developed an autoimmune disorder with similarities to human SLE, including increased levels of circulating antibodies and production of antinuclear antibodies by plasma B cells. Tadashi Yamamoto's group further observed that Lyn<sup>KO</sup> mice developed splenomegaly, with increased numbers of monocytes, granulocytes, and B1 B cells (78).

Several proteins and signaling cascades were subsequently shown to drive autoimmunity in Lyn<sup>KO</sup> mice. Cytokines, such as interleukin (IL)-6, B-cell activating factor (BAFF), and interferon gamma (IFN $\gamma$ ) (79, 80), and cytokine receptors, such as IL-5 receptor subunit alpha (IL5R $\alpha$ ) (81), were shown to promote inflammation and myeloid-cell proliferation in the absence of Lyn. Proteins associated with actin rearrangement, such as Ezrin (82), and signaling proteins downstream of Lyn, such as PI3K and Bruton's tyrosine kinase (Btk), were also contributors. Lyn was found to suppress BCR signaling in mature B cells, increasing B-cell reactivity and antibody production in Lyn<sup>KO</sup> mice (83).

While initial animal studies relied on germline deletion of Lyn, B-cell-specific knockout, using Cre recombinase expressed under the control of the CD79a promoter, was sufficient to induce autoimmunity and myeloproliferation (84). Parallel studies showed that selective deletion of Lyn in dendritic cells (CD11c-driven Cre) was also sufficient to drive disease, with overexpression of the T-cell-costimulatory molecules CD80 and CD86 in Lyn<sup>KO</sup> conventional dendritic cells (85) and increased inflammatory signaling in cultured cells (described in the following discussion).

Lyn suppresses ITAM signaling by phosphorylating immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which inhibit immune activation by recruiting inhibitory protein phosphatases and lipid phosphatases (86-88). Lyn was found to co-immunoprecipitate with and phosphorylate the ITIMs of CD22 and Fc $\gamma$ RIIb in B cells as well as mediate phosphorylation of their associated, SH2-domain-containing phosphatases Shp-1, Shp-2, and Ship-1 (78,87,89). The integrin CD11b was also shown to recruit Lyn following either BCR or toll-like receptor (TLR)2 ligation, leading to Shp-1 activation and dampened B- and dendritic-cell activation (90, 91).

TLR pathways, which trigger inflammatory responses to pathogen-associated molecular patterns (eg, bacterial/viral DNA, bacterial/fungal cell wall) are also regulated by Lyn. This link was first illustrated in 1993, when Lyn was found to co-immunoprecipitate with CD14 and become activated following lipopolysaccharide treatment of human monocytes (92). Downstream of TLRs, the adaptor proteins myeloid differentiation primary response 88



**Figure 2.** Lyn signaling in immune cells. Lyn positively regulates immunoreceptor signaling by phosphorylating and activating ITAM-coupled receptors (eg, the B-cell antigen receptor). Lyn negatively regulates immunoreceptor signaling by phosphorylating ITIMs and suppressive phosphatases (eg, Shp-1). Lyn can also modulate TLR signaling by processes less clearly defined.

(MyD88) (84,85) and caspase recruitment domain family member 9 (CARD9) (91) and the transcription factor IFN regulatory factor 5 (IRF5) (93) generate hyperactivated signaling in  $Lyn^{KO}$  myeloid cells and drive autoimmunity in  $Lyn^{KO}$  mice. These effects, moreover, appear to be cell-type specific. For example,  $Lyn^{KO}$  dendritic cells have elevated signaling responses and secrete more cytokines following treatment with ligands such as lipopolysaccharide and unmethylated CpG nucleotides, which activate MyD88-dependent TLR4 and TLR9, respectively (85,91,94). One study found a negative role for Lyn in macrophage TLR4 signaling (95), while others have reported a minimal role for Lyn in the macrophage response to TLR4 activation (91,96). In mast cells, Lyn may be required for optimal cytokine release and other downstream signaling processes in response to TLR4 activation (97).

TLR signaling is itself linked to autoimmune and inflammatory disease in humans and mice. Patients with severe forms of lupus have increased TLR4, TLR7, and TLR9 expression (98,99), and, in mice, TLR7 overexpression or chronic TLR7 and TLR9 signaling induces autoimmune disease (100-102). TLR4 signaling promotes autoimmune kidney pathology in the pristane hypergammaglobulinemia mouse model of lupus (103). Despite strong associations of Lyn and TLR signaling with autoimmune disease, the

mechanisms by which Lyn both positively and negatively regulates early TLR signaling remain unknown.

Together, these studies revealed a primary function of Lyn in negatively regulating ITAM and TLR signaling pathways, in some cases in a cell-specific manner. Lyn is therefore required to protect against overproduction of proinflammatory cytokines, hyperresponsive B-cell activation, and myeloproliferation. This central suppressive role for Lyn is a key factor that, when dysregulated, drives both myeloid and B-cell contributions to autoimmunity and chronic inflammation.

### Complex Effects of Positive and Negative Lyn Signaling

A clear, mechanistic map of Lyn function is still largely lacking due to the simultaneous participation of Lyn in positive- and negative-regulatory pathways; these effects are both complex and cell-type specific. The pleiotropic functions Lyn in B cells exemplify this duality. Like some other severely immunocompromised/autoimmune KO models (eg,  $Btk^{KO}$  (104),  $Shp-1^{KO}$  motheaten (105)),  $Lyn^{KO}$  mice have a defect in B-cell development, particularly in the transition of progenitor B cells to mature B cells (19). In the absence of Lyn, dampened BCR

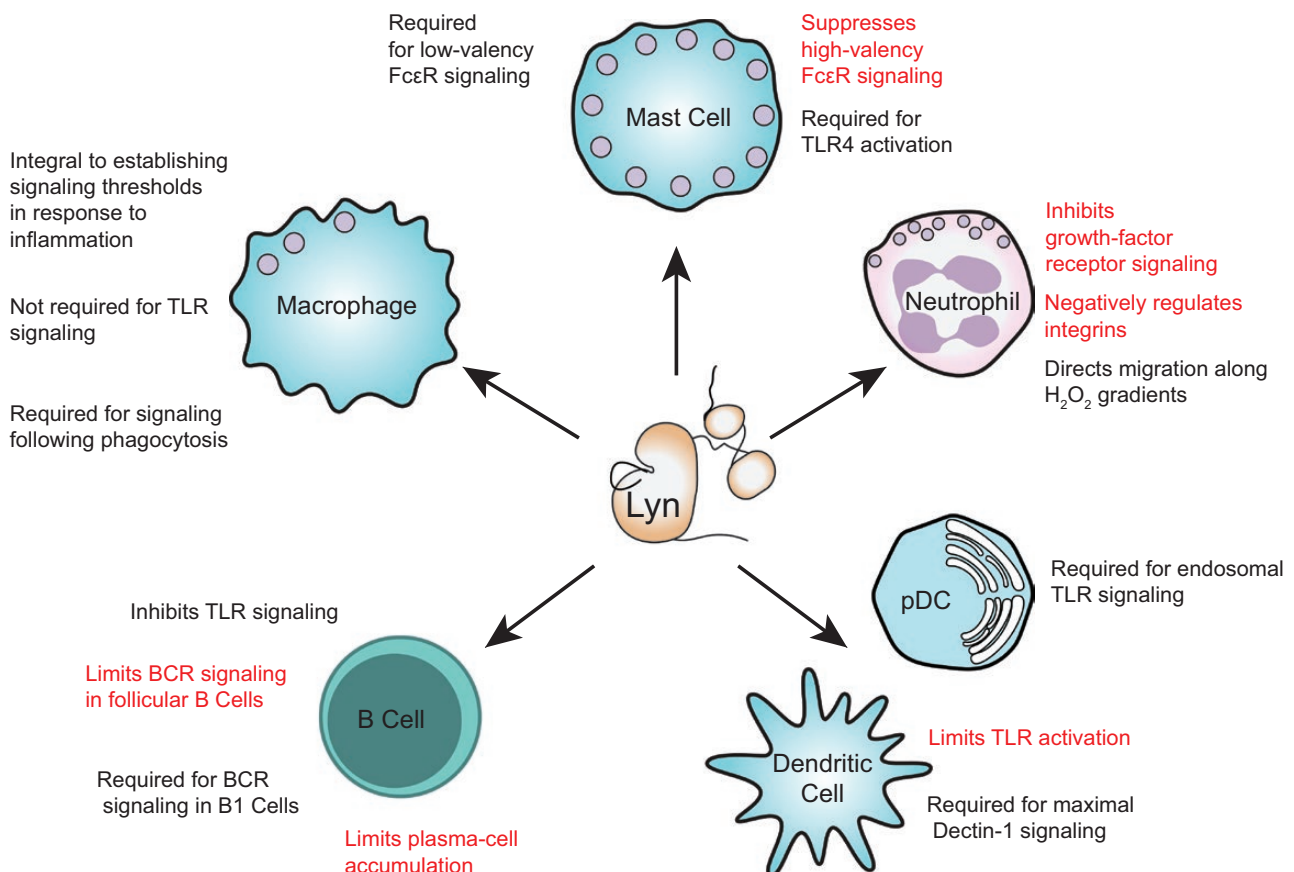
signaling leads to skewing of the B-cell repertoire, with autoreactive and other strongly engaging cells preferentially evading negative selection, surviving positive selection, and going on to produce autoantibodies. B1 B cells, which respond to antigens independently of T cells, also require Lyn for downstream signaling in response to BCR ligation (106). In contrast, Lyn deletion in mature follicular B cells causes both a delay and an amplification of BCR signaling, while simultaneously making cells more resistant to apoptosis. This pushes B cells toward a more activated state and promotes further differentiation to antibody-secreting plasma B cells (20,107). There is therefore a seemingly paradoxical increase in B-cell activation and enrichment of autoreactive and plasma cells, despite the severe B-cell lymphopenia observed in Lyn<sup>KO</sup> mice (84).

Lyn also has both positive and negative roles in mast cells and plasmacytoid dendritic cells (pDCs). In mast cells, the directionality of Lyn signaling seems to vary with stimulus strength (108), with Lyn promoting ITAM-initiated Erk and Akt activation in response to low concentrations of stimulus, which induces poor clustering of FcεR1, but dampening activation in response to strong stimulation. Lyn function in pDCs seems similarly context

specific (85,109). In pDCs, Lyn promotes the trafficking of CpG nucleotides from the extracellular space to internal endosomes, where TLR9 is located, which potentiates the production of proinflammatory cytokines and type 1 IFNs (109).

Lyn seems to have a stronger positive role in neutrophil migration and trafficking. Peroxides, generated at the site of infection, oxidize cysteines in Lyn, leading to Lyn activation and directing neutrophil migration along the peroxide gradient toward the site of infection (110). Moreover, the nonobese diabetic mouse model of type I diabetes harbors a mutation (E393K) in the Lyn activation loop that increases activation-loop phosphorylation. This results in impaired neutrophil chemotaxis along bacterial N-formylated peptide gradients (111).

In respiratory and enteric infection models, Lyn<sup>KO</sup> mice suffer from increased bacterial burden, despite increased cytokine secretion. *Salmonella typhimurium* and *Pseudomonas aeruginosa* infections are more lethal to Lyn<sup>KO</sup> mice (91,112-114). While increased cytokine production in response to pathogens may result from a lack of negative regulation, Lyn simultaneously promotes pathogen phagocytosis and killing (113). The susceptibility of Lyn<sup>KO</sup> mice to infection epitomizes the dual positive and



**Figure 3.** Diverse roles of Lyn in immune cells. Lyn can have positive (black) or negative (red) signaling capabilities, depending on the cell type and context of receptor activation.

negative functions of Lyn in immunoreceptor signaling. Understanding how Lyn balances these functions, especially how Lyn regulates signaling across different cell types (Fig. 3), is critical to understanding the contexts in which cell- and receptor-specific dysregulation of immune-cell signaling promotes diseases such as autoimmunity and cancer.

### Alternative Splicing of *lyn*: LynA and LynB

RNA transcript from the *lyn* gene is alternatively spliced to produce 2 proteins, LynA and LynB, which differ only in a 21-residue LynA insert (115). The 5' alternative splice site within *lyn* exon 2 and the amino acid sequence of the LynA insert are highly conserved in mammals, from monotremes to primates (116) (Fig. 4). Despite the prominent role of Lyn in regulating immune activation, a lack of genetic tools constrained most research to the comparisons between wild-type and total Lyn<sup>KO</sup> mice, probing only the net effects of losing LynA and LynB.

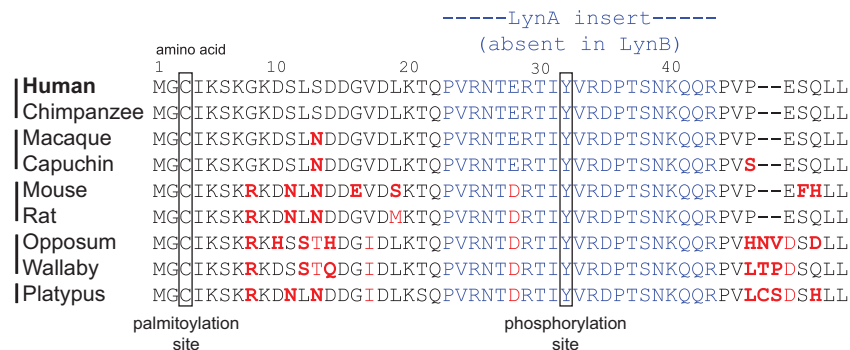
Noting that LynA and LynB proteins could be resolved by immunoblotting, Tadashi Yamamoto's group first demonstrated that they are differentially regulated—LynA protein was selectively downregulated upon BCR ligation (117). Using Lyn<sup>KO</sup> mast cells transduced with either LynA or LynB, Juan Rivera's group showed that LynA more effectively restored FcεR-dependent phospholipase activation, calcium signaling, and degranulation (118).

Our own group has found that LynA in macrophages is selectively degraded upon pan-SFK activation and that this process is extremely fast ( $t_{1/2} = \sim 1$  min) and selective (with at least 5-fold slower targeting of the SFKs Hck and Fgr in macrophages and Lck and Fyn in T cells subjected to parallel treatment) (41,55,119). This loss of macrophage LynA frustrates the transmission of an initially strong burst of SFK signaling, blocking downstream Erk and Akt activation

(41). LynA degradation is triggered by phosphorylation of its unique-region insert at tyrosine 32, which flags LynA for polyubiquitination by the E3 ligase Casitas B-lineage lymphoma (c-Cbl) (55). The aforementioned decay in signaling occurs despite the continued presence of activated LynB, Hck, and Fgr, suggesting that LynA is uniquely capable of potentiating activating signaling in macrophages in the absence of ITAM clustering. Furthermore, expression of Lyn is upregulated in response to the proinflammatory cytokine IFN $\gamma$ , which bypasses the signaling checkpoint and promotes LynA-dependent Erk and Akt activation (41). This tuning process is cell-type specific: mast cells, which express little *cbl* messenger RNA (35) or c-Cbl protein (55), are unable to degrade LynA and, as a consequence, are much more responsive to SFK activation (55). Together these data suggest that LynA is an important environment-sensitive and cell-specific rheostat, tuning myeloid-cell sensitivity and setting the signaling threshold.

Using their overexpression/reconstitution model, Juan Rivera's group showed that LynB was more effective than LynA at restoring phosphorylation of SHIP-1 and ITIM complex formation in mast cells (118).

While analysis of Lyn signaling suggested unique roles for LynA and LynB, further mechanistic study had been hampered by a lack of genetic models in which LynA or LynB could be expressed selectively at a physiological level, with appropriate responsiveness to transcriptional and posttranslational regulation. To address this problem, the Freedman lab generated splice-fixed, germline knockout mice (LynA<sup>KO</sup> or LynB<sup>KO</sup>) that selectively express one or the other Lyn isoform (96). LynB<sup>KO</sup> mice develop more severe autoimmune disease, with profound splenomegaly, antinuclear antibodies, and glomerulonephritis resembling total Lyn<sup>KO</sup> mice and human SLE patients. Moreover, selective loss of LynB increased inflammatory TLR signaling. Together, these data suggest that LynB is the



**Figure 4.** Conservation of the LynA unique region. Sequence alignment of LynA N-termini from ape (human, chimpanzee), monkey (rhesus macaque, capuchin), rodent (mouse, rat), marsupial (opossum, wallaby), and monotreme (platypus). Sequence differences (with reference to human) are in red, with the least conservative substitutions in bold. The LynA insert is shown in blue. Palmitoylation (C3) and tyrosine phosphorylation (Y32) sites are boxed. Sequences obtained from the Universal Protein Resource (UniProt entries: P07948, H2R453, F7BV42, A0A6J3JBZ6, P25911, Q07014) and Suthers and Young (116).

dominant negative regulator of immune function that protects against autoimmune disease, in concert or in competition with LynA signaling. The combined activities of LynA and LynB tune the inflammatory activation threshold of immune cells, parsing the net signaling effect of activating and suppressive signals in the complex tissue environment.

### Lyn Signaling in Nonimmune Cells

Although Lyn is most highly expressed in hematopoietic cells, data from the Human Protein Atlas (120) and other reports suggest that Lyn is expressed in many nonhematopoietic cells and tissues, including the pancreas (120,121), lungs, brain (122,123), and several epithelial cell types (120). Aberrant Lyn signaling has been implicated in several cancers, particularly in breast and prostate. Lyn inhibition and knockdown experiments have revealed that Lyn [and LynA in particular (124)] promotes migration, invasion, and metastasis of breast-cancer cells (124-126). In breast-cancer cells, LynA Y32 phosphorylation by the epidermal growth factor receptor (EGFR) induces proliferation due to increased activation of MCM7, which licenses DNA replication and proliferation (127). Correspondingly, high Y32 phosphorylation, as measured by the intensity of anti-pY32 antibody staining of immunohistochemical sections, correlates with a worse breast cancer survival prognosis (127). Similarly, Lyn expression is increased in aggressive-variant prostate cancer compared to hormone-naïve cancer and benign hyperplasia (128). Lyn is normally expressed in the prostate epithelia, and Lyn<sup>KO</sup> mice have abnormal prostate gland development, characterized by underdeveloped ducts (129). This may be due to interactions between Lyn and androgen receptor (AR) that protect AR from proteasomal degradation and preserve AR transcriptional activity (128). These studies suggest that Lyn may promote positive signaling pathways in both epithelial and cancer cells, particularly those activated by hormones or growth factors.

Lyn is also likely to have complex effects in cancer and immune hormone-receptor signaling. In breast cancer, however, mutations that increase the catalytic activity of Lyn promote resistance to the antiestrogen fulvestrant and the PI3K inhibitor BKM120 in estrogen receptor (ER) + breast cancers (130).

Lyn is also expressed in some untransformed nonhematopoietic cells. In pancreatic acinar cells, Lyn is activated by treatment with growth factors and hormones, including insulin and insulin-like growth factor-1 (IGF-1) (121). The nonobese diabetic (NOD) mouse model of type I diabetes harbors an activating mutation in Lyn, but how this mutation affects pancreatic islet cells and the development of diabetes in this model remain unclear.

Lyn has been reported to have different roles in neuronal activation, depending on the receptor. Lyn is activated by the  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor following glutamate administration, which promotes mitogen-activated protein kinase (MAPK) activation and induces brain-derived neurotrophic factor expression in neuronal cultures (131). Brain-derived neurotrophic factor (BDNF) is important for the development of long-term plasticity and memory formation, which suggests that targeting Lyn signaling in select AMPA-expressing neurons could improve cognitive defects in some conditions, such as aging (132). Interestingly, Lyn<sup>KO</sup> mice have a defect in motor activity that stems from overactive N-methyl-D-aspartate (NMDA) receptor activation (123). Like the AMPA receptor, the NMDA receptor is activated by glutamate. In contrast to its positive role in AMPA receptor signaling, Lyn negatively regulates NMDA receptor, highlighting again its context-specific activities and the complex, integrated effects of genetic or therapeutic loss of Lyn function. Lyn also negatively regulates dopamine release (122), possibly through phosphorylation of synaptophysin proteins (133), which affects reward-seeking behaviors, such as alcohol dependency.

Studies in both cancer and noncancer settings reveal that, as in immune cells, Lyn can have positive and negative regulatory roles in multiple signaling pathways. Because of the broad expression of Lyn in tissues as varied as breast, prostate, pancreas, and brain and the potential impact of Lyn in hormone, growth factor, and neurotransmitter signaling, further research is needed to understand how Lyn impacts the development of endocrine and neuronal disease. Indeed, targeting Lyn signaling in Alzheimer's disease has shown promise (134). A clearer understanding, particularly the roles of LynA and LynB, is needed to develop more therapeutics that tune Lyn signaling to achieve desired outcomes in multiple cell types.

### Concluding Remarks

SFK signaling is necessary for nonhematopoietic and hematopoietic cell survival, proliferation, and migration. This combined with unique functions in the immune system necessitates exquisite control of SFK activation and signaling. Despite the therapeutic potential of modulating SFK signaling, there is still much to discover about how the activities of all the SFKs work together to parse different types of receptor signals and yield an appropriately integrated functional response. This extends to the differential roles of the Src family members, governed in large part by their peculiar unique-region interactions and regulatory mechanisms. Given that the battery of expressed SFKs differs by cell type, it is



tempting to speculate that the unique combination of SFK functions within each cell helps to confer its unique functional identity (in sensitivity, ligand preference, signaling and functional kinetics, and pathway biases). Intriguingly, much of the protein machinery within these signaling pathways is shared, especially in B and myeloid cells, so the top-down value of the SFK interactome may be essential for determining cell-specific outcomes. Importantly, the immune-cell SFKs also add orthogonal immune signaling functionality on top of the universal survival and proliferation pathways of Src, Yes, and Fyn by phosphorylating tandem tyrosines within ITAMs, creating specific docking sites for the dual-SH2 kinases Syk and Zap70 (135,136). The SFK Lyn has emerged as a particularly complex mediator of proinflammatory and immunosuppressive signaling, regulated by cell identity and environment. In immune cells, Lyn has a central regulatory role in phagocytosis and killing of infected and dying cells and release of inflammatory cytokines. Lyn also helps to set immunoreceptor thresholds and modulate the amplitude and kinetics of intracellular signaling. Some, but not all, of these opposing functionalities may be attributed to distinct roles of the splice variants LynA and LynB. The special role of Lyn is exemplified by its regulation by a feedback process that senses expression/function of LynA and LynB but is separate from expression sensors for the other SFKs (96). Despite the complex roles of Lyn in regulating the immune response and the wide expression of Lyn in nonimmune tissues, there is still much unknown about Lyn function. The development of severe autoimmunity in total Lyn<sup>KO</sup> and LynB<sup>KO</sup> mice is an aging-dependent process (18,19,96), and the aging factors that may couple with loss of LynB function are as yet undescribed. Overactive Lyn signaling has also been implicated in Alzheimer's pathology (134) and breast and prostate cancer, while Lyn-activating small molecules have been tested as therapies for type I diabetes (137). Mapping the intra- and intermolecular interactions formed by the unique regions of LynA, LynB, and the other SFKs could inform the development of novel therapies for autoimmune, neurological, and endocrine disorders, allowing selective inhibition of proinflammatory *vs* suppressive pathways and even cell- or tissue-targeted therapies.

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